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EXPERIMENTAL ARTICLES

Ecophysiological Properties of Photosynthetic Bacteria from the Black Sea Chemocline Zone

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Abstract—In May 1998, during the fifty-first voyage on board the research vessel *Professor Vodyanitskii*, a comparative study was conducted of the species diversity of green and purple sulfur bacteria in the water column of the chemocline zone at deep-sea stations and on the bottom surface of the Black Sea shallow regions. At three deep-sea stations, the accumulation of photosynthetic bacteria in the chemocline zone at a depth of 85–115 m was revealed on the basis of the distribution of potential values of carbon dioxide light fixation. The location of the site of potential carbon dioxide light fixation suggests that the photosynthesis may be determined by the activity of the brown *Chlorobium* sp., earlier revealed at these depths. Enrichment cultures of brown sulfur bacteria were obtained from samples taken at the deep-sea stations. By morphology, these bacteria, assigned to *Chlorobium* sp., appear as nonmotile straight or slightly curved rods 0.3–0.5 × 0.7–1.2 μm in size; sometimes, they form short chains. Ultrathin sections show photosynthetic antenna-like structures, chlorosomes, typical of *Chlorobiaceae*. The cultures depended on the presence of NaCl (20 g/l) for growth, which corresponds to the mineralization of Black Sea water. The bacteria could grow photoautotrophically, utilizing sulfide, but the Black Sea strains grew much more slowly than the known species of brown sulfur bacteria isolated from saline or freshwater meromictic lakes. The best growth of the strains studied in this work occurred in media containing ethanol (0.5 g) or sodium acetate (1 g/l) and low amounts of sulfide (0.4 mM), which is consistent with the conditions of syntrophic growth with sulfidogens. The data obtained allow us to conclude that the cultures of brown sulfur bacteria are especially adapted to developing at large depths under conditions of electron donor deficiency owing to syntrophic development with sulfate reducers. The species composition of the photosynthetic bacteria developing in the bottom sediments of shallow stations differed substantially from that observed at deep-sea stations. Pure cultures of the green *Chlorobium* sp. BS 1C and BS 2C (chlorobactin as the carotenoid), purple sulfur bacteria *Chromatium* sp. BS 1Ch (containing spirilloxanthine series pigments), and *Thiocapsa marina* BS 2Tc (containing the carotenoid okenone) were obtained from samples of sediments at shallow-water stations. Brown sulfur bacteria were absent in the sediment samples obtained from the Black Sea shallow-water stations 1 and 2.

Key words: Black Sea, meromictic reservoirs, anoxygenic phototrophic bacteria, relict ecosystems.

Kriss and Rukina were the first to discover photosynthetic bacteria in samples obtained from large depths of the Black Sea [1]. However, no cultures of these microorganisms were isolated, and their properties were not studied. Later, on the basis of electron-microscopic studies and cell count on filters, the presence of purple bacteria morphologically similar to *Thiocapsa* (2.3×10^5 cells/ml) was established below the chemocline zone at a depth of 160–200 m [2, 3]. In 1976, cultures of the purple *Chromatium* sp. and *Thiocapsa* sp., as well as cultures of brown and green sulfur bacteria, were obtained from sediment samples of shallow and deep-sea stations of the Black Sea [4]. According to their morphological properties and pigment composition and the presence of bacteriochlorophyll *e* (BCl *e*) and the carotenoid isorhenierathine, the green sulfur bacteria were defined as *Chlorobium phaeobacteroides*. These microorganisms are strict anaerobes, require sulfide as an electron donor, depend on light, and are incapable of growing in the dark. Their constant presence in deep-sea sediments in the absence of light did not find a convincing explanation. However, it was suggested that both purple and green sulfur bacteria are brought from littoral sediments of shallow regions and then survive in deep-sea silts through a sustaining metabolism—slow fermentation of reserve polysaccharides [4]. This explanation was supported by the fact that the purple bacteria isolated appeared to be freshwater species. Later, evidence that the brown sulfur bacteria of the family *Chlorobiaceae* develop in the deep waters of the Black Sea in the chemocline zone was obtained [5, 6]. The evidence of the presence of anoxygenic phototrophic bacteria was based on the distribution in the water at a depth of 70–100 m of the pigments BCl *e* and the carotenoid isorhenierathene and its homologues specific for this group of photosynthetic

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bacteria. It is important to note that the authors did not identify bacteriochlorophyll *a* and the spirilloxanthine series carotenoids inherent in purple bacteria at depths of up to 250 m. Thus, the occurrence of considerable amounts of purple bacteria in the Black Sea water was questioned, while the existence of a layer of brown sulfur bacteria may be considered an established fact. Later, cultures similar to *Chlorobium phaeobacteroides* were isolated from samples obtained from a depth of 77–83 m [7, 8]. It was shown that, compared to other well-known species of brown sulfur bacteria, the Black Sea strains are the ones best adapted to life at an extremely low illumination (0.25 μEinst m^{-2} s^{-1}). The Black Sea *Chl. phaeobacteroides* strains required sulfide and a strongly reduced medium (Eh –560 mV) and, like other green sulfur bacteria, could not develop aerobically or anaerobically in the dark.

Thus, it was proven that, in deep regions of the Black Sea, in the chemocline zone at a depth of 74–100 m, the development of *Chlorobium* brown sulfur bacteria extremely adapted to life at low illuminations occurs.

However, their contribution to the carbon and sulfur cycles in the Black Sea ecosystem has not been clarified yet. The available data were primarily obtained by the extrapolation of laboratory experiments with isolated cultures of brown sulfur bacteria. The strain *Chlorobium* MN1 was lost, and some of its properties, as well as the exact species affiliation, remain unclear.

The objectives of our investigation were to carry out a comparative study of the species diversity of green and purple sulfur bacteria in the water column of the chemocline zone and in the shallow regions of the Black Sea, to isolate cultures of photosynthetic bacteria, and to study their ecophysiological properties in order to determine their adaptability to the conditions in the Black Sea ecosystem and the possible role of anoxygenic phototrophs in the sulfur and carbon cycles in this unique water body.

MATERIALS AND METHODS

In the course of the fifty-first voyage on board the research vessel *Professor Vodyanitskii* in May 1998, water and sediments were sampled to inoculate selective media for cultivation and enumeration of phototrophic bacteria and sulfur reducers, as well as to determine the total number of microorganisms by using the fluorescent method with DAPI. In addition, the potential activity of photosynthesis and the vertical distribution of phototrophs were determined by measuring fixation of labeled [^{14}C] carbon dioxide. The exposure of light and dark vials from the selected depths was carried out at an artificial illumination of 300–500 lx under luminescent lamps. The exposition time was 12 h.

Pfennig's medium with 2% NaCl, containing additionally 1 g/l of sodium acetate and 100 mg/ml of yeast extract, was used for phototroph quantification. The final pH was 6.8 [9]. Malate–sulfide medium was used for sulfidogen enumeration [10]. The number of water and sediment microorganisms was counted by the serial dilution method in agarized media. Pure cultures were isolated by repeated subculturing of individual colonies from end-point dilutions. The conditions optimal for growth were determined by varying such parameters as light, temperature, pH, and sulfide concentration, using the basal medium for phototrophs [9]. The cultivation was carried out in 30-ml vials with hermetically sealed screw caps. The temperature parameters of the growth of strain BS 5C were determined in a gradient thermostat [11] using two methods: by light fixation of ^{14}C-labeled carbon dioxide and from the optical density of the grown cultures. The morphological characteristics were studied under a light microscope or by electron microscopy of ultrathin sections [12]. The pigment composition of the cultures isolated was determined from the in vivo spectrum and the spectrum of acetone–methanol (2 : 7) extracts on an SF-56 spectrophotometer.

The oxygen content in the Black Sea water was determined by means of Oxy CTD and chlorophyll was determined by its fluorescence. The corresponding equipment was fixed on the samplers. Sulfide was determined spectrophotometrically using the standard technique (from methylene blue formation in the reaction of paraphenylenediamine with sulfide).

RESULTS AND DISCUSSION

Field Studies

In May 1998, during the international Russian–Swiss expedition in the Black Sea on board the research vessel *Professor Vodyanitskii*, the development of phototrophic microorganisms at three shallow and four deep-sea stations was studied.

The surface water temperature during the period of study was 13–14°C. Hydrogen sulfide at the shallow stations 1 and 2 was absent, while below 1 cm, the sediments were dark gray and were characterized by an Eh value varying between –150 and –200 mV, which gave evidence of their reduced state. The hydrogen sulfide layer at the 650-m-deep station 3 at the continental slope began at a depth of 185 m, whereas at the deepest stations 4, 5, and 6, the hydrogen sulfide layer boundary was at 105–115 m. At the same time, oxygen disappeared at a depth of 75–90 m, as measured by Oxy CTD; hence, the 20–25 m chemocline zone was characterized by extremely low oxygen and hydrogen sulfide contents. As noted earlier [5, 6], precisely this zone accounted for the occurrence of the BCl *e* of the *Chlorobium* brown bacteria.

Our investigations coincided with the period of extremely low seasonal activity of phytoplankton in the Black Sea [13]. The potential values of light and dark fixation of carbon dioxide at different stations are shown in Figs. 1a–1d.

The potential photosynthesis curve at station 1a (depth, 26.1 m) coincided with the distribution of chlo-

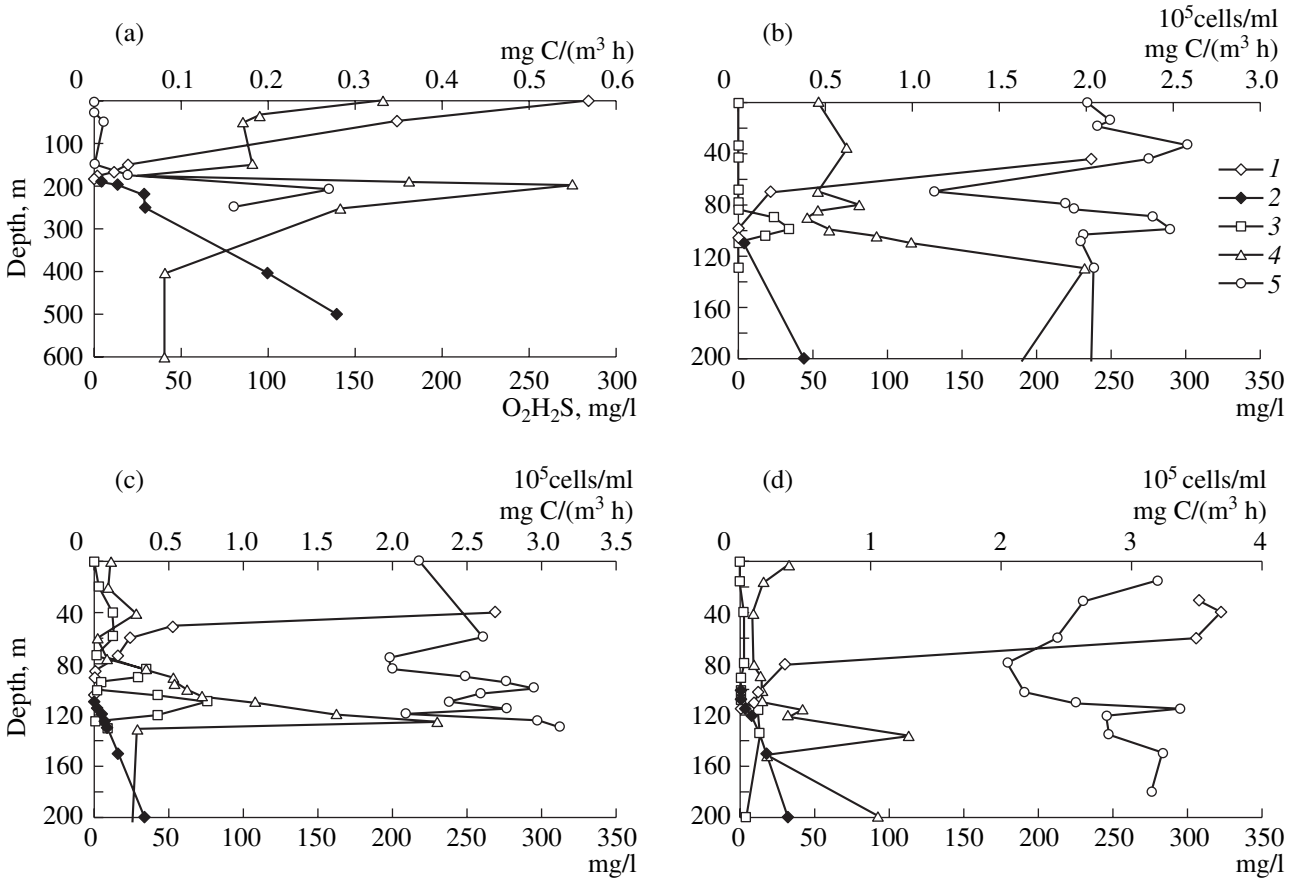

Fig. 1. Profiles of the hydrogen sulfide and oxygen contents, microbial number, and potential activity of carbon dioxide assimilation in the light and in the dark at some deep-sea stations of the Black Sea (May 1998): (a), (b), (c), (d) are stations 3, 4, 5, 6, respectively. (*1*) Oxygen, mg/l; (*2*) hydrogen sulfide, mg/l; (*3*) potential rate of carbon dioxide light fixation, mg C/(m^3 h); (*4*) potential rate of carbon dioxide dark fixation, mg C/(m^3 h); (*5*) number of microorganisms, cells/ml (DAPI).

rophyll *a* fluorescence (data not shown) as shown by the equipment fixed to the winch cable; the maximum was at a depth of 6 m (1.4 mg C/m^3 daily or 0.058 mg C/m^3 per hour; dark fixation was about 0.77 mg C/m^3). At station 1b, which is 12 m deep, photosynthesis smoothly increased toward the bottom, attaining 0.04 mg C/m^3 per hour. At the deep-sea stations 3, 4, 5, and 6, only a slight homogeneous fluorescence was recorded, which was indicative of the small amount of phytoplankton. Only at station 5 was the potential carbon dioxide fixation in the light in the photic zone higher than that in the dark. The phytoplankton light fixation values at this station were the highest at a depth of 40–60 m. This coincided with the salinity jump; the values were 3.12–3.36 mg/m^3 per 24 h or 0.13–0.14 mg/m^3 per 1 h.

At three deep-sea stations (4, 5, and 6), the accumulation of photosynthetic microorganisms was discovered from the distribution of the carbon dioxide light fixation values in the chemocline zone at a depth of 85–115 m. This photosynthesis zone is free of or extremely low in oxygen and hydrogen sulfide. The potential photosynthesis maxima were at the upper boundary of the anaerobic zone, which can easily be determined by the appearance of a hydrogen sulfide odor and a sharp increase in the water content of phosphorus, ammonium, and methane [14]. At station 4, the potential photosynthesis maximum was at 100 m, whereas at station 5, photosynthesis was recorded in a wide zone ranging between 80 and 120 m. At station 6, it was feebly marked at depths of 110 and 130 m.

The site of the potential carbon dioxide light fixation allows us to suggest that the photosynthesis might have been determined by the activity of the brown *Chlorobium*, revealed at these depths earlier [8]. Interestingly, at station 3, situated at the slope (depth, 650 m), the sulfide zone boundary was at a depth of 185–190 m, and the peak of potential photosynthesis was revealed at a depth of 180 m, i.e., beyond the theoretically possible penetration of light into the seawater. It may be suggested that the phototrophic microorganisms appeared at this depth because of sedimentation. At the same time, we cannot but note that it is at these depths (160–200 m) that the accumulation of coccus-like bacteria resembling the purple sulfur bacteria *Thiocapsa* sp. was revealed [2]. However, the purple bacteria were not iso-

lated from the Black Sea water samples, nor was any other convincing evidence of their accumulation at such large depths obtained.

Characteristics of the Cultures of the Microorganisms Isolated

Photosynthetic bacteria of the chemocline zone. Despite a number of factors giving evidence of the development of phototrophic bacteria in the chemocline zone (absence of oxygen, low redox potential, increased activity of carbon dioxide assimilation in light), no growth of brown sulfur bacteria was observed when solid media were directly inoculated with 1–10 ml of the chemocline water or sediment samples. No growth of enrichment cultures was observed when liquid medium was inoculated with fresh samples. Brown sulfur bacteria were detected by us only via the modified method of Winogradsky's columns. Deep-sea (1700 m) sediments from station 5 or 6 were placed (20% of the total volume) in tall 20-ml vials closed with rubber stoppers, and the vials were filled with chemocline (80–115 m) water from the respective stations. Yeast extract (100 mg/l) was added to such vessels, and they were exposed to scattered natural light (in the day–night regime) at room temperature. The development of an anaerobic syntrophic association of phototrophs and sulfidogens was initiated as a result of incubation. In one month, the water in the vials turned dark brown due to the abundant growth of the bacteria. The microorganisms were later cultivated in Pfennig's liquid medium with 2% NaCl [9]. Two morphologically similar monocultures of brown *Chlorobium* were obtained from the sediment and water samples of station 5 (strain BS 5C) and station 6 (strain BS 6C). Strain BS 5C was studied in detail. Both strains could grow independently of other microorganisms in a medium with different reducing agents: 1.5–2 mM sulfide or crystalline or powdered sulfur (2 g per 1 l of medium). The culture could grow for a long time only in liquid medium; however, growth in the form of individual colonies in end-point dilutions in agarized medium became possible when repeatedly washed Difco agar was used. However, we failed to obtain a pure culture because of the unstable growth in agarized medium.

According to morphological characteristics, the *Chlorobium* BS 5C bacteria are tiny nonmotile straight or slightly curved rods, measuring 0.3–0.5 × 0.7–1.2 μm; sometimes they form short chains, which are frequently curved (Fig. 2a). Such a morphology is characteristic of the species *Chl. phaeovibrioides*. The photosynthetic antenna-like structures, chlorosomes, typical of *Chlorobiaceae* bacteria are well seen on ultrathin sections (Figs. 2b, 2e). Chlorosomes allow brown and green sulfur bacteria to grow at low illuminations that cannot be used by other phototrophs. The bacteria exhibit a gram-negative type of cell wall and multiply by nonsymmetrical division (Figs. 2b–2e). Dense polyphosphate granules, characteristic of many green sulfur bacteria, occur in the cells.

The absorption spectra of whole cells, as well as the spectra of the methanol–acetone extracts of our strains BS 5C and BS 6C, virtually coincide with the spectra of strain MN1 of *Chl. phaeovibrioides* isolated by Overmann [8]. The main BCl *e* maximum in strain BS 5C in vivo is at 718 nm (715 nm for MN1); in the extract, it is at 652 nm (648 nm for MN1). The absorption maximum of the carotenoid isorhenierathine in strain BS 5C whole cells is at 517 nm (Figs. 3a, 3b).

The cultures depended on the presence of 20 g/l NaCl in the medium for growth, which was consistent with the mineralization of water in the Black Sea. The optimum growth was recorded at 21–24°C. Thus, the microorganisms isolated are mesophils but are adapted to life at decreased temperatures, as evidenced by the growth curve shape (Fig. 4) and almost complete absence of growth at 36°C. At 15°C, the growth rate of the BS 5C culture decreases by approximately a factor of 2. It was established that the microorganism is capable of anaerobic growth in light in the presence of 2 mM sulfide and 1 g/l of sodium acetate. A higher sulfide concentration inhibits the growth of strain BS 5C. The strains of the brown sulfur bacteria isolated differ from other known isolates of brown *Chlorobium* in the low growth rate under laboratory conditions. The culture attained the maximum biomass with the stationary growth phase, which occurred after approximately one month of growth. The best growth of the Black Sea strains occurred in the syntrophic association, likely with sulfate reducers, in media containing ethanol (0.5 g/l) or sodium acetate (1 g/l) and low amounts of sulfide (0.4 mM); the best growth under these conditions occurred in the presence of ethanol. Long-term culture growth was maintained in the autotrophic medium in the presence of powdered elemental sulfur.

Photosynthetic bacteria from the sediments of shallow stations. The species composition of the photosynthetic bacteria developing in the bottom sediments of the shallow stations 1b (12 m) and 2 (64 m) differed substantially from the deep-sea species composition. From station 1b sediment samples, we isolated pure cultures of the green sulfur bacterium *Chromatium* sp. BS 1C (the carotenoid chlorobactin); the purple sulfur bacterium *Chromatium* sp. BS 1 Ch, containing spirilloxanthene series pigments; and *Thiocapsa marina* BS 1bTc, containing the carotenoid okenone, which distinguishes this species from the widespread freshwater species *Thiocapsa roseopersicina.* The absorption spectra of two strains isolated are shown in Figs. 3b, 3d, and 5. Note that four more *Thiocapsa marina* strains containing the carotenoid okenone are known, and all of them were isolated from sea lagoons of the Mediterranean and White seas [15]. The green sulfur bacteria of the genus *Chlorobium* were also obtained by us in enrichment cultures from 60-m

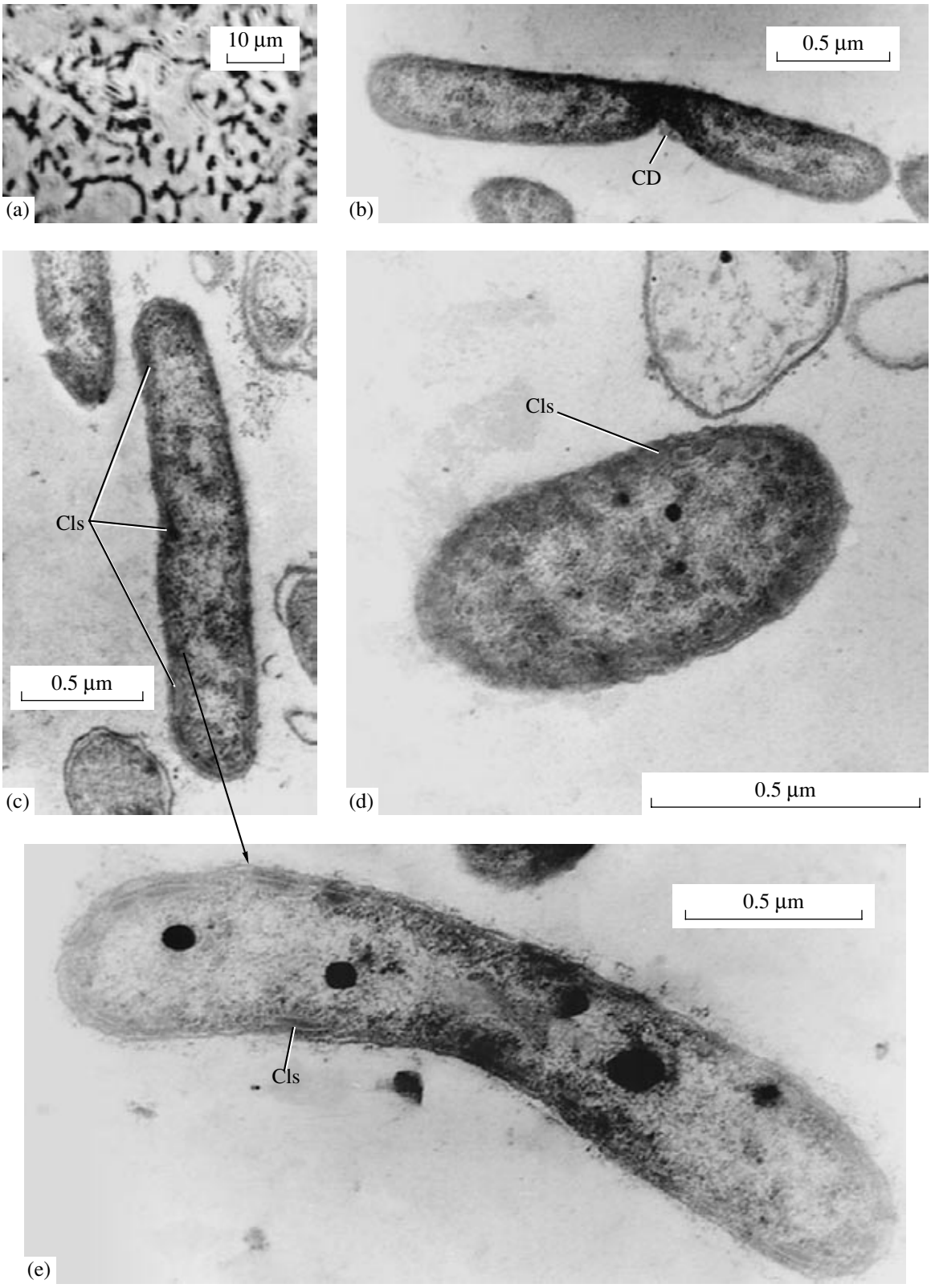

Fig. 2. Morphology and ultrafine structure of brown sulfur bacteria, strain BS 5C. (a) light microscope; (b–e) transmission electron microscope, ultrathin sections. One can see nonsymmetrical cell division (CD), location of antenna-like photosynthetic structures, chlorosomes (Cls), the gram-negative type of the cell wall (CW) structure with a markedly pronounced three-layered external membrane, and dark round polyphosphate (PP) inclusions.

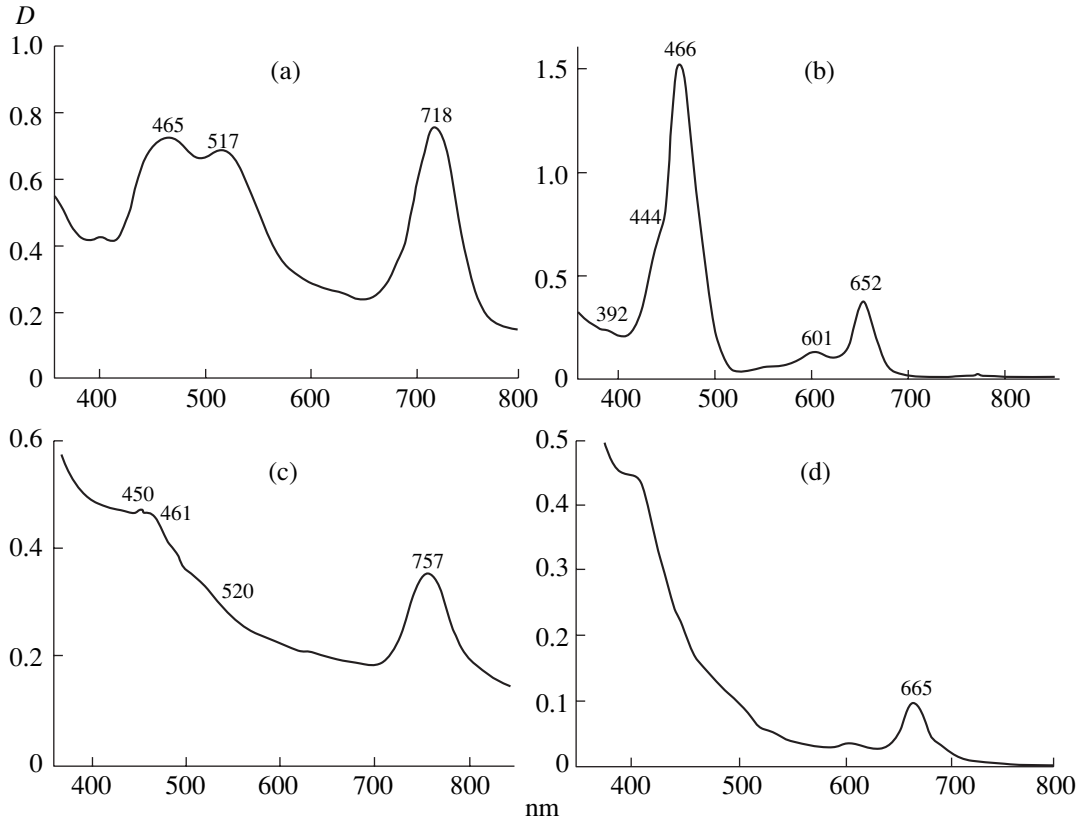

Fig. 3. Absorption spectrum of (a, b) the brown sulfur bacterium, strain BS 5C, isolated from the water and sediment of the deep-sea station 5 and (c, d) the green sulfur bacterium from the sediment of the shallow station 1. (a, c) Whole cells; (b, d) methanol–acetate extracts.

sediment samples (station 2). Thus, the photosynthetic bacteria revealed at the littoral stations are characteristic of shallow regions of different seas. Brown sulfur bacteria were absent in the sediment samples from the shallow Black Sea stations 1 and 2.

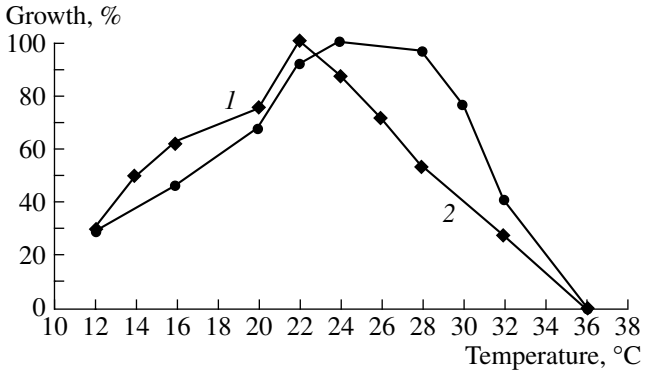

Fig. 4. Influence of temperature on the growth of the brown phototrophic bacteria *Chlorobium* sp. BS 5C. (*1*) Growth assessed by the rate of carbon dioxide light fixation, as the percentage of the maximal value; (*2*) growth assessed by the optical density, as the percentage of the maximal value.

It was established in the course of this work that the brown sulfur bacteria *Chlorobium* occur only at deep-sea stations, whereas green and purple sulfur bacteria are characteristic of shallow sediments at depths within the photic zone (64 m according to our data). We also obtained indirect evidence of the presence of brown sulfur bacteria in the chemocline zone of the deep-sea stations explored during our investigations in May 1998. Apparently, they arrive in the deep-sea sediments (1700 m or deeper) by sedimentation from the chemocline zone but are not brought from the littoral shallow regions, as thought before [4]. In the period of our investigations, the sulfide zone boundary was at a depth of 100–105 m at stations 4 and 5, while at station 6, it was lower, at a depth of 115–120 m. Thus, the illumination conditions for the development of photosynthetic bacteria in the chemocline were less favorable than in May–June 1988 [5, 6]. Nevertheless, we succeeded in recording potential photosynthesis in the chemocline regions at a depth of 80–130 m, where the BCl *e* maxima were earlier revealed. According to Yu.I. Sorokin's calculations, slightly more than 5 lx reaches a depth of 80–100 m [13]. Overmann [8] gives lower illumination values for the Black Sea

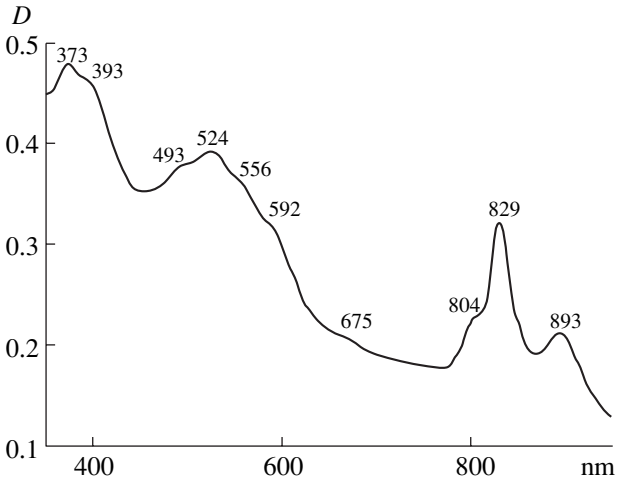

Fig. 5. Absorption spectrum of the whole cells of the purple bacterium *Thiocapsa marina* BS1aTc.

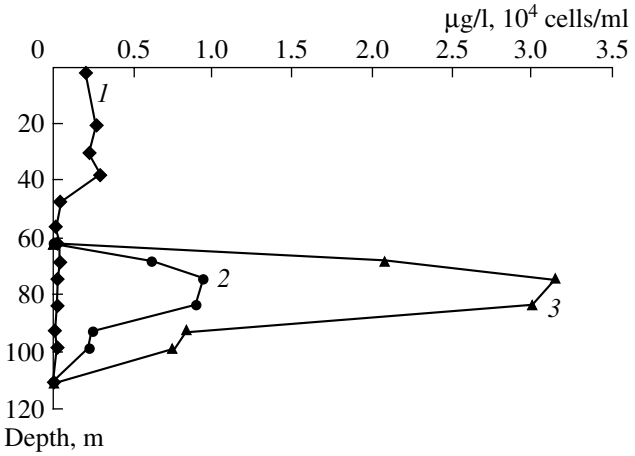

Fig. 6. Distribution of (*1*) chlorophyll *a* and (*2*) bacteriochlorophyll *e* (according to the data of Repeta and Simpson [6]) and the calculated number of anoxygenic phototrophic bacteria in the water column of the Black Sea deep zone. (*1*) Chlorophyll *a* content, μg/l; (*2*) bacteriochlorophyll *e* content, μg/l; (*3*) calculated number of phototrophic bacteria, 10^4 cells/ml. The calculation of the cell number and the wet biomass in the Black Sea water was carried out on the basis of the BCl *e* content, considering the weight of one cell to be 0.5×10^{-6} μg : 1000 : 200 μg BCl *e* = 5 μg BCl *e* = μg protein (Pr). Taking into account that protein makes up about 50% of dry biomass and carbon makes up about 50% of organic matter, *N* cells in 1 ml = Pr (μg/ml) × 6.7 : 0.5×10^{-6} = Pr × 6.7 × 10^6, where Pr × 6.7 = wet biomass.

chemocline—less than 0.5 lx (0.005% of surface illumination). It was proven experimentally [10, 16] that a 5-lx illumination is sufficient for photosynthesis to be carried out by both brown and green bacteria when they grow syntrophically and photoheterotrophically with sulfidogens. In this process, the generation time, according to [10], was 48 h for the green sulfur bacterium *Prostecochloris aesturarii* and 118 h for the brown *Chlorobium phaeovibrioides*. No growth of purple bacteria occurred at such a low illumination.

The data obtained by us also allow a conclusion that the cultures of brown sulfur bacteria isolated from the Black Sea chemocline are adapted to the development at large depths under conditions of electron donor deficiency and are capable of existing at an extremely low intensity of light of a certain spectral composition due to their high content of carotenoids.

Using Overmann's data (according to these data, the brown bacteria isolated from the Black Sea, strain MN1, contain, at low illumination, 210 μg of BCl *e* per 1000 μg of protein) and the data on the BCl *e* distribution in May 1989 [6], we enumerated phototrophs in the Black Sea chemocline (Fig. 6). According to these calculations, the maximal number of the brown *Chlorobium* is 3×10^4 cells per 1 ml in the chemocline zone. Repeta and Simpson [6] calculated that the total biomass of brown bacteria in the Black Sea chemocline at station BS2-2 in May 1989 was 0.5 g/m^2, whereas that of phytoplankton was 0.9 g/m^2; i.e., phototrophic bacteria constituted half of the phytoplankton biomass in the period of study. Assuming that phototrophic bacteria in the Black Sea chemocline inhabit approximately 50% of the water area (the depths below 1000 m account for about 200000 km^2 with the total area being 420000 km^2), it may be calculated that the biomass of the brown *Chlorobium* in spring 1989 constituted about 200000000000 m^2 × 0.125 g = 25×10^9 g or 25×10^3 tons for the whole Black Sea water area.

Since brown sulfur bacteria in the Black Sea seem to occur in the chemocline zone, where sulfide is virtually absent, it may be suggested that, for the photosynthesis to be carried out, they depend on syntrophic relations with sulfur reducers, which provide them with sulfide. The best growth of the cultures isolated occurred in syntrophic associations with sulfidogens. The number of sulfur-reducing and aerobic heterotrophic bacteria in the Black Sea water and bottom sediments is given in Table 2. It varied between single digits and hundreds of thousands in 1 ml of silt water. Note the increase in their number in the chemocline zone, which is indi-

Table 1. Location of the sampling stations

Station number	Depth, m	Coordinates
1a (5456)	26.1	45°12, 05′ N; 29°50, 80 E
1b (5457)	12.6	46°02, 99′ N; 30°29, 22 E
2 (5458)	64.0	44°52, 39′ N; 31°51, 74 E
3 (5459)	650	44°39, 12′ N; 31°45, 62 E
4 (5460)	2002	43°20, 03′ N; 32°09, 75 E
5 (5461)	2172	44°15, 22′ N; 33°59, 92 E
6 (5462)	2123	44°59, 91′ N; 37°30, 06 E

Table 2. Number of sulfate-reducing and aerobic heterotrophic bacteria in the Black Sea water and sediment, determined by the end-point dilution method in malate–sulfide medium

Station no./depth, m	Sulfur reducers, cells/ml
2/64, sediment deposits	10^3
3/190	10^3
3/200	10
3/650, surface sediment (0–2 cm)	10^2
4/80	NR
4/90	1
4/100	10
4/105	10^2
5/90	10^3
5/105	10^2
5/2172, surface sediment (0–2 cm)	10
6/90	10^2
6/100	10
6/115	10
6/120	10

Note: NR means "not revealed".

rectly indicative of their metabolic relations with the brown *Chlorobium* localized in this zone. Thus, the sulfide diffusing from the deep-sea layers does not seem to completely meet the requirements of *Chlorobium* for electron donors. This suggestion allows the geochemical role of the phototrophs in the Black Sea chemocline zone to be reconsidered and indicates the expediency to go ahead with the attempts to obtain by direct methods data on the contribution of phototrophic bacteria to the cycles of sulfur and carbon.

It remains unclear whether purple bacteria are present below the chemocline zone and what their function is. It is known that *Tca. roseopersicina* like the other purple bacteria, does not synthesize pigments under aerobic conditions. At the same time, purple bacteria contain bacteriochlorophyll *a* and carotenoids under strictly anaerobic conditions, and thus the absence of these pigments in the samples obtained in 1989 [7] gives us grounds to question the existence of the layer of purple bacteria at a depth of 160–200 m. Nevertheless, further study of this problem is still topical, since its solution may shed light on the specific features of the sulfur and carbon cycles in the Black Sea.

In conclusion, it should be emphasized that the Black Sea system is the only large-scale model known on the Earth which is suitable for studying sulfur and carbon cycles under light limitation and deficiency or complete absence of oxygen, i.e., under the conditions dominant in the Cambrian and Precambrian periods. The Black Sea brown bacteria should be considered as sulfide-dependent anaerobic phototrophs highly adapted to extreme conditions, which are the first settlers of the lower light boundary of the biosphere.

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REFERENCES

1. Kriss, A.E., *Morskaya mikrobiologiya* (Marine Microbiology), Moscow: Akad. Nauk SSSR, 1959.
2. Dickman, M. and Artuz, I., Mass Mortality of Photosynthetic Bacteria as a Mechanism for Dark Lamina Formation in Sediments of the Black Sea, *Nature*, 1978, vol. 275, no. 5677, pp. 191–195.
3. Caraco, N., The Importance of Photosynthetic Sulphur Bacteria in Microlaminae Formation in the Black Sea, *EOS*, 1985, vol. 66, p. 1302.
4. Hashwa, F.A. and Trueper, H.G., Viable Phototrophic Sulfur Bacteria from the Black See Bottom, *Helgol. Wiss. Meeresunters*, 1978, vol. 31, pp. 249–253.
5. Repeta, D.J., Simpson, D.J., and Jannash, H.W., Evidence for Anoxygenic Photosynthesis from the Distribution of Bacteriochlorophylls in the Black Sea, *Nature*, 1989, vol. 342, pp. 69–72.
6. Repeta, D.J. and Simpson, D.J., The Distribution and Recycling of Chlorophyll, Bacteriochlorophyll and Carotenoids in the Black Sea, *Deep Sea Res.*, 1991, vol. 38, no. Suppl. 2, pp. 969–984.
7. Jorgensen, B.B., Fossing, H., Wirsen, C.O., and Jannash, H.W., Sulfide Oxidation in the Anoxic Black See Chemocline, *Deep Sea Res.*, 1991, vol. 38.
8. Overmann, J., Cypionka, H., and Pfennig, N., An Extremely Low-Light-Adapted Phototrophic Sulfur Bacterium from the Black See, *Limnol. Oceanogr.*, 1992, vol. 37, no. 1, pp. 150–155.
9. Pfennig, N. and Lippert, K.D., Uber das Vitamin B12-bidurfnis Phototropher Schwefelbacterien, *Arch. Microbiol.*, 1966, vol. 55, pp. 245–256.
10. Biebl, H. and Pfennig, N., The Growth Yield of Sulfur Bacteria in Mixed Cultures with Sulfur- and Sulfate-

Reducing Bacteria, *Arch. Microbiol.*, 1978, vol. 117, pp. 9–16.

11. Kuntikov, E.I. and Gorlenko, V.M., Interrelation between Halo- and Thermotolerance in Anoxygenic Phototrophic Bacteria, *Mikrobiologiya*, 1998, vol. 67, no. 3, pp. 298–304.
12. Bryantseva, I.A., Gorlenko, V.M., Kompantseva, E.I., Achenbach, L.A., and Madigan, M.T., *Heliorestis daurensis* gen. nov., sp. nov., a New Alkaliphilic Rod-To-Coil-Shaped Phototrophic Heliobacterium from a Siberian Soda Like, *Arch. Microbiol.*, 1999, vol. 172, pp. 167–174.
13. Sorokin, Yu.I., *The Black Sea: Ecology and Oceanography*, Leiden: Backhuys, 2002, pp. 391–468.
14. Pimenov, N.V., Rusanov, I.I., Yusupov, S.K., Fridrich, Ya., Lein, A.Yu., Wehrli, B., and Ivanov, M.V., Microbial Processes at the Aerobic–Anaerobic Interface in the Deep-Water Zone of the Black Sea, *Mikrobiologiya*, 2000, vol. 69, no. 4, pp. 527–540.
15. Montesinonos, E., Guerrero, R., Abella, C., and Esteve, I., Ecology and Physiology of the Competition for Light between *Chlorobium limicolo* and *Chlorobium phaeovibrioides* in Natural Habitats, *Appl. Environ. Microbiol.*, 1983, vol. 46, pp. 1007–1016.