Limnol. Oceanogr., 37(1), 1992, 150–155

An extremely low-light-adapted phototrophic sulfur bacterium from the Black Sea

Abstract—Five strains of a brown phototrophic sulfur bacterium (*Chlorobium phaeobacteroides*) were isolated from the chemocline of the Black Sea (80-m depth). All contain bacteriochlorophyll *e* as the main photosynthetic pigment. The strains revealed extreme low-light adaptation of growth compared to 12 other green and purple sulfur bacterial strains. At very low light intensities (<4 μEinst m^{-2} s^{-1}), the Black Sea strain MN1 oxidized sulfide faster than the type strain 2430; the latter reached three times higher oxidation rates at light saturation. Low-light adaptation is achieved by an increase of light-harvesting pigments (175% compared to the type strain) and a very low maintenance energy requirement. The efficiency of energy transfer (59%) within light-harvesting structures (chlorosomes) is comparable in other green sulfur bacteria and, therefore, appears to be limited by the molecular organization of the chlorosomes. From data in the literature, a light transmission of 0.0005% of surface irradiance was calculated for the chemocline of the Black Sea. Extrapolation of our laboratory data revealed that anoxygenic photosynthesis could account for 4% of total sulfide oxidation under average light conditions in situ and for 13% at maximal surface irradiance in summer.

Striking changes in the vertical distribution of oxygen and sulfide in the Black Sea were reported recently (Murray et al. 1989). Since 1969 the sulfide-containing zone of this largest anoxic basin in the world has moved upward by 30 m to a depth of 74 m (center of west basin, Repeta et al. 1989). Above the sulfide zone the RV *Knorr* cruises in 1988 revealed a 20–30-m-thick water layer free of oxygen and sulfide. At the upper boundary of the sulfide zone, maxima of sulfide oxidation and carbon assimilation rates were observed, but the roles of microorganisms in these processes remained unclear (Jørgensen et al. 1991).

Earlier investigators did not find any viable phototrophic bacteria in the free water column of the Black Sea (Hashwa and Trüper 1978). Recently, a peak of bacteriochlorophyll (BChl) *e* was detected in the chemocline at 74 m (station BS2-2), indicating anoxygenic photosynthesis. BChl *e*-containing water layers extended from 68 to 98 m deep (Repeta et al. 1989). Anoxygenic photosynthesis could not be demonstrated in chemocline water, possibly because of cell damage during manipulation of the samples or inappropriate laboratory incubation conditions (Jørgensen et al. 1991). Therefore we examined the physiological properties of phototrophic bacterial strains isolated from the Black Sea and evaluated their possible roles in sulfide oxidation and primary productivity under in situ conditions.

Samples from the chemocline (77–83 m, station BS2-2 42°50′N, 32°00′E; center of west basin) were obtained in May 1988 during RV *Knorr* cruise 134. After filtration of water samples through membrane filters (0.2 μm), the latter were transferred to chemocline water and stored under nitrogen in the dark in rubber-stoppered serum bottles.

Five strains of a rod-shaped, brown bacterium were isolated after 10 times repeating a deep agar dilution series (Pfennig 1978). Although various modifications of the basal growth medium (Pfennig and Trüper 1989) were tried (various concentrations of dithionite between 0 and 1,000 μM, pH values between 6.8 and 7.2, addition of various vitamins and growth factors), the cultures still contained low numbers (<2%) of a colorless bacterium, which was isolated and identified as a sulfate-reducing strain.

Bacteriochlorophylls were identified from absorbance spectra of whole cells and acetone extracts (Shimadzu UV-300 spectrophotometer) and carotenoids by HPLC analysis (Perkin-Elmer with column Spherisorb ODS-1, eluent acetonitrile/methanol/isopropanol 90/7/3, photodiode array detector Waters 994). The pigment composition resembled that of other brown-colored

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Acknowledgments
We thank Holger Jannasch for inviting H.C. on the RV *Knorr* cruise and A. Angerhofer and J. Greis for cooperation with energy transfer efficiency measurements.

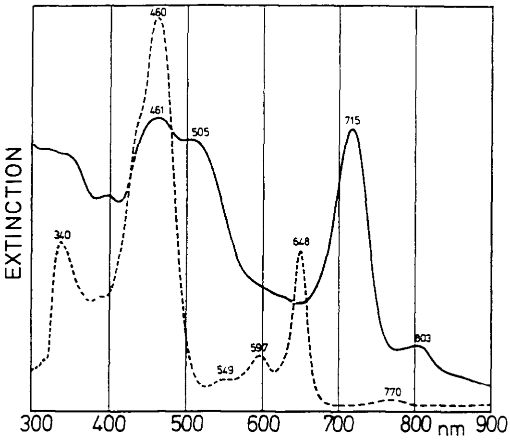

Fig. 1. Absorption spectra of *Chlorobium phaeobacteroides* MN1 (whole cells—solid line; acetone extract—dashed line. Numbers refer to absorption maxima of BChl *e* (in vivo 715, 461 nm; in acetone 648, 597, 549, 460, 340 nm), BChl *a* (803 nm; 770 nm), and the carotenoids isorenieratene/β-isorenieratene (505 nm).

green sulfur bacteria (Fig. 1). Only the carotenoids isorenieratene (79% of total carotenoids) and β-isorenieratene (21%) were detected. On the basis of cell morphology and pigment composition, the isolated strains were identified as *Chlorobium phaeobacteroides.* All strains exhibited extreme low-light adaptation when, in a preliminary experiment, their light-limited growth rates were measured. Therefore one strain (MN1) was chosen for further experiments.

Growth rates at different light intensities were measured in three parallel replicates with 22-ml screwcap tubes or bottles with parallel front and back walls (Meplats bottles) by following optical density at 650 nm. In all experiments, a temperature of 20°C was used. The basal medium of Pfennig and Trüper (1989) was applied. Cultures used for inoculation were grown at 10 μEinst m^{-2} s^{-1} prior to the experiment. Light intensity was measured with a LiCor 185B quantum meter equipped with a LiCor 200SB quantum sensor. Mean light intensity $\bar{I}$ was calculated from intensities in front of (I_1) and behind (I_2) the culture vessels according to

$$\bar{I} = (I_1 - I_2)/\ln(I_1/I_2).$$

During the experiments, the mean light intensity was adjusted at 24-h intervals by increasing the intensity in front of the vessels to compensate for increasing OD values inside. By this method, the range of light intensities around the average intensity will get broader with increasing cell density, so growth rates were determined only from OD 650 values between 0.2 and 0.5. Furthermore, all strains tested were incubated in parallel under exactly the same conditions and growth rates determined in the same OD range. Therefore, results for several strains studied are fully comparable even if there were slight errors in measurement of low light intensities during the experiments.

The Black Sea strain proved to be exceptionally low-light adapted compared to nine brown, one green, and two purple sulfur bacterial strains from various habitats. Similar to strain 2430 (Fig. 2A), five other brown *Chlorobium* strains (*C. phaeobacteroides* 4930, 5230, 9230, Cad1, and *C. phaeovibrioides* B1), brown *Pelodictyon phaeoclathratiforme* BU1, green *Prosthecochloris aestuarii* ML1, and two salt-water purple sulfur bacterial strains (*Amoebobacter purpureus* ML1 and *Thiocapsa roseopersicina* ML1) could not be grown at 0.25 μEinst m^{-2} s^{-1}. Growth rates of *C. phaeovibrioides* strains 2531 and 2631 were 15 and 29% of the MN1 growth rate. At light intensities <1 μEinst m^{-2} s^{-1} growth rates of the Black Sea *Chlorobium* exceeded those of the morphologically similar *C. phaeobacteroides* 2430 (Fig. 2B). At light saturation, strain MN1 grew considerably slower (25–30%) than the reference strains. Above 200 μEinst m^{-2} s^{-1} it was light inhibited. Obviously, the Black Sea *Chlorobium* competes successfully with other sulfur bacteria only under conditions of severe light limitation.

Due to the narrowing of the underwater light spectrum with depth, mainly green or blue-green light will penetrate to the chemocline of the Black Sea. According to Fig. 2B, however, growth rates under these conditions are similar to those in white fluorescent light. Therefore, fluorescent light was used for the following experiments.

Sulfide oxidation rates were determined by monitoring sulfide depletion in washed cell suspensions with a combined sulfide-pH electrode (Cypionka 1989). In the dark,

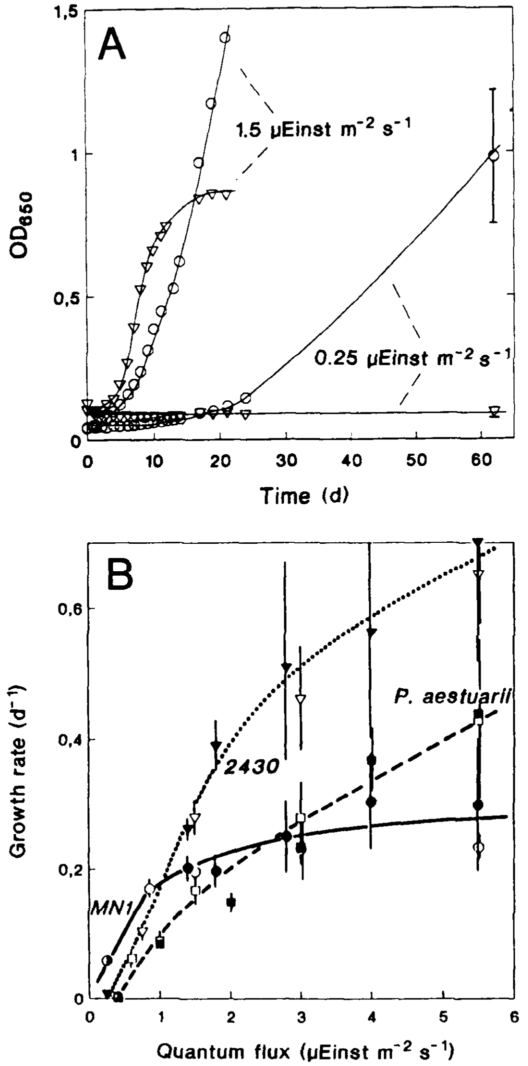

Fig. 2. A. Growth of strains MN1 (●) and 2430 (▽) at two different intensities of fluorescent light (Osram daylight 5000 de luxe) measured by optical density at 650 nm. Each point represents the mean of three replicates. The *SD* for both strains at the end of the 0.25 μEinst m^{-2} s^{-1} experiment demonstrates the significance of growth of MN1. B. Dependence of growth rate on light intensity (open symbols—Osram daylight 5000 de luxe; closed symbols—green light, maximal intensity at 505 nm) for three green sulfur bacterial strains. Vertical lines indicate 95% C.L. Curves fitted to both daylight and green light experiments.

no increase of sulfide was observed, indicating inactivity of the colorless sulfate-reducing strain due to the lack of substrate. Light dependence of sulfide oxidation rates resembled that of the growth rates (Fig. 3). At low light intensities, the Black Sea strain reached significantly higher oxidation rates than strain 2430, while the latter revealed much higher rates at light saturation. Thus photosynthetic reactions in the Black Sea *Chlorobium* must be more efficient at low light intensities. According to Liebig's law of the minimum, increased sulfide affinity would not be of selective advantage under light limitation. The K_M values for sulfide calculated from sulfide depletion curves were virtually the same for strain MN1 (7.1±4.1 μM) and strain 2430 (6.9±2.7 μM).

In a second experiment, the potential of MN1 for chemotrophic growth was examined. Even in the presence of nitrate (100 μM) as an electron acceptor, no sulfide oxidation in the dark was observed. Furthermore, strain MN1 grew only under strongly reducing conditions (400 μM $Na_2S_2O_4$, $E' = -560$ mV) in the absence of oxygen. The Black Sea strain must, therefore, be considered obligately phototrophic.

Conceivable mechanisms of low-light adaptation comprise enhanced light absorption, enhanced efficiency of energy transfer, and a lower maintenance energy requirement. BChl *e* was determined from absorbance of acetone (>99.5%) extracts at 649 nm, applying the specific extinction coefficient of 98.0 ml mg BChl e^{-1} cm^{-1} (Montesinos et al. 1983). Protein was measured after Hartree (1972). The BChl content of the cells varied according to light intensity during growth. At 30 or 1 μEinst m^{-2} s^{-1} the Black Sea *Chlorobium* showed a specific BChl content similar to strain 2430 (Fig. 4). Below 1 μEinst m^{-2} s^{-1} the pigment content of MN1 was further enhanced by a factor of two compared to the reference strain. The ability of enhanced light harvesting is an important selective advantage for survival under light limitation at 70–80-m depth in the Black Sea. The presence of additional photosynthetic pigments (Schlichter et al. 1986) in significant amounts could be excluded by spectrophotometric and HPLC analysis. Correspondingly, only homologs of BChl *e*, and isorenieratene and β-isorenieratene were detected in the natural habitat of MN1 (Repeta et al. 1989).

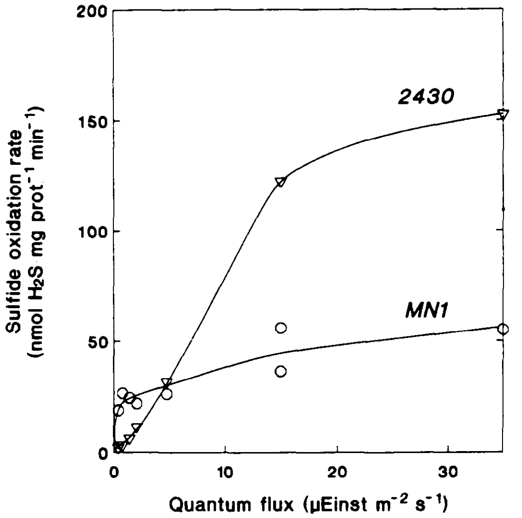

Fig. 3. Dependence of sulfide oxidation on light intensity.

The efficiency of energy transfer from carotenoids to BChl *e* in cells adapted to low light was determined by fluorescence spectroscopy (Angerhofer et al. 1986). Fluorescence excitation was normalized to absorption in vivo. Transfer efficiency was 58.9±8.8 % ($n = 3$) in the Black Sea *Chlorobium* and 46.1±2.1 % ($n = 2$) in reference strain 2430. Energy transfer of antenna pigments in the chlorosomes thus did not show the conspicuous differences observed for growth rates, sulfide oxidation, and specific pigment content. Published values of transfer efficiencies of other photosynthetic green bacteria are in the same range (~40%; Vasmel et al. 1986), while a value of 100% was determined for purple nonsulfur bacteria (Cogdell and Frank 1987) and two algal species of the Phaeophyceae and Dinophyceae (Siefermann-Harms 1987). The considerably lower value of green sulfur bacteria might be due to the specific molecular organization of antenna pigments in chlorosomes in these microorganisms.

Besides increased light harvesting, the Black Sea *Chlorobium* revealed an extremely low maintenance energy requirement μ_e. The latter was calculated from extrapolation of growth curves (Fig. 2B) toward the ordinate (van Gemerden 1980). The value for Black Sea *Chlorobium* was significantly lower (μ_e = 0±0.001 h^{-1}) compared to *C. phaeobacteroides* 2430 (μ_e = 0.0031±0.0016 h^{-1}) and values in the literature (van Gemerden 1980).

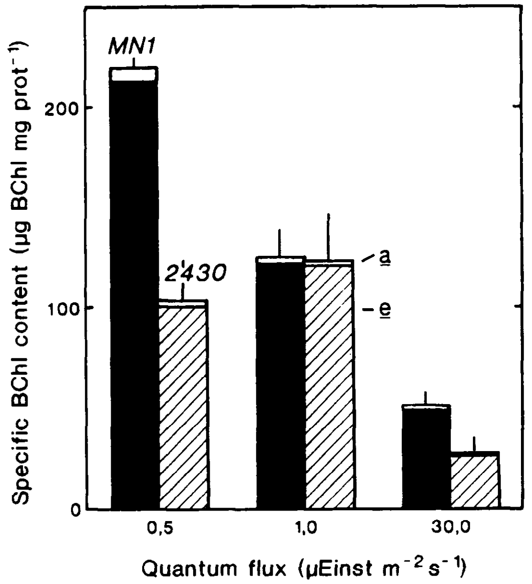

Fig. 4. Specific BChl *e* and *a* content determined for strains MN1 and 2430 grown at various light intensities. Vertical lines indicate ±1 SD.

From our data, the relative contribution of phototrophic bacteria to sulfide oxidation and total primary production in the Black Sea can be estimated. On the basis of irradiance measurements at Odessa (Deutscher Wetterdienst, Meteorol. Hamburg pers. comm.), the average light intensity reaching the BChl *e*-containing water layer at 68 m in May and the maximal intensity reaching the layer in June was calculated. Light attenuation between the sea surface and the bacterial layer was determined from Chl *a* concentrations in the Black Sea (Repeta et al. 1989), the chlorophyll-specific attenuation coefficient (Overmann and Tilzer 1988), and the light transmission reported by Repeta et al. (1989).

This calculation yielded an underwater irradiance available for anoxygenic photosynthesis of 0.003 μEinst m^{-2} s^{-1} (max in June 0.01 μEinst m^{-2} s^{-1}), which is 0.0005% of the surface irradiance. Assuming a BChl-specific attenuation coefficient of 0.02 m^2 (mg BChl $e)^{-1}$ (the value obtained for algae) and a background light attenuation similar to that in upper water layers, we calculated light intensities at different depths in the bacterial layers. Using Fig. 3 and the maximal specific BChl content [210 μg (mg protein)$^{-1}$], we estimated an average value of integral sulfide oxidation in the phototrophic bacterial layer of 0.31 μmol H_2S m^{-2} h^{-1} for May. The calculated maximum of sulfide oxidation in the layer at maximal illumination was 1.08 μmol H_2S m^{-2} h^{-1}. An upward flux of 8.3 μmol H_2S m^{-2} h^{-1} was reported for the chemocline (Jørgensen et al. 1991). According to our estimate, anoxygenic photosynthesis would account for 4% of the total sulfide oxidation under average conditions and for 13% under maximal illumination in situ.

During phototrophic growth, the stoichiometry between complete sulfide oxidation and CO_2 assimilation can be described as

$$17H_2S + 32CO_2 + 8NH_3 + 20H_2O \rightarrow 17H_2SO_4 + 8\langle C_4H_8O_2N\rangle.$$

Thus, a maximal carbon assimilation rate at the top of the bacterial layer of 0.55 μg C m^{-3} h^{-1} (max in June: 1.91 μg C m^{-3} h^{-1}) would be expected at in situ light intensity. Anoxygenic carbon fixation will, therefore, be much lower than phytoplankton productivity (2–15 mg C m^{-3} d^{-1}; Karl and Knauer unpubl.). Extrapolating the results of growth measurements to in situ light conditions yielded a doubling time of 2.8 yr. Growth requirements of strain MN1 obviously were not met completely, however, as demonstrated by the failure to isolate pure cultures. Therefore, contribution of anoxygenic photosynthesis to sulfide oxidation and carbon metabolism in the Black Sea is possibly higher than indicated by our estimates.

Our results prove that the photosynthetic organisms isolated are indeed able to live under the extremely low-light conditions at the chemocline of the Black Sea. The low-light adaptation detected for the Black Sea strain is the most extreme among all phototrophic sulfur bacteria strains tested so far. The light level at the top of the bacterial layer (0.0005% of surface light intensity) is far lower than values cited for other phototrophic bacterial communities (0.015–10%, Parkin and Brock 1980). Further shoaling of the anoxic zone will cause increasing impact of phototrophic bacteria on sulfur and carbon cycling in this ecosystem. It can be assumed that the trophogenic zone of other anoxic waters also extends much deeper than previously thought.

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Submitted: 26 October 1990
Accepted: 16 June 1991
Revised: 5 November 1991

Limnol. Oceanogr., 37(1), 1992, 155–161

Patterns in phytoplankton community structure in Florida lakes

Abstract—We demonstrate, based on examination of phytoplankton communities from 165 lakes located throughout Florida, the existence of smooth gradients of change in phytoplankton community structure with increasing lake trophic status. This change involves a gradation from a tendency toward the dominance of green algae in oligotrophic lakes to dominance of cyanobacteria in eutrophic and hypereutrophic lakes, with a peak in diatom abundance in mesotrophic lakes. The mean biomass of the dominant genera within these groups was not related to the biomass of the communities where they occurred; instead, it was strongly related to their size, the (geometric) mean biomass of the genera increasing as the 0.74 power of their cell volume.

Many lakes in Florida are eutrophic and experience water quality problems derived from extensive algal development (Canfield and Hoyer 1988). Although the highest phytoplankton biomasses attained in Florida lakes are in algal communities dominated by cyanobacteria, they may also be dominant in unproductive Florida lakes (Canfield et al. 1989). Similarly, phytoplankton communities in eutrophic Florida lakes can be dominated by other algal taxa, such as diatoms and green algae (Canfield et al. 1985). Thus, attempts to predict the relative contribution of particular algal taxa to community biomass from trophic characteristics (e.g. N and P concentrations) have been largely unsuccessful in lakes in Florida (e.g. Canfield et al. 1989) and elsewhere (Kalff and Knoechel 1978).

Agustí et al. (1990) showed that, in Florida lakes, there is a tendency for dense phytoplankton communities to be dominated by cyanobacteria, while sparse communities tend to be dominated by green and diatom species. Whether this difference in community structure across lakes of different trophic status develops as a sharp, step shift as the population attains a threshold biomass or is part of a gradual transition in community structure from oligotrophic to eutrophic lakes is unknown. Here we demonstrate the existence of smooth gradients of change in phytoplankton community structure with increasing lake trophic status, from dominance by green algae in oligotrophic lakes to dominance by cyanobacteria in eutrophic and hypereutrophic lakes, with a peak in diatom abundance in mesotrophic lakes.

We examined these patterns during a sur-

Acknowledgments

Florida Agricultural Experimental Station Journal Series R 01908.

This work was supported by a contract from the U.S. Department of the Interior (project 14-16-0009-79-064) and state allocations to the Department of Fisheries and Aquaculture.

We thank Claire L. Schelske for criticisms.