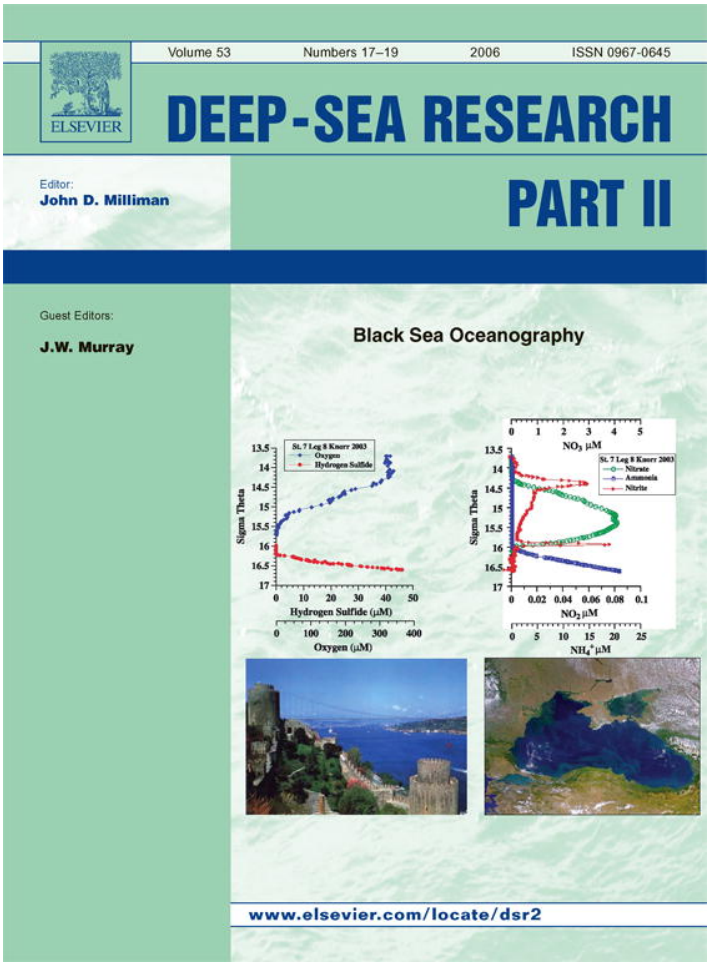

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Diversity and distribution of photosynthetic bacteria in the Black Sea

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Abstract

Using an infrared fast-repetition-rate (IRFRR) fluorometer, we measured bacteriochlorophyll *a* (BChl *a*) fluorescence kinetics to examine the spatial pattern of the distribution of aerobic and anaerobic photosynthetic bacteria in the Black Sea in June 2001. Aerobic photosynthetic bacteria containing BChl *a* were distributed together with chlorophyll-containing phytoplankton in the upper, aerobic portion of the water column, confirming their exclusive requirement for oxygen. The BChl *a* to chlorophyll *a* ratio averaged 10^{-2} within the euphotic zone. Seven strains of aerobic photosynthetic bacteria were isolated and partially characterized. They belong to two subgroups closely related to *Erythrobacter* and *Roseobacter* genera. Green sulfur photosynthetic bacteria were detected in the central part of the basin, at the boundary between suboxic and anoxic (hydrogen sulfide) zone. However, the population of green sulfur bacteria was extremely small, and confined to a very narrow density layer deep in the water column.
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Keywords: Aerobic anoxygenic phototrophs; *Erythrobacter*; *Roseobacter*; *Chlorobium*; Bacteriochlorophyll; Green sulfur photosynthetic bacteria

1. Introduction

In oxygenic photosynthesis chlorophyll *a* (Chl)-containing eukaryotic and prokaryotic phytoplankton utilize light energy to split water and evolve oxygen. The electrons and protons derived from this reaction are used to reduce carbon dioxide to form organic matter. While this process provides the overwhelming majority of fixed carbon on Earth, there is a broad phylogenetic spectrum of anoxygenic (non-evolving oxygen) photosynthetic bacteria containing bacteriochlorophyll (BChl). Anoxygenic photosynthetic bacteria are divided into four major phyla-purple photosynthetic bacteria, green sulfur bacteria, green non-sulfur photosynthetic bacteria, and heliobacteria. These relict organisms first appeared approximately 3.5 Ga (Des Marais, 2000), long before the evolution of an oxidized atmosphere on Earth (Farquhar et al., 2000). Thus, most of them grow and photosynthesize only under strictly anaerobic conditions. Under aerobic conditions, which have characterized the upper ocean for the past ca. 2.4 Ga, these organisms have been restricted largely to small ecological niches, such as anoxic zones or sulfide springs.

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Recently, a significant activity of anoxygenic photosynthesis was discovered in the open ocean by kinetic measurements of BChl *a* fluorescence emission (Kolber et al., 2000). The activity was related to the presence of aerobic anoxygenic phototrophs (AAPs), which constituted about 11% of the total bacterial community in the North East Pacific (Kolber et al., 2001). AAPs were discovered in the end of 1970s in Bay of Tokyo (Harashima et al., 1978; Shiba et al., 1979), but until recently they were thought to be confined only to specialized niches such as sand, seaweed surfaces, etc. These bacteria are related to purple non-sulfur bacteria, but they adapted to oxygenic atmosphere and became obligate aerobes (Shimada, 1995; Yurkov and Beatty, 1998).

The Black Sea is an unique marine environment in which extreme density stratification physically isolates an upper, aerobic layer, inhabited by metazoans including fish and mammals, from a deep anoxic region that supports only anaerobic microbial communities (Leppäkoski and Mihnea, 1996; Özsoy and Ünlüata, 1997; Konovalov and Murray, 2001). The reports of anoxygenic phototrophs in the Black Sea are controversial. Kriss and Rukina (1953) first reported growth of sulfur purple photosynthetic bacteria in enrichment cultures inoculated with water samples collected in the anoxic zone below 500 m. However, Hashwa and Trüper (1978) were not able to find any photosynthetic bacteria in samples below the chemocline. On the other hand, in 1988, a population of green sulfur photosynthetic bacteria was detected at the suboxic/anoxic interface by pigment analysis (Repeta et al., 1989). However, no carotenoid biomarkers of the purple sulfur bacteria were found (Repeta and Simpson, 1991). Later, the presence of green sulfur bacteria was observed using kinetic fluorescence measurements (Karabashev, 1995). This population appears to be dominated by *Chlorobium* sp, as suggested by the presence of BChl *e* (Repeta and Simpson, 1991) and successful cultivation of five *Chlorobium* sp. isolates (Overmann et al., 1992).

We surveyed the distribution of anoxygenic phototrophs in the western part of the Black Sea in June 2001 during US R/V *Knorr Black Sea* cruise voyage no. 162, leg 17(2) (see http://oceanweb.ocean.washington.edu/cruises/Knorr2001/). Our goal was to quantify and characterize the diversity of different types of photosynthetic organisms over sharp oxygen gradients in the water column.

2. Materials and methods

2.1. Kinetic fluorescence measurements (FRRF)

Oxygenic photoautotrophs (Chl *a*-containing), AAPs (containing BChl *a*), and anaerobic anoxygenic phototrophs (containing BChl $a+e$) were detected using a custom-built IRFRR fluorometer (Kolber et al., 1998, 2000) operating at 470 nm, 30 nm bandwidth excitation wavelength. The emission wavelength was selected as follows: RG650 glass and 685 nm interference filters for phytoplankton (Chl *a*), RG695 glass and 880 nm (Intor, 70 nm half-width) interference filters for AAPs (BChl *a*), and RG780 glass and 800 nm (Intor, 65 nm half-width) interference filter for green sulfur bacteria (BChl $a+e$). The instrument generates a train of short (0.6 μs) blue (470 nm) or infrared (795 nm) flashlets in the microsecond to millisecond timescale. Electron transport elicited by these light pulses induces transient changes in the fluorescence emission originating from the functional photosynthetic reaction centers. The signal is detected by a large-area (16 mm diameter) avalanche photodiode detector (630-70-72-631, Advanced Photonix Inc.) and processed by the fluorometer electronics. The fluorescence parameters F_0, F_V, F_V/F_M, $\sigma_{470\,nm}$ were determined as described previously (Kolber et al., 1998).

For field measurements, Chl *a* signals were recorded directly in the bulk water samples collected from Niskin bottles. To measure the much weaker BChl *a* signal, we filtered 100-mL water samples through a GF/F filter (Whatman, 25 mm diameter, 0.7 μm pore size) and acquired the signal from the surface of the filter. The GF/F filter retains about 95% of total BChl *a* signal, and under our experimental conditions the signal was proportional to the BChl *a* concentration within 20% error. The Chl *a* and BChl *a* was estimated from variable fluorescence signal, F_V, using fluorescence/pigment calibration curves (Kolber et al., 2001). In case of green sulfur photosynthetic bacteria, only the relative fluorescence signal was assessed.

2.2. Strain isolation

Seawater samples were collected by Niskin bottles. Immediately after sample recovery, about 5 μL of the sample were streaked on plates containing 1.5% bacteriological agar with f/2 media (Guillard and Ryther, 1962) or the same plates

enriched with 40 mg/L of yeast extract and 50 mg/L streptomycin. In addition to a standard f/2 vitamin mix, all the media were enriched with 2 μM nicotinic acid (vit. B_3) required by the *Roseobacter* (*Rsb.*)-like species. The plates were stored for 7 days in the dark to eliminate oxygenic phototrophs and then exposed to the natural light–dark cycle. After several weeks, the plates were screened for pigmented (pink, yellow, orange and red) colonies. The colonies were resuspended in sterile f/2 seawater medium enriched with 2 μM nicotinic acid. The presence of BChl *a*-containing organisms was tested using IRFRR fluorometer (Kolber et al., 2000). The samples exhibiting the BChl *a* fluorescence transient were repeatedly plated on the f/2 agar plates until pure cultures were obtained.

Pure isolates were typically grown in organic-rich medium composed of f/2 medium supplemented by 0.5 g of peptone and 0.1 g of yeast extract per liter. The isolates could be maintained in liquid or on agar media at 4 °C for several months. For a long-term storage, the cells were resuspended in 30% glycerol containing f/2 medium, frozen in liquid nitrogen and stored at −75 °C.

No attempt was made to cultivate anaerobic anoxygenic phototrophs. *C. phaeobacteriodes* was grown as described by Frigaard and Bryant (2001).

2.3. Strain characterization

The absorption spectra were recorded on cells resuspended in 70% glycerol in f/2 medium using an Aminco DW2000 spectrophotometer in the dual beam mode, at a resolution of 0.33 nm using a 2-nm slit width.

Pigment composition was assayed using a Shimadzu VP HPLC system composed of the SCL-10A controller, SIL-10A autoinjector, two LC-10AT pumps and equipped with the Microsorb MV C8 3 μm 100 A column. Bacterial cell pellets, collected by centrifugation, were extracted in acetone:methanol mixture (7:2) (50 μL sample volume) and analyzed immediately. The pigments were separated by a linear gradient using a modification of the Mantoura and Llewellyn (1983) method: 0.0′ (100% A), 9.0′ (100% B), 17.5′ (100% B), 18.0′ (100% A) and 20.0′ (100% A). Solvent A: 80% methanol in water + 1.5 g tetrabutyl-ammonium acetate per liter and 7.7 g ammonium acetate per liter. Solvent B: 100% methanol. The flow rate was set to 0.75 mL min^{-1}. Pigments were detected with the SPD-10AV absorption detector at 470 nm and the RF-10AXL fluorescence detector using 370 nm excitation and 780 nm emission wavelength.

16S rRNA genes were amplified directly from cells using a eubacterial primer SSEub27F (Giovannoni et al., 1988) and a universal primer SS1492R (Lane, 1991). PCR products were purified by gel electrophoresis. Fragments containing 16S rDNA were extracted using the Zymoclean Gel DNA Recovery Kit™. PCR fragments were directly sequenced using the SSEub27F primer. Partial 16S rDNA sequences (350–450 bp) were used for genetic affiliation.

3. Results

3.1. Field measurements

The presence of anoxygenic and oxygenic phototrophs in the western part of the Black Sea was surveyed in the early June 2001. In this period, the surface water temperatures ranged from 17.5 to 18.5 °C in the southern part of the Black Sea (Stations 1–3), and from 15 to 16.4 °C in the northern part (Stations 5–10). The minimal temperature of 13.7 °C was observed in the Station 12 (the Sevastopol eddy). Surface Chl concentrations at individual stations, estimated from IRFRR fluorescence measurements, reached maximum in the northwestern part of the Black Sea (Station 5) located on the continental shelf close to the estuary of Dnieper, Bug and Dniestr rivers (Fig. 1). At this location, estimated surface Chl values were about 8 nM (~7.1 mg Chl m^{-3}), decreasing from north to south, and reaching the minimum of 0.5 nM in the center of the western gyre (Station 2). About two-fold higher Chl *a* concentrations were found in the Strait of Bosporus (Station 1) and close to the Turkish coast (Station 14). The Chl minimum was observed in the Sevastopol eddy (Station 12), where it decreased to 0.3 nM. These numbers roughly correspond to average Chl *a* values for June retrieved from SeaWIFS remote sensing of ocean color (Oguz et al., 2002).

Typical BChl *a* signal (Fig. 2) was detected at all stations. The highest concentrations were found on the continental shelf (Station 5) where BChl *a* reached ca. 80 pM (~73 μg BChl m^{-3}) (Fig. 3). At this station we were able to record the BChl *a* signal without preconcentration. In the center of the western gyre (Station 2) the BChl *a* surface concentration was ~4 pM. Like Chl *a*, the BChl *a* signal was restricted to the upper 50–60 m of aerobic

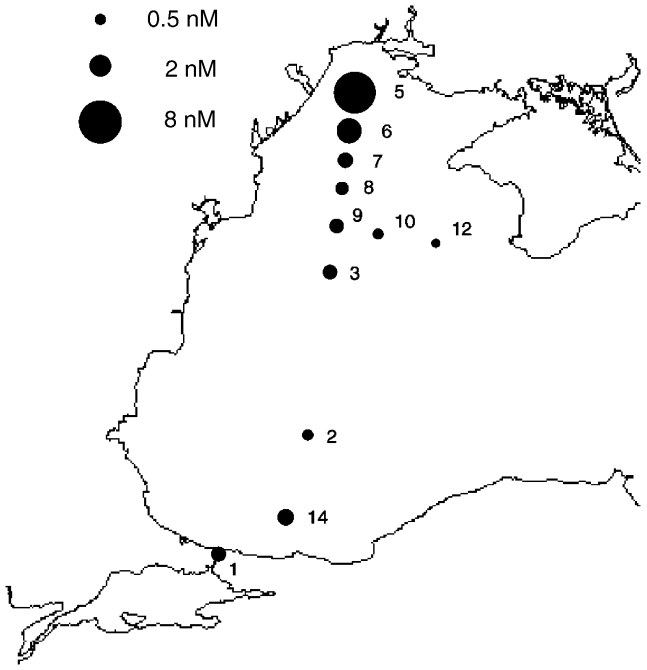

Fig. 1. Positions of sampling stations during the R/V *Knorr 2001 Black Sea* cruise no. 162, Leg 17. The diameter of the circle represents the surface chlorophyll concentration estimate at the station as it was derived from variable chlorophyll fluorescence measurements (FRRF).

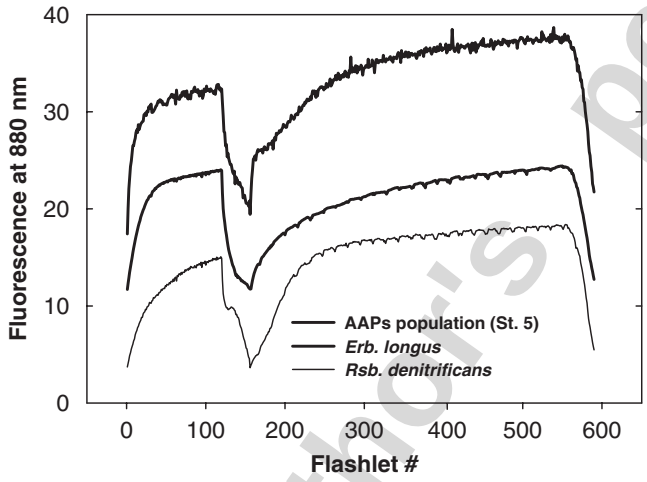

Fig. 2. The IRFRR fluorescence signal of AAP community recorded at Station 5. The sample was not pre-concentrated. The community signal is compared with signals of *Erb. longus* and *Rsb. denitrificans* recorded in the laboratory.

layer (Fig. 4), consistent with AAP's exclusive requirement for oxygen. The Chl *a* and BChl *a* distributions in the water column did not completely overlap. To compensate for uneven distribution of BChl *a* and Chl *a* signals, and to partially reduce the random scatter originating from the experimental error, we calculated integrated water-column numbers for each station (Fig. 3). The Chl *a* integrated values followed the same trend as sea-surface Chl *a* concentrations, reaching the maximum of more than 160 μM Chl $a\,m^{-2}$ on the continental shelf (Station 5) and the lowest value about 15 μM Chl $a\,m^{-2}$ at the Sevastopol eddy (Station 12). The calculated BChl *a*/Chl *a* molar ratio was 0.0103 ± 0.0018.

At Station 2 (= 13), located in the center of the western gyre, another fluorescence signal (800 nm) from anoxygenic phototrophs was observed at the suboxic/anoxic boundary (H_2S chemocline). In accordance with earlier observations (Repeta and Simpson, 1991; Overmann et al., 1992), the signal (800 nm) was tentatively ascribed to the presence of BChl *e* and BChl *a*-containing green sulfur photosynthetic bacteria belonging to genus *Chlorobium*. At Station 2, the signal was located at 106 m depth and isopycnal surface $\sigma_t \sim 16.1$ (Fig. 3). However, the signal was not registered at other stations where the chemocline was deeper than in the center of the gyre.

The identity of the signal (Fig. 5) was confirmed with a laboratory culture of *C. phaeobacteroides*. Both traces display similar features clearly distinct from the fluorescence signal of AAPs (Fig. 2). A strong fluorescence quenching was observed during the initial, 140-μs long, high excitation power portion of the IRFRR protocol (flashlets 1–120, about $3 \times 10^4\,W\,m^{-2}$ excitation power). In contrast, the signals measured in phytoplankton or AAPs, display a rapid induction of the fluorescence yield. In the multiple-turnover phase of the FRR protocol (flashlets 191–590), with about 100 times lower excitation power applied over a millisecond timeframe, the *Chlorobium* specific signal displayed an increase in the fluorescence yield, similar to that observed in Chl *a*, and BChl *a*-containing organisms.

When the natural *Chlorobium* population signal is compared to the laboratory culture of *C. phaeobacteroides*, there is an apparently faster increase of fluorescence in the multiple-turnover phase of the FRR protocol. Such behavior suggests a significantly larger functional absorption cross-section of photosynthetic units of the Black Sea *Chlorobium* when compared to laboratory-grown *C. Phaeobacteriodes*, suggesting that the chlorosome-type antenna in these organisms facilitates efficient light collection under extremely low irradiance levels (see Discussion). During the last relaxation phase of the FRR protocol (flashlets 591–650) the

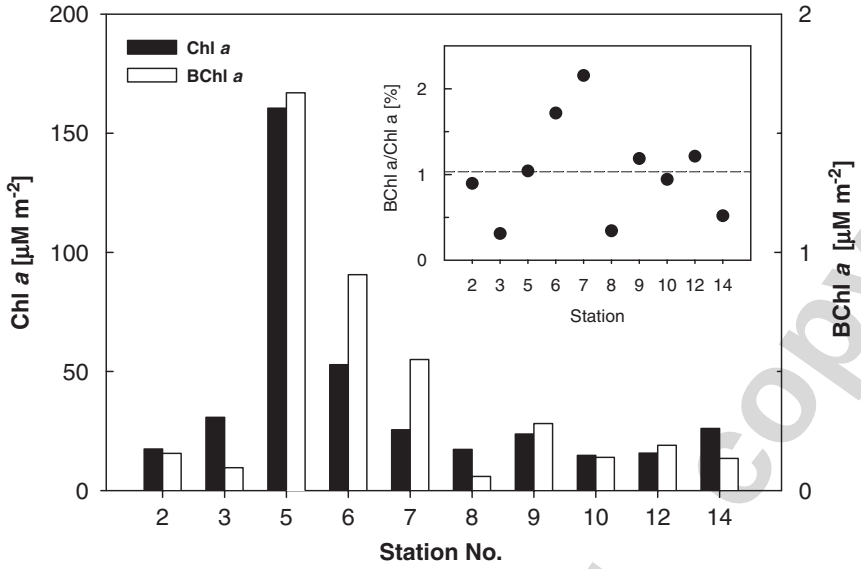

Fig. 3. Integrated water-column values for Chl *a* (empty bars) and BChl *a* (filled bars) as estimated from variable fluorescence measurement. The insert shows BChl a to Chl a ratio calculated from the integrated values for every station. The BChl *a*/Chl *a* has a mean value of $1.03 \pm 0.18\%$.

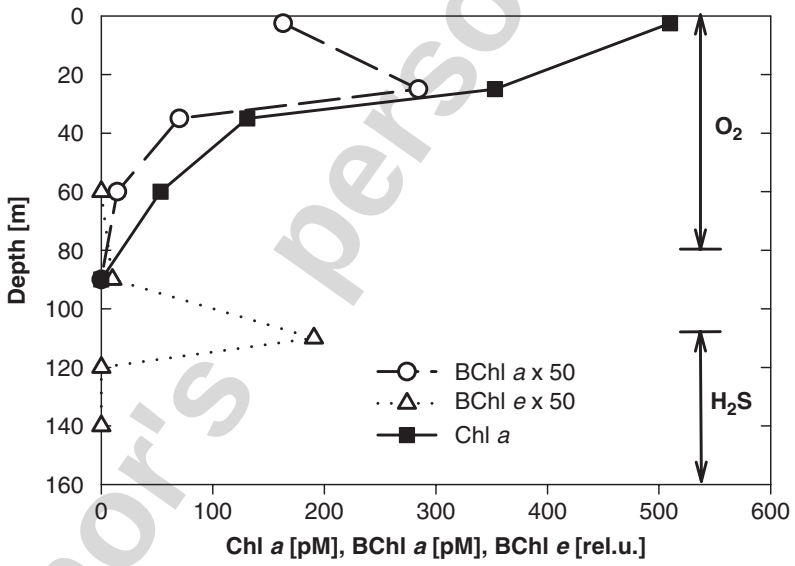

Fig. 4. Water-column profile measured at Station 2 (Cast 8) on June 3, 2001. The values of BChl *a* are 50 times expanded. BChl *e* signal is proportional to that of BChl *a* and it is shown only in relative numbers.

fluorescence signal relaxed with half-life about 50 ms. This rate probably corresponds to turnover time of electron transport in *Chlorobium* population. Moreover, it suggests that the observed fluorescence changes do not reflect redox changes in the type-1 reaction centers, but rather are connected with other processes occurring downstream in the electron transport chain.

We did not detect any signal originating from purple photosynthetic bacteria in the anoxic zone of the Black Sea. This is consistent with the absence of carotenoid biomarkers specific to purple photosynthetic bacteria in the anoxic zone as reported by Repeta and Simpson (1991).

3.2. Isolation and characterization of the Black Sea AAPs

Seven strains containing functional bacterial photosynthetic reaction centers were isolated from the

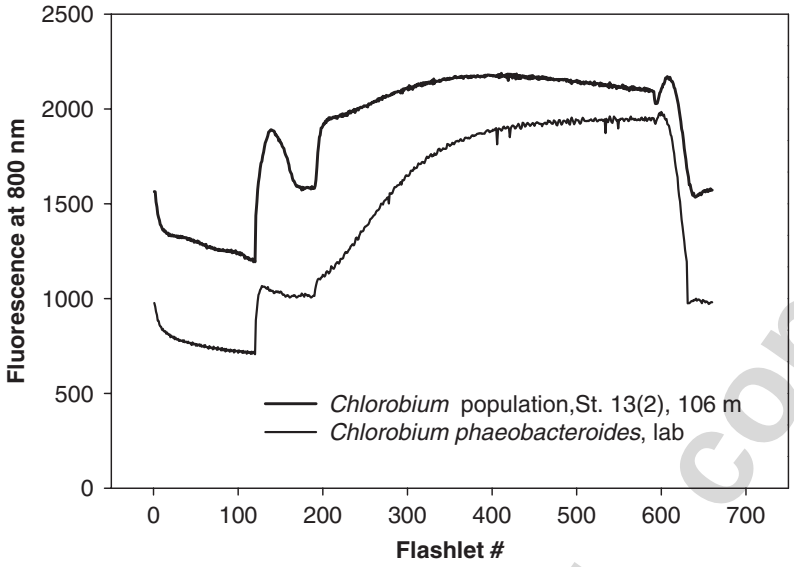

Fig. 5. The IRFRR fluorescence signal of green sulfur photosynthetic bacterial community at the boundary of the anoxic zone (106 m, $\sigma_t = 16.1$). The trace was recorded at Station 13 (= Station 2) in the center of the gyre on June 8, 2001. The trace is compared with signal of laboratory grown culture of *C. phaeobacteriodes*.

Table 1
List of locations where the bacterial isolates were collected

Strain	Date 2001	Station/Cast	Depth (m)	Location	Plate	Color
BS36	June 5	6/18	5	45° 42.7′N, 31° 05.7′E	F/2	Pink
BS53	June 1	1/1	62	41° 12.8′N, 29° 07.4′E	F/2	Orange
BS90	June 6	9/22	15	44° 31.7′N, 30° 58.0′E	F/2	Pink
BS110	June 1	1/1	62	41° 12.8′N, 29° 07.4′E	F/2	Pink
BS130	May 29	—[a]	87	41° 53.0′N, 30° 30.0′E	Strept.	Orange
BS140	June 9	14/34	10	41° 27.5′N, 30° 15.8′E	F/2	Orange
BS150	June 5	7/20	1	45° 23.0′N, 31° 03.3′E	F/2	Pink

[a]The strain was collected during a preceding cruise in the same waters; R/V *Knorr* No. 162, Leg 16(1) Station 7, Cast 38.

western part of the Black Sea (Table 1). Three isolates displayed orange-red color, whereas the other four were pink. All the isolates were capable of growth at room temperature (20 °C) in an organic medium under aerobic conditions; anaerobically cultured strains did not grow. No growth occurred in f/2 medium devoid of an added organic carbon source.

All the isolates exhibited an infrared Q_Y band of BChl *a* LH1 antennae centered between 864 and 872 nm, confirming the presence of bacterial photosynthetic reaction centers. LH2 antennae frequently found in purple photosynthetic bacteria and *Rsb.* type strains were not present (Fig. 6). This is consistent with the field measurements, which failed to detect any BChl *a* signal upon 795 nm excitation targeted to LH2 antennae.

The pigment composition of the AAPs isolates were determined by HPLC. Fig. 7 shows a typical chromatogram recorded for two representatives—isolates BS53 (orange) and BS90 (pink). Both isolates contained BChl *a* with phytyl side-chain (peak 3). However, there was a clear distinction between ''orange'' and ''pink'' isolates in carotenoid composition. The ''orange'' group displayed typical pigment composition of *Erythrobacter* (*Erb.*) isolates, expressing polar erythroxanthin sulfate (peak 1) as a major carotenoid. The minor non-polar carotenoids were bacteriorubixanthinal and zeaxanthin (peak 2), which is in agreement with other members of *Erb.* genus (Koblížek et al., 2003). The peak identities were confirmed with control extract from type strain *Erb. longus* (not shown). All the pink isolates contained spheroidenone as a dominant carotenoid. Its identity was confirmed with the control extract from *Rsb. denitrificans* and semiaerobically grown *Rba. sphaeroides*.

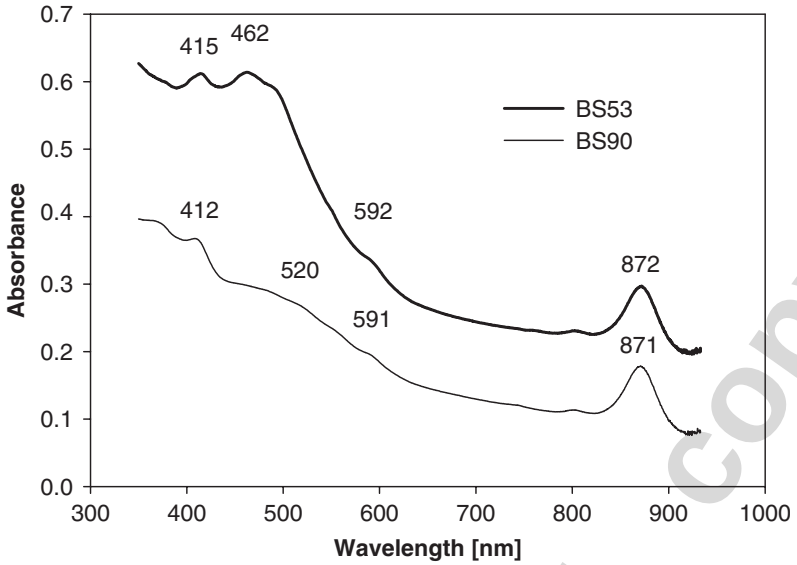

Fig. 6. Absorption spectra recorded for isolates BS53 (orange) and BS90 (pink). The cells were resuspended in 70% glycerol to reduce the scattering, though the scattering profile was not subtracted. The positions of the major absorption bands were determined by means of second derivative analysis and are depicted in the figure.

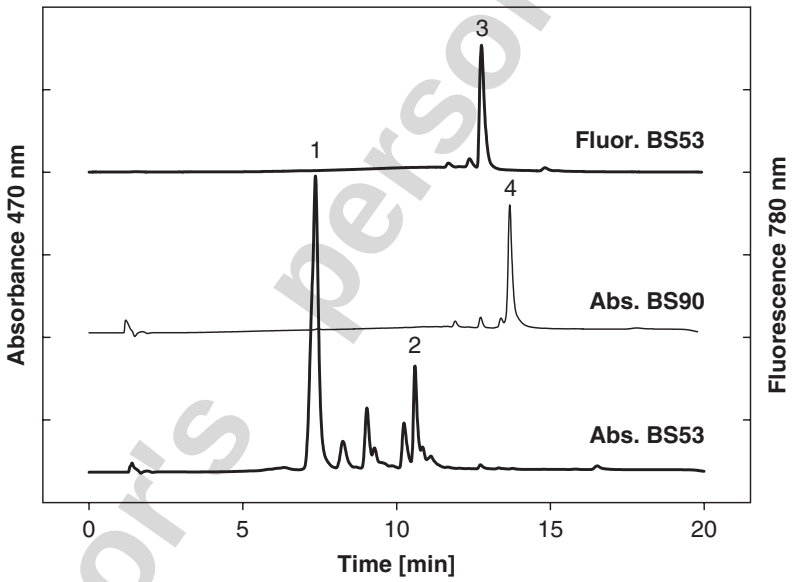

Fig. 7. HPLC chromatogram recorded for two isolates BS53 (orange) and BS90 (pink). Peak identity was determined by comparison with extracted pigments of the type strains. The peaks are (1) erythroxanthin sulfate, (2) zeaxanthin, (3) BChl *a*, (4) sphaeroidenone. The pigments were detected by an absorption detector at 470 nm (lower traces) and a fluorescence detector operating at 370 nm excitation and 780 nm emission wavelength (upper most trace). The recorded fluorescence for isolate BS90 is not shown, since it was identical to that of BS53.

The sensitivity to antibiotics was tested with isolate BS53 and BS90 and compared to that of the type strains (Table 2). Although each isolate exhibited distinct susceptibility towards antibiotics, the general sensitivity pattern of BS53 isolate resembled that of *Erb. longus,* and that of BS90 resembled that of *Rsb. denitrificans*, indicating the genetic relation of the studied species.

16S rDNA of three isolates BS53, BS90 and BS140 were PCR-amplified and the product directly sequenced. Obtained 16S rDNA partial (350–500 bp) sequences were compared with the NCBI sequence database. The closest matches with cultivated organisms were *Erb*. species for isolates BS53 and BS140 and *Rsb*.-like species for strain BS90. The overall similarity among BS53, BS140

Table 2
Comparative characteristics of the isolates BS53 and BS90 and the reference strains *Erb. longus* and *Rsb. denitrificans*

Strain	BS53	BS90	*Erb. longus*	*Rsb. denitrificans*
Subclass of *Proteobacteria*	α4	α3	α4	α3
Color	Orange	Pink	Orange	Pink
In vivo abs. max. LH1	872	871	866	870
LH2	—	—	—	806
Major carotenoid	Erythroxanthin sulfate	Spheroidenone	Erythroxanthin sulfate	Spheroidenone
Erythromycin	+	+	+	+
Gentamycin sulfate	−	+	+	+
Kanamycin	+	+	+	−
Paromomycin	−	+	−	+
Polymyxin B	−	+	−	+
Streptomycin sulfate	−	+	−	+
Vankomycin	+	+	+	−

Antibiotic sensitivity was tested in the organic medium with individual antibiotics added at concentrations of 50 mg/L, the growth was assayed after 4–5 days. '+' indicates the antibiotic sensitivity, '−' antibiotic resistance. LH1, LH2 = light harvesting complex 1 and 2, respectively.

and *Erb. longus* strains was about 99%. The isolate BS90 displayed about 96% similarity to the type strains *Rsb. denitrificans* and *Rsb. litoralis*.

4. Discussion

AAPs appear to account for a significant fraction of the marine microbial community in temperate oligotrophic waters (Kolber et al., 2001). The ratio of anoxygenic to oxygenic phototrophs (phytoplankton), reflected in the BChl *a*/Chl *a* ratios, varies significantly. In the northeastern Pacific Ocean the BChl *a*/Chl *a* ratio was about 0.8% (Kolber et al., 2001), whereas in warm oligotrophic waters of Eastern Pacific Ocean the ratio ranged from 0.7% up to 10% (Kolber et al., 2000). Lower BChl *a*/Chl *a* ratios of 0.1% to 2% were reported in waters off Southern California by Goericke (2002), which is similar to numbers determined in this study ranging from 0.3% to 2.2%. Lower numbers were observed in the eutrophic Baltic Sea where in late summer 2003 the BChl *a*/Chl *a* ratios ranged from 0.12% to 0.65% (Koblížek et al., 2005).

Due to the presence of photosynthetic apparatus, AAPs are capable of satisfying a significant portion of metabolic energy requirements (ATP) by light-derived energy. This allows them to operate with significantly lower carbon requirements than the obligate heterotrophs. When exposed to a natural light–dark regime, AAPs display up to two times higher efficiency in utilizing organic carbon over purely heterotrophic bacteria (Kobližek, unpublished data). Potentially, that represents a strong competitive advantage over strictly heterotrophic bacteria when bacterial growth is limited by the availability of carbon. Theoretically, that should lead to AAPs dominance under carbon limitation. Yet, AAPs represent only a fraction of total bacterial community. On the other hand, the presence of photosynthetic apparatus offers little metabolic advantage under phosphorus or nitrogen limitations. This suggests that the growth of marine heterotrophs is usually limited by other factors than the availability of organic carbon (e.g., phosphorus or inorganic nitrogen).

Little is known about AAPs diversity. All the marine AAP isolates reported to-date were classified as members of four genera *Erb.*, *Rsb.*, *Citromicrobium* and *Roseibium*, although only *Erb.* and *Rsb.*-like isolates has been reported repeatedly (Shiba et al., 1991; Nishimura et al., 1994; Koblížek et al., 2003). The occurrence of AAPs-like 16S rDNA sequences have been repeatedly reported from analyses of uncultured populations of marine bacteria for *Rsb.* (Eilers et al., 2000; Fuhrman et al., 1993; González et al., 2000; Mullins et al., 1995; Suzuki et al., 1997). In contrast, *Erb.*-related 16S rDNA sequences were mostly acquired from cultivated material (Bernard et al., 2000; Pinhassi et al., 1997; Vybiral et al., 1999). However, the 16S rDNA similarity with AAPs does not guarantee the presence of BChl *a* containing reaction centers. In fact, there are known strains, *Rsb.* (*Ruegeria*) *algicola* (Lafay et al., 1995), *Rsb. gallaeciensis* (Ruiz-Ponte et al., 1998), *Erb. citreus* (Denner et al., 2002), and *Erb.* strain SD-21 (Francis et al.,

2001), that align phylogenetically with AAPs, but lack BChl *a*. Such BChl *a*-lacking organisms are unable to utilize light energy and rely exclusively on heterotrophic metabolism.

To overcome the uncertainty related with the 16S rDNA analyses, Béjà et al. (2002) probed directly for the presence of *puf*M gene (encoding the M subunit of the reaction center) in marine bacterial communities. In DNA samples recovered from Monterey Bay, *Rsb.*-like *puf*M sequences together with novel, likely γ proteobacterial *puf*M sequences, were the most dominant. Interestingly, virtually no sequences representing the genus *Erb.* were found. *Rsb.*-like *puf* sequences also were obtained in a recent study from the Red and Mediterranean Sea (Oz et al., 2005). The reason of this ambiguity has yet to be determined. In any case, the results of both molecular and culture analyses indicate that communities of marine AAPs are more diverse than previously suggested (Kolber et al., 2001).

Based on this study, we suggest that the specific classes of AAPs could be distinguished and quantified in the environment on the basis of their specific carotenoid composition. Two pigments, erythroxanthin sulfate and bacteriorubixanthinal, are, to our knowledge, specific for *Erb.* genus (but also are found in the freshwater AAP *Erm. ramosum*). Between these two pigments, bacteriorubixanthinal appears to be a better tracer, since erythroxanthin sulfate is not functionally related to photosynthesis. *Rsb.*-like AAPs could be traced using spheroidenone, which was found to be a major carotenoid in all presented *Rsb.*-like BChl *a*-containing isolates as well as in the type strains.

Interestingly, all the *Rsb.*-like isolates were missing the outer light-harvesting complexes LH2. This is consistent with the field data where we did not detect excitation energy transfer from LH2-specific absorption band at 795 nm. This feature makes our isolates clearly distinct from the type species of *Rsb.* genus—*Rsb. denitrificans* and *Rsb. litoralis*, which both contain LH2. Also, our other field data (Hawai'i, December 2002, Kobližek, unpublished data) lack a 795 nm excitation signal, which strongly suggests that *Rsb.* genus-type species may not be representative for oceanic *Rsb.*-like species.

The IRFFR signal of anoxygenic phototrophs was detected at suboxic/anoxic interface at Station 2 (center of the western gyre). The character of the measured fluorescence transients was similar to that of laboratory grown *C. phaeobacteriodes* and clearly distinct from signals originating from organisms containing type-2 reaction centers. This highly specific fluorescence signal provides a convenient, selective tool for identification and semiquantification of green sulfur bacteria, in the environment. The presence of a Black Sea *Chlorobium* population supports earlier reports of these organisms (Coble et al., 1991; Repeta and Simpson, 1991; Overmann et al., 1992; Karabashev, 1995). Interestingly, the suboxic/anoxic boundary was found much deeper than in end of 1980's (Coble et al., 1991; Repeta and Simpson, 1991). During our measurement, the boundary was located in 106 m and isopycnal surface $\sigma_t = 16.1$. This observation is consistent with recent data showing that the upper boundary of the anoxic zone is located at the $\sigma_t \sim 16.11$ isopycnal surface (Yakushev et al., 2001), despite the fact that its actual depth varies with location and time. Due to inherent problems with fluorescence emission from green sulfur bacteria, we could not quantify rigorously the IRFRR signal in terms of the BChl *e* + BChl *a* abundance. However, we estimate that it was 1–2 orders of magnitude lower than the values (up to 1 nM) reported previously (Repeta and Simpson, 1991). A plausible explanation is the recent deepening of the suboxic/anoxic interface, from 75 to 90 m in 1988, to 106 m observed during this study. The *Chlorobium* population at those depths is likely to be severely light-limited, and the recent deepening of the chemocline probably has reduced the population of *Chlorobium.* Moreover, we were not able to detect *Chlorobium* signals at other stations, where the interface was deeper than in the center of the western gyre (Station 2). This hypothesis might explain a report from 1975, when Hashwa and Trüper (1978) were not able to find any viable anaerobic photosynthetic bacteria. The suboxic/anoxic interface was then at a depth of 140 m.

The *Chlorobium* population receives a very small portion of solar radiation. We estimate that only about 10^{-6} of the surface irradiance can reach 106 m. Assuming an average surface radiation of 200 μmol quanta $m^{-2} s^{-1}$, we estimate about 2×10^{-10} mol quanta $m^{-2} s^{-1}$ at the chemocline. Assuming the optical cross-section of one *Chlorobium* cell about 1 μm^2 and dry weight (DW) of about 10^{-13} g DW, we calculate an energy flux of 2×10^{-9} mol quanta g $DW^{-1} s^{-1}$. The synthesis of one ATP molecule requires about 3 quanta. Hence, the photon flux at depth yields an ATP production of about 2.4 μmol ATP g $DW^{-1} h^{-1}$ during the photoperiod. This is roughly comparable

with published data about cell maintenance energies in bacteria ranging from 0.3 to 8 μmol ATP $g\,DW^{-1}\,h^{-1}$ (Lengeler et al., 1999). From these calculations we infer that depths around 106 m are the lower limit for *Chlorobium* population survival under light conditions present in the Black Sea. It is likely that the long-term changes in the position of suboxic/anoxic interface might lead to periods of *Chlorobium* blooms (shallow chemocline) and extinctions (deep chemocline) driven by physical factors governing the Black Sea hydrography.

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