Pigment variation in the photosynthetic sulfur bacterium *Chlorobium phaeobactereoides* from Lake Kinneret

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Abstract The photosynthetic sulfur bacterium *Chlorobium phaeobactereoides* found in Lake Kinneret harbors three homologues of bacteriochlorophyll *e* (Bchl *e*). The ratios between the Bchl *e* homologues found in samples from the metalimnion are approximately constant and perennially stable. The proportion of Bchl *e* homologues in a laboratory isolate of *Ch. phaeobactereoides* was different from that found in the lake population, but resistant to changes in light, even if cultures were exposed to light intensity 2-3 orders of magnitude higher than the intensity experienced by the bacterium under natural conditions. Exposure of cultures in the lake did not induce changes in the proportion of pigments, indicating that the isolate maintained in the culture collection is intrinsically different from the genotypes that build up most of *Ch. phaeobactereoides* in Lake Kinneret. We found a positive relationship between light intensity and carotenoid content in laboratory experiments implying the photoprotective role of those compounds. The laboratory isolate also showed two unknown carotenoids, never found before in lake samples. Those carotenoids are less polar than β-carotene, and showed absorption peaks at 427 and 451 nm, and 429 and 451 nm, in methanol.

Keywords Bacteriochlorophyll *e*; carotenoids; photoprotection

Introduction

Bacteriochlorophyll *e* (Bchl *e*) is the light-harvesting pigment of the green sulfur bacterium *Chlorobium phaeobactereoides* Pfennig, as well as of other species of brown-colored photosynthetic sulfur bacteria (Imhoff, 1995). Separation of pigment extract of that group of bacteria by high-performance liquid chromatography (HPLC) showed the presence of homologues of Bchl *e*, that possess identical spectral characteristics and elute as a cluster of three peaks (Korthals and Steenbergen, 1985). In other studies isolates with 3 (Yacobi et al., 1990; Borrego and Garcia-Gill, 1994; Borrego et al., 1997) and 4 homologues (Repeta et al., 1989; Hurley and Watras, 1991; Borrego et al., 1997) were found. The quantitative relationships between homologues differ conspicuously in different isolates. Borrego et al. (1997) studied those relationships in several European and American populations of brown-colored photosynthetic sulfur bacteria, and found that patterns of relationships between Bchl *e* homologues are related to the light regime under which different populations were grown and the physiological status of the cells.

*Ch. phaeobactereoides* forms a dense population in the metalimnion of Lake Kinneret between July and October (Bergstein et al., 1979; Butow and Bergstein-Ben Dan, 1992). Samples from the metalimnion were analyzed by HPLC, and showed that the relationships between Bchl *e* homologues were stable temporarily (Yacobi et al., 1990; Eckert et al., 1990). The goals of the present work were: (1) to compare the current composition of *Ch. phaeobactereoides* pigments to the composition found in previous years in Lake Kinneret, and (2) to investigate the impact of light on cellular pigmentation in that species in cultures and natural conditions.
Methods
A culture of Chlorobium phaeobacteroides, isolated from Lake Kinneret, is kept in a Pfennig medium under strict anaerobic conditions at a photon flux density of 10 mol m\(^{-2}\) sec\(^{-1}\). Cells were transferred to fresh medium, and exponentially growing bacteria were exposed to photon flux density (PFD) of 1, 10 or 100 mol m\(^{-2}\) sec\(^{-1}\), and temperature of 17ºC. In another experiment, cultures were exposed to PFD that ranged between 0.2 and 30 \(\mu\)mol m\(^{-2}\) sec\(^{-1}\), and the temperature was 28ºC. In a third experiment, cultures were established in the laboratory, and after four days were deployed in the lake, at depth of 3 and 16 m. They were incubated at Station A, which is located at the deepest part of Lake Kinneret, and taken back to the laboratory for analysis after seven days.

Water samples from the lake were withdrawn from the metalimnion at Station A. The protocols used for water sampling, collection of particulate matter and pigment separation by HPLC and quantification were applied as described elsewhere (Yacobi et al. 1996). Pigment identification was facilitated by the use of ChromaScope (BarSpec, Israel), a spectral peak analyzer that allows scanning of pigments separated by the HPLC system, in the range from 360 to 700 nm. The spectral data of the separated peaks, and their retention times were used as the parameters for peak identification, using published data (Korthals and Steenbergen, 1985). The quantification of the chromatograms was facilitated by injection of known pigment concentrations into the HPLC system and calculating the response factor based on the area under the peak. The concentration of two unidentified carotenoids (see Results) was determined using an extinction coefficient of 2500. All the calculations of pigment concentration are presented as the mean of duplicate measurements. Individual measurements did not differ by more than 10%.

Results and discussion
Three Bchl e homologues and the carotenoids isorenieratene and \(\beta\)-isorenieratene were consistently identified in all Chlorobium phaeobacteroides cultures and water samples drawn from Lake Kinneret. In cultures, two unidentified carotenoids showed, which displayed very unusual spectral characteristics as their maximal absorption showed at 427 and 451, and 429 and 451 nm, in the first and second unidentified carotenoid, respectively (Figure 1). In the cultured isolate of Ch. phaeobacteroides the homologues Bchl e1 or Bchl e2, were conspicuously more abundant than Bchl e3. In samples drawn from the lake metalimnion Bchl e3 was invariably more abundant than Bchl e2 and the latter was more abundant than Bchl e1 (Figure 2).

![Figure 1: Spectral characteristics of isorenieratene and an unidentified carotenoid from a laboratory grown isolate of Chlorobium phaeobacteroides](https://iwaponline.com/wst/article-pdf/42/1-2/423/427714/423.pdf)
In this study, lake samples which contained *Ch. phaeobacteroides* pigments were taken from strictly anoxic layers. The light available in the uppermost layer sampled in the current experiment, at 16.5 m, was lower than 0.1% of the photon flux density at the lake surface (data not shown). The highest Bchl e concentration, of 27 μg·l⁻¹, was found at 18.5 m, where the available light declined to less than 0.05% of the surface value. At a depth of 20.5 m the Bchl e concentration dropped to 1.8 μg·l⁻¹. The ratios between Bchl e homologues were approximately constant along a vertical gradient sampled from the water column. Those ratios are similar to our previous study, done nine years ago (Yacobi *et al.*, 1990). However, we found variation in quantitative relationships between Bchl e homologues in samples taken from sediments (Ostrovsky and Yacobi, 1999). In samples taken from sediments overlaid by anoxic waters the relationships between Bchl e homologues were similar to those found in the water column, i.e., about 1.5, 2.0 and 0.75 for the ratios of Bchl e₂/e₁, Bchl e₃/e₁ and Bchl e₂/e₃, respectively. On the other hand, in samples collected from shallow sediments we were able to detect other relationships between Bchl e homologues. Thus, in a sample collected from a sediment located at the depth of 12 m the mentioned ratios were: 0.90, 1.65 and 0.55, indicating that uniformity of *Ch. phaeobacteroides* pigment composition is changed in some niches of the lake.

In the current sampling we found an exceptionally high ratio of carotenoids to Bchl e in the uppermost sample, but otherwise the ratio of carotenoids (both forms of isorenieratene) to Bchl e (the three homologues combined) although varied vertically, did not show a defined pattern with depth.

Cultures of *Ch. phaeobacteroides* were exposed to continuous light in the laboratory, and subsamples harvested 6 and 13 days after the initiation of the experiment. Bchl e₂ was the most conspicuous Bchl homologue in cultures, with the exception of the cultures grown

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**Figure 2** Photosynthetic pigments of *Chlorobium phaeobacteroides*, grown at several photon flux density intensities. (a) ratios between the three Bchl e homologues; (b) Ratio between carotenoids (both forms of isorenieratene and two unidentified carotenoids) and Bchl e. Left group: after six days in culture; Center group: after 13 days in culture; Right: (LK) Lake Kinneret sample from depth of 18.5 m, where the available light was <0.01 μmol m⁻² sec⁻¹
at 1 μmol m$^{-2}$ sec$^{-1}$ and checked after six days, where Bchl $e_1$ was the most abundant pigment. After 13 days of exposure, all cultures assumed almost similar proportions of Bchl $e$ homologues (Figure 2a). On the other hand, the ratio of carotenoids (both isorenieratene forms and the two unidentified carotenoids) to Bchl $e$ increased, with the increase of photon flux density, on both occasions on which cultures were sampled (Figure 2b). The ratio of the unidentified carotenoids varied between 0.37 and 0.51 of the total concentration of carotenoids found in the cultures, with the highest proportions in cultures exposed to the highest light. In an additional experiment light intensity varied between 0.2 and 30 μmol m$^{-2}$ sec$^{-1}$. Bchl $e$ concentration of cultures grown at 0.2 μmol m$^{-2}$ sec$^{-1}$ was less than 30% of those grown under higher photon flux density, but the ratios between Bchl $e$ homologues were similar in all light regimes and to the previously described experiment.

The ratio of Bchl $e$ homologues in cultures incubated in the lake were similar to those found in laboratory cultures after six days, and grown at photon flux density of 10 and 100 μmol m$^{-2}$ sec$^{-1}$, both at the depth of 3 m and 16 m. The ratio of carotenoids to Bchl $e$ was 0.09 in the cultures incubated at the depth of 3 m, and 1.5 times higher than in cultures incubated at the depth 16 m. In both depths the unidentified carotenoids comprised approximately one half of the carotenoid bulk. The pigment composition of cultures deployed in the lake were not significantly different from cultures maintained in the laboratory, despite the obvious difference in light intensity, and spectral quality, between those locations. Although the *Ch. phaeobacteroides* maintained in the Kinneret Laboratory culture collection was isolated from the lake, it apparently does not represent the prevalent genotype(s) thriving in the lake water column. Several attempts were made, in different years, to culture *Ch. phaeobacteroides* from Lake Kinneret and the resulting culture consistently harbors similar combination of Bchl $e$ homologues. Therefore, we assume that isolation acted in favor of a certain genotype of *Ch. phaeobacteroides*.

Borrego and Garcia-Gil (1995) found that the proportion of two Bchl $e$ homologues of *Ch. phaeobacteroides* increased and two decreased with the increase of photon flux density from 0.1 to 1 μmol m$^{-2}$ sec$^{-1}$. However, in higher light intensities, the proportion of different homologues was pretty stable. It seems that adaptation to variation in light intensity of *Ch. phaeobacteroides*, by differential synthesis of Bchl $e$ homologues was apparently restricted to very low light intensities, in the work of Borrego and Garcia-Gil (1995). Those low light intensities are actually close to those *Ch. phaeobacteroides* is exposed to in the metalimnion in Lake Kinneret (Bergstein et al., 1979), but we did not found vertical differentiation of Bchl $e$ homologues ratios in Lake Kinneret, neither in natural samples, nor in deployed cultures in situ.

The increased synthesis of carotenoids with light increase is a well known phenomenon in oxygenic phototrophs, and is also found in cultures of *Ch. phaeobacteroides*, and other green sulfur bacteria (Borrego and Garcia-Gil, 1995). The results of our experiments indicate the same trend, but the relative concentration of carotenoids was conspicuously lower and the increase with the increase of light was higher than in the former example. That difference may be explained by the very nature of the habitat where the bacterium thrives, as the light penetrating down to the metalimnion in Lake Kinneret, is below the intensities demanding the permanent existence of photoprotective apparatus.

Conclusions

The distinctive difference between the relative abundance of the three Bchl $e$ homologues in the laboratory-maintained strain of *Chlorobium phaeobacteroides* and the lake samples, and the relative stability of those quantitative relationships within each source, indicate a possible genetic difference.
Differential synthesis of Bchl e homologues is apparently not a mechanism for optimization of the light-harvesting capacity of *Ch. phaeobacteroides*, as the ratio between Bchl e homologues changed only slightly in response to changing light intensity, both in the laboratory and in the lake.

The capability to increase the relative content of carotenoids in response to elevation of light intensity, indicates that *Ch. phaeobacteroides* may grow successfully in waters layers exposed to a relatively high photon flux density. However, photoprotection is not required in deep water layers, therefore that option is not utilized by *Ch. phaeobacteroides* in the metalimnion of Lake Kinneret.

References


