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EXPERIMENTAL ARTICLES

Spectral Properties of the Green Alga *Trebouxia*, a Phycobiont of Cryptoendolithic Lichens in the Antarctic Dry Valley

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Abstract—An algologically pure culture of the green alga *Trebouxia*, a phycobiont of cryptoendolithic lichens, was isolated from sandstone samples collected in the high-altitude polar regions of Antarctica. The absorption and second-derivative absorption spectra of acetone extract of the Antarctic phycobiont cells were studied in comparison with those of a *Trebouxia* phycobiont isolated recently from a *Parmeliaceae* lichen in the Mid-European climatic zone. The cells of the Antarctic phycobiont were characterized by a lower content of chlorophyll *a* and a higher ratio of chlorophyll *b* and carotenoids to chlorophyll *a* as compared to the Mid-European phycobiont. Furthermore, the carotenoids of the Antarctic phycobiont were more diverse. The low-temperature fluorescence spectra of the Antarctic phycobiont were characterized by an increased intensity of the short-wavelength fluorescence peak of chlorophyll *a* and a diminished intensity of fluorescence in the long-wavelength spectral region.

Key words: Antarctica, sandstone, phycobiont, cryptoendolithic lichens, *Trebouxia*, absorption spectra, second-derivative absorption spectra, low-temperature fluorescence spectra.

Among the five cryptoendolithic lichens of the Antarctic Dry Valley that have been described thus far, three contain cyanobacteria (predominantly of the genus Gloeocapsa) and two contain the green algae Trebouxia and Hemichloris antarctica. The cyanobacteria grow in different zones of lichens, whereas the green algae grow in the upper zones of lichens [1, 2]. The microbiological analysis of samples of Beakon sandstones with pigmented zones of lichen algal growth, which were collected in the Antarctic Dry Valley, has led to the isolation of three algologically pure cyanobacteria of the genus Gloeocapsa (blue-green, brown, and red-orange in color) and one greenish brown cyanobacterium, Chroococcidiopsis sp.

The study of the absorption, second-derivative absorption, and low-temperature fluorescence spectra of these cyanobacterial phycobionts allowed us to suggest that the *Gloeocapsa* phycobionts grow in the upper zone of lichens [3], which is in agreement with the data available in the literature [1]. These cyanobacteria are characterized by a low content of chlorophyll *a* and relatively high contents of phycobiliproteins and carotenoids, which are involved in light harvesting and the transfer of excitation energy to photosystem II (PSII). The carotenoids of these cyanobacterial phycobionts are quite diverse.

We succeeded in the isolation of an algologically pure culture of a green phycobiont from the cryptoendolithic lichens. This phycobiont was found to be a member of the genus *Trebouxia* [4].

The aim of the present work was to study the content and composition of photosynthetic pigments in the *Trebouxia* phycobiont based on absorption, second-derivative absorption, and low-temperature fluorescence spectra.

MATERIALS AND METHODS

The samples of Beakon sandstones with the pigmented zones of lichen algal growth were collected in the Antarctic Dry Valley. The samples were ground aseptically and placed in petri dishes with BG-11 mineral medium [5]. The dishes were incubated for 60 days in two variants. In variant I, which was proposed by Meyer *et al.* [2] for the cultivation of the Antarctic cryptoendolithic lichen phycobionts *Trebouxia* sp. and *Hemichloris antarctica*, the enrichments were incubated at 8°C under white light from luminescent lamps at an intensity of 400–600 lx. In variant II, the enrichments were incubated at 20°C under 15000-lx illumination. The color and size of cells grown under these conditions did not differ, although the number of cells was greater in the latter case. To obtain the phycobiont

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in an algologically pure culture, cells grown in the enrichment culture were transferred to the liquid BG-11 medium and incubated at 20°C at 15000-lx illumination in an atmosphere containing 2% CO_2 for 30 days. The isolated Antarctic cryptoendolithic lichen phycobiont was designated *Trebouxia* sp. 1, whereas a *Trebouxia* phycobiont isolated recently from a *Parmeliaceae* lichen in the Mid-European zone and used in this work for comparison was designated *Trebouxia* sp. 2.

The low-temperature fluorescence spectra of the phycobionts were recorded at liquid nitrogen temperature (–196°C) using a Hitachi 850 spectrophotometer (Japan). The wavelength of excitation light was 434 nm. The optical density of the samples did not exceed 0.1–0.2.

The absorption and second-derivative absorption spectra of the phycobionts were recorded at room temperature using a Shimadzu UV-160 PC spectrophotometer (Japan). For this purpose, cell suspensions were dried in a flow of compressed air to give films about 0.5 mm in thickness. Control experiments showed that the absorption spectra of the cell suspensions and the films prepared from them were identical, suggesting that photosynthetic pigments remained intact in the films.

The content of chlorophyll *a*, chlorophyll *b*, and total carotenoids in cold 100% acetone extract of cells was estimated by using the following Maclachlan equations [6]:

$$C_a = 12.3E_{663} - 0.86E_{645}; \quad C_b = 19.3E_{645} - 3.6E_{663};$$

$$C_a + C_b = 8.7E_{663} + 18.4E_{645};$$

$$C_{car} = 4.695E_{440.5} - 0.268C_{a+b}.$$

The relative content of chlorophyll *a* in algal cells was estimated from the ratio of the absorbance of the cell suspension or film in the absorption maximum of chlorophyll *a* (A_{680}) to the background absorbance at 730 nm. The relative content of chlorophyll *b* in algal cells was estimated from the ratio of the absorbance of the cell suspension or film in the absorption maximum of chlorophyll *b* (A_{650}) to the absorbance in the absorption maximum of chlorophyll *a* (A_{680}), i.e., as the A_{650}/A_{680} ratio. Similarly, the relative content of total carotenoids in algal cells was estimated as the A_{500}/A_{680} ratio.

All the spectra were recorded in triplicate. Since the difference between the replicate spectra did not exceed 7%, the figures show typical spectra.

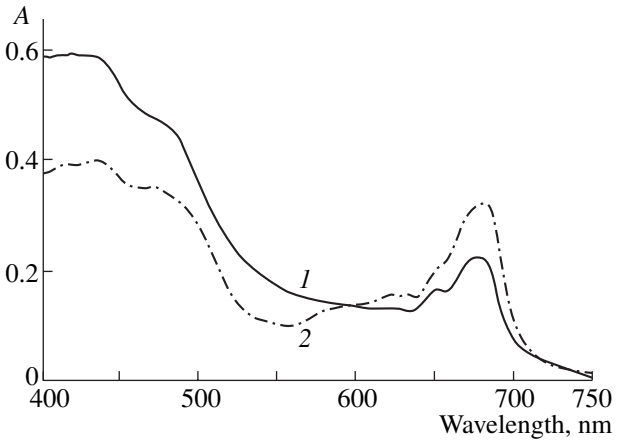

Fig. 1. The absorption spectra of (*1*) *Trebouxia* sp. 1 and (*2*) *Trebouxia* sp. 2.

RESULTS

As shown in Fig. 1, the absorption spectra of *Trebouxia* sp. 1 and *Trebouxia* sp. 2 are similar in the position of the absorption peaks of chlorophylls *a* and *b*. Both spectra exhibited a wide asymmetric band of chlorophyll *a* in the red spectral region, with two shoulders at 680 nm and 672 nm, which appeared as two distinct peaks at 684 and 670 nm in the second-derivative absorption spectra (Fig. 2, Table 1). In the absorption spectrum of *Trebouxia* sp. 1, the absorbance at 672 nm was higher than at 680 nm. Conversely, in the absorption spectrum of *Trebouxia* sp. 2, the absorbance at 672 nm was lower than at 680 nm. The absorbance A_{680} of the *Trebouxia* sp. 1 and sp. 2 cells was equal to 0.22 and 0.32, respectively (Fig. 1). Consequently, the relative content of chlorophyll *a* in the *Trebouxia* sp. 1 cells was 1.45 times lower than in the *Trebouxia* sp. 2 cells.

The second-derivative absorption spectra of the algal cells had the chlorophyll *a* peaks located at 623, 440, and 418 nm (*Trebouxia* sp. 1) and at 620, 441, and 415 nm (*Trebouxia* sp. 2) (Fig. 2, Table 1). Therefore, the Antarctic phycobiont differed from the Mid-European phycobiont in that the former contained less chlorophyll *a*, with a prevalence of the short-wavelength spectral forms of this chlorophyll.

The absorption spectra of the *Trebouxia* sp. 1 and *Trebouxia* sp. 2 cells also had an absorption maximum of chlorophyll *b*, located at 650 nm (it was more distinct in the absorption spectrum of *Trebouxia* sp. 1). In the second-derivative spectra (Fig. 2, Table 1), the absorp-

Table 1. Peaks in the second-derivative absorption spectra of the Antarctic (*Trebouxia* sp. 1) and Mid-European (*Trebouxia* sp. 2) phycobionts

Phycobiont	Wavelength, nm
Trebouxia sp. 1	418, 440, 487, 498, 508, 533, 543, 578, 593, 623, 648, 669, 684
Trebouxia sp. 2	415, 441, 487, 497 577, 594, 620, 649, 670, 684

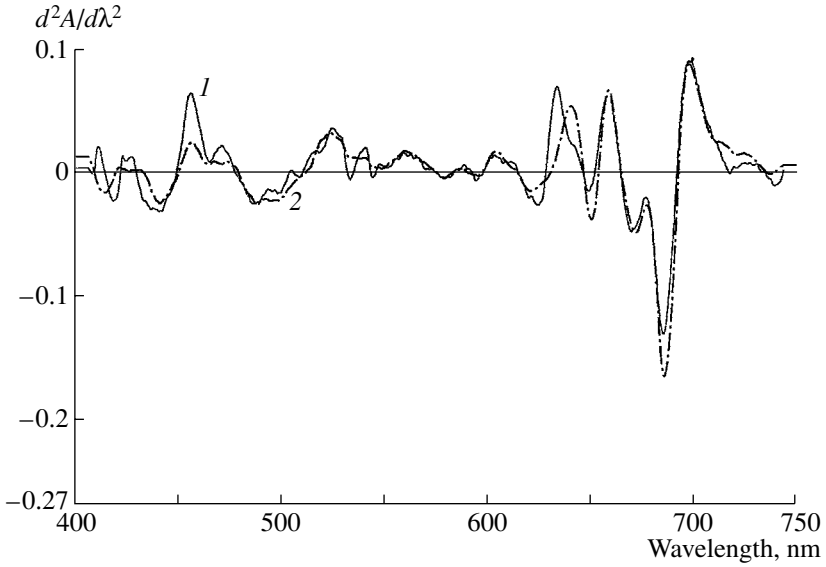

Fig. 2. The second-derivative absorption spectra of (*1*) *Trebouxia* sp. 1 and (*2*) *Trebouxia* sp. 2.

tion peaks of chlorophyll *b* were located at 648–649 and 487 nm. In the absorption spectrum of *Trebouxia* sp. 1, the values A_{650} (chlorophyll *b*) and A_{680} (chlorophyll *a*) were equal to 0.17 and 0.22, respectively, with the A_{650}/A_{680} ratio being equal to 0.77. In the absorption spectrum of *Trebouxia* sp. 2, the values A_{650} (chlorophyll *b*) and A_{680} (chlorophyll *a*) were equal to 0.2 and 0.32, respectively, with the A_{650}/A_{680} ratio being equal to 0.62. Consequently, the relative content of chlorophyll *b* (with reference to chlorophyll *a*) in the *Trebouxia* sp. 1 cells was 1.24 times higher than in the *Trebouxia* sp. 2 cells.

The absorption spectra of the *Trebouxia* sp. 1 and *Trebouxia* sp. 2 cells differed considerably in the spectral region typical of carotenoids (at 500 nm) (Fig. 1). In the second-derivative spectra (Fig. 2, Table 1), the absorption peaks of carotenoids were located at 497–498 nm. In the absorption spectrum of *Trebouxia* sp. 1, the values A_{500} (carotenoids) and A_{680} (chlorophyll *a*) were equal to 0.35 and 0.22, respectively, with the A_{500}/A_{680} ratio being equal to 1.6. In the absorption spectrum of *Trebouxia* sp. 2, the values A_{500} (carotenoids) and A_{680} (chlorophyll *a*) were equal to 0.28 and 0.32, respectively, with the A_{500}/A_{680} ratio being equal to 0.8. Consequently, the relative content of carotenoids (with reference to chlorophyll *a*) in the *Trebouxia* sp. 1 cells was two times higher than in the *Trebouxia* sp. 2 cells.

Table 2. The relative content of chlorophyll *a*, chlorophyll *b*, and carotenoids in the cells of the Antarctic and Mid-European phycobionts

Phycobiont	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
Trebouxia sp. 1	100	48	56
Trebouxia sp. 2	100	27	15

In addition to the absorption peak of carotenoids located at 497–498 nm, the second-derivative absorption spectrum of *Trebouxia* sp. 1 had peaks with maxima at 508, 533, and 543 nm (Fig. 2, Table 1). These peaks might belong to the carotenoid zeaxanthin, which reportedly has absorption maxima at 501 nm [7] and 535 nm [8]. Thus, the carotenoids of the Antarctic phycobiont *Trebouxia* sp. 1 differed from the Mid-European phycobiont *Trebouxia* sp. 2 in not only the content but also the composition of carotenoids.

The differencies in the relative content of chlorophyll *a*, chlorophyll *b*, and carotenoids in the *Trebouxia* sp. 1 and sp. 2 cells revealed from the analysis of their absorption spectra were confirmed by the analysis of the relative contents of these pigments in the cold 100% acetone extracts. The content of chlorophyll *a* in either of the two extracts was taken to be 100%. The relative content of chlorophyll *b* with reference to the content of chlorophyll *a* in the extracts of the *Trebouxia* sp. 1 and sp. 2 cells was found to be 48 and 27%, respectively. Consequently, the relative content of chlorophyll *b* with reference to chlorophyll *a* in the *Trebouxia* sp. 1 cells was 1.77 times higher than in the *Trebouxia* sp. 2 cells (Table 2). The relative content of total carotenoids with reference to chlorophyll *a* in the extracts of the *Trebouxia* sp. 1 and sp. 2 cells was found to be 56 and 15%, respectively. Consequently, the relative content of carotenoids with reference to chlorophyll *a* in the *Trebouxia* sp. 1 cells was 3.7 times higher than in the *Trebouxia* sp. 2 cells (Table 2). Thus, the Antarctic phycobiont *Trebouxia* sp. 1 differed from the Mid-European phycobiont *Trebouxia* sp. 2 in higher relative contents of chlorophyll *b* and carotenoids and in more complex composition of the carotenoids.

The low-temperature fluorescence spectra of the two phycobionts differed considerably (Fig. 3).

The low-temperature fluorescence spectrum of the *Trebouxia* sp. 1 cells had two peaks, at 686 and 695 nm, in the near-red region and a shoulder near 715 nm in the far-red region. The ratio of the fluorescence intensities at 686 and 715 nm was equal to 1.6. The low-temperature fluorescence spectrum of the *Trebouxia* sp. 2 cells had a peak at 686 nm and a shoulder near 717 nm, with the ratio of the fluorescence intensities at 686 and 717 nm being equal to 0.77. Consequently, the *Trebouxia* sp. 1 and sp. 2 phycobionts differed in the relative intensity of the short-wavelength and long-wavelength fluorescence maxima of chlorophyll *a* in their low-temperature fluorescence spectra. The higher intensity of the short-wavelength fluorescence maximum in the low-temperature fluorescence spectrum of *Trebouxia* sp. 1 (Fig. 3) is in agreement with the prevalence of the short-wavelength spectral forms of chlorophyll *a*, as is evident from the absorption spectrum of the Antarctic phycobiont (Fig. 1).

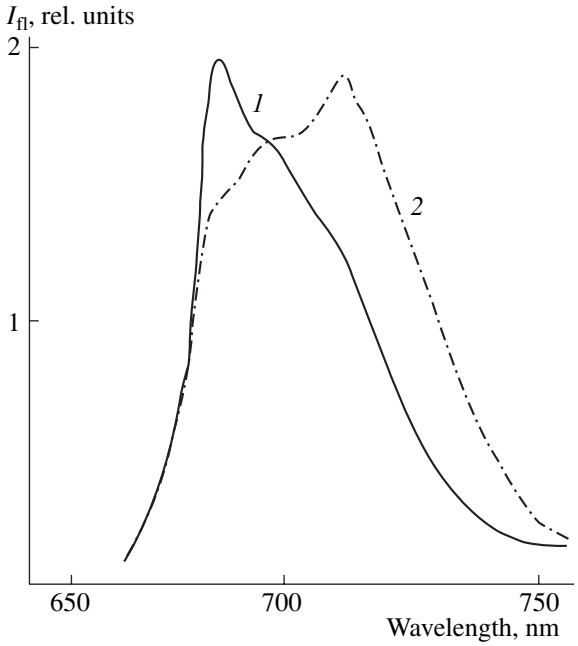

Fig. 3. The low-temperature fluorescence spectra of (*1*) *Trebouxia* sp. 1 and (*2*) *Trebouxia* sp. 2.

DISCUSSION

Photosynthetic organisms, particularly phycobionts occurring in the upper zones of lichens in the Antarctic Dry Valley, can grow under the extreme conditions of high insolation and very low temperatures due to the specific organization of their photosynthetic apparatus, which provides, first of all, the protection of these organisms against photoinhibition [9].

Photoinhibition is mostly observed in the PSII structures, particularly in the peripheral antenna complexes [7, 8], which contain more chlorophyll *b* and carotenoids than the photosystem I structures [10]. Photosynthetic pigments (chlorophylls and carotenoids) play an important part in photoprotection by inhibiting the photodegradation of some specific proteins and polypeptides of PSII [7, 8, 11]. To prevent the inhibiting action of excessive light, the size of light-harvesting complexes (LHCs) is diminished by means of reducing the relative content of chlorophyll *a* and increasing the relative content of carotenoids [8–10].

The activation of the xanthophyll cycle plays a key part in the photoprotection of cells. In LHC2, zeaxanthin interacts with excited chlorophyll molecules (predominantly of chlorophyll *a*), quenching the triplet state of this chlorophyll [7, 8, 11]. On the other hand, neoxanthin interacts with chlorophyll *b*, promoting the removal of singlet oxygen [7].

The data obtained in this study show that the cells of the Antarctic phycobiont *Trebouxia* sp. 1, living under extreme conditions [1], contain less chlorophyll *a* than do the cells of the Mid-European phycobiont *Trebouxia* sp. 2. At the same time, the relative content of carotenoids and chlorophyll *b* in the Antarctic phycobiont is higher than in the Mid-European phycobiont. The second-derivative absorption spectra of the Antarctic phycobiont had additional absorption peaks in the region 500–540 nm, which may belong to zeaxanthin [7, 8]. These changes in the cellular content of photosynthetic pigments in the Antarctic phycobiont may be due to active processes aimed at stabilizing the structure and preventing the photoinhibition of PSII. This suggestion is in agreement with the data of Koroleva [12] that PSII of Antarctic photosynthetic organisms is tolerant to excessive insolation and extremely low temperatures.

The increased intensity of the short-wavelength maximum at 686 nm (it corresponds to LHC2) in the low-temperature fluorescence spectrum of *Trebouxia* sp. 1 can be accounted for by the emissive deactivation of the electron excitation of LHC2 in order to remove excess absorbed solar energy. On the other hand, the low intensity of long-wavelength fluorescence in the low-temperature fluorescence spectrum of the Antarctic phycobiont *Trebouxia* sp. 1 may indicate a diminished content of LHC1, as was observed in some algae under excessive insolation [13].

The spectral analysis described in this paper is an important step in more comprehensive studies that are necessary to investigate the molecular organization of the photosynthetic apparatus of Antarctic phycobionts, which has evolved over a period of tens of thousands of years under the extreme insolation and temperature conditions of Antarctica [14].

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