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SHORT COMMUNICATIONS

Spectral Properties of Ancient Green Algae from Antarctic Dry Valley Permafrost

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Algologically pure cultures of ancient viable green algae were isolated for the first time from Antarctic Dry Valley permafrost (presumably 8.1 billion years old [1]).

Forty-eight samples were taken from seven wells from depths of 0.1 to 18.7 m. To detect and isolate ancient algae, enrichment cultures were incubated in petri dishes in mineral BG-11 medium [2] either at 5–8°C under illumination at an intensity of 400–600 lx or at 18–20°C under 20000-lx illumination. The latter conditions proved to be more optimal for algal growth, and after seven months of incubation, two samples taken from well 1/99 from depths of 14.5 and 14.8 m gave rise to green algal colonies that could be classified as belonging to the order *Chlorococcales*, family *Chlorococcaceae* [3]. A sample taken from the depth of 14.9 m from the same well produced green algal colonies that, according to [3], could be assigned to the same order *Chlorococcales*, family *Chlorellaceae*. These two algae were designated as *Chlorococcum* sp. and *Chlorella* sp. Algologically pure cultures of these algae were obtained by standard microbiological methods. For experiments, they were grown in liquid BG-11 medium for 30 days at 20°C and 20000-lx illumination in an atmosphere containing 2% CO_2.

The aim of this work was to study the content and composition of photosynthetic pigments in the cells of these ancient green algae based on their absorption spectra, the second-derivative absorption spectra, and low-temperature fluorescence spectra. For comparison, we used the laboratory alga *Chl. vulgaris* grown under the same cultivation conditions (20°C, 20000-lx illumination, an atmosphere with 2% CO_2) for 7 days.

The spectral methods used in this study were described elsewhere [4]. The relative content of chlorophyll *a* in algal cells was estimated from the absorbance at the absorption maximum of this pigment at 680 nm (A_{680}) in the absorption spectra of cell suspensions or films with reference to the absorbance at 730 nm. The relative content of chlorophyll *b* was estimated by the ratio of the absorbance at the absorption maximum of this pigment at 650 nm to that of chlorophyll *a* (A_{650}/A_{680}). The relative content of carotenoids was also estimated with reference to the content of chlorophyll *a* from the ratio of the absorbance at the absorption maximum at 480 nm to A_{680} (A_{480}/A_{680}).

As shown in Fig. 1, the absorption spectrum of *Chlorococcum* sp. exhibited a wide asymmetric band of chlorophyll *a* in the red spectral region with two shoulders at 680 nm and at 673 nm, which appeared as two distinct peaks at 682 and 671 nm in the second-derivative absorption spectrum. The wide band at 680 nm in the absorption spectrum of *Chlorella* sp. was represented by two peaks at 682 and 673 nm in the second-derivative absorption spectrum. The peak at 678 nm in the absorption spectrum of *Chl. vulgaris* was resolved into the 682- and 671-nm peaks in the second-derivative absorption spectrum of this alga. The short-wavelength bands of chlorophyll *a* at 430–440 nm in the absorption spectra of *Chlorococcum* sp. and *Chlorella* sp. were resolved into peaks at 440–438 and 413–415 nm in the second-derivative spectra. The peak at 437 nm in the absorption spectrum of *Chl. vulgaris* was resolved into two peaks (at 437 and 415 nm) in the second- derivative spectrum. The absorption bands of chlorophyll *b* in the absorption spectra of *Chlorococcum* sp., *Chlorella* sp., and *Chl. vulgaris* were observed at 650 nm. In the second-derivative absorption spectra of these algae, chlorophyll *b* was represented by two peaks, at 650–649 nm in the long-wavelength spectral region and at 467–470 nm in the short-wavelength region. Thus, the positions of the peaks of chlorophylls *a* and *b* in the absorption spectra and in the second-derivative spectra of *Chlorococcum* sp. and *Chlorella* sp. were virtually the same and differed slightly from their positions in the absorption spectra of *Chl. vulgaris* [5]. The short-wavelength regions of the absorption spectra of all the algal species studied exhibited broad bands at 480–486 nm, i.e., in the region of the predominant absorption of carotenoids. These bands corresponded to peaks at 486–488 nm in the second-deriva-

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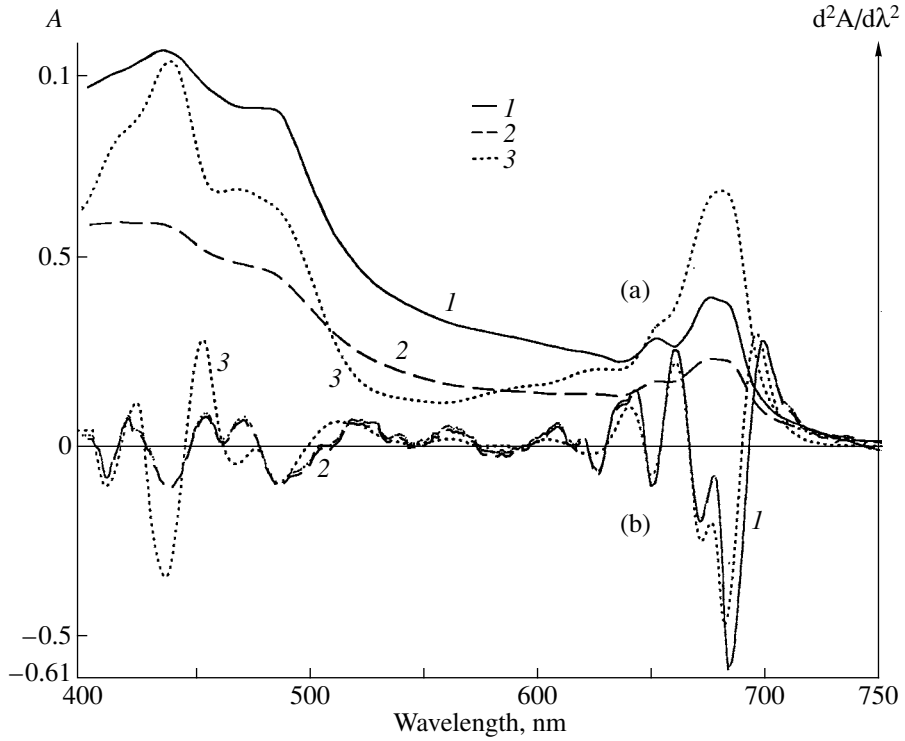

Fig. 1. (a) The absorption spectra and (b) the second-derivative absorption spectra of (*1*) *Chlorococcum* sp., (*2*) *Chlorella* sp., and (*3*) *Chl. vulgaris.*

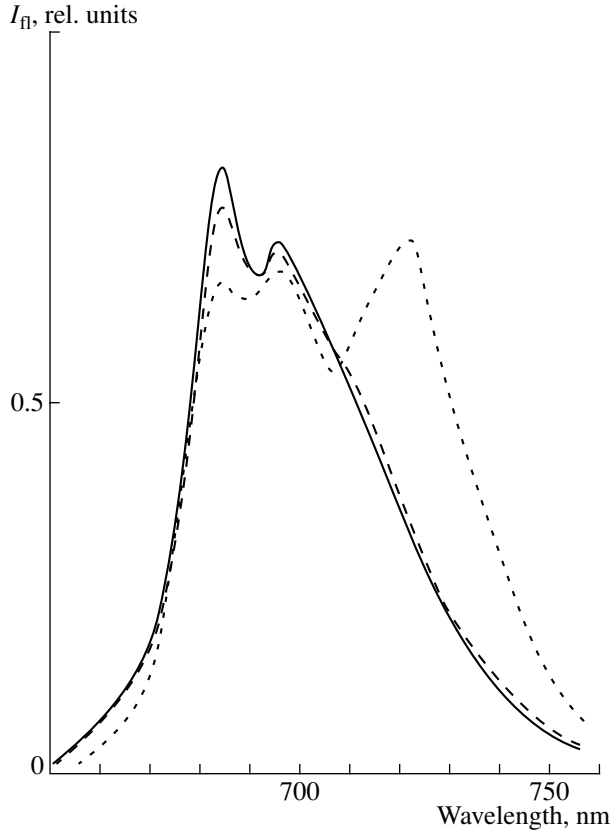

Fig. 2. The low-temperature fluorescence spectra of (*1*) *Chlorococcum* sp., (*2*) *Chlorella* sp., and (*3*) *Chl. vulgaris* excited at 434 nm.

tive absorption spectra of *Chlorococcum* sp., *Chlorella* sp., and *Chl. vulgaris.* In addition to this peak, which was common to all the algal species studied, the second-derivative spectra of *Chlorococcum* sp. and *Chlorella* sp. exhibited peaks at 585, 546, 508, and 496 nm in the spectral regions typical of carotenoids.

The A_{680} value in the absorption spectra of *Chlorococcum* sp., *Chlorella* sp., and *Chl. vulgaris* amounted to 0.37, 0.23, and 0.67, respectively, indicating that the content of chlorophyll *a* in *Chlorococcum* sp. and *Chlorella* sp. cells was, respectively, 1.8 and 2.9 times lower than in the cells of *Chl. vulgaris.* The A_{650} value in the absorption spectra of *Chlorococcum* sp., *Chlorella* sp., and *Chl. vulgaris* comprised 0.35, 0.28, and 0.15, respectively. The A_{650}/A_{680} ratio in the absorption spectra of these algae was equal to 0.75, 0.65, and 0.52, respectively. Consequently, in *Chlorococcum* sp. and *Chlorella* sp. cells, the content of chlorophyll *b* with reference to the content of chlorophyll *a* was, respectively, 1.4 and 1.25 times higher than in *Chl. vulgaris* cells. The A_{480} value in the absorption spectra of *Chlorococcum* sp., *Chlorella* sp., and *Chl. vulgaris* was 0.92, 0.45, and 0.62, and, accordingly, the A_{480}/A_{680} ratio comprised 2.48, 1.95, and 0.92, respectively. This implies that the content of carotenoids in *Chlorococcum* sp. and *Chlorella* sp. cells with reference to the content of chlorophyll *a* was, respectively, 2.7 and 2.1 times higher than in *Chl. vulgaris* cells.

The low-temperature fluorescence spectrum of *Chl. vulgaris* cells with excitation at 434 nm showed two peaks located at 686 and 698 nm in the short-wavelength red spectral region, the major peak at 725 nm, and a small shoulder at 715–717 nm in the long-wavelength red spectral region (Fig. 2). This agrees well with the data of Govindjee and Satoh [5]. The fluorescence spectra of *Chlorococcum* sp. and *Chlorella* sp. also exhibited short-wavelength peaks at 686 and 698 nm, the intensity of the major peak (at 686 nm) being 1.2 times higher than that in the fluorescence spectrum of *Chl. vulgaris*. The fluorescence spectra of *Chlorococcum* sp. and *Chlorella* sp. cells exhibited a drastic decrease in the fluorescence intensity in the long-wavelength spectral region and the presence of a shoulder at 715–717 nm.

Thus, the cells of ancient viable green algae recovered from Antarctic Dry Valley permafrost showed a low content of chlorophyll *a*, a high relative content of chlorophyll *b* and carotenoids with reference to the content of chlorophyll *a*, and a more complex composition of carotenoids. The low-temperature fluorescence spectra of these algae exhibited an increase in the intensity of the short-wavelength peak of chlorophyll *a* and a drastic decrease in the fluorescence intensity in the long-wavelength spectral region. Further investigations along this line may contribute to an understanding of the molecular organization of the photosynthetic apparatus of ancient viable algae recovered from Antarctic permafrost.

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