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SHORT COMMUNICATIONS

Spectral Properties of Viable Ancient Green Algae from Arctic Permafrost

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The recent detection, isolation, and obtaining of algologically pure cultures of viable ancient photosynthetic organisms from the Arctic and Antarctic permafrost bedrock represent an important achievement in cryobiology [1, 2]. The study of the spectral properties of viable ancient green algae from the deep ice layers in the high-altitude polar regions of Antarctica showed that the concentrations of the principal photosynthetic pigments contained in their cells differed significantly from those of the laboratory culture of *Chlorella vulgaris* [2].

The goal of this work was to obtain algologically pure cultures of viable ancient algae from the Arctic permafrost rocks of different ages, as well as to carry out a comparative investigation of the photosynthetic pigment compositions and their concentrations in cells, using absorption spectra, the second derivatives of absorption spectra, and spectra of low-temperature fluorescence.

A total of 154 rock samples of different age were taken from nine bor holes for microbiological analysis according to the previously described procedure [2]. Green algae of the order *Chlorococcales* were found in 29 samples. The samples which contained members of the family *Chlorellaceae* (according to [3]) were selected for further investigation. Several algologically pure cultures of algae identified as *Chlorella* sp_1, *Chlorella* sp_2, and *Chlorella* sp_3 were isolated from rocks dated as up to 5000–10000 years old from depths of 2.5 (borehole 1/95) and 24.3 and 46.9 m (borehole 2/94). Algologically pure cultures identified as *Chlorella* sp_4, *Chlorella* sp_5, and *Chlorella* sp_6 were isolated from 2–3 Ma-old rocks (borehole 6/90; 1.6, 17.3, and 28.3 m).

The algologically pure cultures were obtained by traditional microbiological methods. The cultures were grown for 30 days in BG-11 medium [4] at 20°C, under illumination (20000 lx), in the presence of 2% CO_2. A laboratory culture of *Chl. vulgaris*, grown under the same conditions for seven days, was used for comparison.

The sample preparation procedure and the spectral methods employed in this study are described in [5]. The chlorophyll *a* concentrations in the cells of various algal species were assayed as the optical density at its absorption maximum, 680 nm, (A_{680}) in absorption spectra of the suspensions adjusted to equal optical density at 730 nm. The ratio of chlorophyll *b* to chlorophyll *a* was calculated as the ratio between the optical density values at 650 nm (chlorophyll *b* absorption maximum, A_{650}) and at 680 nm ($A_{650} : A_{680}$). The ratio between carotenoids and chlorophyll *a* was calculated as the ratio between the optical density values at 487 nm (wavelength of primary carotenoid absorption) and at 680 nm ($A_{487} : A_{680}$. The dispersion between the optical density values of the algal absorption spectra in duplicates did not exceed 10%.

The absorption spectra and the second derivative absorption spectra of *Chlorella* sp_1, *Chlorella* sp_2, and *Chlorella* sp_3 (Fig. 1 A, B), and *Chlorella* sp_4, *Chlorella* sp_5, and *Chlorella* sp_6 (Fig. 2) did not show any significant differences in the absorption band positions. A wide dissymmetric chlorophyll *a* band in the red spectral region with a maximum at 680 nm and a shoulder at 677 nm was detected. In the second derivative spectra, this band was represented by two maximums at 683 and 671 nm. In the *Chl. vulgaris* absorption spectrum, we observed a wide band with a maximum at 678 nm, represented by two maximums at 683 and 671 nm in the second derivative spectra. In all investigated cultures, the positions of chlorophyll *a* short wavelength maximums in both absorption spectra and in the second derivative spectra at 437 nm and 413 nm were similar. In the absorption spectra and the second derivative absorption spectra of the ancient algae and *Chl. vul-*

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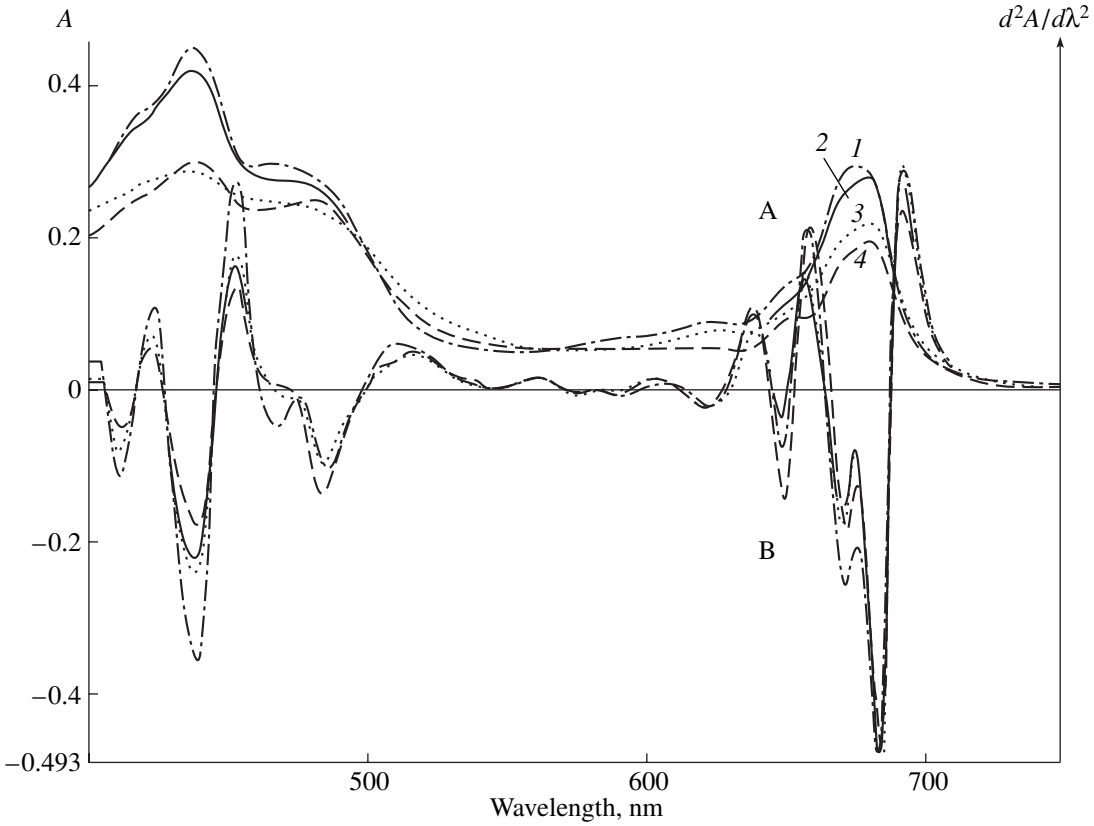

Fig. 1. Absorption spectra (A) and second derivative absorption spectra (B) of the cells of various algal species: (*1*) *Chl. vulgaris*; (*2*) *Chlorella* sp_1; (*3*) *Chlorella* sp_2; (*4*) *Chlorella* sp_3.

garis cells, the chlorophyll *b* band position was similar at 650 nm. In the region of carotenoid primary absorption, both in absorption spectra and in the second derivative spectra of ancient algae, a wide band was present at 487 nm.

In the absorption spectrum of *Chl. vulgaris* cells, broad maximums, represented by maximums at 469 and 487 nm in the second-derivative spectra, were observed at 466 and 487 nm.

The data shown in Fig. 1A indicate that the A_{680} values in the absorption spectra of *Chlorella* sp_1, *Chlorella* sp_2, and *Chlorella* sp_3 were 0.26, 0.21, and 0.19, respectively; they were thus 1.07, 1.23, and 1.36 times less than this value for *Chl. vulgaris*. In the *Chlorella* sp_4 absorption spectrum, the A_{680} value was 0.3 and matched with that in the *Chl. vulgaris* absorption spectrum (Fig. 2A). In the absorption spectra of *Chlorella* sp_5 and *Chlorella* sp_6, the A_{680} values were 0.26 and 0.22 respectively, i.e., 1.15 and 1.36 times less than the *Chl. vulgaris* absorption spectrum. Hence, a decrease in the chlorophyll *a* content in algal cells isolated from deep-seated rocks, whose age can be measured as 5000–10 000 or 2–3 million years, was detected.

In the absorption spectra of *Chlorella* sp_1, *Chlorella* sp_2, and *Chlorella* sp_3, the $A_{650} : A_{680}$ ratios, comprising 0.45, 0.47, and 0.47 respectively, are comparable to the value of 0.46 for the absorption spectrum of *Chl. vulgaris* (Fig. 1A). In the *Chlorella* sp_4 absorption spectrum, this ratio, amounting to 0.46, is comparable to that of the *Chl. vulgaris* absorption spectrum as well. In the absorption spectra of *Chlorella* sp_5 and *Chlorella* sp_6, the ratio $A_{650} : A_{680}$, which was equal to 0.66, is 1.46 greater than that in the *Chl. vulgaris* absorption spectrum (Fig. 2A). The results obtained suggest that the chlorophyll *b* content was higher in ancient algae from deeper-seated rocks, whose age can be measured as up to 2–3 million years.

In the absorption spectra of *Chlorella* sp_1, *Chlorella* sp_2, and *Chlorella* sp_3, the $A_{487} : A_{680}$ ratios were equal to 0.96, 1.1, and 1.1, respectively, i.e., 1.2, 1.37, and 1.37 times higher the value of 0.8 for the *Chl. vulgaris* absorption spectrum (Fig. 1A). These ratios in the absorption spectra of *Chlorella* sp_4, *Chlorella* sp_5, and *Chlorella* sp_6 were 1.18, 1.62, and 1.71 times higher than the $A_{487} : A_{680}$ value for the *Chl. vulgaris* absorption spectrum and comprised 1.07, 1.46, and 1.54, respectively (Fig. 2A). Therefore, the increase in caro-

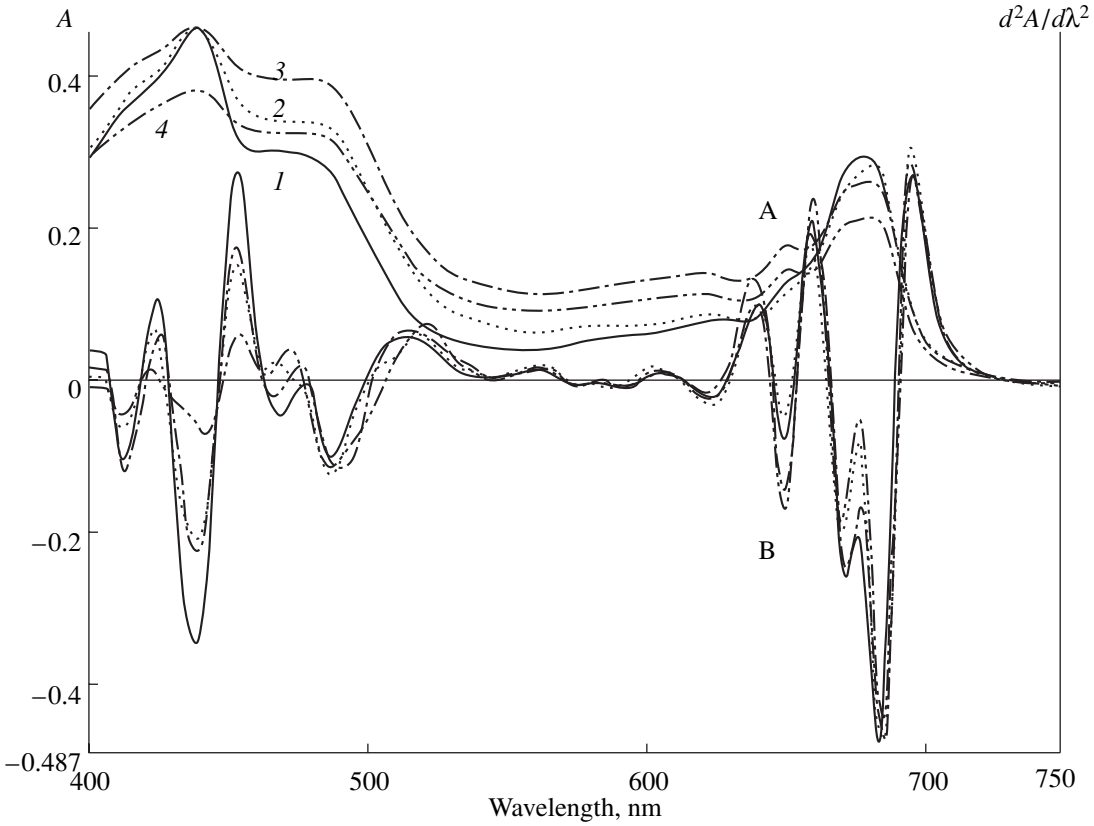

Fig. 2. Absorption spectra (A) and second derivative absorption spectra (B) of the cells of various algal species: (*1*) *Chl. vulgaris*; (*2*) *Chlorella* sp_4; (*3*) *Chlorella* sp_5; (*4*) *Chlorella* sp_6.

tenoid content in ancient viable algae cells depended on the sampling depth, as well as on the age of rocks.

It is well-known [6] that in the low-temperature fluorescence spectrum of *Chl. vulgaris*, two short-wave maximums are present at 686 and 696 nm; a maximum at 725 nm, which is the main predominant maximum in the fluorescence spectra of this species, as well as a shoulder at 715 – 717 nm, are present as well in the far-red region of the spectrum. The fluorescence spectra of ancient algae in the long-wavelength spectral region also had two maximums at 686 and 696 nm, but, unlike for *Chl. vulgaris*, the short-wave maximum at 686 nm was the main one. Fluorescence decreased significantly in the far-red region of the fluorescence spectra of ancient algae and its 725 nm maximum shifted to 715 nm (Fig. 3A, 3B). Among the ancient algae *Chlorella* sp_1, *Chlorella* sp_2, and *Chlorella* sp_3, the values of fluorescence intensity in the 725 nm region were 1.36, 1.56, and 1.8 times lower in comparison with the fluorescence maximum in the *Chl. vulgaris* fluorescence spectrum. In the fluorescence spectra of *Chlorella* sp_4, *Chlorella* sp_5, and *Chlorella* sp_6, the fluorescence intensity at 725 nm decreased more markedly (by 1.43, 1.67, and 3.0 times respectively). These measurements indicated that the main difference between the low-temperature fluorescence spectra of ancient algae and *Chl. vulgaris* was in the fluorescence redistribution between the long- and short-wave maximums of chlorophyll *a* fluorescence. This redistribution was significant in the low-temperature fluorescence spectra of ancient algae in the deepest rock samples taken from the 2-million-year-old permafrost.

Similar changes in the spectral properties of ancient viable algae from Arctic permafrost and from the deep ice of internal Antarctic regions were revealed. They include decreased chlorophyll *a* concentration, higher chlorophyll *b* and carotenoid concentrations compared to chlorophyll *a*, an increase in the intensity of the short-wave fluorescence maximum of chlorophyll *a*, and a sharp decrease in the fluorescence intensity in the long-wavelength spectral region [2]. The nature and extent of these changes point to the structural and functional peculiarities of the photosynthetic apparati of these algae, responsible for the photosystem II stability under the different cryoconservation conditions in the Arctic and Antarctica.

One can suggest that ancient photosynthetic organisms may remain viable for 13 million years in Antarctic permafrost (according to a number of scientists) and for 3 million years in the Arctic at extremely low tem-

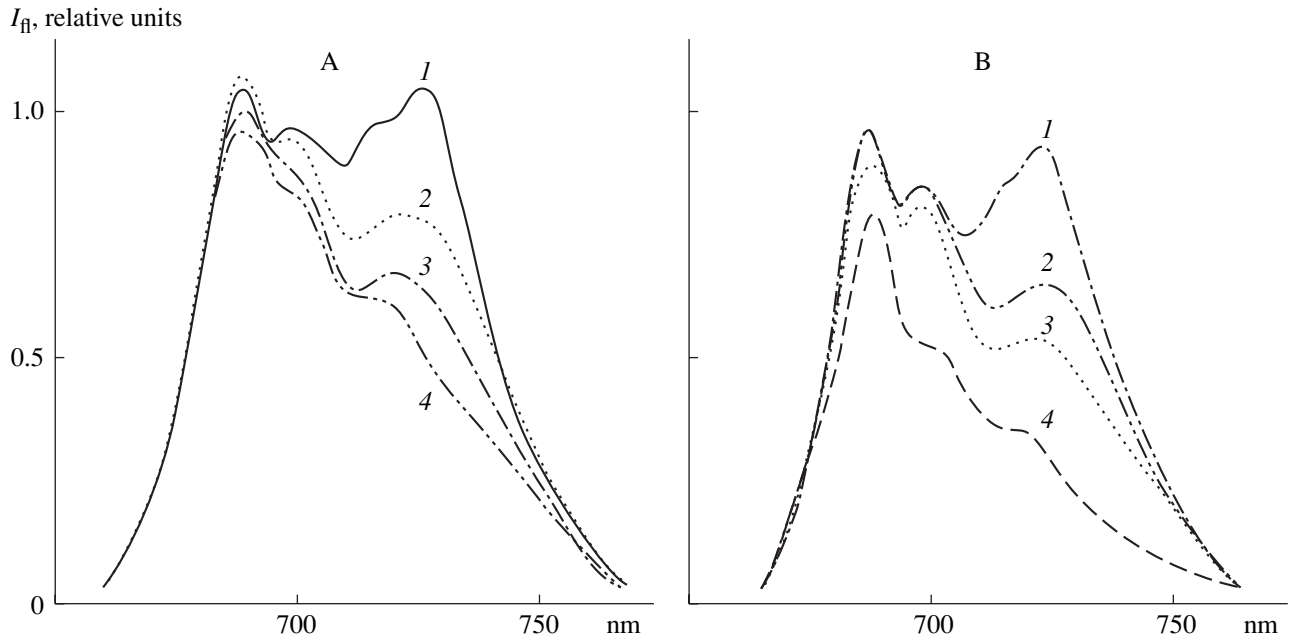

Fig. 3. Low-temperature fluorescence spectra of the cells of various algal species (excitation 434 nm); A: (*1*) *Chl. vulgaris*; (*2*) *Chlorella* sp_1; (*3*) *Chlorella* sp_2; (*4*) *Chlorella* sp_3; B: (*1*) *Chl. vulgaris*; (*2*) *Chlorella* sp_4; (*3*) *Chlorella* sp_5; (*4*) *Chlorella* sp_6.

peratures due to some properties of their photosynthetic apparatus selected and fixed in the course of evolution.

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