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## Chromatic Adaptation of Viable Ancient Cyanobacteria from Arctic Permafrost

L. G. Erokhina, E. V. Spirina, A. V. Shatilovich, and D. A. Gilichinskii

*Institute of Fundamental Problems of Biology, Russian Academy of Sciences, Pushchino, Moscow oblast, 142290 Russia*

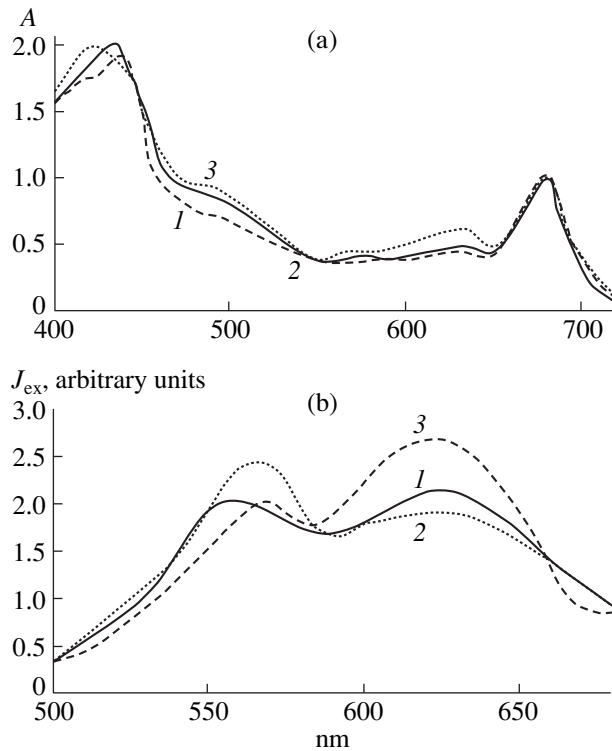
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Chromatic adaptation of cyanobacteria involves the regulation of the synthesis of the main cellular phyco-bilins, C-phycoerythrin and phycocyanin, by red and green light [1, 2]. The chromatic adaptation of modern nitrogen-fixing cyanobacteria was most pronounced when they were grown on nitrogen-free media. The addition of nitrogen sources to the cultivation media reduces the effect of green light and increases the effect of red light on the accumulation of C-phycoerythrin and phycocyanin [3]. The organisms studied in this work was the ancient and viable heterocystous cyanobacterium *Nostoc* sp., taken from a depth of 10 m in 5000-year old Holocene lake sediments [4]. In this study of the ancient bacterium, as in modern nitrogen-fixing bacteria, the amount of C-phycoerythrin is the greatest with growth on a nitrogen-free medium and decreases in the presence of various nitrogen sources [5]. To discern the capacity of this cyanobacterium for chromatic adaptation, we studied the effect of various nitrogen sources on the levels of C-phycoerythrin and phycocyanin in cells grown under red and green light. The amount of pigments in the cells was estimated from the absorption and fluorescence excitation spectra.

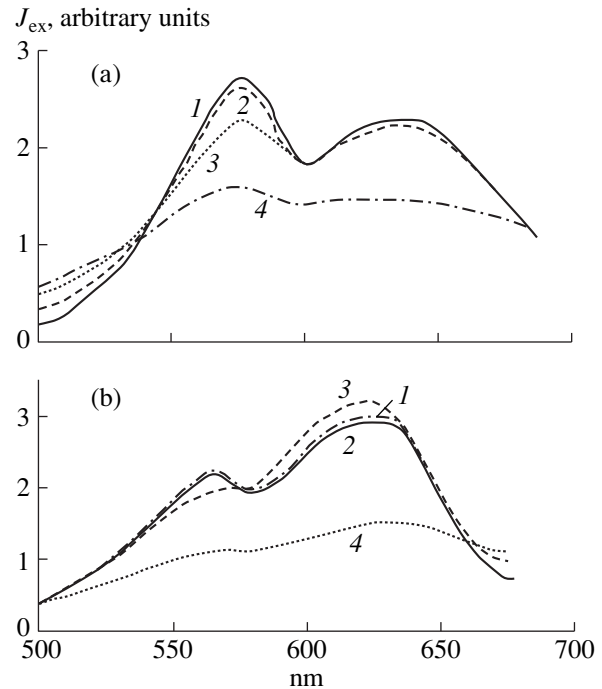
The cyanobacterium was cultivated either on a nitrogen-free BG-11 medium [6] or in the presence of various nitrogen sources, such as 1.5 mM KNO<sub>3</sub> (Reakhim, Russia), 1.5 mM asparagine, and 1.5 mM glycine (Reanal, Hungary), for 30 days at 25°C under illumination [3]. The absorption and fluorescence excitation spectra of cells were recorded using the SF-18 (LOMO, Russia) and UV-160 PC (Shimadzu, Japan) spectrophotometers, respectively. For comparative analysis of the changes in the cellular levels of C-phycoerythrin and phycocyanin, the absorption and fluorescence excitation spectra of cells were normalized to the value of absorption and fluorescence of chlorophyll *a* at 680 and 686 nm, respectively. The relative content of phycocyanin in the cells was calculated by the formula  $A_{\text{phc}}/A_{\text{chl}}$ , where  $A_{\text{phc}}$  is the optical density of cells at 622 nm (the absorption maximum of phycocyanin) and  $A_{\text{chl}}$  is the optical density of cells at 680 nm (the absorption maximum of chlorophyll *a*). The C-phycoerythrin content of cells was calculated by a similar formula, representing the ratio of the intensity of fluorescence of

the cells at 565 nm (the fluorescence maximum of C-phycoerythrin) to the intensity of fluorescence of the cells at 625 nm (the fluorescence maximum of phycocyanin). The ratio  $A_{\text{phc}}^{\text{phc}}/A_{\text{chl}}^{\text{chl}}$  of cells grown on a nitrogen-free medium under illumination with white light, and the intensities of fluorescence of phycocyanin and C-phycoerythrin in the fluorescence spectra of cells grown on this medium under illumination with green and red light, were taken to be 100%. The deviation of these values in three replicated experiments was within 5–7%.

The comparison of absorption spectra of cells grown on nitrogen-free medium under illumination with different light wavelengths (Fig. 1a) revealed that the relative amount of phycocyanin increased by 24% in cells grown under red light and decreased by 10% in cells grown under green light, when compared to the amount of cells grown under white light. Analysis of the fluorescence spectra of cells (Fig. 1b) showed that the phycocyanin content of cells increased by 23% and decreased by 5–7% during cultivation in red and green light, respectively. The content of C-phycoerythrin was 20% higher and 5–7% lower in cells grown in green and red light, respectively, than in the cells exposed to white light. In the next series of experiments, the effect of different nitrogen sources on the content of phycocyanin and C-phycoerythrin in the cells was studied. As seen from the fluorescence spectra (Figs. 2a, 2b), the phycocyanin content of cells grown in green light was not influenced by the addition of 1.5 mM KNO<sub>3</sub> to the cultivation medium, was slightly decreased (by 3–5%) in the presence of 1.5 mM asparagine, and was considerably dropped (by 47%) in the presence of 1.5 mM glycine. In the cells grown in red light, the amount of phycocyanin increased by 3 and 7–8% in the presence of 1.5 mM KNO<sub>3</sub> and 1.5 mM asparagine, respectively, while it decreased by 47% in the presence of 1.5 mM glycine. The content of C-phycoerythrin in cells grown in green light was not influenced by 1.5 mM KNO<sub>3</sub>, while it decreased by 15 and 47% in the presence of 1.5 mM asparagine or 1.5 mM glycine, respectively (Figs. 2a, 2b). When the cyanobacterium was cultivated in red light, the amount of C-phycoerythrin in the cells decreased by 15 and 47% in the presence of 1.5 mM asparagine and 1.5 mM glycine, respectively.



**Fig. 1.** (a) Absorption and (b) fluorescence excitation spectra of the cyanobacterium *Nostoc* sp. grown on a nitrogen-free medium in (1) white light, (2) green light, and (3) red light. The excitation spectrum was recorded at the fluorescence maximum of chlorophyll *a* (686 nm).



**Fig. 2.** Fluorescence excitation spectra of the cyanobacterium *Nostoc* sp. grown in (a) green light and (b) red light on (1) a nitrogen-free medium (control), or in the presence of (2) 1.5 mM  $\text{KNO}_3$ , (3) 1.5 mM asparagine, and (4) 1.5 mM glycine. The excitation spectrum was recorded at the fluorescence maximum of chlorophyll *a* (686 nm).

In conclusion, the ancient and viable cyanobacteria, like modern cyanobacteria, appear to be capable of chromatic adaptation, and their spectral characteristics were found to depend on the nitrogen source in the cultivation medium.

#### ACKNOWLEDGMENTS

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