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Nitrogen-Fixing Cyanobacterium with a High Phycoerythrin Content

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The elemental and molecular composition, pigment content, and productivity of a phycoerythrin-rich nitrogen-fixing cyanobacterium—an *Anabaena* strain isolated from the coastal lagoon Albufera de Valencia, Spain—has been investigated. When compared with other heterocystous species, this strain exhibits similar chlorophyll *a*, carotene, and total phycobiliprotein contents but differs remarkably in the relative proportion of specific phycobiliproteins; the content of C-phycoerythrin amounts to 8.3% (versus about 1% in the other species) of cell dry weight. Absorption and fluorescence spectra of intact phycobilisomes isolated from this *Anabaena* sp. corroborate the marked contribution of phycoerythrin as an antenna pigment, a circumstance that is unusual for cyanobacteria capable of fixing N_2. The pigment content of cells is affected by variations in irradiance and cell density, these adaptive changes being more patent for C-phycoerythrin than for phycocyanins. The *Anabaena* strain is clumpy and capable of rapid flocculation. It exhibits outdoor productivities higher than 20 g (dry weight) m^{-2} day^{-1} during summer.

Algal biomass represents a source of protein and of a variety of chemical and pharmaceutical products. Photosynthetic pigments such as chlorophylls, carotenes, xanthophylls, and phycobiliproteins have commercial value as natural dyes, antioxidants, and vitamins (3, 4, 18). Of special interest are phycobiliproteins, antenna pigments unique to certain algal groups, which in cyanobacteria and red algae are assembled into macromolecular aggregates called phycobilisomes (13). These water-soluble pigments have recently been used in fluorescence immunoassays and fluorescence microscopy for diagnostics and biomedical research because they fluoresce with a high quantum yield and emission is basically unaltered over a broad temperature and pH range (14). Phycobiliproteins are, moreover, stable and can be readily conjugated to a variety of biomolecules which confer specificity in binding (phycofluors), without sensible alterations in light absorption and emission characteristics. These and other properties make phycobiliproteins, and particularly phycoerythrins, candidates of choice for use as fluorescent tracers in a variety of immunoassays (5, 14).

Among microalgae, the N_2-fixing, heterocystous, filamentous cyanobacteria are particularly attractive for the production of high-quality biomass (15). Nevertheless, little has been done with regard to the establishment of cultures of these cyanobacteria on a large scale (10, 15).

This work deals with the characterization of a phycoerythrin-rich, filamentous, nitrogen-fixing, blue-green alga (an *Anabaena* sp.) isolated from a brackish coastal lagoon (Albufera de Valencia, Spain). Emphasis is placed on elemental and molecular composition, pigment content, and productivity.

The *Anabaena* sp., *Nostoc paludosum*, and an *Anabaenopsis* sp. came from the lagoon Albufera de Valencia. The *Anabaena* sp. and *N. paludosum* were a gift from V. Vidal (E.T.S., Ingenieros Agrónomos, Valencia) and have been provisionally classified by M. Hernández (Facultad de Farmacia, Universidad Autónoma, Barcelona, Spain). The *Anabaenopsis* sp., from a liquid sample supplied by E. Vicente (Facultad de Biología, Universidad de Valencia), was isolated and classified by us. *Anabaena variabilis* ATCC 29413 came from the American Type Culture Collection, Rockville, Md. The culture medium of Arnon et al. (1) was used without modification for *A. variabilis* and was modified to contain 4 mM K_2HPO_4 for the rest of the strains. The medium was supplemented with 10 mM $NaHCO_3$ in the case of *N. paludosum* and with 10 mM $NaHCO_3$ and 30 mM NaCl in the case of the *Anabaenopsis* sp. Cells were grown autotrophically by bubbling air containing 0.4% CO_2 as sources of carbon and nitrogen through the cell suspension. The air flow rate was 100 liters liter of culture medium^{-1} h^{-1} for the *Anabaena* sp. and *A. variabilis* and 50 liters liter of culture medium^{-1} h^{-1} for *N. paludosum* and the *Anabaenopsis* sp. Cells were grown at 35°C in 5-cm-deep 1-liter glass containers. Unless otherwise indicated, cultures were laterally illuminated with fluorescent lamps at an irradiance of 40 W m^{-2}. The *Anabaena* sp. and *A. variabilis* were grown in semicontinuous culture and were subjected to alternating 12-h light and dark cycles. The cultures were partially renewed with fresh medium to a cell density below 10 mg (chlorophyll *a*) liter^{-1} at the beginning of the light period, at which time samples were withdrawn for analysis. *N. paludosum* and *Anabaenopsis* sp. were grown in continuous culture at a density of 10 to 12 mg of chlorophyll *a* liter^{-1}.

Elemental analysis, as well as chlorophyll *a*, dry weight, protein, carbohydrate, lipid, and nucleic acid determinations, was carried out as described by Fontes et al. (9). Protein measurements were performed on sonicated cells (75 W, 40 to 60 s), with lysozyme as a standard. Phycobiliprotein levels were estimated by the method of Siegelman and Kycia (19), and carotene levels were estimated by the method of Davies (8). Ash content was determined for dry matter as described by Larsen (16). Phycobilisomes were prepared from the *Anabaena* sp. as described by Gantt et al. (11, 12). Phycoerythrin was partially purified from phycobilisomes by the method of Gantt et al. (12) but without the acrylamide gel electrophoresis step.

Table 1 shows the elemental and molecular composition of *Anabaena* sp. cells grown in semicontinuous culture. Especially significant is the high nitrogen content (about 10% of dry weight) and net protein (about 47%). These levels are in

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TABLE 1. Elemental and molecular composition of *Anabaena* sp. cells

Component	% of dry weight[a]
Elements	
C	46.4 ± 1.1 (6)
O	27.6 ± 0.1 (2)
N	9.5 ± 0.5 (6)
H	6.6 ± 0.3 (6)
Proteins	47.0 ± 3.5 (6)
Carbohydrates	26.8 ± 4.0 (6)
Lipids	7.9 ± 1.2 (4)
RNA	8.4 ± 0.1 (2)
DNA	0.6 ± 0.1 (6)
Ash	9.1 ± 0.6 (3)

[a] Mean values ± standard deviations. The number of measurements is indicated in parentheses.

the range of those reported for several *Spirulina* (2, 7) and *Scenedesmus* (2) strains, microalgae of recognized nutritional interest. The *Anabaena* sp. studied in this paper has the substantial advantage of being a nitrogen-fixing organism, thus requiring no combined nitrogen. In addition, this blue-green alga exhibits a high productivity which amounts to about 40 g (dry weight) m^{-2} day^{-1} when grown in semicontinuous culture at high irradiance (300 W m^{-2}) under alternating 12-h light and dark cycles. Preliminary outdoor experiments carried out in summer (20 to 25 MJ m^{-2} day^{-1}) in miniponds (1-m^2 surface, 10-cm depth) provided with a paddlewheel system yielded productivities higher than 20 g (dry weight) m^{-2} day^{-1}. Another attractive peculiarity of this *Anabaena* sp. strain is the wide margin of temperature (30 to 40°C) and pH (6.2 to 8.8) for optimal productivity. Finally, this algal strain is clumpy and capable of rapid flocculation, especially in the presence of calcium ions and at high pH values, a property that allows easy harvesting of biomass.

Table 2 shows the pigment composition of the *Anabaena* sp. in comparison with *A. variabilis* and two other species of nitrogen-fixing cyanobacteria. All these strains exhibit similar cellular contents of chlorophyll *a* (about 1.5% of dry weight), carotenes (0.2 to 0.4%), and total phycobiliproteins (18 to 23%), but they differ in the relative proportion of specific phycobiliproteins. Whereas C-phycoerythrin is less than 2% of the dry weight in *A. variabilis*, *N. paludosum*, and the *Anabaenopsis* sp., it amounts to about 8% of the dry weight in the *Anabaena* sp. The C-phycoerythrin and C-phycocyanin contents of the *Anabaena* sp. are similar, although generally the C-phycoerythrin level is somewhat higher. By contrast, C-phycocyanin is the major phycobiliprotein (12 to 13%) in *A. variabilis*, *N. paludosum*, and the *Anabaenopsis* sp., as is usually the case for cyanobacteria.

TABLE 2. Pigment composition of the *Anabaena* sp. and three other strains of nitrogen-fixing blue-green algae[a]

Pigment	% of dry weight in:			
	Anabaena sp.	*A. variabilis*	*N. paludosum*	*Anabaenopsis* sp.
Chlorophyll *a*	1.4	1.4	1.6	1.4
Carotenes	0.3	0.2	0.4	0.4
C-phycoerythrin	8.3	1.0	0.6	1.6
C-phycocyanin	7.1	13.4	11.6	12.2
Allophycocyanin	3.1	8.8	6.0	5.4

[a] Cultures were illuminated with mercury halide lamps at an irradiance of 300 W m^{-2}. Data are means of at least two measurements.

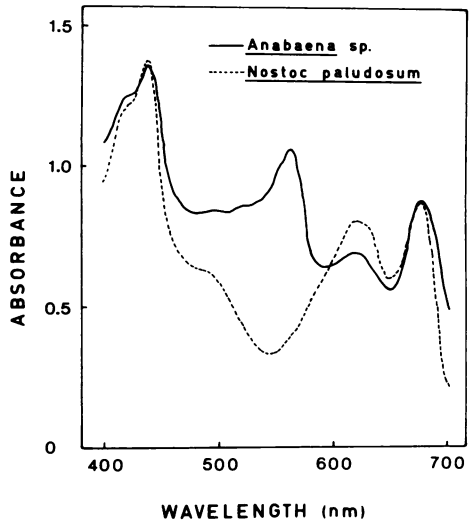

FIG. 1. Absorption spectra of sonicated *Anabaena* sp. and *N. paludosum* cells.

The exceptionally high C-phycoerythrin content of the *Anabaena* sp. is also evident when the absorption spectra of sonicated cell suspensions of this and other blue-green algae such as *N. paludosum* are compared (Fig. 1). The most striking difference between these spectra is precisely in the green region of visible light where the *Anabaena* sp. has an absorbance maximum at 565 nm. Solar spectral irradiance distribution also has its maximum photon flux in this region (6). This unusual light-absorbing capacity may explain the high productivity of this phycoerythrin-rich *Anabaena* sp., which is about twice that of other cyanobacterial species considered in this work.

Figure 2A shows the absorption and fluorescence spectra of intact *Anabaena* sp. phycobilisomes. The absorption spectrum (Fig. 2A-1) exhibits a very pronounced maximum at 565 nm, which is proper to C-phycoerythrin. Another maximum at 615 nm and a shoulder at 645 nm, corresponding to C-phycocyanin and allophycocyanin, respectively, can also be observed. Figure 2A-2 shows the fluorescence spectra of *Anabaena* sp. phycobilisomes. The excitation spectrum, monitoring emission at 652 nm, exhibits a maximum for C-phycoerythrin at 566 nm, with shoulders at 484, 532, and 544 nm. Maxima at 616 and 638 nm, corresponding, respectively, to C-phycocyanin and allophycocyanin, are also noticeable. The emission spectrum presents a major band at 652 nm resulting from the overlap of C-phycocyanin and allophycocyanin. A minor band corresponding to C-phycoerythrin can also be distinguished at 580 nm. This emission spectrum illustrates the integrity of the phycobilisome preparation, with most of the energy absorbed by phycoerythrin being effectively transferred to C-phycocyanin and allophycocyanin. Spectra analogous to those reported here, exhibiting a marked contribution of phycoerythrin, are rare for cyanobacterial phycobilisomes, especially among nitrogen-fixing strains (11, 20). The absorption spectrum of phycoerythrin purified from the *Anabaena* sp. (Fig. 2B-1) exhibits a major peak at about 562 nm, the absorption maximum of C-phycoerythrin. The minor absorption in the red region indicates contamination by C-phycocyanin and allophycocyanin. Figure 2B-2 shows the fluorescence spec-

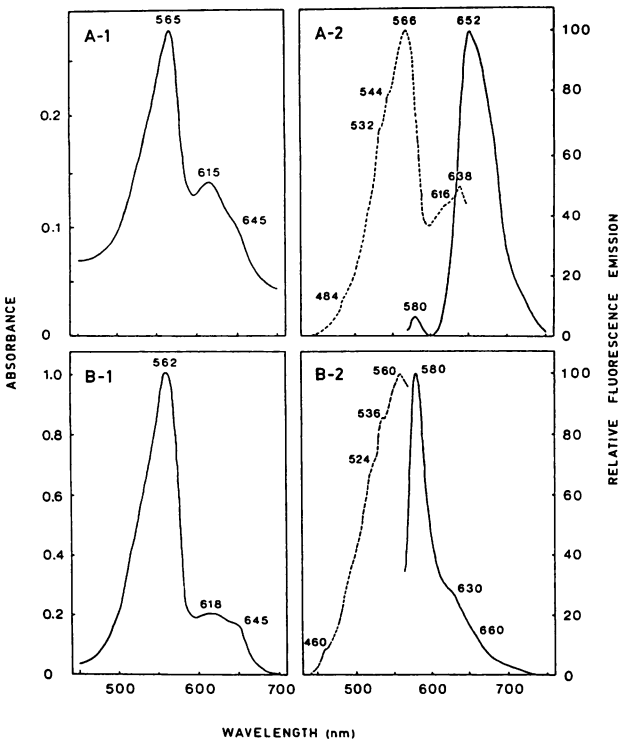

FIG. 2. Absorption and fluorescence spectra of intact phycobilisomes (A) and C-phycoerythrin (B) from the *Anabaena* sp. (A-1 and B-1) absorption spectra; (A-2 and B-2) fluorescence excitation (- - - - -) and emission (——) spectra.

tra of the C-phycoerythrin preparation. The excitation spectrum, monitoring emission at 580 nm, presents a maximum at 560 nm and shoulders at about 460, 524, and 536 nm. The emission spectrum shows a maximum at about 580 nm, which is characteristic of C-phycoerythrin. Shoulders at 630 and 660 nm are probably due to contamination by C-phycocyanin and allophycocyanin (17).

Irradiance and cell density affect the pigment composition of the *Anabaena* sp. Cells exposed to low irradiance (40 W m^{-2}) exhibit a higher phycobiliprotein content than those exposed to high irradiance (300 W m^{-2}), i.e., 19.2 versus 16.5% of cell dry weight. This effect is particularly pronounced for C-phycoerythrin (8.4 versus 6.0%) and to a lesser degree for C-phycocyanin and allophycocyanin. On the other hand, increasing cell density from 6 to 15 mg of chlorophyll $liter^{-1}$ results in a marked increase in total phycobiliproteins (from 16.5 to 21.9%), the effect being likewise more evident for C-phycoerythrin (from 6.0 to 9.3%) than for C-phycocyanin. Irradiance and cell density also affect chlorophyll *a* content. These modifications represent adaptive responses to changes in the availability of light to cells, either directly, by variations in the irradiance to which the cultures are exposed, or indirectly, as a consequence of mutual shading of the cells concomitant with increasing cell density. The results agree with other reports of enhanced phycobiliprotein synthesis at low irradiance in blue-green algae. Phycoerythrin appears to be the most flexible phycobiliprotein, facilitating adaptation to environmental light changes (17).

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