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Picocyanophyte (cyanobacterium) from the boreal inland water accumulates phycoerythrin as a major biliprotein

By Alexander V. Pinevich[1], Hans C. P. Matthijs[2], Svetlana G. Averina[1] and Olga V. Gavrilova[1]

[1] Institute of Biology, University of St. Petersburg, Russia
[2] Laboratory of Microbiology, University of Amsterdam, The Netherlands

With 5 figures in the text

Abstract: Picocyanophyte (cyanobacterium) *Synechococcus* sp. CALU strain 1054 isolated from an enrichment of water sample of a small eutrophic lake in Isthmus Karelia, Baltic East, has purple pigmentation due to a high content of phycoerythrin (PE) and carotenoids. The significance of this finding issues from (i) rare occurrence, especially in freshwater habitats, of PE-rich unicellular cyanophytes; (ii) the isolate's active growth in cultures, which is unattainable with PE-rich chroococcacean cyanophytes examined so far. Ellipsoidal, 1 µm-mean diameter, sheath less cells possess 2–3 cortical layers of thylakoids; the content of PE (50 µg mg^{-1} dry cell weight) is fivefold that of phycocyanin (PC). Essential cultivation properties of new isolate include optimum growth at moderate temperature and weak illumination. Among the chromatic characteristics is, in accordance with HPLC and visible absorption spectrometry data, the presence of large quantity of zeaxanthin and trace amount of a Chl*b* -like pigment. Strain 1054 is similar to marine picoplankters in cell size, ultrastructure, and PE concentration, and to terrestrial (freshwater or soil) strains – in the type of PE. By this combination of diagnostic features, the new isolate should be placed in a separate subgroup of chroococcacean cyanophytes.

Key words: Picoplankton, cyanophytes (cyanobacteria), *Synechococcus*, phycoerythrin, chlorophyll.

Introduction

The presence of large quantity of phycoerythrin (PE), responsible for 550 nm-peak absorption and purple colour of cells and pigment preparations, is infrequent in cyanophytes (Pinevich et al. 1994). In contrast to the photoacclimating PE-upshift in chromatically adapting strains (Grossman et al. 1993), constitutive maintenance of high levels of this pigment needs functional explanation. In particular, for large-water picoplankters which accumulate within the lower,

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badly penetrable by red light, layers of euphotic zone, a mechanism of "permanent" chromatic adaptation is plausible (see: WILBANKS et al. 1991).

The majority of filamentous PE-rich cyanophytes belongs to genera *Oscillatoria, Pseudanabaena, Phormidium, Nostoc, Calothrix*, and associates itself with solid surface or aquatic niches in both marine and terrestrial environments (PHLIPS & ZEMAN 1990, DE CHAZAL & SMITH 1994). Unicellular PE-rich cyanophytes are principally marine; in accordance with the specificity of DNA nucleotide sequences and possession of unique PEs, they constitute a heterogeneous and deeply diverged assemblage within the genus *Synechococcus* (DOUGLAS & CARR 1988, ONG & GLAZER 1991, WEISSE 1993). Freshwater counterparts of marine purple chroococci are frequent in large lakes (KOMÁREK & HIDÁK 1988, ERNST et al. 1995), and only sporadic in small lakes, thermal springs, and fens (KOMÁREK 1976, HINDÁK 1983, STOCKNER & SHORTREED 1991, MAEDA et al. 1992).

In this study, we report on the purple-pigmented unicellular cyanophyte which is unusual with regard to its habitat and non-problematic cultivation; the article has a bearing on essential aspects of cytology, pigments, and growth in new isolate.

Material and methods

Plankton samples were collected in the summertime from sub surface layer of the small eutrophic lake in Isthmus Karelia, Baltic East, cotton-filtered, and supplemented with 1/50 volume of modified BG-11 medium (iron sulfate instead of citrate; 1/5 of nitrate conc.). After two-months incubation in dim light at room temperature, the enrichments were inoculated in slants of 0.8% Bacto-Agar (Difco) with modified BG-11. Cells from a local constellation of purple punctiform colonies, serendipitously found in one of Petri dishes, were axenically cloned. Test material was cultivated in modified BG-11 in airlift 500 ml-fermentor at 20 °C, with continuous illumination of 10μE $m^{-2}s^{-1}$ of photon white light. Light microscopy was monitored with a KARL ZEISS phase contrast (× 1,500). For electron microscopy, cells were pelleted at 1.000 g for 5 min, resuspended in 5 mM Na-cacodylate (pH 6.8), treated at 10 °C with 2.5% glutaraldehyde (12 h), and finally fixed with 0.5% osmium tetraoxide (2 h). The material was immobilized in 1% agar, dehydrated, embedded in Spurr resin, and sectioned with a REICHERT ultramicrotome. REINOLDS-contrasted specimen were observed in a Magiscan at 75 kV.

Absorption spectra of whole cells were recorded with an Aminco model DW-200 TM spectrophotometer. Disintegration of cells suspended in 5 mM Na-phosphate (pH 7.0) was attained with a French pressure at 2.000 psi. The

particulate fraction was removed by centrifugation at 10.000 g for 15 min and filtration through a Millipore (0.1 μm).

The content of phycocyanin (PC) was estimated by spectrophotometry of the soluble fraction of cell homogenate using specific absorbance (625 nm) 7.2 $1\,g^{-1}\,cm^{-1}$ (RÜDIGER et al. 1980). In the case of PE, specific absorbance 7.0 (560 nm) was deduced from molar extinction 47.000 cm^{-1} for bound erythrobilin and $(\alpha\beta)_3$ – aggregation state for apoproteins which carry, respectively, 2 and 3 chromophores (GLAZER 1988).

The content of Chl and carotenoids was determined by means of reversed phase high-performance liquid chromatography (HPLC) of cell pigment extract using Hypersil-ODS column (2.50×4.6 mm i. d., 5 μm particles). For pigment isolation, 90% acetone (MERCK) was added to vacuum freeze-dried cells. After vortexing at 4 °C for 30 s in dim light, the suspension was spun at 12.000 g for 3 min; 0.3 vol of anion-pairing mixture (tetrabutyl ammonium acetate, 1.5% w/v; ammonium acetate, 7,7% w/v) was added to the supernatant 3 min prior to the run. A portion of pigment extract was injected into the column cooled to 4 °C in an ice-water bath. The pigments were allowed to separate along a 31-min chromatographic run, with a 15-min linear gradient elution from 100% degassed phase A (methanol: water, 90:10 v/v) to 100% phase B (methanol : acetone, 80:20 v/v) at a flow rate of 1.5 ml min^{-1}. The pigments were detected at 455 nm, and identified by their retention times and on-line absorption characteristics (obtained with a built-in scan mode). The instrument was calibrated with authentic pigment standards.

Results

The natural occurrence of strain CALU 1054 is sparse, compared to a relatively high population density of its PE-rich unicellular counterparts which dominate in large-water picoplankton (WEISSE 1993). The cells measure ca. 1 μm, possess peripheral/parallel thylakoids, and multiply by symmetric binary fission in one plane; no sheath formation is observed (Fig. 1). In accordance with these criteria, new isolate can be classified as *Synechococcus* sp. (KOMÁREK 1976, KOMÁREK & ANAGNOSTIDIS 1986). Strain 1054 is identical in appearance to those in marine *Synechococcus* spp. (CHISHOLM et al. 1988). In both cases, thylakoids are arranged in widely separated (ca. 100 nm) cortical rows which have a horseshoe-like pattern, while the cell centre is occupied by ribosomes and fibrils of DNA (Fig. 1A).

The strain is easily culturable in both the liquid and solid medium, which contrasts with generally poor growth demonstrated by marine PE-rich chroococci (BRAHAMSHA 1996). Analagous problems of cultivation prevent the study of PE-rich *Synechococcus* spp. from freshwater sources (ERNST et al. 1995). During

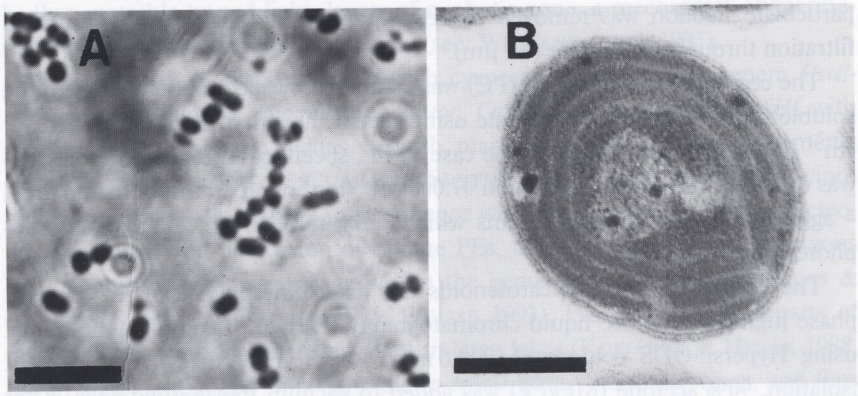

Fig. 1. Microscopic images of cells of *Synechococcus*, strain 1054. **A** – light micrograph [bar = 5 mm], **B** – electron transmission micrograph [bar = 0.5 mm].

growth on agar, punctiform colonies are formed indicating the absence of gliding motility. After prolonged cultivation, ample slime masses are produced; there is a tendency to form microaggregates stabilized by mucilaginous bridges between the neighbouring cells (Fig. 1A). Some of them resemble pseudofilaments in *Cyanodictyon* (see: HICKEL 1981). Aged colonies change the original purple

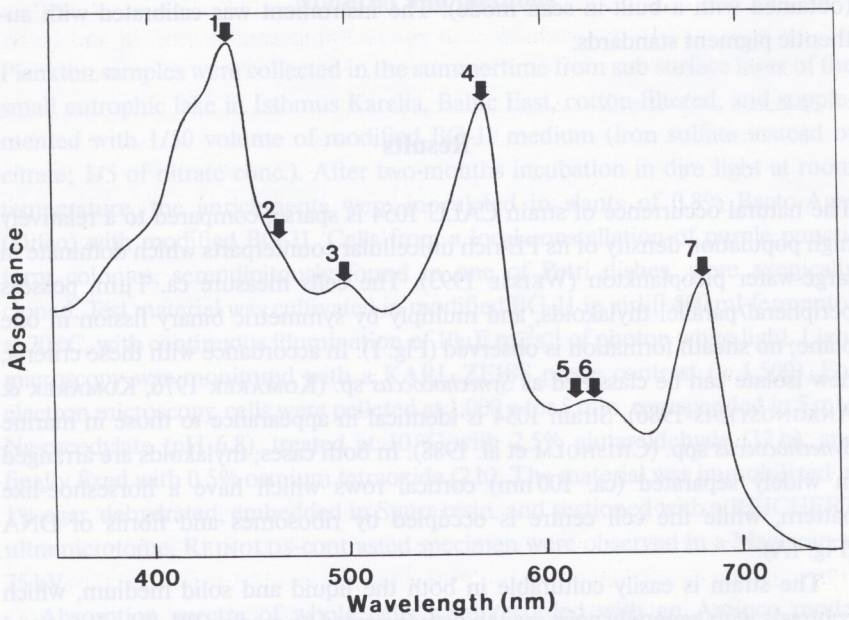

Fig. 2. Absorption spectrum of cells of *Synechococcus*, strain 1054 suspended in culture medium. Positions of maxima and shoulders, nm: 439 **(1)**; 466 **(2)**; 498 **(3)**; 569 **(4)**; 622 **(5)**; 624 **(6)**; 681 **(7)**.

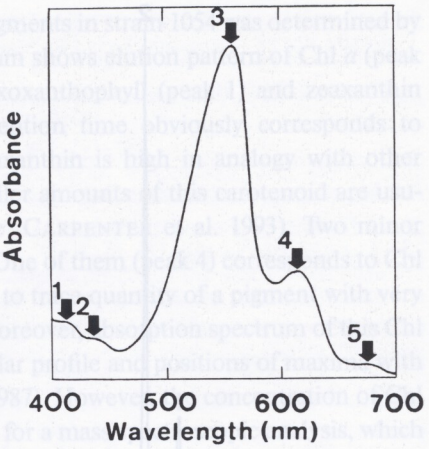

Fig. 3. Absorption spectrum of biliprotein containing fraction of *Synechococcus*, strain 1054 obtained after cell disruption. Positions of maxima and shoulders, nm: 416 **(1)**; 439 **(2)**; 560 **(3)**; 615 **(4)**; 680 **(5)**.

pigmentation, via pale green, to creamy. In liquid medium, non-viscous homogeneous cell suspensions with a population density of ca. $5 \cdot 10^8$ ml^{-1} are formed after 5-day cultivation. Optimum growth is promoted by moderate temperature and weak illumination (see: "Material and methods").

Absorption spectrum of cell suspension (Fig. 2) shows a variety of photosynthetic pigments including Chl *a* (peaks 1, 7), carotenoids (shoulder 2, peak 3), PE (peak 4), and PC (shoulder 5, peak 6). The predominance of PE and brown carotenoids (peak 3) among the *Synechococcus* sp. pigments is responsible for a deep purple colour of low light grown cultures.

The spectrum of soluble fraction of cell homogenate (Fig. 3) is dominated by absorption of PE (peak 3) which is ca. 5 times the one of PC (peak 4). The relative content of allophycocyanin (APC) is low, which issues from apparent absence of a shoulder at 650 nm. Because the strain 1054 resisted freeze-thawing and those chemicals, which permeabilize surface structures, the biliprotein fraction was obtained from French-pressured cells; correspondingly, shoulders 1,2, and 5 in Figure 3 are due to the residual fragments of thylakoids. The bulk of spectrum is occupied by 560 nm-maximum which corresponds to C-PE bearing only phycoerythrobilin chromophores (Glazer 1984). This type of the pigment is regular in filamentous cyanophytes, whereas marine picoplankter strains are characterized by phycourobilin-containing PE (Ong & Glazer 1991). The calculated concentration of PE (50 µg mg^{-1} dry cell weight) matches the values which were previously demonstrated for PE-rich chroococcacean cyanophytes (Wyman et al. 1985), although it is lesser than in filamentous strains because of a difference in the size of lamellar system. PE:PC ratio of 5:1 is between those in other unicellular (10–20:1) and most filamentous (2–3:1) PE-rich cyanophytes (see: Pinevich et al. 1994).

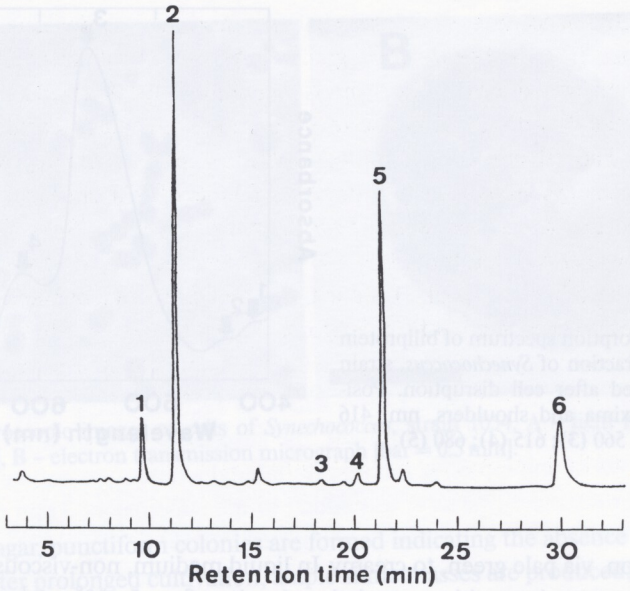

Fig. 4. HPLC tracing of an acetone extract of cells of *Synechococcus*, strain 1054. Peaks identities: myxoxanthophyll **(1)**; zeaxanthin **(2)**; Chl *b* - like pigment **(3)**; Chl *a* allomer **(4)**; Chl *a* **(5)**; β-carotene **(6)**. Detection wavelength, 455 nm.

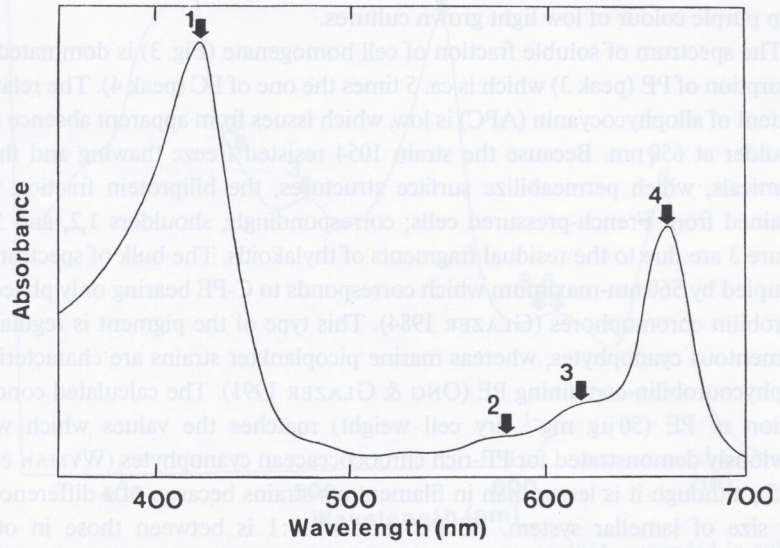

Fig. 5. Absorption spectrum of Chl *b* - like pigment of *Synechococcus*, strain 1054 in methanol: acetone, 80:20 v/v. Positions of maxima, nm: 421 **(1)**; 577 **(2)**; 616 **(3)**; 661 **(4)**.

The identity of acetone-extractable pigments in strain 1054 was determined by HPLC analysis (Fig. 4). The chromatogram shows elution pattern of Chl *a* (peak 5) and two major oxycarotenoids – myxoxanthophyll (peak 1) and zeaxanthin (peak 2). Peak 6 with the longest retention time obviously corresponds to β-carotene. The relative content of zeaxanthin is high in analogy with other chroococcacean cyanophytes, while smaller amounts of this carotenoid are usually revealed in filamentous strains (see: CARPENTER et al. 1993). Two minor peaks were also eluted in front of Chl *a*. One of them (peak 4) corresponds to Chl *a* allomer. The other one (peak 3) is due to trace quantity of a pigment with very similar retention time to that of Chl *b*. Moreover, absorption spectrum of this Chl *b* – isochronous peak (Fig. 5) shows similar profile and positions of maxima with authentic Chl *b* (see: LICHTENTHALER 1987). However, the concentration of Chl *b* -like pigment in eluate was not enough for a mass spectroscopic analysis, which is necessary to further substantiate its identity with Chl *b*.

Discussion

Marine *Synechococcus* spp. which are recognized as main component of phytoplankton, have two different types of PE. One of them (PEII) contains an additional chromophore which is bound to (αβ)-apoprotein monomer. Both PE, beside 550 nm-peak which is due to phycoerythrobilin, show a second maximum around 500 nm caused by phycourobilin. The optimized absorbance of PE(II), as well as high concentration of total PE, represent the mechanism of photoacclimation in these cyanophytes (ONG & GLAZER 1991).

Unlike marine PE-rich picoplankters, freshwater and soil unicellular strains examined so far have only PE(I) in which phycourobilin is usually missing (WILBANKS et al. 1991). Strain 1054 also has PE composition, different from marine synechococci, although it resembles them in cell size, ultrastructure, and PE concentration. In accordance with this combination of properties, strain 1054 should be attributed to a separate subgroup of chroococcacean cyanophytes.

A rationale(s) of constitutive accumulation of PE in cyanophytes, which develop in environments distinct from large-water ones, is not clear. In natural habitats, such strains cannot take the photoacclimating advantage of high PE content (cf. ONG & GLAZER 1991, ERNST et al. 1995). Hence, it is most probable that their phycobilisomes (with PE as the main phycobiliprotein) represent a rudimentary type with respect to the structure and biogenesis. In fact, ancestral cyanophytes presumably localized themselves deep under water, in order to escape the harmful dose of UV. In these niches, photosynthetic pigments with short-wavelength maxima would have been selectively advantageous (OLSON 1981). In phylogeny of cyanophytes, labile patterns of PE synthesis including chromatic adaptation were secondarily acquired. Another possibility to explain

the adaptive accumulation of PE is related to metabolic role of this pigment in the deposition of combined nitrogen (see: WYMAN et al. 1985).

Finally, HPLC data suggest that strain 1054 has a pigment of Chl *b*-type; its quantity is in a range of several per cent Chl *a*. In accordance with the recent demonstration of Chl *c* in the prochlorophytes (Chl *b* -containing cyanophytes) *Prochlorococcus marinus* and *Prochloron* sp., ancestor cyanophytes possessed the genes for and expressed components of more than one light-harvesting complex (CHISHOLM et al. 1992, LARKUM et al. 1994). A distinction between these antennae is based on the nature of their apoproteins and chromophores. The evolutionary divergence was hallmarked by a mosaic of deletions, that explains the variety of pigmentation in extant cyanophytes (BHATTACHARYA & MEDLIN 1995). The large scale study of light-harvesting complexes in oceanic picoplankters, using a free-flow cytospectrofluorimetry method and HPLC, has revealed the long-quested organisms in which PE coexists with Chl *b* (HESS et al. 1996). Our data suggest that "cyanoprochlorophytes" may have a wider distribution, and should also be looked for in freshwater habitats.

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