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Phycoerythrin production by a marine *Oscillatoria* (Cyanophyta)

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ABSTRACT The production of the commercially important pigment phycoerythrin by the marine cyanobacterium *Oscillatoria* UMACC 216 was investigated. The cultures from different stages of growth (day 2, 4, 6, 8 and 10) were harvested for the determination of phyocerythrin. Cells from the exponential phase contained the highest amounts of phycoerythrin (66.7 mg g^{-1} dry weight). The cultures changed from red to green then yellow colour after attaining stationary phase. A separate batch of cultures was grown at salinities ranging from 5, 10, 15, 20 to 25 (control) parts per thousand (ppt). Cells grown at 15 ppt contained the highest amounts of phycoerythrin (114.7 mg g^{-1} dry weight). The phycoerythrin content of *Oscillatoria* UMACC 216 was much higher than that reported for other cyanobacteria. Further studies to optimise phycoerythrin production by this alga are worthwhile.

ABSTRAK Fikoeritrin merupakan sejenis pigmen alga yang mempunyai nilai komersial. Penghasilan pigmen ini oleh alga biru-hijau *Oscillatoria* UMACC 216 telah dikaji. Kandungan fikoeritrin dalam sel daripada peringkat berlainan dalam kitar hidup alga ini dibanding. Kandungan fikoeritrin (66.7 mg g^{-1} berat kering) adalah tertinggi pada fasa eksponen. Kultur tersebut berubah daripada merah ke hijau, kemudian ke kuning apabila fasa pegun tercapai. Bila alga tersebut dikulturkan pada julat saliniti antara 5, 10, 15, 20 hingga 25 ppt (kawalan), kandungan fikoeritrin adalah tertinggi (114.7 mg g^{-1} berat kering) pada 15 ppt. Berbanding dengan alga biru hijau lain yang dilaporkan, kandungan fikoeritrin *Oscillatoria* UMACC 216 adalah lebih tinggi. Kaijian lanjutan untuk mengoptimumkan penghasilan fikoeritrin adalah wajar dilakukan.

(oscillatoria, phycoerythrin, cyanobacteria, carotenoids, salinity)

INTRODUCTION

Phycobiliproteins are water-soluble fluorescent pigments that function as accessory pigments for photosynthesis in algae, especially blue-green algae (cyanobacteria) and rhodophytes (red algae). The three major types of phycobiliproteins are phycocyanin, allophycocyanin and phycoerythin; the distribution of such pigments varies among the different classes of algae. Phycoerythrin is a red phycobiliprotein found in cyanobacteria, rhodophytes, cryptophytes and prochlorophytes.

All cyanobacteria contain phycocyanin and allophycocyanin, but not all contain phycoerythin [1]. Cyanobacteria are blue-green in colour when phycocyanin is the major component, and red-brown to purple-red when phycoerythrin is dominant. Some cyanobacterial strains, which contain phycoerythin may undergo chromatic adaptation, when grown under different light conditions. In those cyanobacteria, the cellular ratio of phycoerythrin to phycocyanin is much higher when grown in green light when compared with red light [2].

Apart from light, factors such as salinity and nutrient levels can also affect phycobiliprotein production in algae. For instance, phycocyanin content in the freshwater alga *Nostoc muscorum* decreases in salt-grown cells [3]. As phycobiliproteins also serve as storage proteins, the pigment is broken down for energy when the alga is under nitrogen limitation [4]. In addition, iron starvation has also been shown to depress synthesis of phycobiliproteins in *Anacystis nidulans* and *Synechococcus cedorum* [5].

Phycobiliproteins have biotechnological applications as natural pigments for the food, drug and cosmetic industries to replace currently used synthetic pigments [6]. Phycoerythrin is also used as fluorescent probes in biomedical research, especially in techniques such as flow cytometry, fluorescent microscopy and fluorescent immunoassay [7]. The market value for phycoerythrin is more than US$ 1000 per kg [8]. Due to the potential commercial applications of phycobiliproteins, there have been efforts to screen cyanobacteria for potential producers of such pigments. For example, Moreno *et al.* (1995) screened ten strains of nitrogen-fixing cyanobacteria and found that phycobiliproteins account for about 50% of the total cell proteins, with the dominant type being phycocyanin, followed by allophycocyanin [9]. Phycoerythin is dominant in some strains of *Nostoc*, reaching 10% dry weight.

The objective of this study was to characterise the pigmentation, especially phycobiliprotein composition of *Oscillatoria* UMACC 216. We also investigated the production of phycoerythrin throughout the growth cycle of this alga, and the effect of salinity on the production of this pigment.

MATERIALS AND METHODS

Oscillatoria UMACC 216 is a red-pigmented cyanobacterium originally growing as an epiphyte on the green seaweed *Ventricaria ventricosa* collected from Tioman Island (Figure 1). The red filaments are very fine (~ 2 μm), forming mats in non-shaken cultures and spherical colonies in shake flasks. The culture is deposited in the University of Malaya Algae Culture Collection (UMACC).

The cultures were grown in conical flasks containing 100 mL Prov 50 Medium [10] and placed in a controlled environment incubator shaker (150 rpm). The cultures were grown at 28 °C and subjected to irradiance of 50 μmol m^{-2} s^{-1}, with 12 :12 h light-dark cycle. The inoculum was from exponential phase cultures with an optical density at 620 nm (OD_{620}) of 0.2, and the inoculum density used was 10%. Three batches of cultures were grown for pigment extraction. In the first batch, six flask cultures were grown for 5 days before being harvested by filtration (Whatman GF/C, 0.45 μm) for pigment extraction. Three cultures were used for phycobiliprotein extraction and another three for carotenoid and chlorophyll a extraction. In the second batch, three cultures were harvested on day 2, 4, 6, 8 and 10 respectively for phycobiliprotein extraction. In the third batch, the cultures were grown at salinities ranging from 5, 10, 15, 20 to 25 (control) parts per thousand (ppt). On day 4, three cultures grown at each salinity were harvested for phycobiliprotein extraction.

Phycobiliproteins were extracted by mashing the filtered cells in 0.15 M NaCl in 0.01 M Na_2HPO_4 (pH 7) using a glass homogeniser. The concentration of phycobiliproteins was determined by spectrophotometric method after centrifugation at 3500 *g* for 10 min [11]. Carotenoids and chlorophyll a were determined by HPLC method as described in Chu *et al.* (1995) [12]. Dry weight was determined after drying the filtered samples (in triplicate) at 100 °C for 24 h.

RESULTS

Table 1 shows the pigment composition of *Oscillatoria* UMACC 216 from the first batch of cultures harvested on day 5. Phycoerythrin was the predominant pigment in *Oscillatoria* UMACC 216 and the content was almost doubled that of phycocyanin. The absorption spectra of the phycobiliprotein extract showed a peak at 569 nm (Figure 2). The cells contained very low amounts of carotenoids (1.29 mg g^{-1} dry weight), consisting of zeaxanthin (1.07 mg g^{-1} dry weight), β-carotene (0.13 mg g^{-1} dry weight) and oscillaxanthin (0.09 mg g^{-1} dry weight).

In the second batch, the cultures changed from reddish during exponential phase (day 2 – 4) to greenish upon reaching stationary phase (day 6) and became yellowish in old cultures (day 8 – 10) (Figure 3). The cells from exponential phase cultures (day 4) contained the highest amounts of phycoerythrin (66.7 mg g^{-1} dry weight) [Figure 4] and the concentration was also highest (12.8 mg L^{-1}) in such cultures (Table 2). In old cultures (day 8 and 10), allophycocyanin instead of phycoerythrin was the dominant phycobiliprotein (Figure 4).

In the third batch, biomass was highest (165 mg dry weight L^{-1}) from the cultures grown at 25 ppt (Figure 5), and there was very little growth at 5 ppt. Phycoerythrin content was highest (114.7 mg

g^{-1} dry weight) in cells grown at 15 ppt (Figure 6). The phycoerythrin concentrations of cultures grown at 15 and 25 ppt were similar (Table 3). Allophycocyanin was the dominant phycobiliprotein in cells grown at 5 ppt.

Table 1. Pigment composition of *Oscillatoria* UMACC 216 harvested during exponential phase (day 5). Figures are mean (± standard deviation) values from triplicate samples.

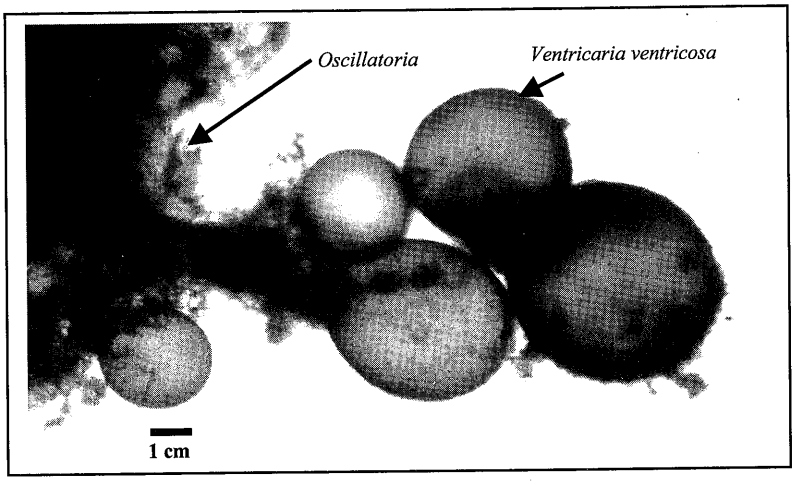
Pigment	Content (mg g^{-1} dry weight)
Total phycobiliproteins	68.0 ± 1.3
Phycoerythrin (PE)	33.8 ± 0.6
Phycocyanin (PC)	18.1 ± 0.8
Allophycocyanin (APC)	16.1 ± 1.2
Chlorophyll a (chl-a)	5.7 ± 0.3
Total carotenoids (car)	1.3 ± 0.2
Ratio	
PBP: car: chl-a	11.9: 0.23: 1
PE: PC	1.9

Table 2. Phycobiliprotein concentrations of *Oscillatoria* UMACC 216 cultures harvested at different stages of growth. Figures are mean (± standard deviation) values from triplicate samples.

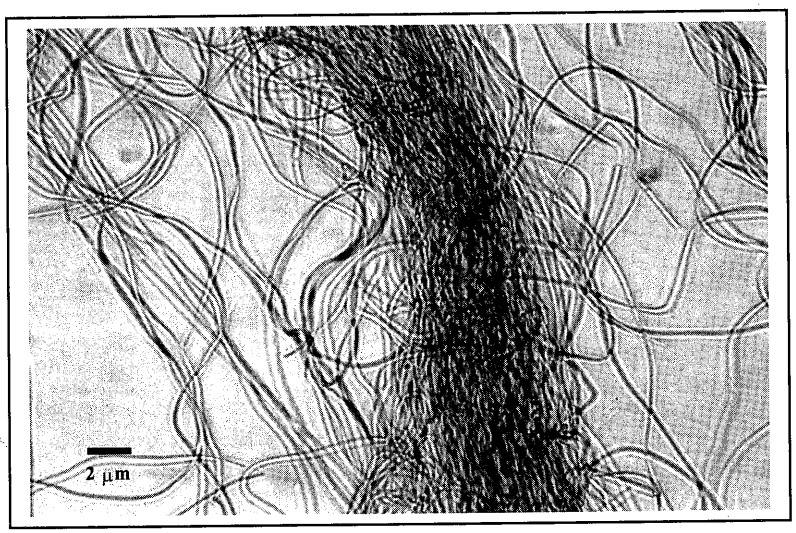
Day	Total phycobiliproteins (mg L^{-1})	Phycocyanin (mg L^{-1})	Allophycocyanin (mg L^{-1})	Phycoerythrin (mg L^{-1})
2	1.4 ± 0.5	0.3 ± 0.1	0.3 ± 0.1	0.8 ± 0.1
4	12.8 ± 0.3	2.1 ± 0.4	2.0 ± 0.1	8.7 ± 0.4
6	9.7 ± 0.4	2.0 ± 0.3	3.0 ± 0.2	4.7 ± 0.5
8	5.2 ± 0.2	1.1 ± 0.2	2.4 ± 0.1	1.7 ± 0.3
10	2.7 ± 0.1	0.7 ± 0.1	1.4 ± 0.2	0.6 ± 0.1

Table 3. Phycobiliprotein concentrations of cultures grown at different salinities. Figures are mean (± standard deviation) values from triplicate samples.

Salinity (ppt)	Total phycobiliproteins (mg L^{-1})	Phycocyanin (mg L^{-1})	Allophycocyanin (mg L^{-1})	Phycoerythrin (mg L^{-1})
5	2.5 ± 0.2	0.8 ± 0.1	1.2 ± 0.1	0.5 ± 0.1
10	12.9 ± 1.4	4.4 ± 0.2	3.9 ± 0.2	4.7 ± 0.2
15	20.2 ± 1.3	5.5 ± 0.1	4.9 ± 0.3	9.8 ± 0.2
20	16.3 ± 0.5	5.5 ± 0.2	4.5 ± 0.2	6.3 ± 0.2
25	18.8 ± 1.2	5.6 ± 0.3	2.8 ± 0.4	10.4 ± 0.5



(a)



(b)

Figure 1. a) *Oscillatoria* UMACC 216 grows as epiphytes on the green seaweed *Ventricaria ventricosa*. b) Red filaments of *Oscillatoria* UMACC 216.

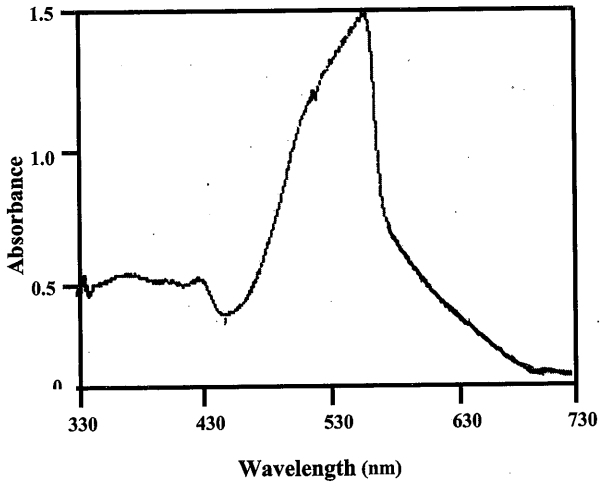


Figure 2.

Absorption spectra of crude phycobiliprotein extract from *Oscillatoria* UMACC 216.

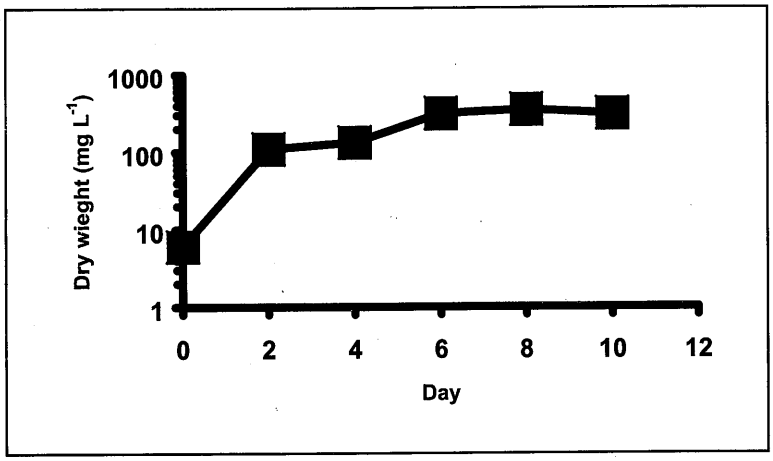


Figure 3.

Semi-logarithmic growth curve based on dry weight (mg L^{-1}) of *Oscillatoria* UMACC 216.

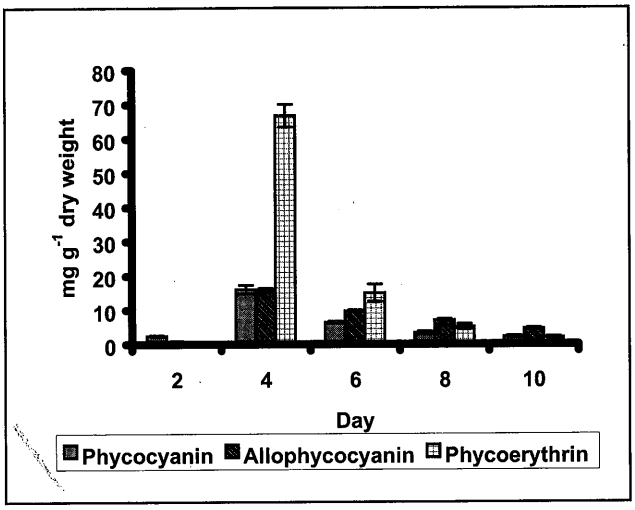


Figure 4.

Phycobiliprotein composition of *Oscillatoria* UMACC 216 harvested at different stages of growth. Vertical bars denote standard deviations from triplicate samples.

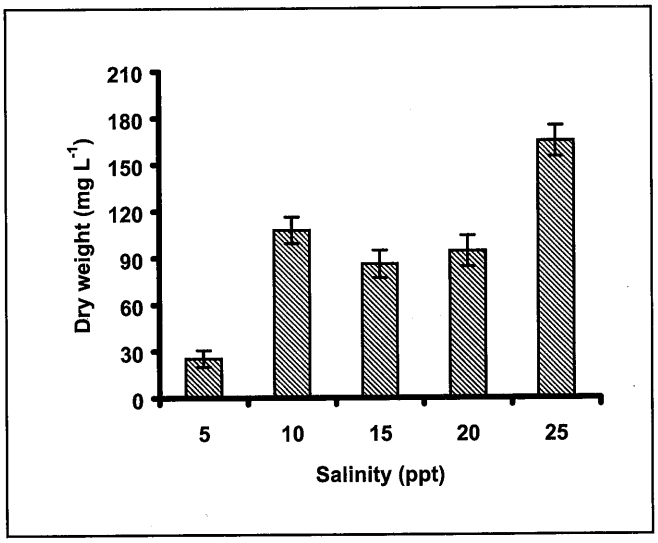


Figure 5.

Biomass of *Oscillatoria* UMACC 216 grown at different salinities and harvested on day 4. Vertical bars denote standard deviations from triplicate samples.

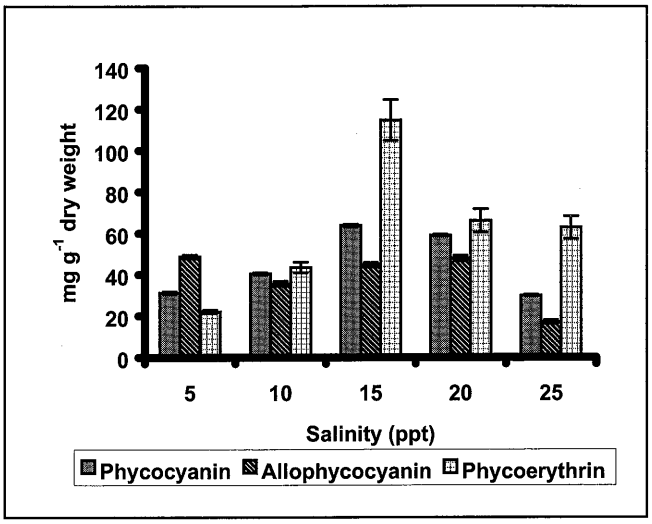


Figure 6.

Phycobiliprotein composition of *Oscillatoria* UMACC 216 grown at different salinities. Vertical bars denote standard deviations from triplicate samples.

DISCUSSION

The red colour of *Oscillatoria* UMACC 216 is due to the dominance of phycoerythrin. Strains of red *Oscillatoria* are found to grow in freshwater, marine and thermal habitats, as planktonic, benthic or symbiotic algae [13]. The phycoerythrin of *Oscillatoria* UMACC 216 is of the C-PE (cyanophyceae-phycoerythrin) type, as indicated by its absorption maximum at 569 nm. This contrasted with other species of red *Oscillatoria*, which contain phycoreythrourobilin (CU-PE) with absorption maxima of 494-498 and 540-567 nm [13].

The dominance of phycoerythin in *Oscillatoria* UMACC 216 occurs only under non-stressed conditions, as shown by its high content during exponential phase and at 25 ppt NaCl. In general, stressed conditions such as nutrient limitation and high irradiance can reduce phycobiliprotein production in cyanobacteria [4, 14].

Optimum growth of *Oscillatoria* UMACC 216 occurred at 25 ppt, but there was considerable growth even at 10 ppt. This finding is similar to that reported for another epiphytic *Oscillatoria*, which grows best at 100% seawater but also tolerates a salinity down to 50% seawater [13]. At low salinity, the total phycobiliprotein content of *Oscillatoria* UMACC 216 decreased markedly, and allophycocyanin dominated instead of phycoerythrin. The osmotic stress could have caused the destruction of the phycobilisomes, which would affect the photosynthetic efficiency, as shown in another cyanobacterium, *Nostoc muscorum* [3].

The phycoerythin content of *Oscillatoria* UMACC 216, reaching up to 114.2 mg g^{-1} dry weight, was much higher than that in cyanobacteria such as *Nostoc* sp. (101 mg g^{-1} dry weight), *Nodularia* sp. (16.0 mg g^{-1} dry weight) and *Anabaena* sp. ATCC 33017 (13.0 mg g^{-1} dry) [9]. The cultures of *Oscillatoria* UMACC 216 can be easily harvested as they form spherical clumps in shake flasks. The cultures should be harvested at exponential phase and grown at 25 ppt for maximum yield of phycoerythrin. Further studies to optimise biomass and phycoerythrin production by manipulating factors such as irradiance and nutrient levels are worthwhile.

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REFERENCES

1. Rosinski, J., Hainfeld, J.F., Rigbi, M. and Siegelman, H.W. (1981). Phycobilisome ultrastructure and chromatic adaptation in *Fremyella diplosiphon*. *Ann. Bot.* **47**: 1–12.
2. De Marsac, N.T. (1977) Occurrence and nature of chromatic adaptation in cyanobacteria. *J. Bacteriology* **130**: 82 – 91.
3. Blumwald, E.D. and Tel-Or, E. (1982). Osmoregulation and cell composition in salt-

adaptation of *Nostoc muscorum*. *Arch. Microbiol.* **132**: 168 – 172.

4. Levy, I. and Gantt, E. (1990). Development of photosynthetic activity in *Porphyridium purpureum* (Rhodophyta) following nitrogen starvation. *J. Phycol.* **26**: 62 – 68.
5. Guikema, J.A. and Sherman, L.A. (1983). Organization and function of chlorophyll in membranes of cyanobacteria during iron starvation. *Plant Physiol.* **73**: 250 – 256.
6. Cohen, Z. (1986). Products from microalgae. In: *CRC Handbook of Microalgal Mass Culture* (ed. Richmond A.), CRC Press, Boca Raton, Florida, pp. 421 – 454.
7. Telford, W.G., Moss, M.W., Morseman, J.P. and Allnutt, F.C.T. (2001). Cryptomonad algal phycobiliproteins as fluorochromes for extracellular and intracellular antigen detection by flow cytometry. *Cytometry* **44**: 16-23.
8. Borowitzka, M.A. (1994) Products from algae. *Proceedings of the First Asia-Pacific Conference on Algal Biotechnology* (eds. Phang S.M., Lee Y.K., Borowitzka M.A. and Whitton B.A.), University of Malaya, Kuala Lumpur, pp. 5-15.
9. Moreno J., Rodriguez H., Vargas M.A., Rivas J. and Guerrera M.G. (1995) Nitrogen-fixing cyanobacteria as source of phycobiliprotein pigments. Composition and growth of ten filamentous heterocystous strains. *J. Appl. Phycol.* **7**: 17-23.
10. Phang, S. M. and Chu, W. L. (1999) *University of Malaya Algae Culture Collection (UMACC): Catalogue of Strains*. Institute of Postgraduate Studies and Research, University of Malaya, 77 pp.
11. Bennett, A. and Bogorad, L. (1973). Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell Biol.* **58**: 419-435.
12. Chu, W.L., Phang, S.M. and Goh, S.H. (1999). Influence of carbon source on growth, biochemical composition and pigmentation of *Ankistrodesmus convolutus*. *J. Appl. Phycol.* **7**: 59-64.
13. Hoffman, L., Talarico, L. and Wilmotte, A. (1990). Presence of CU-phycoerythrin in the marine benthic blue-green alga *Oscillatoria* cf. *coralllinae*. *Phycologia* **29**: 19-26.
14. Rodriguez H., Rivas J., Guerrero M.G. and Losada, M. (1991). Enhancement of phycobiliprotein production in nitrogen-fixing cyanobacteria. *J. Biotechnol.* **20**: 263 – 270.