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Different Light Spectral Qualities Influence Sterol Pool in *Porphyridium cruentum* (Rhodophyta)

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Abstract: Detailed sterol analysis of *Porphyridium cruentum* (Ag.) Nägeli, grown under different light spectral qualities, was carried out using TLC and GLC techniques. The total sterol amount along with the profile (22-dehydrocholesterol, cholesterol, brassicasterol, campesterol and cycloatenol) weights and their concentration inside different classes (free, steryl esters, steryl glycosides and acyl steryl glycosides) were determined. The results revealed a gradual decrease in the total sterol amount in the cells grown under different light spectral qualities according to the order: white>yellow>green>red>blue, however the composition of the sterol pool was not affected. Whatever the light conditions, cholesterol was the major component detected, mostly as steryl glycoside. Furthermore, our data demonstrated that the amount of different sterol profiles and their distribution among the free and conjugated forms strongly depend on the light spectral quality.

Key words: Light spectra, *Porphyridium cruentum*, sterol

INTRODUCTION

Light availability is a function of the geographic location and the local climatic parameters (Duffie and Beckman, 1980). The spectral quality of the available light may vary as a result of selective absorption by various materials suspended or dissolved in the water column (Kirk, 1983). The variability of light components such as red, green, blue (Talarico and Maranzana, 2000) and ultraviolet radiations (Villafane *et al*., 2005) seems to play a role of regulating algal metabolism at the cellular and molecular levels (Bhandari and Sharma, 2006). Sterol is integral component of eukaryotic cell membrane lipid which serves in different vital physiological processes. Therefore the concentration of the sterol is a very important characteristic to determine the physiological state of microalgae (Fabregas *et al*., 1997) hence the study of the influence of light spectral qualities on the sterol pool is of interest.

Porphyridium cruentum (Ag.) Nägeli is a primitive rhodophycean microalga (Bangiophycidae) with spherical cells (5-8 μm) that lacking a cell wall (Ramus *et al*., 1989). The alga has an economic value since it is a source of sulphurized polysaccharides (Arad *et al*., 1985), tocopherol, vitamin K, isoprenoid quinines, carotenes (Antia *et al*., 1970) and phycoerythrin (Gantt, 1981) which is responsible for the characteristic red color of the alga. *P. cruentum* accumulates large amount of lipid (Yongmanitchai and Ward, 1992), it is an excellent source of different fatty acids especially arachidonic acid (C20:4ω6) and ecosapentaenoic acid (C20:5ω3) (Lee and Tan, 1988). Beastall *et al*. (1971) confirmed the presence of sterol in the alga followed by the detection of relatively high proportion of 4α-methylsterol fractions (Beastal *et al*., 1974). Therefore, the objective of this study was to evaluate the influence of different light spectral qualities on the concentration of the total, free and conjugated sterolic forms and their composition in *P. cruentum*.

MATERIALS AND METHODS

Biological Material and Culture Conditions

Porphyridium cruentum was obtained from culture collection of algae laboratory, Faculty of Science, Alexandria University, Egypt. The alga was grown xenically in triplicate 1 L glass flasks with sterilized and enriched sea water (1g 100 mL KNO_3; 0.1g 100 mL K_2HPO_4; 0.1g 100 mL $MgSO_4$. $7H_2O$; 10 g/100 mL yeast extract) according to Jones *et al*. (1963). pH was 7.6 and the cultures were bubbled with air enriched with 2% (v/v) CO_2 and incubated at 24±1°C. Light intensity was 100 μmol photons m^{-2} s^{-1} with a 12/12 h light/dark cycle provided from white (visible, 350-750 nm) and the monochromatic spectrum of yellow, green, red and blue fluorescent lamps (ROLA T12, China), corresponding to the wavelengths ~580, ~550, ~680 and ~470 nm, respectively. Cells grown under the monochromatic spectrum were collected at the concentration adjusted to those grown under the visible light of about 12×10^6 cells mL^{-1} at the beginning of the stationary growth phase. All Cultures were harvested by centrifugation and the pellets were immediately lyophilized.

Sterol Isolation and Analysis

One g dried samples of *P. cruentum* grown under different light spectral qualities was extracted in a Soxhlet apparatus for 4 h with diethyl ether. A part of total lipid was analyzed for total sterols according to Nadal (1971). The remaining part of total lipid was fractionated according to Véron *et al*. (1996b) by preparative Thin Layer Chromatography (TLC) developed in the first dimension in hexane: Ethyl acetate 92:2 (v/v) to separate the Steryl Esters (SE). And in a second dimension in dichloromethane: methanol: water, 90:10:0.5 (v/v) to separate Free Sterols (FS), Steryl Glycoside (SG) and Acyl Steryl Glycoside (ASG) fractions. FS, SE, SG and ASG bands were located according to R_f values of a commercial plant sterol mixture standards (Plant Sterol Mixture, Matreya Inc.). Spots of standards were visualized with Libermann-Burchard reagent. The different bands were scraped off and eluted with dichloromethane for FS and SE and with 2:1 (v/v) dichloromethane: Methanol for SG and ASG. SE were saponified by a 1 h reflux with methanolic KOH (6% w/v) and 0.5% (w/v) pyrogallol. SG and ASG were separately hydrolyzed by a 4 h reflux with ethanolic H_2SO_4 (1% v/v). Sterols were recovered by partition into hexane and acylated at room temperature in the dark for 48 h using acetic anhydride in anhydrous pyridine. Acetyl derivatives were purified by TLC on silica gel plates developed in dichloromethane with cholesterol acetate as the standard. Location was determined as described above. Sterols generated from preceding fractions were identified by comparison of their RR_t with those of commercial standards using Gas Liquid Chromatography (GLC). Quantification was done by comparison of the sterol peaks area with the area of a known quantity of 5α-cholestane used as an internal standard.

Statistical Analysis

All analyses were made in three replicas, the means and the Standard Deviations (SD) were calculated.

RESULTS

Effect of Light Spectral Qualities on Total and Sterol Profile Contents

The total sterol contents (expressed as μg/g dry wt) of *P. cruentum* grown under different light spectral qualities are shown at Fig. 1. The results revealed a gradual decrease in the total sterol amount in the cells grown under different light spectral qualities according to the order: white>yellow>green> red>blue. The slow down in the sterol production in the cells grown under blue light was approximately 4-fold reduction of those grown under white light.

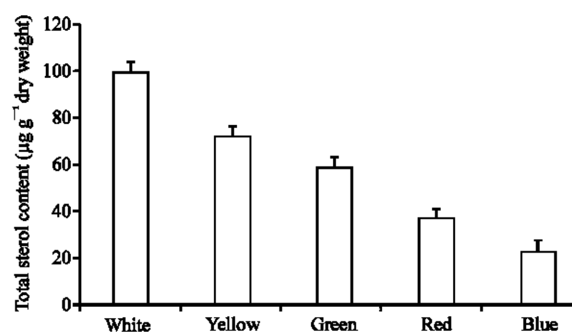

Fig. 1: Total sterol content in *Porphyridium cruentum* grown under different light spectral qualities

Table 1: Concentration of sterol profiles in *Porphyridium cruentum* grown under different light spectral qualities (data expressed as µg g^{-1} dry weight±SD)

	Sterol profile				
Light spectrum	22-dehydrocholesterol	Cholesterol	Brassicasterol	Campesterol	Cycoartenol
White	21±0.3	40±0.3	28±0.4	7±0.1	4±0.4
Yellow	5±0.1	29±0.2	18.54±0.3	12±0.2	8±0.2
Green	2±0.1	42±0.6	13±0.1	1±0.01	1±0.1
Red	1±0.01	19.6±0.1	10±0.1	5±0.2	1.4±0.01
Blue	2±0.01	15±0.4	3.7±0.01	0.3±0.1	4±0.2

As described above sterol profiles have been isolated and identified according to their R_f values using TLC and the comparison of their RR_t with those of commercial standards using GLC. In cells grown under visible light, the results revealed the presence of five sterol molecules; cholesta-5,22-diene-3β-ol (22-dehydrocholesterol); cholest-5-en-3β-ol (cholesterol); 24-Methylcholesta-5,22-diene-3β-ol (brassicasterol); 24-Methylcholest-5-en-3β-ol (campesterol) and cycloartenol.

In cells grown under different light spectral qualities, no change in the composition has been observed, but the amounts were strongly affected by the type of the spectrum used. In this study, whatever the spectral quality of light used, cholesterol was the dominant individual detected (Table 1).

Effect of Light Spectral Qualities on the Content of Different Sterol Classes

Our results demonstrated the presence of four sterol classes in *P. cruentum*; FS, SE, SG and ASG in cells grown under white illumination (Fig. 2). SG compounds were dominated (41% of the total sterols) followed by FS compounds (33%). SE and ASG compounds were 15 and 11%, respectively. In cells grown under yellow and green illuminations, SG compounds were the majors, while in cells grown under both red and blue light, FS compounds were dominated. The major amount of SE and ASG compounds was detected in cells grown under white and yellow light, respectively. The repartition of sterols between the different classes was found to be strongly dependent on the type of illumination.

Effect of Light Spectral Qualities on the Concentration of Sterol Composition of Each Class

Cholesterol was the dominant profile and mainly found as SG (Table 2-6). While brassicasterol was the major constituent of FS in cells grown under white, yellow and red light. Twenty two-dehydrocholesterol was found as SG under yellow, red and blue light, while under

green light, it was found as ASG. Campesterol was found as ASG in the cells grown under blue light, but under green and red light, it detected as SE. Cycloartenol, the first molecule formed in the sterol biosynthesis, was distributed among different classes depending on the type of illumination.

Table 2: Concentration of sterol profiles of free and conjugated classes in *Porphyridium cruentum* grown under white light (data expressed as $\mu g\ g^{-1}$ dry weight±SD)

	Sterol distribution[a]			
Sterol fraction	FS	SE	SG	ASG
22-Dehydrocholesterol	6.3±0.1	4.2±0.5	10.5±0.7	-
Cholesterol	8.4±0.1	2.8±0.1	28±0.6	0.8±0.1
Brassicasterol	14±0.3	0.84±0.1	2.24±0.3	10.9±0.4
Campesterol	2.1±0.2	4.9±0.4	-	-
Cycloartenol	2.4±0.1	1.6±0.1	-	-

Table 3: Concentration of sterol profiles of free and conjugated classes in *Porphyridium cruentum* grown under yellow light (data expressed as $\mu g\ g^{-1}$ dry weight±SD)

	Sterol distribution[a]			
Sterol fraction	FS	SE	SG	ASG
22-Dehydrocholesterol	-	-	5±0.4	-
Cholesterol	4.2±0.2	5.8±0.3	16±0.2	3±0.3
Brassicasterol	14±0.8	0.42±0.1	1.12±0.1	3±0.3
Campesterol	-	6±0.5	1±0.1	5±0.2
Cycloartenol	-	-	6±0.2	2±0.1

Table 4: Concentration of sterol profiles of free and conjugated classes in *Porphyridium cruentum* grown under green light (data expressed as $\mu g\ g^{-1}$ dry weight±SD)

	Sterol distribution[a]			
Sterol fraction	FS	SE	SG	ASG
22-Dehydrocholesterol	-	-	-	2±0.2
Cholesterol	16±0.2	-	24±0.6	2±0.2
Brassicasterol	6±0.1	-	2±0.2	5±0.1
Campesterol	-	1±0.3	-	-
Cycloartenol	1±0.2	-	-	-

Table 5: Concentration of sterol profiles of free and conjugated classes in *Porphyridium cruentum* grown under red light (data expressed as $\mu g\ g^{-1}$ dry weight±SD)

	Sterol distribution[a]			
Sterol fraction	FS	SE	SG	ASG
22-Dehydrocholesterol	-	-	1±0.1	-
Cholesterol	4.2±0.1	3±0.1	6±0.4	2.4±0.1
Brassicasterol	10±0.1	-	-	-
Campesterol	-	5±0.3	-	-
Cycloartenol	-	-	1.4±0.1	-

Table 6: Concentration of sterol profiles of free and conjugated classes in *Porphyridium cruentum* grown under blue light (data expressed as $\mu g\ g^{-1}$ dry weight±SD)

	Sterol distribution[a]			
Sterol fraction	FS	SE	SG	ASG
22-Dehydrocholesterol	-	-	1±0.1	-
Cholesterol	6±0.5	-	7±0.5	2±0.1
Brassicasterol	4.6±0.2	3±0.1	-	0.1±0.02
Campesterol	-	-	-	0.3±0.01
Cycloartenol	4±0.1	-	-	-

[a]FS, free sterols; SE, steryl esters; SG, steryl glycosides; ASG, acyl steryl glycosides, -not detected

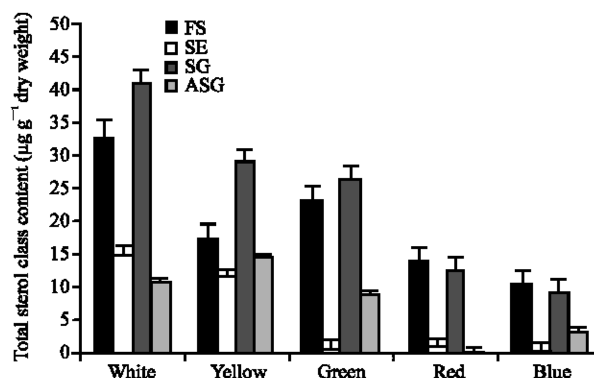

Fig. 2: Concentration of free and conjugated sterol classes in *Porphyridium cruentum* grown under different light spectral qualities. (FS, free sterols; SE, steryl esters; SG, steryl glycosides; ASG, acyl steryl glycosides)

DISCUSSION

Our data here showed a dramatic decrease in the total sterol amount (25% of those grown under white light) in cells grown under blue light. The results agree with those obtained by Véron *et al.* (1996a) on the diatom *Phaeodactylum trocornutum* grown at 23°C. In this respect, Ma *et al.* (2001) concluded that the regulated genes in all light qualities were estimated to account for approximately one-third of the genome. Analysis of those light-regulated genes revealed more than 26 cellular pathways and they were distributed among with three-fifths up regulated and two-fifths down regulated by light. However, genes for sterol biosynthesis pathway were classified of down regulated type by light.

Sterol pattern in *P. cruentum* supports its systematic position among the primitive types of rhodophytes; the presence of cycloartenol with absence of stigmasterol and β-sitosterol. As demonstrated by Cvejic and Rohmer (2000), sterol biosynthesis in *P. cruentum* takes place in the cytoplasm via the mevalonic acid pathway, which is strongly influenced by the light (Bush and Grunwald, 1973). According to our results, cholesterol was the most abundant profile (40% of the total sterol content) produced in cells grown under white illumination, followed by brassicasterol. The profile concentration was found to be depending on the type of spectrum used; however, sterol composition was not affected. As explained by Fabregas *et al.* (1997) the differences in the sterol profile concentrations could be attributed to their dissimilar response towards the difference in the light availability.

The concentration and distribution of sterol profiles among the free and conjugates were shown here to be closely dependent on subjected light quality. The slowing down of the biosynthesis is accompanied by the decrease in the sterol amounts. In contrast to higher plants (Wojciechowski, 1991) and other species of *Porphyridium* (Dupéron *et al.*, 1980), FS compounds are not the dominant class in control culture, since 52% of the total sterols are glycosylated. However, FS slightly increased in cells grown under red and blue light. The data here showed a shift in the sterol amounts and distribution, this alteration, as postulated by Tuckey *et al.* (2002), mainly depend on the light type. Therefore, the sterol metabolism is greatly influenced by the chloroplast system (Anding *et al.*, 1971).

Whatever the light conditions, cholesterol was the major component and mainly detected as SG form. This result go parallel with a decrease in the concentration of brassicasterol indicating, as explained by Véron *et al.* (1996a), an inhibition of the first methylation step at C-24. SG and ASG

sterols are biosynthesized by a UDP-glucose: Sterol glucosyltransferase in eukaryotic organisms and the genes encoded the enzyme can use different sterols such as cholesterol as sugar acceptors. The biosynthesis of this enzyme is extremely affected by environmental stress (Warnecke *et al*., 1999). SE compounds detected in a minor amount in the cells grown under green, red and blue light; while this could reflect differences in responses to environmental variables (Jensen-Pergakes *et al*., 2001).

Ballantine *et al*. (1979) attributed the alteration in sterol pool by different spectral light qualities is a result of switching between 27C Δ^7sterol and 28CΔ^7 sterol in marine phytoplankton.

Jensen-Pergakes *et al*. (2001); Wentzinger *et al*. (2002); Laule *et al*. (2003) reported that, conjugation of sterols is a critical homeostatic response by all eukaryotic cells to an excess of either resource. The intracellular esterification reaction is mediated by enzymes known collectively as *O*-acyltrnsferases. The biosynthesis and activity of acyltransferases is closely correlated with light induction and it is completely neglected in cells grown under complete darkness (Jelsema *et al*., 1982).

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