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MODIFICATION OF STEROL CONCENTRATION IN MARINE MICROALGAE

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Key Word Index—*Phaeodactylum tricornutum*; Phaeodactilaceae; *Tetraselmis suecica*; Prasinophyceae; renewal rate; semicontinuous culture; biosynthesis; phytosterols.

Abstract—Semicontinuous cultures of the marine microalgae *Phaeodactylum tricornutum* and *Tetraselmis suecica* were carried out with renewal rates (RR) of 10, 20, 30, 40 and 50%. The concentration of (24*S*)-24-methylcholesta-5,22*E*-dien-3β-ol in *P. tricornutum* followed a progressive fall from a maximum of 29.1 fg cell^{-1}, in the culture with the 10% RR, to a minimum of 20 fg cell^{-1} in the culture with the 50% RR. In *Tetraselmis suecica*, 24-methylcholest-5-en-3β-ol ranged from 137 fg cell^{-1} with a 10% RR to 40 fg cell^{-1} with a 40% RR and 24-methylcholesta-5,24(28)-dien-3β-ol ranged from 403 fg cell^{-1} with a 10% RR to 80 fg cell^{-1} with a renewal rate of 50%. Renewal rate is a good tool to modify the sterol concentration in marine microalgae.

INTRODUCTION

Different microalgae contain different types of sterols which are characteristic of each species [1–3]. Moreover, sterols may be useful in taxonomic studies of microalgae [4]. Changes in the sterol composition of microalgae can be produced by chemical compounds such as petrol [5]. The possibility of changing the sterol concentration of microalgae is a very important characteristic that can be used to determine the physiological state of a microalgal population, as a pollution biomarker, or to define the nutritive requirements in microfeeders such as molluscs. It is possible to change the sterol concentration of microalgae by the culture conditions. Changes in the daily renewal rate of semicontinuous cultures could provoke changes in the sterol concentration of microalgae, as well as in that of proteins and lipids [6]. As the possibility of changing the sterol concentration in microalgae remains unclear, semicontinuous cultures in cyclostate regimen of *Tetraselmis suecica* and *Phaeodactylum tricornutum* were carried out with daily renewal rates (RR) of 10, 20, 30, 40 and 50% to determine the changes in the concentration of the sterols of these microalgae.

RESULTS AND DISCUSSION

The epibrassicasterol concentration of *P. tricornutum* decreased when the renewal rate increased ranging from a maximum of 29.1 fg cell^{-1} with the RR of 10%, to a minimum of 20 fg cell^{-1} with the RR of 50% (Table 1). In the same way, the sterols of *T. suecica*, decreased from a maximum of 137.2 fg cell^{-1} of methylenecholesterol and 540.2 fg cell^{-1} of campesterol with a RR of 10%, to a minimum of 42.5 fg cell^{-1} of methylenecholesterol and 207 fg cell^{-1} of campesterol with the RR of 50% (Table 2). Sterol concentration in *P. tricornutum* and *T. suecica* decreases with increasing renewal rate, though lipid concentration tends to fall in *P. tricornutum* and rise in *T. suecica*. The concentration of total lipids of microalgae, followed different patterns, decreasing with the higher renewal rates in the case of *P. tricornutum* (Fig. 1) and increasing in the case of *T. suecica* (Fig. 2). Changes in the sterol concentration seem not to be related to cell volume, because cell volume follows a U-shaped curve in semicontinuous cultures with different renewal rates [6]. The sterol concentration of

Table 1. Sterols (fg cell^{-1}), total lipids and dry wt (pg cell^{-1}) of *Phaeodactylum tricornutum*

Renewal rate	10%	20%	30%	40%	50%
Epibrassicasterol	29.1	27.9	22.7	20.4	20.0
Total lipids	4.2	2.8	2.4	2.2	2.5
Dry wt	31.6	24.5	21.3	20.5	33
% Sterol/lipids*	0.69	0.99	0.94	0.85	0.8
% Sterol/dry wt†	0.09	0.11	0.1	0.09	0.06
$\times 10^6$ cell ml^{-1}	42.3	42.2	36.4	32	20.2

* % of sterols with regard to total lipids. † % of sterols with regard to total dry wt.

Table 2. Sterols (fg cell^{-1}), total lipids and dry wt (pg cell^{-1}) of *Tetraselmis suecica*

Renewal rate	10%	20%	30%	40%	50%
Methylenecholesterol	137	68	66.6	40	127
Campesterol	403	234	180	168	80
Total lipids	19.6	21.4	24.1	34.1	33.7
Dry wt	260	199	140	197	176
% Sterols/lipids*	2.75	1.41	1.02	0.72	0.61
% Sterols/dry wt†	0.21	0.15	0.17	0.11	0.12
$\times 10^6$ cell ml^{-1}	5.1	4.6	3.9	2.7	2.5

* % of sterols with regard to total lipids. † % of sterols with regard to total dry wt.

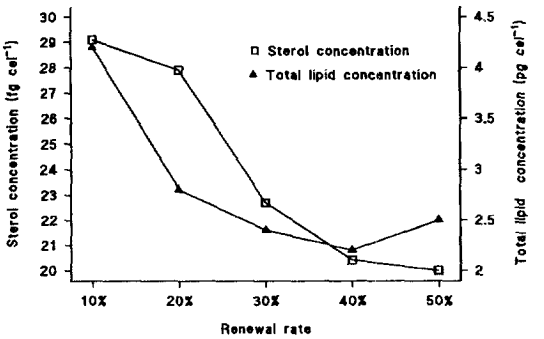

Fig. 1. Sterol and total lipid concentration of *Phaeodactylum tricornutum*.

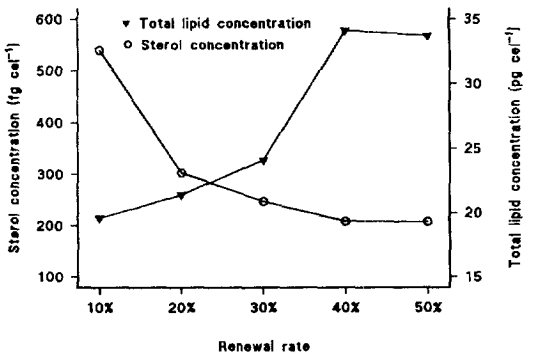

Fig. 2. Sterol and total lipid concentration of *Tetraselmis suecica*.

T. suecica decreased with the increase of the RR, but, total lipids, as in most microalgae, increased with the increased availability of nitrogen with the higher renewal rates. It seems that sterols accumulate mainly in nitrogen deficient conditions. The percentages of methylenecholesterol and campesterol of *T. suecica* varied with the different RR, with a strong change noted in the culture with a 50% RR. (Table 2). These differences in the amounts of these two sterols could be due to the effect of the different light availability [7] of the cells in the cultures. The lowest cell density of the cultures (Table 2) with a higher RR implies a higher light availability due to a lower cellular mutual shading of cells. The different sterol concentration could also be due to the different physiological stage of the cell, because each culture has different renewal rates. This implies that the higher the renewal rate, the bigger is the percentage of the cellular population growing actively, this is, with cell divisions taking place every cellular cycle. On the other hand, the lower the renewal rate, the smaller is the percentage of cellular division, yielding an older population. The relative percentage of the sterols, with regard to the total lipid fraction, ranged from a minimum of 0.69 to a maximum of 0.99% in *P. tricornutum* and from 0.61 to 2.75% in *T. suecica*, both in accordance with the range obtained by other authors [7, 8]. Changes in sterol concentration in the microalgae according to culture conditions, can change its nutritive value when they are used in mollusc feed. Despite the low weight of the sterol fraction relative to total dry wt of the microalgae, 0.11–0.06% in *P. tricornutum* and 0.21–0.10% in *T. suecica*, plays a critical role in molluscs nutrition [9]. Thus, renewal rate of cultures could be an important technique to produce microalgae with different sterol concentrations in order to see its influence in the growth and survival of molluscs during their critical stages of development.

EXPERIMENTAL

Microalgae culture. Semicontinuous cultures of *Phaeodactylum tricornutum* and *Tetraselmis suecica* were started from an inoculum of 2 and 0.5×10^6 cell ml^{-1}, respectively, in sterile sea H_2O (3.5% salinity) with nutrients ($NaNO_3$, 4 mM; NaH_2PO_4, 0.2 mM; $ZnCl_2$, 2 μM; $MnCl_2$, 2 μM; Na_2MoO_4, 2 μM; $CoCl_3$, 0.2 μM; $CuSO_4$, 0.2 μM; $FeC_6H_5O_7$, 40 μM; thiamine, 70 μg l^{-1}, biotin, 10 μg l^{-1}, B_{12}, 6 μg l^{-1}, EDTA, 56.8 μM) [10]. When the beginning of the stationary state was achieved, the semicontinuous cultures were started, with a daily renewal rate of 10, 20, 30, 40 and 50%. These cultures were grown in 100 ml glass tubes with constant aeration of 250 ml min^{-1} supplemented with CO_2 pulses of 10 s every 10 min in order to maintain the pH between 7 and 8. Each culture was carried out in triplicate in a culture chamber at 22° with a light intensity of 166 μE m^{-2} s^{-1} and with a light:dark regime of 12:12 hr. Once cultures were stabilised, the microalgal biomass was harvested by centrifugation and frozen at −30° until the biochemical analysis were done.

Analytical procedures. Total lipids analysis were done [11] as well as dry wt and the ashes of each culture. For dry wt determinations, a known vol. of each culture was filtered through pre-weighed and carbonised glass fibre filters. The pellet was washed with 0.5% (w/v) isotonic NH_4 formate to withdraw the salt. Filters were dried and weighed to find out the microalgae wt, then, they were carbonised again and weighed to find out the ashes.

Isolation and identification of sterols. Starting from the frozen biomass, total lipids were extracted [12], followed by saponification for 1 hr at 80° in KOH at 6% of MeOH (80%). Successive extractions with

Et_2O, hexane and CH_2Cl_2 yielded the neutral lipids. These, were sepd by TLC on silicagel plates (0.5 mm thickness). Sterols were recovered with hexane and Et_2O and, finally, they were redissolved in CH_2Cl_2 and analysed by GC-MS with a Supelco SAC^{TM-5} 30 m × 0.25 mm capillary column. Compounds were identified by comparing their mass spectra with the Nist and Wiley library ones. A commercial plant sterol mixt. served as a reference to complement the identification (Plant Sterol Mixture, Matreya Inc.). RR_t of the sterols were compared with the RR_t of commercial standards for further identification. Quantification was done by comparison of the sterols peaks area with the area of a known quantity of cholesterol used as int. standard [13]. The column was operated at a head pres. of 13 psi of He and the temp. was programmed to raise from 35 to 280° at 35° min^{-1}, held at 280° for 10 min, raise again from 280 to 300° at 5° min^{-1} and held 10 min at 300°. Electron impact mass spectra were measured at 70 eV.

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