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Effect of glycerol on the lipids in the red alga *Gratelupia doryphora*

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RESUMEN

Efecto del glicerol sobre los lípidos de la alga roja *Gratelupia doryphora.*

Se cultivó alga roja *Gratelupia doryphora* en agua de mar enriquecida con Provasoli y en glicerol. La incubación en agua de mar condujo a un aumento en el porcentaje de los ácidos grasos poliinsaturados, mientras que el glicerol incrementó el contenido de lípidos totales. Por tanto, si la alga está siendo producida como un cultivo heterotrófico, es posible aumentar la biomasa de la misma así como el contenido de ácidos grasos biológicamente activos.

PALABRAS-CLAVE: Alga roja - Glicerol (efecto del) - Gratelupia doryphora - Lípido.

SUMMARY

Effect of glycerol on the lipids in the red alga *Gratelupia doryphora.*

The red alga *Gratelupia doryphora* was cultivated in Provasoli enricher (plain) seawater and in a glycerol media. The incubation in seawater leads to an increase in the percentage of polyunsaturated acids, while the glycerol increase the total lipid content. If the alga is being grown as a heterotrophic culture, it is possible to increase the alga biomass as well as the content of biologically-active fatty acids.

KEY-WORDS: Glycerol (effect of) - Gratelupia doryphora - Lipid - Red alga.

1. INTRODUCTION

The need for sea food and drugs is due to the increasing human population and to the biological potential of marine products. A reasonable way of meeting this deficiency is trough cultivation and production of valuable marine algae for food and pharmaceutical industry. As stated by Cheng (1996), heterotrophic culture may provide an alternative method of cultivation for some algae that utilise organic carbon substances.

Recently we have reported that glycerol stimulates vegetative growth and shoot emission with effects on morphogenesis and oxygen uptake and evolution. Furthermore, there are evidences of an intense accumulation of lipid and polisaccharidic substances when glycerol is added to the cultures (Robaina *et al.,* 1990a, b; Robaina *et al.,* 1995; García-Jiménez *et al.,* 1996, 1998).

The aim of the present work was to study the changes in the main lipid classes and their fatty acid (FA) profiles in *G. doryphora* when glycerol was used in the culture medium.

2. EXPERIMENTAL

Thalli of *Gratelupia doryphora* (voucher specimen deposited in the herbarium of the Jardin Canario, Gran Canaria, Canary Islands, Spain as sheet 129) were collected from the upper intertidal pools in the rocky littoral of San Cristóbal (Gran Canaria, Canary Islands). They were immediately transferred to the laboratory. Disc fragments of 3 mm diameter were excised within 2 h. The fragments were disinfected and tested for sterility as previously described (Robaina *et al.,* 1990 a, b). Explants were then cultivated in 100 ml Pyrex flasks with 50 ml of autoclaved seawater (20-25 explants per flask). The glycerol media was prepared with plain seawater diluted with distilled water (70:30 v/v) and 0.3 M glycerol. Seawater dilution is necessary to avoid osmotic stress due to the addition of glycerol. The osmolality of both culture media was 1.0 osmol kg^{-1} as checked out with an osmometer (Robaina *et al.,* 1990 b). All culture media were supplemented with nutrients formulations and vitamins of the Provasoli enriched seawater medium (PES, Provasoli 1968).

Cultures were placed in an orbital shaker for 5 days. They were subjected to 16 h light - 8 h dark photoperiod at 19 ± 2 °C and irradiance of 30 mmol photon m^{-2} s^{-1}. Two grams (fresh weight) of thalli was also used to perform analysis as a control of the contents in natural samples (non cultivated). They were epiphyte-free thalli cleaned further with brushes to avoid the contamination with organism associated with the thallus.

The total lipids were extracted according to Bligh and Dyer (1959). The main lipid classes: triacylglycerols (TAG), monogalactosyl diacylglycerols (MGDG), digalactosyl diacylglycerols (DGDG) and phospholipids (PL) were separated by preparative thin-layer chromatography (Silica gel G, *Merck,* layer thickness 0.5 mm). The lipid spots were vizualized under UV-light after spraying with fluorescent indicator, scrapped off into small glass containers with teflon screw caps. After addition of internal standard (heptadecanoic acid) all lipid classes were transesterified with 15 % acetyl chloride in methanol. The analysis of the obtained fatty acid methyl esters (FAME) was carried out by flame-ionization detector - gas-liquid chromatography (FID-GLC) on a glass capillary column (30 m, 0.2 mm i.d. coated with SILAR 10 C). The column temperature was increased from 165 °C to 220 °C (2 deg min^{-1}) with nitrogen as a carrier gas at a flow rate 14 mL s^{-1}. The amount of each lipid class was determined on the basis of the FAME weight using converting factors as follows: 1.0 for TAG; 1.4 for MGDG and PL and 1.8 for DGDG (Elenkov *et al.,* 1993).

3. RESULTS AND DISCUSSION

Table I represents the effect of culture with plain seawater or glycerol media on the amounts of the main lipid classes in *G. doryphora* compared to natural samples. The plain seawater in the culture medium leads to a decrease of the amount of the total lipids and especially of the PL. The PL are substantial components of the cell membranes and their decrease may leads to changes in the membrane fluidity. There is a slight increase of the MGDG.

However the addition of glycerol in the culture medium leads to substantial increase of the total lipids and glycolipids. The amount of PL leaves constant.

The cultivation of the algae in plain seawater leads to substantial changes in the FA-profiles of the alga (Table II). The amount of the 20:5 acid increase almost twice, and 16:0 acid decrease. These changes are more expressed in the TAG and PL. As a result the FA-profiles of plain seawater-incubated algae are similar to those from a sample of *Grateloupia turuturu* Yam. from Japan Sea (Khotimchenko and Vaskovsky, 1990). In contrary, the incubation with a glycerol media has not significant effect on the FA-composition of the alga.

It is evident that changes in the culture medium lead to changes of the lipid content of the alga. If this alga being grown in heterotrophic cultures, it is possible to increase the algal biomass as well as to exploit the increase in biologically-active fatty acids. It remains to investigate whether the alteration of the nutrient or other paramethers of culture conditions (Falkowsky, 1988) could drive to even better yield in some lipid fractions.

Table I

Effect of glycerol and PROVASOLI on the main lipid classes in *G. doryphora*

Culture medium and lipid classes	mg g^{-1} DW	% of total
Control (collected in the nature)		
TAG	8.7 ± 0.7	35.9
MGDG	3.4 ± 0.3	14.1
DGDG	4.2 ± 0.3	17.5
PL	7.8 ± 0.6	32.5
Total	24.1 ± 1.9	100.0
Plain seawater		
TAG	7.3 ± 0.6	40.7
MGDG	5.5 ± 0.4	30.5
DGDG	2.4 ± 0.2	13.5
PL	2.8 ± 0.2	15.3
Total	18.0 ± 1.4	100.0
Glycerol media		
TAG	11.5 ± 0.9	38.1
MGDG	5.5 ± 0.4	18.2
DGDG	6.0 ± 0.4	19.9
PL	7.2 ± 0.6	23.8
Total	30.2 ± 2.3	100.0

Table II
Effect of glycerol and PROVASOLI on the fatty acid composition of *G. doryphora*

Culture medium and lipid classes	Fatty acids (% on total FAME)								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	20:5
Control (collected in the nature)									
TAG	5.7	43.4	2.1	15.1	3.4	0.9	1.7	14.1	13.5
MGDG	11.3	53.2	2.4	1.8	0.9	—	—	14.0	16.1
DGDG	1.2	63.0	1.3	0.7	—	—	—	17.0	16.2
PL	7.3	60.9	2.2	1.1	2.3	—	—	12.8	13.2
Total	6.2	53.8	2.0	6.1	1.7	0.3	0.6	14.2	14.2
Plain seawater									
TAG	4.3	28.0	1.7	13.1	5.0	0.4	1.8	14.0	31.4
MGDG	11.4	45.8	—	5.0	—	3.8	—	20.0	13.7
DGDG	14.5	29.5	3.8	2.2	—	—	—	14.9	32.5
PL	12.5	40.0	—	—	—	4.2	—	13.7	25.6
Total	9.1	35.5	1.2	7.1	2.0	2.0	0.7	15.9	25.3
Glycerol media									
TAG	6.6	48.8	1.3	13.2	4.1	—	0.8	12.4	12.6
MGDG	7.3	54.1	0.6	8.4	—	—	0.9	12.7	15.2
DGDG	6.1	51.0	2.0	5.5	—	—	0.3	14.6	18.5
PL	8.3	48.2	5.3	1.6	—	4.2	5.3	10.7	15.3
Total	7.0	50.1	2.3	8.0	1.6	1.0	1.8	12.5	14.9

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