J. Aqua., **21** (2013): 1-8

BIOCHEMICAL VARIATIONS OF MICROALGA *ISOCHRYSIS GALBANA* CULTURED IN DIFFERENT CULTURE MEDIA

K. Naseera, Gijo Ittoop, K. A. Anisha, P. Lakshmi Devi and Aneykutty Joseph*

Dept. of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin-16, Kerala, India

**Corresponding author: aneykuttyj@yahoo.co.in*

The dianoflagellate, *Isochrysis galbana*, grown in different culture media was analysed to study the biochemical variations. The species was cultured in four different growth media, viz. Walne's, Miquel's, Chu's and f/2, keeping the physicochemical factors such as temperature (23.0 - 25.0 ^{o}C), light intensity (2000 lux), pH (8.0) and salinity (35.0 ppt) constant. Harvesting was done in exponential phase to carry out the biochemical analyses. The results of the study revealed that the nutritional status of *I. galbana* varies with different culture media. The best growth rate, cell density, protein (22.33±1.54%), carbohydrate (23.24±2.6%) and lipid (27.71±5.55%) contents were obtained in Chu's medium. The colour intensity and chlorophyll *a* content were observed highest in the microalgae grown in Miquel's medium. The amounts of protein, carbohydrate and lipid showed significant ($P < 0.05$) variations in different culture media. Therefore, from the study it is inferred that biochemical compositions of *I. galbana* varies with the media used for its culture.

INTRODUCTION

The success of any hatchery system whether of crustaceans, oysters, sea cucumbers or fishes, entirely depends on the availability of the suitable microalgae as feed. Hence culture of microalgae and its nutritional content are essential prerequisite for the rearing operations of economically important cultivable organisms. Manipulation of the nutritional content of the microalgae is possible using modulations in the media and culture strategies (Otero *et al*., 2006; Lincymol *et al*., 2012). Research work in this line has great significance to improve the survival rate of the larvae, which feeds on microalgae. Studies conducted in other countries have revealed that even the essential fatty acid composition of the algae could be modulated using these strategies (Martínez-Fernández *et al*., 2006; Rivero-Rodríguez *et al*., 2007).

One of the most important species of microalgae used for the larval rearing in the aquaculture industry throughout the world is *Isochrysis galbana*. It is of substantial interest in aquaculture, principally to feed mollusc larvae, as well as fish and crustaceans in their

early stages of growth (Sukenik and Wahnon, 1991). It has been cultured successfully either indoor or outdoor (Kaplan *et al.*, 1986). It is rich in docosahexaenoic acid (DHA) and is used to enrich zooplankton such as rotifers and *Artemia,* which are used as feed for larval stages of different fish and shellfish. In the present study, the variations in growth and nutritional status of this microalga in different media were investigated so as to identify a medium that supports good growth and at the same time can provide a larval feed rich in fat and protein.

MATERIALS AND METHODS

Pure culture of *I. galbana* in f/2 medium was procured from the marine hatchery complex of the Central Marine Fisheries Research Institute, Kochi. In the algal culture laboratory of the Department of Marine Biology, Cochin University of Science and Technology, it was subcultured in fresh f/2 medium prepared in filtered and sterilized seawater of 35.0 ppt salinity. The culture was maintained at a temperature of 23.0 to 25.0 ^{o}C, pH 8.0 and a light intensity of 2000 lux with a photoperiod of 12L:12D. Satisfactory growth occurred in 9 to 10 days. The culture was tested for contamination once in three days throughout the experiment.

Walne's (Walne, 1974), Miquel's (Miquel, 1892), Chu's (Chu *et al*., 1942) and f/2 (Guillard, 1975) media were used in the present study to understand the influence of media composition of algae. The compositions of the media are given below.

1. **Miquel's Media**

Solution A

Potassium nitrate	20.2 g
Distilled water	100 ml

Solution B

Sodium ortho phosphate	20.2 g
Calcium chloride	2 g
Ferric chloride	2 g
Hydrochloric acid	2 ml
Distilled water	100 ml

0.55 ml of A and 0.5 ml of B were added to 1 litre of filtered and sterilized seawater.

2. **Walne's or Conway media**

Solution A

Potassium nitrate	100 g

Sodium ortho phosphate	20 g
Sodium EDTA	45 g
Boric acid	33.4 g
Ferric chloride	1.3 g
Manganese chloride	0.36 g
Distilled water	1000 ml

Solution B

Zinc chloride	4.2 g
Cobalt chloride	4.0 g
Copper sulphate	4.0 g
Ammonium molybdate	1.8 g
Distilled water	1000 ml

Solution C

Vitamin B1	200 mg/100 ml
Vitamin B12	10 mg/100 ml

1 ml of A, 0.5 ml of B and 0.1 ml of C were added to 1 litre of filtered and sterilized seawater.

3. f/2 media

Sodium nitrate	7.5 g/100 ml
Sodium ortho phosphate	500 mg/100 ml
Sodium silicate	3 g/100 ml
Ferric chloride	0.32 g/9.5 ml
Sodium EDTA	0.44 g/9.5 ml
Manganese chloride	18 g/100 ml
Zinc sulphate	2.2 g/100 ml
Cobalt chloride	1 g/100 ml
Copper sulphate	0.98 g/100 ml
Sodium molybdate	0.63 g/100 ml
Thiamine	200 mg/l
Biotin	1 mg/l
Cyanocobalamin	1 mg/l

1 ml of sodium nitrate, 1 ml of sodium ortho phosphate, 1 ml of sodium silicate, 1 ml of trace metal and 0.5 ml of vitamin solution were added to 1 litre of filtered and sterilized seawater.

4. Chu's (CHU#10) media

A.	Calcium nitrate	5.76 g/100 ml
B.	Potassium ortho phosphate	0.5 g/100 ml
C.	Magnesium sulphate	2.5 g/100 ml
D.	Sodium carbonate	2 g/100 ml
E.	Sodium silicate	2.5 g/100 ml
F.	Ferric chloride	0.08 g/100 ml

1 ml each of A, B, C, D, E and F were added to 1 litre of filtered and sterilized seawater.

Standard algal culture procedures were used throughout the study (Lee and Shen, 2004). The microalgae were harvested using flocculation method by increasing the pH using sodium hydroxide (Lee and Shen, 2004). Haemocytometer was used to estimate cell density. Chlorophyll *a* content was determined by using the method described by Lee and Shen (2004). Lipid content was determined using sulpho-phospho vaniline method (Barner and Blackstock, 1973). Protein content of the microalgae was estimated using the dye binding method (Bradford, 1976) and amount of carbohydrate was estimated using phenol-sulphuric acid method (DuBois *et al*., 1956). The analyses were done using the cultures with a growth of 11 days at the late exponential phase. Cell count, cholorophyll *a* content, protein, lipid and carbohydrate content of microalgae were determined in four replications of each media.

RESULTS AND DISCUSSION

The cell counts of the algae in the different culture media are depicted in Fig. 1. The figure reveals that in all the media tested the exponential phase in the growth of the algae was reached in day 9 and the decline of the culture started after day 12. The maximum cell count was obtained in Chu's medium, followed by f/2 medium. Miquel's medium and Walne's medium showed similar performance with values lower as compared to the other two media. The result in the present study is in accordance with Fábregas *et al*. (1986), who revealed that different nutrient composition of media can influence the biomass production in microalgae. The Chu's medium doesn't contain

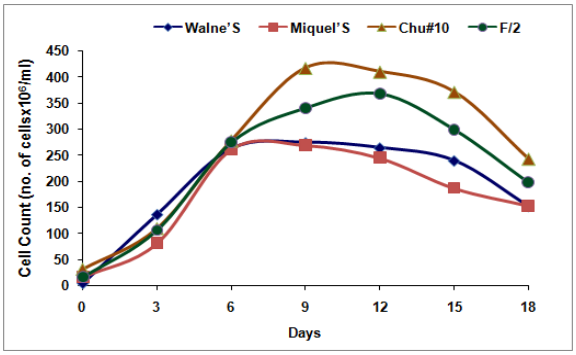

Fig. 1. The cell count of *I. galbana* in different media

vitamins and minor mineral supplements. The result thus revealed that these are not essential nutrients for *I. galbana* and hence growth was not influenced by these supplements.

The chlorophyll *a* content was also determined as a part of this study. The results obtained are given in Fig. 2. Miquel's medium gave maximum values for chlorophyll *a*, quite contrary to the result obtained for cell count. The change of colour of the culture during the culture period was also observed and the data is given in Table 1. The highest colour intensity was shown in Miquel's medium. This is also a medium without any protein supplement and minor nutrients.

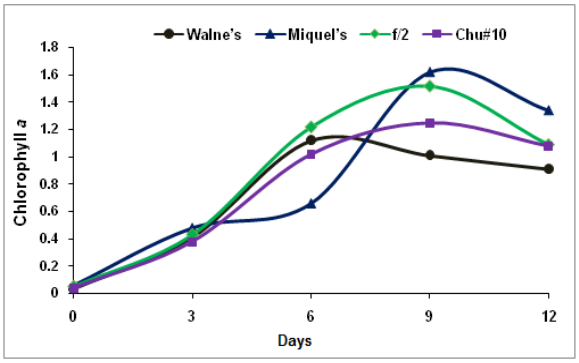

Fig. 2. The chlorophyll *a* content of *I. galbana* in different media

Table 1. Colour pattern of *I. galbana* grown in different media

MEDIA	DAYS											
	1	2	3	4	5	6	7	8	9	10	11	12
WALNE'S	PB	PB	BY	BY	BY	BY	BY	DBY	DBY	DBY	DBY	DBY
MIQUEL'S	PB	PB	BY	BY	BY	BY	B	B	B	DB	DB	DB
CHU#10	PB	PB	BY	BY	BY	BY	BY	DBY	DBY	DBY	DBY	DBY
F/2	PB	PB	BY	BY	BY	BY	BY	DBY	DBY	DBY	DBY	DBY

PB - Pale brown, BY - Brownish Yellow, DBY - Dark Brownish Yellow, B - Brown, DB - Dark Brown

The biochemical composition of the algae in different culture media is given in Fig. 3. Statistical analysis of the protein content of *I. galbana* in different culture media was carried out using analysis of variance technique using the software available in Microsoft windows. Comparison of means using 't' test was done using the method given by Snedecor and Cochran (1968). The analysis revealed that there is statistical difference

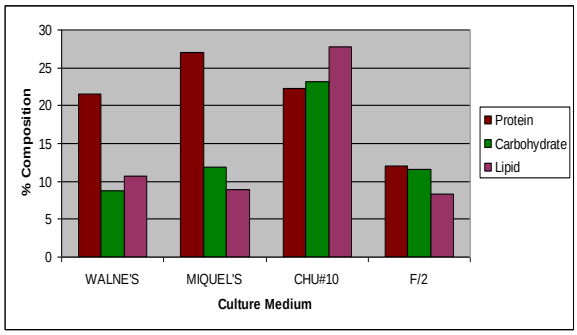

Fig. 3. Biochemical variation of *I. galbana* in different media

($p<0.05$) between the values obtained in cultures raised using different media. The protein content of *I. galbana* raised in f/2 medium was significantly lower compared to that obtained from other three media.

The result obtained for lipid content revealed that the lipid content is almost twice in Chu's medium compared to all the other media tested. The statistical analysis using ANOVA and comparison of means using 't' test revealed that there is significant difference between the lipid content in Chu's medium and the other media used in the study. The results in the other three media showed no significant difference ($P<0.05$).

The carbohydrate content was also found highest in microalgae grown in Chu's medium. The result was statistically significant at 5% level. All the other media tested showed no significant difference in carbohydrate content.

The present study revealed that media could certainly influence the growth as well as biochemical composition of the microalga *I. galbana.* The protein content was best in the algal culture obtained from Miquel's medium (27.01±6.08) followed by Chu's medium (22.33±1.54) and Walne's medium (21.58±1.58). The carbohydrate (23.24±2.6) and lipid (27.71±5.55) contents were highest in Chu's medium. The lipid was twice in this medium compared to all the other media tested. So the amount of essential fatty acid would also be highest in the cultures obtained from this medium. Even though Chlorophyll *a* content was highest in Miquel's medium, it did not have any influence in the nutritional make up of the algae. Cho *et al.* (1998) also has revealed the influence of media on the biochemical composition of *I. galbana*.

Thus in the present study the Chu's medium was proved to be the best medium for the culture of *I. galbana*, since it supported maximum growth and best nutritional value. This may be because only Chu's medium among the selected media for analysis contains CO_3^{2-}. The previous studies reveal that in the absence of CO_2, microalgae can utilize CO_3^{2-} (Wang *et al.,* 2008) and *I. galbana* utilized the CO_3^{2-} ions present in the Chu's medium to build up the biomass and other biomolecules as there was no external source of CO_2 provided. However, Chu's medium gives maximum amount of lipid and carbohydrate and Miquel's medium supported the production of high amount of protein. Therefore the selection of medium for algal culture should be based on the individual requirement of the larvae, which feed on it for growth and survival. It is also revealed that modulation of biochemical composition of microalga is possible by using different culture media.

ACKNOWLEDGEMENTS

The authors are deeply indebted to the Kerala Biotechnology Commission, Government of Kerala, Trivandrum, University Grants Commission, New Delhi and Department of Marine biology, Cochin University of Science and Technology, for the facilities provided.

REFERENCES

Barner, H. and J. Blackstock, 1973. Estimation of lipids in marine mammals and tissues. Detailed investigation of Sulpho-phospho vaniline method for total lipids. *J. Exp. Biol. Ecol.*, **12**: 103-118.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal. Biochem*., **72**: 248 – 254.

Cho, J. Y., J. Hyung-Joo, J. L. Hyun, N. C. W. John and Y-K. Hong, 1998. Growth activation of the microalga *Isochrysis galbana* by aqueous extract of the seaweed *Monostoma nitidum. J. Appl. Phycol.*, **10**: 561-567.

Chu, F. E., J. L. Dupuy and K. L. Webb, 1942. Polysaccharide composition of five algal species used as food for larvae of American oyster. *Aquaculture,* **29**: 241-252.

DuBois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, 1956. Colorimetric determination for sugars and related substances. *Anal. Chem*., **28**: 350-356.

Fábregas, J., C. Herrero, B. Cabezas and J. Abalde, 1986. Biomass production and biochemical composition in mass cultures of the marine microalga *Isochrysis galbana* Parke at varying nutrient concentrations. *Aquaculture*, **53**: 101-113.

Guillard, R. L, 1975. Culture of phytoplankton for feeding marine invertebrates. *In:* Culture of marine invertebrate animals (Eds. W. L. Smith and M. H. Chanley). Plenum Press, New York, pp. 29-60.

Kaplan, D., Z. Cohen and A. Abeliovich, 1986. Optimal growth conditions of *Isochrysis galbana*. *Biomass*, **9**: 37-48.

Lee, Y-K. and H. Shen, 2004. Basic culturing techniques. *In*: Handbook of microalgal culture: Biotechnology and Applied Phycology (Ed. A. Richmond). Blackwell Science Ltd, UK, pp. 40-56.

Lincymol, K. P., G. Ittoop, P. Lakshmi Devi and A. Joseph, 2012. Modulation in nutitional quality of microalgae, *Chaetoceros calcitrans* in different culture media. *Fish. Technol.,* **49**: 120-124.

Martínez-Fernández, E., H. Acosta-Salmón and P. C. Southgate, 2006. The nutritional value of seven species of tropical microalgae for black-lip pearl oyster (*Pinctada margaritifera*, L.) larvae. *Aquaculture*, **257**: 491-503.

Miquel, P., 1892. de la culture artificielle des diatomees. *C.R. Academic Science*, **94**: 1-780.

Otero, A., D. García and J. Fábregas, 2006. Factors controlling eicosapentaenoic acid production in semicontinuous cultures of marine microalgae. *J. Appl. Phycol.*, **9**: 23-36.

Rivero-Rodríguez, S., A. R. Beaumont and M. C. Lora-Vilchis, 2007. The effect of microalgal diets on growth, biochemical composition, and fatty acid profile of *Crassostrea corteziensis* (Hertlein) juveniles. *Aquaculture*, **263**: 199-210.

Snedecor, G. W. and W. G. Cochran, 1968. Statistical Methods (6th Edn.). Iowa State University Press, Ames, Iowa, 593 pp.

Sukenik, A. and R. Wahhnon, 1991. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. I. *I. galbana. Aquaculture*, **97**: 61-72.

Walne, P. R., 1974. The culture of marine bivalve larvae. *In*: Physiology of mollusca (Eds. K. M. Wilbur and C. N. Yonge). Academic Press, New York, pp. 197-210.

Wang, B., Y. Li, N. Wu and C. Q. Lan, 2008. CO_2 bio-mitigation using microalgae. *Appl. Microbiol. Biotechnol.*, **79**: 707–718.