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Modulation in Nutritional Quality of Microalgae, *Chaetoceros calcitrans* in Different Culture Media

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Abstract

The present study was initiated to identify the best medium for culture of *Chaetoceros calcitrans* that could provide better protein and lipid content while supporting good growth of the algae. The media selected were Walne's, Miquel's, Chu and f/2 media. The algae cultured in Walne's, Miquel's and f/2 media showed significantly higher content of lipid ($P<0.05$) in the late exponential phase. Protein content was the highest in Walne's medium (32.53 ± 0.28% dry weight) as compared to other media tested. Maximum cell density was recorded in f/2 medium. Miquel's medium gave the maximum carbohydrate content (16.92 ± 1.54% dry weight). It was observed that media could certainly influence the biochemical composition of microalgae and therefore selection of media should be based on the larval requirement of the target species. Results obtained in the present study revealed that microalgae cultured in Walne's medium gave high values of protein and lipid content. Although cell count was the highest in f/2 medium, Walne's medium provided the best nutritional quality of algae.

Keywords: Algal culture, *Chaetoceros calcitrans*, culture media, biochemical variation

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Introduction

Microalgae contribute half of the globe's photosynthetic activity and form the basis of the food chain for more than 70% of the world's biomass (Raja et al., 2008). About 25 000 species of microalgae are reported, but only a few are used for various purposes beneficial to humans. One of the most important applications of the microalgae is its use in the larval rearing of a variety of fin fish and shellfish (Brown et al., 1997). Advancement in biotechnology has paved way for enrichment of microalgae using various media as well as culture strategies, so that the percentage of larval survival can be improved (Noue & Pauw, 1988). Otero et al. (2006) had developed culture techniques for *Phaeodactylum tricornutum, Isochrysis galbana* and *Porphyridium cruentum* with high polyunsaturated fatty acid (PUFA) content. Different nutrient concentrations and aeration rates were tried to improve nutritional status of microalgae by Fábregas et al. (1986a, 1986b, 1998). Eicosapentaenoic acid and docosahexaenoic acid emulsions were used in the media for improving polyunsaturated fatty acid content in *Dunaliella tertiolecta* (Nevejan, 2003). Much advancement has been made by other countries in the manipulation of biochemical content of the microalgae in accordance with the requirement of the cultured species. This field of study is still in its infancy in India. Even though different media are used for algal culture, their effect on the biochemical composition of microalgae has not yet been studied. The present study attempts to find the best medium which supports good growth with improved nutritional value for the culture of *Chaetoceros calcitrans,* an important live food used in mariculture.

Materials and Methods

Pure culture of *C. calcitrans* maintained in f/2 medium was obtained from Central Marine

Fisheries Research Institute, Cochin, India. The media tested in the present study were Miquel's (Miquel, 1892), Chu (Chu *et al*., 1942), Walne's (Walne, 1974) and f/2 media (Guillard & Provasoli, 1975), the composition and preparation/procedure of which are given in Table 1.

Sea water of salinity between 28-35 ppt was used for the preparation of the media. Standard algal culture procedures were used throughout the study (Lee & Shen, 2004). Hafkin's flasks of 3000 ml capacity were used for testing each media in triplicate. A temperature of 23 to 25^{0}C and illumination of 1000 to 2000 lux for 12 h were provided for the algal growth. Inoculation of each media was done at the rate of 9 to 10 x 10^4 cells ml^{-1}. Algal cell counting was done every day using haemocytometer. Preliminary experiments showed that algal culture reached exponential phase in 10 to 12 days and the decline phase started by day 15 in all the media tested. So it was decided to analyze the biochemical composition on day 12, the late exponential phase. Throughout the culture period, strict anti-contamination procedures were followed (Lee & Shen, 2004) and the culture was checked on every sampling for contamination. For harvesting, flocculation was done by increasing the pH using sodium hydroxide. Protein analysis was done using dye binding method (Bradford, 1976), carbohydrate analysis using phenol-sulphuric acid method (Dubois et al., 1956) and lipid content using sulpho-phospho vaniline method (Barner & Blackstock, 1973). The differences in the means of cell count and biochemical compositions among various treatments were compared using ANOVA and the variations between individual treatments, if any, was brought out following post hoc analysis.

Results and Discussion

Cell count of the algae in the different culture media during the study period is given in Fig. 1. The results showed that the maximum cell count was in f/2 medium (145 cells ml^{-1}), followed by Walne's medium. Miquel's medium showed the poorest performance. Statistical analysis of the data revealed significant difference (*P<0.05*) between cell density in different media. The f/2 medium gave a significantly high cell count compared to all other media. The result obtained is in accordance with Fábregas et al. (1986a), who reported that different nutrient composition can influence the biomass production in microalgae.

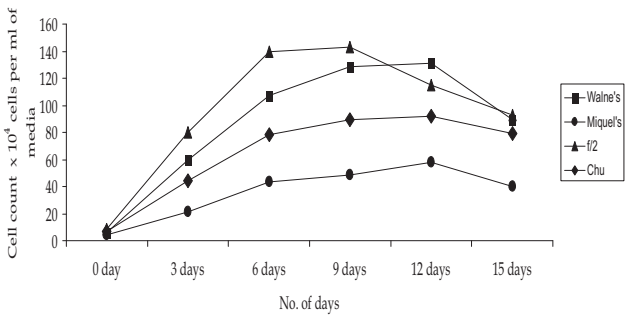

Fig. 1. Cell count of *Chaetoceros calcitrans* in different media against time

Mean percentage of protein, lipid and carbohydrate content of *C. calcitrans* in different media are given in Table 2. Statistical analysis of the data showed significant differences *(P<0.05*) between the protein content in microalgae cultured in different media. The Walne's medium gave a significantly high (*P<0.05*) content of protein (32.53 ± 0.28% dry weight). This was followed by f/2 medium (25.69 ± 0.30% dry weight). The influence of nutrient content of the media in determining protein content of the microalgae produced has been elucidated by Fábregas et al. (1986a).

In the case of lipid content, the results revealed that it was almost the same in all the media tested except the Chu medium. There was no significant difference between the lipid content in Walne's, Miquel's and f/2 media whereas in the Chu medium there was significantly lower (*P<0.05*) lipid content in the microalgae produced.

Miquel's media which gave microalgae with lower protein and fat content, provided a higher content of carbohydrate (16.92 ± 1.54% dry weight). The Walne's, Chu and f/2 medium showed results with no significant difference at (*P>0.05*). But at the same time, these results were significantly lower (*P<0.05*) compared to the values obtained in Miquel's medium. Growth of algae and level of protein and lipid content were lower in the Miquel's medium. But the carbohydrate content was maximum in this medium. The change in the carbohydrate content in different nutrient composition of the culture media is reported by Fábregas et al. (1986b).

From Table 2, it can be seen that protein and lipid content were higher in media such as Walne's and f/2 media where trace elements and vitamins are included. In case of Miquel's and Chu media,

Table 1. Composition of media selected for the study

Sl. No.	Media	Composition/procedure	Quantity
1	Miquel's media (Miquel, 1892)	A. Potassium nitrate	20.2 g
		Distilled water -	100 ml
		B. Sodium ortho phosphate	4 g
		Calcium chloride	2 g
		Ferric chloride	2 g
		Hydrochloric acid	2 ml
		Distilled water	100 ml
		0.55 ml of A and 0.50 ml of B were added to 1 litre of filtered and boiled sea water	
2	Conway or Walne's media (Walne, 1974)	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
		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
		C. Vitamin B1	200 mg 100 ml^{-1}
		Vitamin B12	10 mg 100 ml^{-1}
		A, B and C were prepared in different bottles. One ml of A, 0.5 ml of B and 0.1 ml of C were added to 1 litre filtered and boiled sea water	
3	f/2 medium (Guillard and Provasoli, 1975)	Sodium nitrate	7.5 g 100 ml^{-1}
		Sodium ortho phosphate	500 mg 100 ml^{-1}
		Sodium silicate	3 g 100 ml^{-1}
		Ferric chloride	0.32 g 9.5 ml^{-1}
		Sodium EDTA	0.44 g 9.5 ml^{-1}
		Manganese chloride	18 g 100 ml^{-1}
		Zinc sulphate	2.2 g 100 ml^{-1}
		Cobalt chloride	1 g 100 ml^{-1}
		Copper sulphate	0.98 g 100 ml^{-1}
		Sodium molybdate	0.63 g 100 ml^{-1}
		Thiamine	200 mg l^{-1}
		Biotin	1 mg l^{-1}
		Cyanocobalamin	1 mg l^{-1}
		1 ml of sodium nitrate, 1 ml of sodium orthophosphate, 1 ml of sodium silicate, 1 ml of trace metal and 0.5 ml of vitamin solution added to 1 liter of filtered and boiled sea water	
4	Chu's medium (Chu et al., 1942)	A. Calcium nitrate	5.76 g 100 ml^{-1}
		B. Potassium ortho phosphate	0.5 g 100 ml^{-1}
		C. Magnesium sulphate	2.5 g 100 ml^{-1}
		D. Sodium carbonate	2 g 100 ml^{-1}
		E. Sodium silicate	2.5 g 100 ml^{-1}
		F. Ferric chloride	0.08 g 100 ml^{-1}
		Add 1 ml of A, B, C, D, E, F to 1 liter of filtered and boiled sea water	

Table 2. Mean percentage and SD of protein, lipid and carbohydrate in *Chaetoceros calcitrans* cultured in different media*

Nutritional parameters	Media			
	Walne's	Miquel's	f/2	Chu
Protein (%)	32.53 ± 3.97 [a]	13.84 ± 4.07 [b]	25.69 ± 2.89 [c]	18.23 ± 2.15 [b]
Lipid (%)	16.07 ± 3.28 [a]	15.89 ± 2.35 [a]	16.09 ± 3.22 [a]	9.39 ± 1.85 [b]
Carbohydrate (%)	11.29 ± 1.99 [a]	16.92 ± 1.54 [b]	11.88 ± 1.76 [a]	9.47 ± 5.89 [a]

* Values which are differently superscripted in each row are significantly different ($P<0.05$)

growth as well as nutritional quality of the algae produced were low because of the absence of trace elements and vitamins in the media. According to Croft et al. (2006), most groups of algae require thiamine, cyanocobalamin and biotin as growth factors in media for increased ability of photosynthesis. The role of trace elements such as iron, copper and manganese in the growth of microalgae has been elucidated by Spencer (1957).

From the above results and discussion, it is evident that media have certainly influenced the growth as well as biochemical composition of the microalgae *C. calcitrans.* The f/2 medium supported maximum growth of the algae while Walne's medium gave maximum content of protein and lipid. Miquel's media though did not support good growth of algae the carbohydrate content was higher in algae grown in this medium. The biochemical variability of algae in Walne's and f/2 medium was similar to that obtained by Brown et al. (1997) and Phatarpekar et al. (2000). Thus the present study has proved the superior efficiency of Walne's medium for producing *C. calcitrans* with higher protein and lipid content for better performance in larval nutrition of cultured species.

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