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Alterations in Body Fatty acid Composition in Microalga, *Pavlova lutheri*, grown in different standard Culture Media

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Abstract

The present work monitored variations in fatty acid profile and analysed the nutritional status of the live feed *Pavlova lutheri*, a marine microalga which was grown in three different culture media. Pure cultures of microalga were collected from the marine hatchery complex of the ICAR-Central Marine Fisheries Research Institute, Kochi. Standard algal culture conditions *viz.,* medium, in sterilized seawater of salinity 33 ppt, standard pH of 8.0, culture room temperature of 25ºC, light intensity of 1500 lux with a photoperiod of 12h.L:12h.D etc. were maintained. Lipids were extracted and were subjected to gas chromatography in an attempt to ascertain their fatty acid profile. Total saturated fatty acids ranged from 54.88% (Miquel's medium), 76.54% (Chu#10 medium) to 75.8% (F/2). In the case of monounsaturated, only a slight variation is seen among the three. *ie.* minimum for Chu#10 (16.54%) and maximum for F/2 (19.99%). For cultures in Miquel's, medium the value was 18.42%. Polyunsaturated fatty acids (PUFAs) accounted for over 26.70% of the total fatty acids in Miquel's medium whereas it was only 6.87 and 4.21% in Chu#10 and F/2 media respectively. Statistical analysis of data brought to light that the composition of the medium can influence the fatty acid pattern of microalgae significantly ($p \leq 0.05$). By analyzing the results it can be confirmed that the nutritional value of *Pavlova lutheri* can be enhanced by culturing in Miquel's medium.

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Introduction

Medium screening and optimization is one of the most important preconditions for photoautotrophic cultivation of microalgae. Generally the quality of the culture medium ascertains the growth performance of the microalgae (Prathima et al., 2011; Lam & Lee, 2012; Li et al., 2012). Vital variations in growth dynamics, biochemical composition and in fatty acid layouts of microalgae have been noticed in response to growth media and time of harvest (Lincymol et al., 2012; Naseera et al., 2013; Lidiya et al., 2018; Aswathy et al., 2020). Manipulation of the dietary standing of the microalgae is possible by changing media composition and culture methods (Otero et al., 2006; Rivero-Rodriguez et al., 2007; Ilavarasi et al., 2011; Neethu et al., 2016; Prabha et al., 2016).

Changes within the carboxylic acid moiety within a given species in response to nutrient accessibility are also reported (Volkman et al., 1989; Fernandez et al.,1989; Dunstan et al.,1993). Since the composition and availableness of macro and micronutrients within the media directly influence algal cultures, they should be supplied in optimum level (Carvalho et al., 2006; Liu et al., 2008). As macro nutrients such as nitrogen, potassium, magnesium, sulfur, and sodium are harmless to microalgal cells they can be used at larger concentrations. In distinction, essential trace elements such as Fe, Cu, Mn, Zn, Co and Mo are noxious at higher concentrations and growth-retarding at lower amounts (Spotte et al., 1979; Becker et al., 1994). Actually the growth kinetics of microalgal cultures is affected by the

availability of these trace elements as they play a major role in many metabolic channels (Sunda et al., 2005).

The *Pavlova lutheri*, a marine phytoflagellate is being used in marine aquaculture industry especially in bivalve hatcheries to nurture bivalves at all stages of growth in the same way; as zooplankton (rotifers, copepods and brine shrimps), which are used to nourish larvae of crustaceans and fishes (Walne, 1974; Volkman et al., 1989; Coutteau et al., 1992; Rico-Villa et al., 2006). The genus Pavlova is distinguished by its high nutritional value, particularly in terms of its content of polyunsaturated fatty acids (PUFA) of the omega-3 series, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) which are essential for seawater fauna (Kanazawa et al., 1979; Tatsuzawa & Takizawa,1995; Brown et al., 1997; Carvalho et al., 2006; Ponis et al., 2008).

It ought to be noted that the optimum concentration of nutrients in the culture media varies depending on the microalgal species and need to be optimized for every microalgal strain prior to use for mass cultivation. A detailed understanding of biochemical parameters and fatty acid expression patterns in various microalgal species can help to estimate their dietary potential and provide scope for the manipulation of culture conditions to improve their nutrition. The present investigation is aimed to profile the total fatty acid in *P. lutheri*, when grown in 3 different standard culture media for improving the nutritional status of microalga.

Materials and Methods

Pure culture of microalgae was obtained from ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi and was maintained in standard F/2 medium (Table1) so that maximum production of biomass may well be ensured. Since no single medium is suitable for higher growth and biochemical production in microalgal species, the alga was cultured in F/2 medium, Miquel's medium and Chu#10 medium, prepared as per standard published methodologies. The chemical composition of the 3 different media used are given in Tables 1-3.

1. F/2 medium (Guillard, 1975) (Table 1)

2. Miquel's medium (Miquel, 1892) (Table 2)

3. Chu # 10 medium (Chu et al., 1942) (Table 3).

Table 1. F/2 medium (Guillard, 1975)

A	Sodium nitrate	7.5 g 100 ml^{-1}
	Sodium orthophosphate	500 mg 100 ml^{-1}
	Sodium silicate	3 g 100 ml^{-1}
	Ferric chloride	0.3 gm 9.5 ml^{-1}
	Sodium EDTA	0.44 gm 9.5 ml^{-1}
B	Manganese chloride	18 gm 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}
C	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 to 1 L of filtered and boiled sea water.

Table 2. Miquel's Media (Miquel, 1892)

A.	Pottassium nitrate	20.2 gm
B.	Sodium orthophosphate	4 gm
C	Calcium chloride	2 gm
	Ferric chloride	2 gm
	Hydrochloric acid	2 ml
	Distilled water	100 ml

0.55 ml of A and 0.50 ml of B were added to 1 L of filtered and boiled sea water.

Table 3. Chu # 10 medium (Chu et al.,1942)

A	Calcium nitrate	5.76 gm
B	Potassium orthophosphate	0.5 gm
C	Magnesium sulphate	2.5 gm
D	Sodium carbonate	2 gm
E	Sodium silicate	2.5 gm
F	Ferric chloride	0.08 gm

Add 1 ml of A,B,C,D,E,F to 1 L filtered and boiled sea water

The experiment was conducted using Completely Randomised Design (CRD) and each measurement was done in three replications. About 600 ml (20% of the culture medium) of microalgal isolate at an

initial inoculum density of 20-30x10^4 exponentially growing cells ml^{-1} (Pavlo et al., 2016) was transferred into previously autoclaved, properly capped and aerated borosilicate 4 haffkine flasks (a set of 3 for each treatment) under aseptic conditions. For illumination of the environment, fluorescent tubes having intensity 1500 lux were used (Hoff et al., 1987). A light/dark (L/D) cycle of 12 h of lightness and 12 h of darkness was used for maintaining the stock as well as major cultures controlled by an auto timer (E-Tech Digital Timer) (Barsanti et al., 2006) and maintained at 25°C. For cultures, seawater of salinity 33-34 ppt was used (Barsanti et al., 2006).

The duration of lag phase, log phase and stationary phases of the microalga were monitored based on cell count, following the method of Andersen et al. (2005). After determining cell concentration (Hoff et al., 1987), the fully grown culture was harvested during the late exponential phase using High Speed Refrigerated Centrifuge (Himac CR22G Country) at 10000 rpm for one minute. The pellets were collected after multiple washing with seawater.

Total lipids were extracted as per the method of Bligh & Dyer (1959). About 1 to 2 gm of wet microalgal sample and a pinch of Butylated Hydroxy Toluene (to prevent oxidation) was homogenized well in 5-10 ml distilled water using mortar and pestle. The pulp was transferred to a 250 ml conical flask and mixed with 20-30 ml chloroform methanol (2:1 v/v) mixture and shaken well. The mixture was kept overnight at 4°C in the dark for complete extraction. A further addition of 20 ml chloroform and 20 ml distilled water was made and the resulting solution was subjected to centrifugation (1000 rpm for 5 min at room temperature) to obtain three layers. Then the mixture was transferred to a separating funnel and the lower chloroform layer was carefully collected free of interface by filtering through sodium sulphate using a filter paper. It was concentrated in a pre-weighed round bottom flask at 40-45°C using a rotary vacuum evaporator (Heidolph Hei-Vap Silver 6), allowed to cool and the weight (w_1) was noted.

Total lipid was calculated using the formula,
Lipid = $(w_1\text{-}w_2/w_3) \times 100$

(wherein w_1=weight of flask +lipid, w_2= weight of flask, w_3= weight of sample taken).

Fatty acids were analyzed as fatty acid methyl esters (FAMEs). For this 5 ml of 0.5 N methanolic alkali was added to the lipid (150 to 250 mg) and reflexed for five minutes in a boiling water bath under a nitrogen atmosphere to break and saponify the content. After cooling, 5ml BF_3 Methanol solution was slowly added into it, refluxed for another five minutes in boiling water bath under nitrogen atmosphere and the mixture was kept for cooling to get Fatty Acid Methyl Esters (FAMEs) (Metcalf et al., 1966). About 5-6 ml saturated NaCl was added to it and mixed well to separate the fame and the separated fame was extracted thrice with petroleum ether. Finally the fame was filtered through anhydrous Na_2SO_4, evaporated and concentrated which was used for injecting in GC. For injection, 1 µl of sample was used. FAMEs were identified by comparison of retention times with the authentic commercially available FAME standards (Supelco TM 37 Component FAME Mix, Catalog No. 47885-U), and the results were expressed as % Total Fatty Acid. The area of each component was obtained from computer generated data.

The experiment was conducted using Completely Randomised Design (CRD). Each measurement was done with 3 replications. Statistical evaluation (to compare means) was carried out after applying Arcsin transformation with SPSS ver. 22.0. The differences between transformed mean values were compared according to Tukey Post- Hoc test taking at $p \leq 0.05$ as significant.

Results and Discussion

The amount of lipid produced in percentage from the harvested algal biomass of all experimental treatments is shown in Fig. 1. The highest lipid content (27.06%) was obtained from *P. lutheri* cultures in Miquel's medium followed by Chu #10 medium (17.67%) and the lowest was therein of F/2 medium (14.3%). Analysis of data revealed that

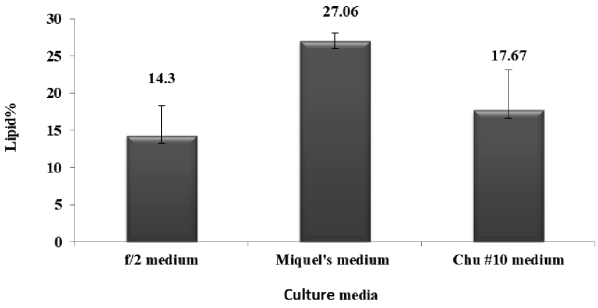

Fig. 1. Lipid content obtained in percentage from *Pavlova lutheri* in 3 different culture media

there is notable variance ($p \leq 0.05$) in the total lipid content of *P. lutheri* cultures in the three different media.

A close scrutiny of Table 1 indicates F/2 medium features fine balance of trace elements relating to each their quantity and proximity. Several of those trace elements are absent in other two media (Tables 2 and 3). Since F/2 medium contains optimum level of trace elements, growth and lipid production balance each other for the investigated algal species.

Anantharaj et al. (2011) studied the impact of F/2 mediums having different concentrations of copper (Cu) on the production of lipid in *Amphora coffeaeformis*. They reported a disproportionate relationship between the amount of Cu in the medium and lipid production. Another observation is that Manganese (Mn) scarcity hinders photosynthetic activities in algae and thereby enhances lipid synthetic activity (Constantopoulos, 1970). There are reports about high concentrations of zinc (Zn), negatively affecting cellular division, total chlorophyll content, ATPase activity etc. in microalgae (Omar et al., 2002). Besides, high concentration of Zn found to increase lipid peroxides in some species (Li et al., 2006), resulting in lipid degradation. Omar et al. (2002) noted that *Botryococcus sudeticus* are found to produce more lipids (30%) at a low concentration of Zn. Our result is in alignment with the above findings as these trace elements are absent

Table 4. Result of Percentage of prominent Fatty Acids in *P. lutheri* cultures grown in 3 different culture media

Sl. No.	Fatty acids		F/2 medium	Miquel's medium	Chu#10 medium
1	Lauric acid	C12	52.15±0.001	31.17±0.002	53.16±0.001
2	Tridecanoic acid	C13	2.38±0.004	nd	2.93±0.003
3	Myristic acid	C14	6.76±0.001	10.79±0.080	5.91±0.070
4	Pentadecanoic acid	C15	14.13±0.01	2.58±0.010	5.95±0.020
5	Palmitic acid	C16	0.38±0.220	9.98±0.001	8.64±0.001
6	Stearic acid	C18	nd	0.35±0.106	nd
7	Oleomyristic acid	C14:1 n-5	1.46±0.031	1.1±0.020	nd
8	Penta decenoic acid	C15:1 n-5	15.64±0.001	0.46±0.010	2.21±0.022
9	Palmitoleic acid	C16:1 n-7	nd	nd	1.27±0.001
10	Vaccenic acid	C18:1 n-7 *cis*	nd	1.35±0.000	nd
11	Oleic acid	C18:1 n-9 *cis*	1.21±0.001	11.13±0.001	13.02±0.002
12	Paullinic acid	C20:1 n-7	0.48±0.000	nd	nd
13	Eicosaenoic acid	C20:1 n-9	0.23±0.020	nd	nd
14	Brassidic acid	C22:1 n-9 *cis*	nd	0.83±0.010	nd
15	Nervonic acid	C24:1 n-9	0.98±0.100	nd	0.034±0.002
16	Linoleic acid	C18:2 n-6 *cis*	nd	4.87±0.001	1.36±0.002
17	Linoleic acid	C18:2 n-6 *trans*	nd	1.51±0.001	nd
18	α linolenic acid(ALA)	C18:3 n-3	0.48±0.020	9.35±0.015	2.69±0.013
19	Gamma linolenic acid (GLA)	C18:3 n-6	0.98±0.010	4.78±0.020	1.28±0.021
20	Stearidonic acid	C18:4 n-3	0.44±0.001	nd	nd
21	Eicosapentaenoic acid (EPA)	C20:5 n-3	2.23±0.200	1.06±0.170	0.33±0.110
22	Docosapentaenoic acid (DPA)	C22:5 n-3	nd	0.96±0.001	nd
23	Docosahexaenoic acid (DHA)	C22:6 n-3	0.07±0.320	4.17±0.120	1.21±0.230
Total SFA			75.80±3.05	54.88±6.90	76.54±3.05
Total MUFA			19.99±2.00	18.42±1.05	16.54±2.98
Total PUFA			4.21±1.19	26.70±7.08	6.87±0.99

Data represent Mean values ± STDnd = not detected

in Chu #10 and Miquel's media in which *P. lutheri* produced maximum lipid content.

Iron (Fe) is taken into account as one among the foremost vital trace elements in microalgal growth media. Actually the highest amount of Fe source (2 g 100 ml^{-1} stock solution) can account for the high lipid content of pavlova cultures in miquel's medium among others. This finding is supported by Liu et al. (2008), where lipid contents in *Botryococcus sudeticus*, *Chlorella sorokiniana*, *Chlorella vulgaris*, and *Ettlia oleoabundans* showed 10, 60, 18 and 36% increase at high concentrations of Fe, respectively. It is expected that Fe will increase overall production of lipid owing to down-regulation of iron utilizing fatty acid desaturase enzymes. *Chlorella vulgaris* under laboratory conditions showed a considerable elevation in lipid radicals of the membranes when Fe was added, up to 500 μM (Estevez et al., 2001).

Being a constituent of cytochromes, Fe has a key role in nitrogen assimilation as a functional part of ferredoxin and controls the synthesis of pigments like phycocyanin, chlorophyll etc. The use of Fe in the form of chelated complex (Fe EDTA) is more useful (Becker, 1994). As the F/2 medium contains Fe source in the form of chelated complex, proper uptake of macronutrients especially nitrogen (N) happens leading to high performance of photosynthetic pathway instead of lipid synthetic pathway. Addition of Fe in the form of inorganic salts will tend to precipitate and become unavailable to algae. Since Miquel' s medium furthermore as Chu #10 mediums contain Fe in the form of ferric chloride, it may shorten N assimilation thereby leading to lipid induction.

Table 4 shows mean values of different saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid components in all experimental cultures and are conferred as percentage of total fatty acids (%TFA). Upshots of post-hoc test (Tukey) showing transformed mean values of total lipid, SFAs, MUFAs and PUFAs are shown in Table 5 and that for lipid profile in Table 6. Saturated Fatty acids comprised more than half of the total fatty acids in all treatments (54.88% - Miquel's medium to 76.54% - Chu #10 medium). The findings of the current investigation showed that among saturated fatty acids, lauric acid (C12) is the major SFA in cultures of almost all media which ranged from 31.17% (Miquel's), 52.15% (F/2), to 53.16% (Chu#10). Data analysis showed that there is remarkable variations regarding SFA content among the cultures.

Close examination of Table 4 shows that the value of saturated fatty acids (SFA) in F/2 (75.8%) and in Chu #10 (76.59%) media for *P. lutheri* cultures are almost the same as that found for a similar strain analysed by Fernandez et al. (1989). In their study, they observed that 70-100% of the total lipid of *P. lutheri* was SFA. The quantity of lauric acid (C12) obtained was maximal for cultures in Chu#10 (53.16%) and minimum in Miquel's medium (31.17%). For myristic acid (C14), palmitic acid (C16) and for stearic acid (C18) the values obtained were highest for those in Miquel's medium (10.79%, 9.98% and 0.035% respectively). The value of C14 obtained during this experiment is in tune with studies of Langdon et al. (1981) who obtained a value of 11.2% for *P. lutheri*.

In the current evaluation the proportion of monounsaturated fatty acids (MUFA) varied from 16.54% (Chu #10) to 19.99% (F/2). Volkman et al. (1991) conducted many experiments in microalgal lipids especially in *P. lutheri strains* and reported the presence of MUFAs with similar patterns of abundance (17.4%, 20.8% etc.) as to those ascertained here. Among MUFAs, the amount of oleic acid (C18:1 n-9 cis) obtained is almost equal in Chu#10 (13.02% and in Miquel's (11.13%) media.

Table 5. Comparison of transformed mean values of percentage of total lipid and fatty acid classes of *P. lutheri* in three different culture media based on Post-Hoc Test (Tukey) and their significance in Analysis of Variance.

Culture media	Total Lipid%	Total SFA%	Total MUFA%	Total PUFA%
F/2	0.3877^{a}	1.0565^{b}	0.4634^{b}	0.2057^{a}
Miquel's	0.5469^{b}	0.8343^{a}	0.4435^{ab}	0.5430^{c}
Chu#10	0.4330^{a}	1.0652^{b}	0.4187^{a}	0.2648^{b}

Transformed Means values having the same superscripts in the same column belong to the same homogeneous subsets.

Several previous studies have clearly demonstrated oleic acid as the major MUFA present in marine organisms (Walkowiak, 1979, Chen et al., 2007, Barrento et al., 2010). Ackman et al. (1968) reported the abundance of C18:1n-9 over C18:1n-7 in some prymnesiophytes. Cultures in the F/2 medium possess a minimum amount of oleic acid (1.21%) which is similar to findings of Volkman et al. (1991) where only 1.6 and 1.8% for different strains of *P. lutheri* was reported. Univariate analysis showed that there is notable deviance ($p \leq 0.05$) in the amount of MUFA among the three different treatments.

The current work observed nine categories of polyunsaturated fatty acids (PUFA) which is presented in Table 4. It is seen that the highest value of PUFA obtained when cultures were grown in Miquel's medium (26.70%) and the lowest in F/2 medium (only 4.21%). Miquel's medium scored for almost all PUFAs which includes linoleic acid (C18:2 n-6) (4.87%), gamma linolenic acid (C18:3 n-6) (4.78%), alpha linolenic acid (C18:n-3) (9.35%) docosapentaenoic acid (C22:5 n-6) (0.96%) and also for docosahexaenoic acid (C22:6 n-3) (4.17%). A marked variance ($p \leq 0.05$) is evident in the polyunsaturated fatty acid radicals among cultures in different media. Even though the level of SFA (54.88%) and MUFA (18.42%) were minimal for *P.lutheri* cultures in Miquel's medium, it produced the highest level of polyunsaturated fatty acids (26.7%). Table 2 shows that it has sufficient quantities of macronutrients like N, P, K, Ca etc. When N is adequate in the medium, microalgae synthesise membrane glycerolipids which occupy in the cellular membrane systems (Piorreck & Pohl, 1984). These glycerol based membrane lipids are constituted particularly of long-chain unsaturated

Table 6. Comparison of transformed mean values of fatty acid components of *P. lutheri* in three different culture media based on Post-Hoc Test (Tukey) and their significance in Analysis of Variance.

Fatty acids		F/2 medium	Miquel's medium	Chu#10 medium	Sig.
Lauric acid	C12	0.8069^{b}	0.5923^{a}	0.8170^{b}	0.000
Tridecanoic acid	C13	0.1549^{b}	0.0000^{a}	0.1642^{b}	0.003
Myristic acid	C14	0.2626^{a}	0.3338^{b}	0.2456^{a}	0.006
Pentadecanoic acid	C15	0.3848^{b}	0.1511^{a}	0.2464^{a}	0.002
Palmitic acid	C16	0.0613^{a}	0.3204^{b}	0.2980^{b}	0.000
Stearic acid	C18	0.0000^{a}	0.0588^{b}	0.0000^{a}	0.000
Oleomyristic acid	C14:1 n-5	0.1155^{b}	0.1050^{b}	0.0000^{a}	0.003
Penta decenoic acid	C15:1 n-5	0.4065^{c}	0.0679^{a}	0.1465^{b}	0.000
Palmitoleic acid	C16:1 n-7	0.0000^{a}	0.0000^{a}	0.1129^{b}	0.000
Vaccenic acid	C18:1 n-7 cis	0.0000^{a}	0.1164^{b}	0.0000^{a}	0.000
Oleic acid	C18:1 n-9 cis	0.1102^{a}	0.3399^{b}	0.3685^{b}	0.000
Paullinic acid	C20:1 n-7	0.0693^{b}	0.0000^{a}	0.0000^{a}	0.000
Eicosaenoic acid	C20:1 n-9	0.0479^{b}	0.0000^{a}	0.0000^{a}	0.000
Brassidic acid	C22:1 n-9 cis	0.0000^{a}	0.0912^{b}	0.0000^{a}	0.000
Nervonic acid	C24:1 n-9	0.0978^{c}	0.0000^{a}	0.0184^{b}	0.000
Linoleic acid	C18:2 n-6 cis	0.0000^{a}	0.2193^{c}	0.1169^{b}	0.000
Linoleic acid	C18:2 n-6 trans	0.0000^{a}	0.1232^{b}	0.0000^{a}	0.000
α linolenic acid (ALA)	C18:3 n-3	0.0688^{a}	0.3105^{c}	0.1628^{b}	0.000
Gamma linolenic acid (GLA)	C18:3 n-6	0.0992^{a}	0.2196^{b}	0.1134^{a}	0.000
Stearidonic acid	C18:4 n-3	0.0664^{b}	0.0000^{a}	0.0000^{a}	0.000
Eicosapentaenoic acid (EPA)	C20:5 n-3	0.1472^{a}	0.0906^{a}	0.0575^{a}	0.087
Docosapentaenoic acid (DPA)	C22:5 n-3	0.0000^{a}	0.0981^{b}	0.0000^{a}	0.000
Docosahexaenoic acid (DHA)	C22:6 n-3	0.0263^{a}	0.2015^{c}	0.1102^{b}	0.001

Transformed mean values having the same superscript in the same row belong to the same homogeneous subset.

fatty acids containing various kinds of PUFAs and perform a structural role in the cell (Hu et al., 2008). Thus, accumulation of PUFAs occurs in the exponential growth phase (Hu et al., 2008). Presence of a large amount of PUFA in Miquel's medium similarly testifies the above mentioned findings. Fernandez et al. (1989) found abnormally low levels of PUFA in *P. lutheri* and other microalgae known to be good sources of PUFA. Our observations are similar results as by Fernandez et al. (1989) in *P. lutheri*. However, the present values contradict with those pronounced by Volkman et al. (1991) and Langdon et al. (1981) wherein they detected 50.4% and 30.5%. of PUFAs in *P. lutheri* respectively.

Eicosapentaenoic acid (C20:5 n-3) was maximum in F/2 medium (2.23%). Camacho-Rodriguez et al. (2014) noticed that growth medium with low amount of Zn would be having lesser value of EPA content in *Nannochloropsis gaditana*. They also stated that biotin (Vitamin B7) was important for EPA production whereas thiamine (Vitamin B1) and cyanocobalamin (Vitamin B12) were not. Another reason for the production of EPA in F/2 medium cultures is the presence of biotin in it. Since Miquel's and Chu #10 media are lacking certain micro elements, the current investigation is in harmony with that of Richmond (1986) who unveiled that the absence of some trace elements such as Zn, B, Mo and Cu in the medium promoted increased production of some PUFA.

The causes for the exceptional results are not known. But the findings ought not be taken as typical of this species. Alterations in all probability arise from the culture conditions, analytical strategies or in the developmental phase sampled, make it difficult to compare the results presented by various authors and also to compare our upshots with those in the literature.

The main purpose of the present investigation was to analyse variations in fatty acid configuration of *P. lutheri* when grown in different standard microalgal culture media and thereby selecting the medium in which the unsaturation level of microalgae is maximum. Since Miquel's medium enriches *P. lutheri* with more PUFA content, it is more suitable for optimised larval rearing in the aquaculture Industry and thereby provides job opportunities for rural area people. As *P. lutheri* is rich in neutral lipids (TAGs) when grown in F/2 medium, it can be used as a potential source of biofuel which is a promising industry in future. The chosen microalga is a promising candidate for biofuel production likewise in aquaculture practices.

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