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Full Length Research Paper

Production of polyunsaturated fatty acid (DHA) by mangrove-derived *Aplanochytrium* sp.

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This study optimized the production of docosahexaenoic acid (DHA) by *Aplanochytrium* sp. The microbe was cultured under different conditions, which were optimized by using the central composite design model of the response surface methodology (RSM). The interaction and individual effects of important factors for DHA production by *Aplanochytrium* sp., was analysed. DHA production was attained at the maximum of 29.42% of total fatty acids under culture medium consisting of maida powder (6.32 mg.l^{-1}), yeast powder (8.35 mg.l^{-1}), vitamin B (12.08 mg.l^{-1}) and vitamin C (10.08 mg.l^{-1}) and at optimal culture conditions of pH 7.04, salinity (34.05 ppt) and temperature (30°C). The present study proved that *Aplanochytrium* sp. is promising to be a source of DHA for large scale production.

Key words: Mangroves, *Aplanochytrium* sp., docosahexaenoic acid, process optimization, response surface methodology.

INTRODUCTION

Thraustochytrids are unique to produce, have high biomass in culture, a high proportion of lipid as part of its biomass, and a high proportion of polyunsaturated fatty acids (PUFAs) particularly omega-3 series for example docosahexanoic acid (DHA 22:6N-3) (Jasuja et al., 2010). The importance of DHA in human and animal nutrition has received a great deal of research attention (Simopoulos, 1989; Takahata et al., 1998). DHA is absolutely essential to humans owing to its functions in the brain and retina. The current commercial source of DHA is fish and fish oils. However, the DHA in fish oil and the problems encountered in extraction, purification and large scale production of DHA is difficult. Therefore, efforts have been made to find alternative sources such as thraustochytrids and marine diatoms for omega-3 fatty acid production (Yokochi et al., 1998).

In this regard, thraustochytrids may be promising microbial source for commercial production of DHA that is important in human health, aquaculture and other emerging technologies (Adams et al., 2006; Burja and Radianingtyas, 2005; Raghukumar, 2008). Most reports concerning the production of PUFA by thraustochytrids have dealt almost exclusively with DHA production, as this fatty acid is often reported to be the most abundant PUFA produced by thraustochytrids (Fan et al., 2001). A thraustochytrid strain (*Thraustochytrium roseum* ATCC 28210) cultured under different conditions is reported to show marked difference in DHA production A fed-batch flask culture yields DHA at 2100 mg.l^{-1} (Singh and Ward, 1996), Thus, the culture conditions and media composition may have a decisive role in DHA production by thraustochytrids. However, only a few studies are available on the factors that influence PUFA production. These works did not optimize the conditions for the maximum DHA production. Optimization and manipulation of culture conditions to produce the amounts and types of PUFA required for specific applications are definitely areas that will require extensive research for each strain taken toward commercial production (Adams et al., 2006). Therefore the present work was undertaken to study the production of DHA by *Aplanochytrium* sp., and to find the optimal conditions for its maximum production.

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MATERIALS AND METHODS

Microorganism

Aplanochytrium spp., was isolated from mangroves soil by pour plate method on glucose peptone agar medium prepared in 50% seawater with the addition of antibacterial agent (Nalidixic acid 1µg.l^{-1}) to prevent bacterial contamination. The pure cultures were obtained after 5 times of sub-culturing once in 7 days. The pure culture stocks were maintained on agar slants. Further, the rDNA region *Aplanochytrium* sp*.,* was extracted (Pennanen et al., 2001; Anderson et al., 2003) and successfully sequenced (Pennanen et al., 2001), finally this sequence was submitted to NCBI; accession number JQ284385.

Docosahexanoic acid (DHA) production

DHA production was tested in the biomass of *Aplanochytrium* sp., at culturing conditions of pH 7.04, salinity of 34.05 ppt and temperature of 30°C for 7days. After incubation, the biomass was harvested to evaluate DHA produced in biomass by using a gas chromatography (Byung-Ki Hur et al., 2001; Jasuja et al., 2010).

Fatty acid analysis by using gas chromatography

Aplanochytrium sp. was cultured under laboratory conditions in different media compositions and its biomass was harvested after specific incubation period. The biomass was prepared for fatty acid methyl ester (FAME) analyses by using Gas Chromatography as described by the method of Sasser (1990).

Augmentation of the DHA production in *Aplanochytrium* sp. by response surface methodology (RSM)

The optimization study for DHA production was carried out with a statistical tool [the central composite design model of the response surface methodology (RSM)]. The interaction and individual effects of important factors for DHA production by *Aplanochytrium* sp. was analysed. The present optimization experiment considered the constituents of culture medium such as maida powder, yeasts powder and vitamin C and vitamin B (1:1). In this model, single response that was DHA production was studied. The important factors along with the code values are presented in Table 1. These experiments were conducted in the batch culture technique. The coded values and actual factor values were calculated by using the following equation.

$$\text{DHA production } Y1 = \beta_0 + \Sigma_i\ \beta_i X_i + \Sigma_i\ \beta_{ii} X_i^2 + \Sigma_{ij}\ \beta_{ij} X_i X_j \quad 1$$

Where Yi is the predicted response, X_iX_j are independent variables, β_0 is the offset term, β_i is the i^{th} linear coefficient, βii is the ii^{th} quadratic coefficient, and β_{ij} is the ij^{th} interaction coefficient.

The experimental design is presented in Table 2 along with experimental and predicted responses. In this study, the independent variables were coded as X_1, X_2, and X_3. Thus, the second order polynomial equation can be presented as follows in Equation 2:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1X_2 + \beta_{13}X_1X_3 + \beta_{14} X_1X_4 + \beta_{23} X_2X_3 + \beta_{24} X_2X_4 + \beta_{34} X_3X_4 \quad 2$$

Statistical model fitness was analyzed for their variables of individuals and their interactions. It was significant to analyze the interaction and individual effects on the DHA production by drawing the surface plot and perturbation plot.

RESULTS

Optimization of factors for DHA production

The important constituents of culture media on DHA production under the culture conditions of pH 7.04, salinity of 34.05 ppt and temperature of 30°C were optimized by using the RSM. It was made by using the 30 runs using central composite design (Table 1) and tested by a quadratic model (Figure 1a to d). Analysis of variance (ANOVA) of the regression model, interaction and combined effect of factors on the DHA production was tested. The model was found significant for the DHA production (Table 2) as evident by Fisher's F-test with a very low probability value ($P < 0.0001$). The quality of the model was tested for DHA production by the determination of coefficient R^2 and multiple correlation coefficients R^2. The value of adjusted R^2 (0.68) suggested that 68% of the total variations could be expected by the model. The value of obtained R^2 (0.80) and R^2 (0.88) in the present experiment revealed the goodness of correlations between the experimental and predicted values of DHA production.

A plot of the standard errors in DHA production of responsible factors (maida powder and yeast powder) is shown in Figure 1a. The shape of the low level of standard error plot exhibited circular contours and symmetrical shape around the centre and this was used to generate the standard error plot for design evaluation. A similar standard error value was fit with the model with the standard error value of 18.61. It was the best value for the acceptable RSM statistical model. Further confirmation of the model fitness was plotted with experimental values and predicted values of model. This proved that model was fit with the experimental values for DHA production (Figure 1b, c). The final detection of the optimized value for the maximum DHA production was detected from perturbation plot and is presented in Figure 1d.

Individual and combined effects of important constituents on DHA productions by *Aplanochytrium* sp.

The interaction and combined effects of four important constituents-maida powder, yeasts powder, vitamin B, and vitamin C was tested on DHA production. Whereas yeasts powder and vitamin B and vitamin C were significant on the DHA production, other individual and combined effects were not significant. It confirmed the importance of vitamins and yeasts powder as the suitable substrate for the maximum production of DHA by *Aplanochytrium* sp. (Table 2). The optimal levels of media

Table 1. Centre composite design for the DHA production along with experimental and predicted responses.

Std	A	B	C	D	DHA production (%)	
					Experimental	Predicted
1	1	0	1	1	2.394	7.811
2	10	0	1	1	2.394	4.839
3	1	10	1	1	26.634	19.998
4	10	10	1	1	23.694	22.1782
5	1	0	20	1	23.634	22.056
6	10	0	20	1	23.724	18.130
7	1	10	20	1	26.127	25.521
8	10	10	20	1	25.827	26.749
9	1	0	1	20	5.694	3.514
10	10	0	1	20	5.694	8.993
11	1	10	1	20	5.094	13.381
12	10	10	1	20	23.694	24.014
13	1	0	20	20	8.634	12.844
14	10	0	20	20	11.994	17.371
15	1	10	20	20	17.694	13.991
16	10	10	20	20	26.394	23.670
17	3.5	5	10.5	10.5	14.061	13.172
18	14.5	5	10.5	10.5	20.427	19.879
19	5.5	5	10.5	10.5	19.467	14.485
20	5.5	15	10.5	10.5	29.427	32.971
21	5.5	5	8.5	10.5	11.034	7.032
22	5.5	5	29.5	10.5	18.369	20.933
23	5.5	5	10.5	8.5	11.427	15.716
24	5.5	5	10.5	29.5	14.067	8.340
25	5.5	5	10.5	10.5	25.734	24.485
26	5.5	5	10.5	10.5	24.327	24.485
27	5.5	5	10.5	10.5	23.694	24.485
28	5.5	5	10.5	10.5	23.694	24.485
29	5.5	5	10.5	10.5	24.594	24.485
30	5.5	5	10.5	10.5	24.867	24.485

A, maida powder ($mg.l^{-1}$); B, yeast powder ($mg.l^{-1}$); C, vitamin B ($mg.l^{-1}$); D, vitamin C ($mg.l^{-1}$).

constituents on maximum DHA production was obtained by using surface and perturbation plots (Figure 2a to f and 1d) as maida powder (6.32 $mg.l^{-1}$), yeasts powder (8.35 $mg.l^{-1}$), vitamin B (12.08 $mg.l^{-1}$) and vitamin C (10.08 $mg.l^{-1}$).

DISCUSSION

The present study proved that *Aplanochytrium* sp. could synthesize DHA which varied with culture conditions. DHA production was the maximum at the culture medium constituents of maida powder (6.32 $mg.l^{-1}$), yeasts powder (8.35 $mg.l^{-1}$), vitamin B (12.08 $mg.l^{-1}$) and vitamin C (10.08 $mg.l^{-1}$) under the culture conditions (pH 7.04, salinity of 34.05 ppt and temperature of 30°C). The maximum DHA production was 29.42% of total fatty acids (Figure 1d). In the scientific literature, the DHA production by thraustochytrids has been reported in a range of 1.7 to 51% under the temperature range of 25 to 28°C and incubation time of 4 to 12 days (Bajpai et al., 1991a, b; Li and Ward, 1994; Nakahara et al., 1996; Iida et al., 1996; Singh and Ward, 1996; Singh et al., 1996; Weete et al., 1997; Yaguchi et al., 1997; Vazhappilly and Chen, 1998). The maximum DHA production is reported in *Thraustochytrium aureum* ATCC34304 and the minimum in *Schizochytrium aggregatum* ATCC 28209 (Bajpai et al., 1991b; Vazhappilly and Chen, 1998). There is large variation in DHA yields of thraustochytrid strains, and the yields depend on the strains used and their culture conditions. However, optimization and manipulation of culture conditions to produce the

Table 2. Analysis of the variance on docosahexaenoic acid (DHA) production by *Aplanochytrium* sp.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1614.111	14	115.293	4.502	0.001**
A-maida powder (mg.l^{-1})	67.47577	1	67.475	2.635	0.223^{NS}
B-Yeast powder (mg.l^{-1})	512.598	1	512.598	20.011	0.0001***
C-Vitamin B (mg.l^{-1})	289.856	1	289.856	11.319	0.004**
D-Vitamin C (mg.l^{-1})	81.608	1	81.608	3.187	0.012*
AB	26.548	1	26.548	1.036	0.324^{NS}
AC	0.907	1	0.907	0.0354	0.768^{NS}
AD	71.444	1	71.444	2.790	0.198^{NS}
BC	76.055	1	76.055	2.970	0.109^{NS}
BD	5.377	1	5.377	0.210	0.897^{NS}
CD	24.147	1	24.147	0.943	0.232^{NS}
A^2	108.599	1	108.599	4.241	0.051*
B^2	0.980	1	0.980	0.038	0.123^{NS}
C^2	189.063	1	189.063	7.383	0.009*
D^2	265.985	1	265.985	10.387	0.005*
Residual	384.092	15	25.606		
Lack of Fit	381.098	10	38.109	63.640	12.98^{NS}
Pure Error	2.994	5	0.598		
Core Total	1998.203	29			

P<0.0001=***; P<0.001 =**; P<0.05 = *significant and NS-Not significant.

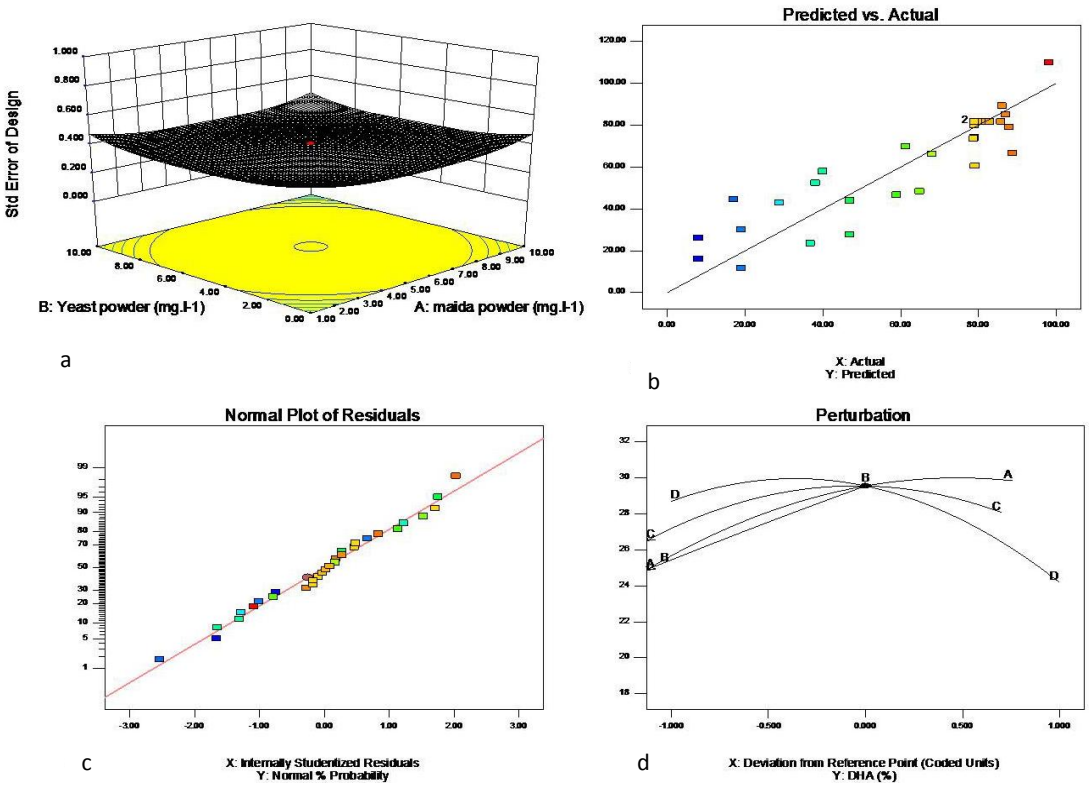

Figure 1. Three-dimensional standard error plot for fatty acid production of *Aplanochytrium* sp. (a) Standard error plot. (b) Normal plot for the residuals and normal percentage of probability for the response of predicted and experimental values. (c) Predicted and actual experimental response for the biomass production. (d) Perturbation plot for *Aplanochytrium* sp. on DHA production.

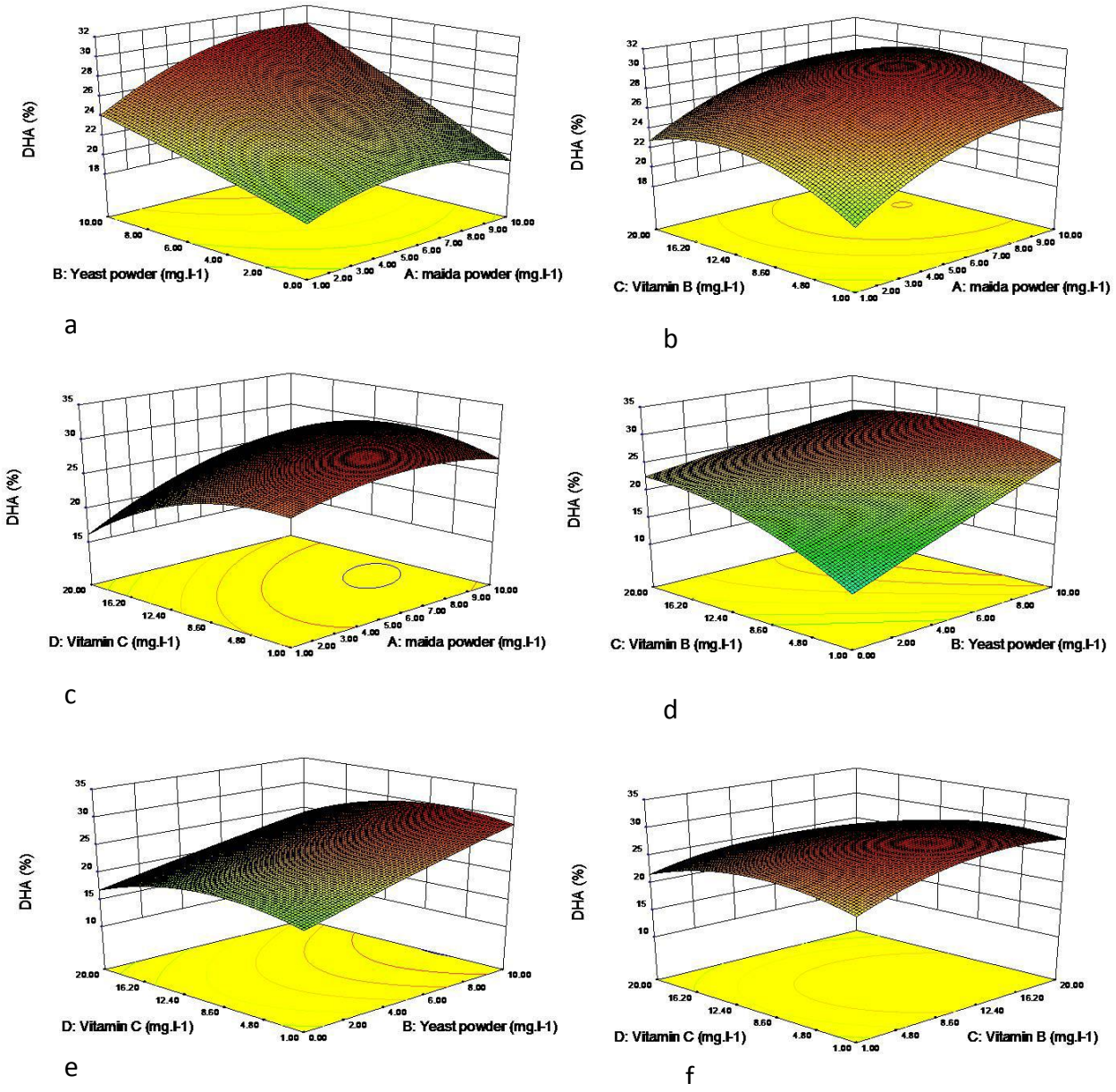

Figure 2. Three-dimensional response surface plot for the (a) effect of maida powder and yeasts powder, (b) effect of maida powder and vitamin B, (c) effect of maida powder and vitamin C (d) effect of yeasts powder and vitamin B, (e) effect of yeast powder and vitamin C and (f) effect of vitamin C and vitamin B on DHA production of *Aplanochytrium* sp.

maximum DHA production by thraustochytrids have not been attempted earlier. This knowledge gap is filled by the present study. Optimization and manipulation of culture conditions to produce the amounts and types of PUFA required for specific applications are definitely areas that will require extensive research for each strain taken toward commercial production. Development of economically viable technologies for the production of microbial PUFA for aquaculture, livestock, and human diets is the subject of intense worldwide research at present. Thraustochytrids are clearly a new and potentially competitive player in the PUFA market. Considerable work is required before the production of oil from these organisms significantly increases its share of the market for PUFA-rich products. Recent research has clearly demonstrated thraustochytrids as potential source of PUFA-containing biomass and oils.

DHA constitutes more than 25% of lipid (Bajpai et al., 1991a; Singh and Ward, 1996; Yaguchi et al., 1997; Yokochi et al., 1998). However, the biological role of DHA to thraustochytrid cells is to be addressed. DHA in the form of membrane phospholipids is generally known to

play an important role in membrane functions. However, only up to 5% of lipids in thraustochytrids occur as phospholipids, 70 to 98% of lipids being generally present as triacylglycerols in storage lipids (Nakahara et al., 1996; Yaguchi et al., 1997). Apparently, accumulation of DHA in the form of triacylglycerols or neutral lipids is important for thraustochytrids. One of the possible roles of DHA in storage lipids is that of antioxidants when a cell is subjected to oxidative stress as during starvation (Mukherjee et al., 2004).

REFERENCES

Adams LK, Lyon DY, Alvarez PJJ (2006). Comparative eco-toxicity of nanoscale TiO_2, SiO_2, and ZnO water suspensions. Water Res. 40:3527-3532.

Anderson IC, Colin D, Campbell, James I (2003). Prosser potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. Environ. Microbiol. 5:36-47.

Bajpai P, Bajpai PK, Ward OP (1991a). Production of docosahexaenoic acid by *Thraustochytrium aureum*. Appl. Microbiol. Biotechnol. 35:706-710.

Bajpai PK, Bajpai P, Ward OP (1991b). Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum*. J. Am. Oil. Chem. Soc. 68:509-514.

Burja AM, Radianingtyas H (2005). Marine microbial-derived nutraceutical biotechnology: an update. Food Sci. Technol. 19:14-16.

Byung-Ki Hur1, Dae-Won Cho1, Ho-Jung Kim, Chun-Ik Park, Hyung-Joon Suh (2002). Effect of Culture Conditions on Growth and Production of Docosahexaenoic Acid (DHA) using *Thraustochytrium aureum* ATCC 34304. Biotechnol. Bioprocess. Eng. 7:10-15.

Fan KW, Chen F, Jones EBG, Vrijmoed LLP (2001). Eicosapentaenoic and docosahexaenoic acids production by and okara-utilizing potential of thraustochytrids. J. Indust. Microbiol. Biotechnol. 227:199-202.

Iida I, Nakahara T, Yokochi T, Kamisaka Y, Yagi H, Yamaoka M, Suzuki O (1996). Improvement of docosahexaenoic acid production in a culture of *Thraustochytrium aureum* by medium optimization. J. Ferment. Bioeng. 81:76-78.

Jasuja N, Djain, Joshi SC (2010). Microbial production of docosahexaenoic acid (ω3 pufa) and their role in human health. Asian J. Pharm. Clin. Res. 3(4).

Li ZY, Ward OP (1994). Production of docosahexaenoic acid by *Thraustochytrium roseum.* J. Ind. Microbiol. 13:238-241.

Mukherjee P, Laura EA, Seyfried TN (2004). Antiangiogenic and proapoptotic effects of dietary restriction on experimental mouse and human brain tumors. Clin. Cancer Res. 10:5622-5629.

Nakahara T, Yokochi T, Higashihara T, Tanaka S, Yaguchi T, Honda D (1996). Production of docosahexaenoic and docosapentaenoic acids by *Schizochytrium* sp. isolated from Yap Islands. J. Am. Oil. Chem. Soc. 73:1421-1426.

Pennanen T, Paavolainen L, Hantula J (2001) Rapid PCR-based method for the direct analysis of fungal communities in complex environmental samples. Soil Biol. Biochem. 33:697-699.

Raghukumar S (2008). Thraustochytrid marine protists: production of PUFAs and other emerging technologies. Mar. Biotechnol. 10:631-640.

Sasser M (1990). Identification of bacteria by gas chromatography of cellular fatty acids. Newark, DE: Microbial ID. Tech. Note. 101.

Singh A, Ward OP (1996). Production of high yields of docosahexaenoic acid by *Thraustochytrium roseum*. J. Ind. Microbiol. 16:370-373.

Singh A, Wilson S, Ward OP (1996). Docosahexaenoic acid (DHA) production by *Thraustochytrium* sp. World. J. Microbiol. Biotechnol. 12:76-81.

Vazhappilly R, Chen F (1998). Heterotrophic production potential of omega) 3 polyunsaturated fatty acids by microalgae and algae-like microorganisms. Bot. Mar. 41:553-558.

Weete JD, Kim H, Gandhi SR, Wang Y, Dute R (1997). Lipids and ultrastructure of *Thraustochytrium* sp. Lipids 32:839-845.

Yaguchi T, Tanaka S, Yokochi T, Nakahara T, Higashihara T (1997). Production of high yields of docosahexaenoic acid by *Schizochytrium limacinum* strain SR21. J. Am. Oil. Chem. Soc. 74:1431-1434.

Yokochi T, Honda D, Higashihara T, Nakahara T (1998). Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. Appl. Microbiol. Biotechnol. 49:72-76.