Int J Pharm Bio Sci 2014 April ; 5 (2) : (B) 27 - 35

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

BIOCONVERSION OF ALPHA-LINOLENIC ACID INTO LONG CHAIN POLYUNSATURATED FATTY ACIDS BY OLEAGINOUS FUNGI

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ABSTRACT

Long-chain polyunsaturated fatty acids (LCPUFA) i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have established role in the prevention of several diseases like atherosclerosis, cardiovascular disorders, and arthritis. However, LCPUFA sources are limited for availability and acceptability problems of fish and flax oil. Microbial sources of n-3 PUFA are of merit being cheaper, contamination free and vegan. We here report fungi that can convert ∝-linolenic acid (ALA**)** from flax seed meal (FSM) into EPA and DHA in dose-dependent manner and therefore serve as valuable LCPUFA sources. *Mucor* sp. and *R. oligosporous* cultivated on FSM supplemented medium accumulated 34 and 36% lipids of dry biomass. Wherein, *Mucor* sp produced 6.01mg DHA per gram of lipid at 10% FSM at 28°C, while *Rhizopus oligosporus* produced 14.32 mg of DHA/gm of lipid at 3% FSM at 20°C. These novel yet exploitable sources of LCPUFA are valuable to ensure EPA/DHA for human supplementation.

KEY WORDS: Oleaginous fungi, LCPUFA, flax, ALA, bioconversion.

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INTRODUCTION

Polyunsaturated fatty acids are of significant commercial interest as important dietary essentials known especially for preventing arteriosclerosis and coronary heart disease. They are also known to alleviate tissue inflammation and retard growth of tumor cells[1]. PUFAs and their derivatives have considerable nutraceutical and pharmaceutical importance[2]. It is important to have a balance of omega-3 and omega-6 fatty acids in the diet. Currently, the commercially available dietary source of omega-3 LCPUFA is fish oil. Consequently, large quantities of fish oil is processed and encapsulated for sale as dietary supplements. However, there are several problems associated with fish oil supplements, including bioaccumulation of fat-soluble contaminants and high levels of saturated and omega-6 fatty acids, both of which have deleterious health effects[3]. However, over-fishing and concerns about pollution of the marine environment indicate a need to develop alternative, sustainable sources of LCPUFA. On the other hand flax seed oil is considered as cheaper agricultural source of PUFA. However it has limited acceptance due to the presence of antinutritional factors and lack of long chain PUFAs like EPA and DHA. Microbial systems reportedly convert unsaturated fatty acids to monohydroxy, dihydroxy and trihydroxy fatty acids. Zygomycetes are able to sequester and accumulate various LCPUFA within the hyphae. Some of the species are able to synthesize molecules with 12 to 24 carbons, such as saturated fatty acids (palmitic acid) and unsaturated fatty acids (oleic and linolenic acids). The fungal biomass contains 5 to 40% lipids, depending on environmental conditions and developmental stage [4]. Several studies on fungi have shown that media variables can modulate growth and lipid accumulation in oleaginous fungi[5-9]. Members of order Mucorales like *Mortierella*, *Mucor* and *Cunninghamella* have demonstrated potential to produce GLA at concentrations of 15 – 25% of total fatty acids[10]. Production of single cell oil (SCO) containing polyunsaturated fatty acids from fungi is the most attractive target for biotechnological production of LCPUFA, and due to the growing awareness of the health benefits of PUFA, SCO are occupying place in market[2]. Microbes such as *Yarrowia lipolytica, Mucor circinelloides, Mortierella alpine, Schizochytrium* and *Crypthecodinium cohnii* are commercially being used for SCO production. These facts justify the search for novel, renewable and low-cost methods for PUFA production[11]. A large range of lipid compounds have been produced using metabolic engineering or biotransformation approaches[12]. It has been shown that, under appropriate conditions, filamentous fungi are able to transform variety of cheaper substrates into high value products containing LCPUFAs[11]. In the present study, we have demonstrated bioconversion of flax ALA into EPA and DHA mediated by commonly occurring fungi. Since oleaginous fungi have been reported to sequester and accumulate various LCPUFA by using oils[10], we have used flax seed meal as a source of ALA, and have assessed its conversion to EPA and DHA. To our knowledge, this is the first report of use of oleaginous filamentous fungi, for bioconversion of flax meal ALA into EPA and DHA.

MATERIALS AND METHODS

Microorganisms

The oleaginous fungal cultures were procured from National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India. The procured cultures included *Beauveria tenella* NCIM 1216, *Cunningamella echinulata* NCIM 691, *Mucor* sp NCIM 881, *Mucor hiemalis* NCIM 873, *Mucor plumbeus* NCIM 984, *Penicillum notatum* NCIM 1206, and *Rhizopus oligosporus* NCIM 1215, *Rhizopus oryzae* NCIM 877. Stock cultures were maintained on potato dextrose agar (PDA) slants at 4°C and sub cultured after every 3 months.

Culture media and conditions for bioconversion

Fungal spores were inoculated at 20°C and 28°C in potato dextrose broth for preparation of mycelial suspension. After 48 h 0.1mL of this suspension was spread on PDA media supplemented with different concentration of flax seed meal (3%, 6% and 10% FSM). After 72 h, profuse cottony hyphal growth was observed. Two hundred mg of the fungal biomass was harvested by careful scraping, washed with distilled water thrice to remove adhering media if any and used further for fatty acid analysis. All experiments were performed in triplicate.

Lipid Extraction

For extraction of lipids from fungi, mycelia were dried for 10 h at 45°C to get dry biomass. Dried mycelial mass was then subjected to solvent extraction in a Soxhlet apparatus with 250 ml chloroform / methanol (2:1, vol/vol) and concentrated by Rotary evaporator at 40°C

Fatty acid analysis

For analysis of fatty acids, the fungal biomass was mixed with 5 mL of 3 N methanolic-HCL for esterification, incubated at 80°C for 2 h and extracted in 3 mL of hexane. The hexane extracts were dried in argon current and reconstituted in 200 µL of chloroform[13.] The fatty acid methyl esters (FAMEs) were analyzed in Auto System XL Gas Chromatograph (Perkin Elmer, USA) equipped with SP-2330, Supelco capillary column 30 meter long and 0.32 mm in diameter. The temperature program was 150°C for 10 min, followed by 10°C rise/min up to 220°C and hold for 10 min. Helium (1mL/min) was used as a carrier gas, injector port was maintained at 240°C and FID detector temperature was maintained at 275°C. Appropriate fatty acid standards were purchased from Sigma (MO, U.S.A.) and the fatty acid peaks were identified by integrating them with the standard profile. The area under the peak was expressed as percent fatty acid content. Estimation of each sample was repeated minimum three times.

Statistical Analysis

All GC analysis was carried out in triplicate and experimental data was subjected to analysis of variance and Tukey – Kramer multiple comparison test (at P = 0.05 or P = 0.001).

RESULTS AND DISCUSSIONS

Micro-organisms that are able to accumulate over 25% lipid on a dry cell biomass basis are referred as oleaginous organisms[14]. In the present study, the oleaginous fungal cultures were selected based on their lipid production from NCIM, NCL catalogue (2010). It is evident from the literature that filamentous fungi are instrumental in transformation of numerous cheaper materials into bio-products including n-6 and n-3 PUFAs[11,15,16]. Oil added in the medium is utilized by microorganisms primarily as source of energy as well as, the fatty acid skeletons are utilized to build various different compounds, for example, oils (sunflower oil) rich in linoleic acid stimulate generation of n-6 fatty acids in fungi from order Mucorals[17]. *Mucor hiemalis* when grown in medium containing vegetable oil could accumulate unsaturated fatty acids[10]. So plant oil can be used as the precursor of short chain PUFA and can be microbially converted into LCPUFA using a suitable fungal strain[18]. Flax seed contains 30 to 40% oil of which 50 to 60% is ALA, which is a valuable source of ALA for human consumption. However, use of flax oil is limited in sense that higher plants do not produce very long chain fatty acids like, EPA and DHA. In this context, microbes having ability to convert oils into LCPUFA effectively were of great interest[19], viz. flax oil rich in n-3 precursor could be added as a substrate to enhance the microbial LCPUFA production[14]. Only few studies are available reporting fungi like *Pythium acanthium* grown in medium containing flax oil, accumulates increased docosapentaenoic acid content[20]. Our study attempts use of oleaginous filamentous fungi exploiting flax seed meal as source of ALA, to convert it into LCPUFA (EPA and DHA).

Out of eight oleaginous fungi screened for the conversion of flax ALA to LCPUFA at increasing concentration of FSM, (Table 1 and

2) only two species, *Mucor* sp. (NCIM 881) and *Rhizopus oligosporus* (NCIM 1215) could effectively convert flax ALA to EPA and DHA. Primarily, a dose-dependent increase in ALA was observed in many cultures suggesting an efficient uptake of fatty acids from the medium. Correspondingly, (Fig 1) a dose dependent increase in DHA accumulation in *Mucor* sp. at 28°C reiterates ability of these cultures to metabolize the available ALA into DHA. At 20°C most of the fungal cultures exhibited higher ALA accumulation with increasing FSM, however that was not converted to any of the PUFAs (Table 2). A similar trend was observed by Nan *et al*[21], lower LCPUFA production at 20°C in 20% molasses medium as compared to 15% molasses supplemented medium. In *Mucor* sp., (Table 3) grown at 20°C, there was 1.44 fold increase in the production of DHA with 3% FSM medium. This further decreased to 0.8 and 0.9 fold at 6% and 10% FSM, respectively. This may suggest either increased utilization of LCPUFA for growth at lower temperature or a feedback inhibition. Interestingly, as could be seen from Fig. 1, we observed a steady increase in DHA production at 28°C by 1.08 fold at 3% FSM, 1.75 fold at 6% FSM and 2.24 fold at 10% FSM. Similarly, there was an increase in the content of EPA in *Mucor* sp. at 28°C, the highest being observed by 1.99 fold for 10% FSM in the medium. This is in contrast with the earlier reports, where low temperature favors the LCPUFA production[21],[22]. Moreover, the *Mucor* sp presented here may have a technological benefit being able to produce LCPUFAs at room temperature. Unlike *Mucor* sp, and in concurrence with the published literature *R. oligosporous* showed increased DHA content at lower temperature in dose dependent manner (At 1.70 fold increase DHA content with 3% FSM, 1.64 at 6% FSM and 1.47 at 10% FSM). As shown in Table 4, *R. oligosporous* could also effectively accumulate ALA from the medium and could convert it to DHA. The highest DHA production observed in our study was *R. oligosporous* (3.98% at 20°C) while it was followed by *Mucor* sp. (1.77% at 28°C), which were more than *Rhizomucor miehei* (0.82% DHA) as reported by Srianta et al[23].

We could not see appreciable accumulation of EPA in either of the cultures, apparently the levels of EPA are transitory and any EPA produced is probably efficiently converted to other molecules including DHA. Although, in the present investigation we are focusing on conversion of flax ALA into EPA/DHA, it will be interesting to note here that significant amount of GLA was produced by *Mucor heimalis* (42.87%) and *Mucor plumbus* (50.55%). As reported, *T. elegans* produced maximum 13-14% GLA in cereal based medium[15]. Taken together the total omega- 3 LCPUFAs (EPA, DHA) up to 13% increase was noticed in *R. oligosporous,* which is followed by 6% in *Mucor* sp, which is in the range of 15.2% increase reported earlier after soybean oil supplementation in *Labyrinthulids*[18]. The increased DHA accumulation in both cultures was associated with corresponding decrease in levels of GLA and AA. This could be attributed to the increased availability of ALA in the medium, which is preferentially metabolized further, this is expected since both n-6 and n-3 fatty acids are metabolized by the same enzymes. This also reiterates that the fungal cultures are utilizing the flax ALA as the increased availability of ALA suppress production pathway of n-6 fatty acids. These cultures will find application as agents that can add value to flax cake by improving its LCPUFA content.

Lipid production from Mucor sp and R. oligosporous

Nan *et al*[21], optimized fermentation with *Mucor recurvus* that produced 5.74g/lit of total PUFA, which breaks up as, 0.17gm ALA, 1.35gm of GLA, 0.257gm of ARA, 0.46gm of EPA and 0.34 gm of DHA. On the other hand, Jang *et al*[24] reported *Mortierella alpina* to produce 17.4 mg of linoleic acid, 17.0 mg of γ -linolenic acid, 103.0 mg of arachidonic acid and 194.2 mg of total PUFAs at 20°C for each gram of carbon supplied. Although, these oleaginous fungi were promising producer of LCPUFA, the production cost of single cell oil (SCO) is very high. An alternative method for LCPUFA production

could be solid state fermentation[16]. *Mucor* sp. and *R. oligosporous* cultivated in FSM containing medium accumulated 34 and 36% lipid (table 5 and 6) with measurable amount of LCPUFA. *Mucor* sp. produced 18.19 mg of GLA, 17.61 mg of ALA, 1.93 mg of ARA, 19.17 mg of EPA and 6.01 mg of DHA per gm of lipid in 10% FSM containing medium. *R. oligosporous* produced 29.30 mg of GLA, 56.73 mg of ALA, 4.96 mg of ARA, 13.21 mg EPA and 14.32 mg DHA per gm of lipid in 3% FSM containing medium. Optimization of culture conditions may further increase LCPUFA production in these fungal strains.

Table 1
LCPUFA production by eight fungal cultures at temp. 28°C

Fungi Name	% polyunsaturated Fatty Acids				
	GLA	**ALA**	**AA**	**EPA**	**DHA**
Mucor sp Control	10.25±0.73	1.175±0.03	0.25±0.08	2.92±0.37	0.79±0.13
Mucor sp 3 % flax	10.40.955	1.69±0.95	0.215±0.08	3.72±0.69	0.86±0.19
Mucor sp 6 % flax	6.75±0.67	2.65±0.13	0.37±0.29	4.78±0.22	1.39±0.80
Mucor sp 10 % flax	5.35±0.22	5.48±1.08	0.575±0.13	5.82±0.26	1.77±0.09
Mucor heimalis control	0	7.245±.85	0.455±0.16	0.07±0.0	0.605±0.09
Mucor heimalis 3 % flax	8.535±0.07	10.75±0.31	0.8±0.6	0.505±0.43	0.255±0.16
Mucor heimalis 6 % flax	3.915±1.8	13.19±3.9	0.235±0.26	0.49±0.26	0.735±0.27
Mucor heimalis 10 % flax	3.41±0.2	29.01±2.02	0.3±0.46	0.045±0.007	0.19±0.04
Mucor plumbus control	33.72±0.14	0.85±0.01	0.29±0.00	1.25±0.18	0
Mucor plumbus 3 % flax	28.59±0.04	25.34±0.17	0.22±0.02	0.67±0.07	1.15±0.12
Mucor plumbus 6 % flax	24.53±0.49	25.43±1.3	0.20±0.02	0.36±0.04	1.15±0.08
Mucor plumbus 10 % flax	25.32±0.91	32.16±0.70	0.28±0.07	0.07±0.007	1.27±0.02
Rhizopus oryzae control	4.45±0.12	0.61±0.66	0.78±0.007	0.035±0.007	0.075±0.04
Rhizopus oryzae 3 % flax	3.94±0.96	3.05±0.05	0.86±0.06	0.22±0.18	0.06±0.007
Rhizopus oryzae 6 % flax	2.94±0.12	8.92±0.4	0.56±0.01	0.03±0.01	0.12±0.01
Rhizopus oryzae 10 % flax	3.65±0.44	15.92±0.72	0.68±0.01	0.065±0.02	0.16±0.007
R. oligosporus control	6.05±0.32	0.14±0.04	0.2±0.02	0.31±0.10	0
R. oligosporus 3 % flax	5.68±0.05	2.65±0.07	0.25±0.02	0.18±0.04	0.18±0.007
R. oligosporus 6 % flax	6.17±0.28	6.71±0.95	0.27±0.00	0.25±0.21	0.21±0.02
R. oligosporus 10 % flax	4.73±0.33	11.91±0.47	0.26±0.02	0.07±0.007	0.19±0.01
C. echinulata control	9.82±0.72	1.12±0.02	0.42±0.15	0.56±0.13	0
C. echinulata 3 % flax	12.59±0.85	3.79±0.11	0.24±0.04	0.43±0.05	0.08±0.007
C. echinulata 6 % flax	9.54±0.70	3.38±0.17	0.35±0.07	0.21±0.17	0.24±0.09
C. echinulata 10 % flax	11.5±0.32	11.25±0.05	0.28±0.14	0.05±0.01	0.08±0.007
Beauveria tenella control	0	1.56±0.69	0	0	0
Beauveria tenella 3 % flax	0	29.30±0.16	0	0	0
Beauveria tenella 6 % flax	0	35.05±1.8	0	0	0
Beauveria tenella 10 % flax	0	35.27±0.17	0	0	0
Penicillium notatum control	0.98±0.04	0.3±0.01	0.77±0.02	0.21±0.007	0
Penicillium notatum 3 % flax	0	14.49±0.46	0.86±0.14	0.44±0.14	0
Penicillium notatum 6 % flax	0	13.26±0.75	0.83±0.01	0.25±0.07	0
Penicillium notatum 10 % flax	0	32.81±1.01	0.78±0.08	0.17±0.03	0

GLA - γ-linolenic acid; ALA - α-linolenic acid; AA - Arachidonic acid; EPA - Eicosapentanoic acid; DHA - Docosahexanoic acid

Table 2
LCPUFA production by eight fungal cultures at temp. 20°C

Fungi Name	% polyunsaturated Fatty Acids			
	GLA	ALA	AA	DHA
Mucor sp Control	13.91±0.14	2.77±0.29	1.14±0.02	1.3±0.23
Mucor sp 3% flax	4.14±0.12	13.05±0.32	2.49±0.16	1.94±0.04
Mucor sp 6% flax	3.77±0.38	29.79±0.82	1.47±0.10	1.13±0.11
Mucor sp 10% flax	3.14±0.07	35.23±0.67	1.16±0.17	1.26±0.15
Mucor heimalis control	14.745±0.94	1.62±0.33	0.43±0.01	0
Mucor heimalis 3% flax	42.87±1.2	3.01±0.2	0.585±0.02	0
Mucor heimalis 6% flax	39.765±0.48	4.38±0.48	0.585±0.06	0
Mucor heimalis 10% flax	40.44±1.06	5.38±0.21	0.645±0.07	0
Mucor plumbus control	50.55±0.67	15.975±0.28	0.11±0.08	0
Mucor plumbus 3% flax	38.96±1.94	33.35±1.18	0.14±0.12	0
Mucor plumbus 6% flax	34.53±0.92	39.96±0.52	0.21±0.02	0
Mucor plumbus 10% flax	34.84±0.28	39.94±0.89	0.22±0.19	0
Rhizopus oryzae control	6.65±0.02	1.12±0.02	0.76±0.02	0.52±0.73
Rhizopus oryzae 3% flax	4.48±0.26	8.75±0.74	0.88±0.09	1.34±0.11
Rhizopus oryzae 6% flax	3.36±0.20	18.86±0.87	1.09±0.19	1.82±0.19
Rhizopus oryzae 10% flax	3.36±0.34	25.67±0.73	0.41±0.27	1.36±0.09
R. oligosporus control	10.59±0.63	2.06±0.04	1.27±0.02	2.34±0.28
R. oligosporus 3% flax	8.14±0.57	15.76±0.75	1.38±0.07	3.98±0.49
R. oligosporus 6% flax	7.66±0.07	17.67±0.55	1.42±0.02	3.85±0.43
R. oligosporus 10% flax	5.33±0.44	28.15±0.31	0.98±0.04	3.44±0.31
C. echinulata control	35.01±0.70	29.63±0.55	0.43±0.02	0
C. echinulata 3% flax	33.79±0.29	31.79±0.52	0.46±0.007	0
C. echinulata 6% flax	15.17±0.69	37.33±1.78	0.74±0.24	0
C. echinulata 10% flax	9.55±0.75	45.53±0.88	0.63±0.05	0
Beauveria tenella control	0	7.65±0.46	0	0
Beauveria tenella 3% flax	0	14.64±0.02	0	0
Beauveria tenella 6% flax	0	17.63±0.50	0	0
Beauveria tenella 10% flax	0	21.27±1.70	0	0
Penicillium notatum control	0	5.62±0.09	0	0
Penicillium notatum 3% flax	0	28.78±0.19	0.31±0.08	0
Penicillium notatum 6% flax	0	37.48±0.23	0.41±0.15	0
Penicillium notatum 10% flax	0	39.15±0.09	0.4±0.18	0

GLA - γ-linolenic acid; ALA - α-linolenic acid; AA - Arachidonic acid; EPA - Eicosapentanoic acid; DHA - Docosahexanoic acid

Table 3
*LCPUFA production by Mucor sp at different temperatures**

	GLA		ALA		AA		DPA	EPA	DHA	
	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
Control	(13.91±0.064)^{a}	(10.25±0.303)^{a}	(2.77±0.121)^{a}	(1.17±0.014)^{a}	(2.14±0.011)^{a}	(0.25±0.036)^{a}	(3.03±0.171)^{a}	(2.92±0.163)^{a}	(1.35±0.104)^{a}	(0.79±0.058)^{a}
3%	(4.14±0.052)^{b}	(10.46±0.406)^{b}	(13.05±0.210)^{b}	(1.69±0.059)^{b}	(2.49±0.071)^{b}	(0.22±0.010)^{a}	(3.623±0.263)^{b}	(3.73±0.283)^{b}	(1.95±0.020^{a}	(0.86±0.083)^{a}
6%	(3.77±0.171)^{c}	(6.75±0.293)^{c}	(29.79±0.338)^{c}	(2.64±0.056)^{c}	(1.47±0.043)^{c}	(0.37±0.121)^{a}	(2.31±0.083)^{c}	(4.78±0.092)^{c}	(1.13±0.047)^{b}	(1.39±0.331)^{a}
10%	(3.14±0.047)^{d}	(5.35±0.162)^{d}	(35.23±0.287)^{d}	(5.48±0.469)^{d}	(1.16±0.073)^{d}	(0.57±0.059)^{a}	(1.34±0.171)^{d}	(5.82±0.116)^{d}	(1.26±0.065)^{c}	(1.77±0.043)^{b}

PDA - Potato Dextrose Agar; GLA - γ-linolenic acid; ALA - α-linolenic acid; AA - Arachidonic acid; DPA – Docosapentanoic acid; EPA - Eicosapentanoic acid; DHA - Docosahexanoic acid

All readings are a mean of three replicates. Values represent the corresponding percentage of fatty acid ± standard error.

Figures followed by different superscript letters are statistically different at P = 0.05 or P = 0.001 (Tukey – Kramer multiple comparison test).

Table 4
*LCPUFA production by Rhizopus oligosporus at different temperatures**

	GLA		ALA		AA		DPA	EPA	DHA	
	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C	20°C	28°C
Control	(10.59±0.290)^{a}	(6.05±0.310)^{a}	(2.06±0.100)^{a}	(0.14±0.092)^{a}	(1.27±0.071)^{a}	(0.20±0.244)^{a}	(2.62±0.060)^{a}	(0.32±0.050)^{a}	(2.34±0.116)^{a}	0^{a}
3%	(8.14±0.254)^{b}	(5.68±0.055)^{a}	(15.76±0.316)^{b}	(2.63±0.052)^{b}	(1.38±0.030)^{a}	(0.25±0.049)^{a}	(3.67±0.057)^{a}	(0.19±0.046)^{a}	(3.98±0.202)^{b}	(0.19±0.006)^{b}
6%	(7.66^{c}±0.031)^{c}	(6.17±0.148)^{a}	(17.67±0.257)^{c}	(6.71±0.475)^{c}	(1.42±0.015)^{a}	(0.27±0.017)^{a}	(3.95±0.087)^{a}	(0.25±0.093)^{a}	(3.85±0.177)^{c}	(0.22±0.023)^{c}
10%	(5.34±0.276)^{d}	(4.73±0.312)^{b}	(28.15±0.301)^{d}	(11.91±0.844)^{d}	(0.98±0.035)^{b}	(0.27±0.010)^{a}	(3.58^{a}±0.067)^{a}	(0.08±0.042)^{a}	(3.44±0.127)^{d}	(0.19±0.018)^{d}

PDA - Potato Dextrose Agar; GLA - γ-linolenic acid; ALA - α-linolenic acid; AA - Arachidonic acid; DPA – Docosapentanoic acid; EPA - Eicosapentanoic acid; DHA - Docosahexanoic acid

All readings are a mean of three replicates. Values represent the corresponding percentage of fatty acid ± standard error.

Figures followed by different superscript letters are statistically different at P = 0.05 or P = 0.001 (Tukey – Kramer multiple comparison test.

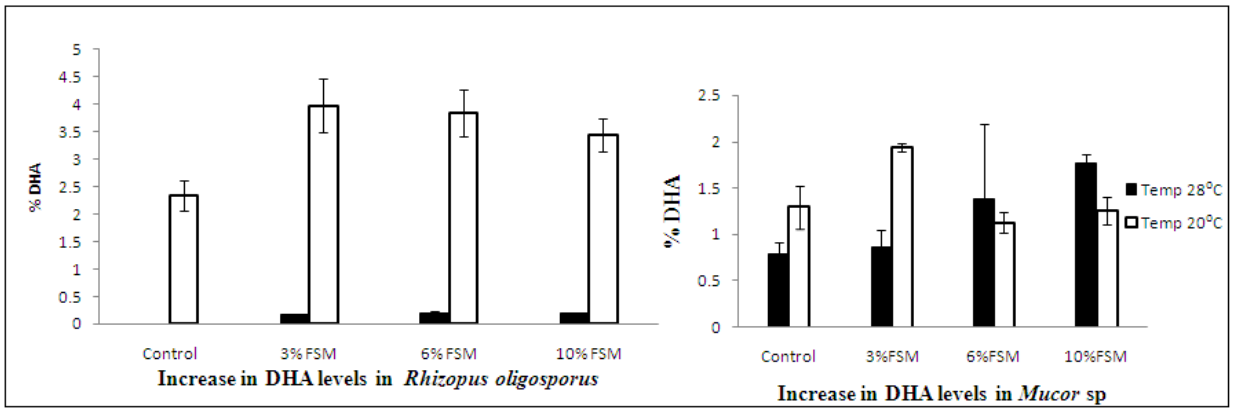
Table 5
Lipid and fatty acid production by Mucor sp at temp. 28°C

Medium used	Lipid (mg/gm of biomass)	Polyunsaturated fatty acid (mg/gm of lipid)				
		GLA	ALA	AA	EPA	DHA
Mucor sp Control	140	14.35	1.63	0.35	4.08	1.1
Mucor sp 3% flax.	150	15.6	2.53	0.33	5.58	1.29
Mucor sp 6% flax	210	14.17	5.56	0.77	10.03	2.91
Mucor sp 10% flax	340	18.19	17.61	1.93	19.78	6.01

Table 6
Lipid and fatty acid production by Rhizopus oligosporus at temp. 20°C

Medium used	Lipid (mg/gm of biomass)	Polyunsaturated fatty acid (mg/gm of lipid)				
		GLA	ALA	AA	EPA	DHA
R. oligosporous control	360	38.12	7.41	4.57	9.43	8.42
R. oligosporous 3% flax	360	29.3	56.73	4.96	13.21	14.32
R. oligosporous 6% flax	270	20.68	47.7	3.83	10.66	10.39
R. oligosporous 10% flax	240	12.81	67.56	2.35	8.59	8.25

Figure 1
Effect of different concentration of flax seed meal (3%, 6% and 10% FSM) on Rhizopus oligosporus and Mucor sp at 28ºC (■) and 20ºC (□).



Control – Potato dextrose agar (PDA) medium
3% FSM- PDA with 3%flax seed meal
6% FSM- PDA with 6%flax seed meal
10% FSM- PDA with 10%flax seed meal

CONCLUSION

We have described a simple and inexpensive process for LCPUFA production using oleaginous fungi. This value-added bio-product may be used as feed supplement in poultry, aqua and dairy and may increase perspectives for nutritional supplementation of PUFA in human food chain.

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