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Production of medium chain fatty acid rich single cell oil using whole de-oiled mustard meal from soil fungus

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Screening of fungal strain was done from soil collected from the rice bran oil industry to search for important fatty acids with health beneficial effects. This study investigates the exceptional production of microbial oil medium-chain fatty acids like caproic acid (6:0), caprylic acid (8:0), capric acid (10:0), by the cultivated fungal culture in a media containing whole mustard meal as a supplement. This paper presents the result on the production of oil with such type of fatty acid composition which makes the oil more stable during cooking and gives fewer calories after consumption which is beneficial for health.

Keywords: Behenic acid, Long chain fatty acids, Low-calorie oil, Meal supplementation, Oleaginous fungi

Fatty acids, the major component of triacylglycerides (TAGs) are the major contributors to dietary fat in human. Fatty acids have a specific metabolic role as a source of energy, functional role as the component of cell membrane phospholipids and signaling role as they constitute many molecules involved in cell signaling processes[1]. Thus fatty acids have a wide range of general and specific biological activities that influence health, well being and disease risk[2]. One of the alternative sources of TAGs is microbial oils or single cell oils (SCOs) because many of which have a similar fatty acid profile as vegetable oil[3]. SCOs are produced by oleaginous microorganisms with the ability to accumulate lipids more than 20% (*w/w*) of their total dry biomass weight[4]. Accumulation of lipids in cells of oleaginous microorganisms including bacteria, yeast, mold, and microalgae during the secondary metabolic growth under conditions where carbon is in excess and another essential nutrient such as nitrogen is limiting[5]. Lipids of oleaginous fungi have attracted great worldwide attention as they can accumulate lipids as high as 80% of their cell biomass[6]. They possess some biotechnological advantages for SCO production such as short life, pelleted growth for easier downstream, easy to scale up, and their ability to use a wide range of carbon sources such as lignocellulosic carbon, agro-industrial residues[7]. Increasing attention has been paid to develop new oil resources by using microbes over the conventional plant and animal resources. There are several filamentous fungi that can produce some value-added fatty acids such as long-chain fatty acids that are comparable to conventional plant oils[8] and some fungi are also capable of accumulating polyunsaturated fatty acids (PUFAs) such as Docosahexaenoic acid (DHA), Alpha-linoleic acid (ALA), Gamma-linoleic acid (GLA), Eicosapentaenoic acid (EPA) and Arachidonic acid (ARA)[9]. Long-chain PUFAs have been reported to exhibit a multifunctional role in preventing or curing a variety of diseases in the human body[10]. Along with their role as energy supplying fuel, short-chain saturated fatty acids (SCFA), from C1 to C5 and medium-chain saturated fatty acids (MCFAs), from C6-C12, are important food constituents and also play an important role in controlling cell metabolism and intracellular signalling[11]. MCFAs have gained attention as an important diet because they exhibit unique structural and physiological characteristics. They are relatively soluble in water, absorbed and transported directly *via* the portal system, have more rapid β-oxidation, augmenting diet-induced thermogenesis and have little affinity to store as body fat. During the last few years, MCFAs have gained attention as part of a healthy diet for the patients suffering from lipid mal-absorption, mal-digestion, obesity, and carnitine deficiency disease. TAGs

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containing very-long-chain fatty acids are poorly absorbed due to their high melting point than the body temperature. This unique property makes LCFAs an important constituent for the synthesis of low-calorie structured lipids. In many medicinal and functional foods, a mixture of medium-chain and long-chain triglyceride is used to provide both rapidly metabolized and slowly metabolized diet as well as essential fatty acids. The amount of naturally produced SCFAs, MCFAs, and LCFAs from plant sources have been found insufficient to meet the great demand of these fatty acids in functional food industries. Increasing health-related concerns and worldwide energy demands, the current research focus has shifted to the de novo biosynthesis of SCFAs and MCFAs from different oleaginous fungi[12]. However, the high cost of the feedstock for the cultivation of oil oleaginous microbes makes SCOs less economically competitive. Microorganisms can degrade various waste products and utilize the carbon compounds present in them as a source of energy, for the production of oil[13]. The use of locally available agro-industrial residues or renewable materials which do not compete with food production has been employed to improve the process economy of SCOs. Production of biofuel and cooking oil leads to the generation of large quantities of meals which are by-products of oil extraction from seeds. Until now, meals from several oilseed crops[14,15] have been used as low-cost substrates for the cultivation of oleaginous microorganisms to produce value-added SCOs. Bioconversion of oilseed meals to value-added products improves the commercial value of meals, reduce the environmental issues and a cost-effective application in animal feeding. De oiled mustard meal consists up to 28-30% (*w/w*) protein, 10-12% (*w/w*) ash and 12-15% (*w/w*) dietary fibre. Low cost, easy availability in India throughout the year and renewable carbon source without any pre-treatment makes this agro-industrial residue (defatted mustard meal) as a potential substrate for oleaginous fungal growth and may offer an economical benefit by reducing the production cost. Very little information is available regarding the growth and SCO production of oleaginous fungi in fermentation media supplemented with mustard meal to ascertain its effect on the fatty acid profile of the fungal strains[16].

Based on the aforementioned findings, the present work aimed to screen different filamentous fungi from the soil of vegetable oil extraction plant and to examine how the lipid yields of the selected fungal strains varied under the influence of untreated mustard meal and to characterize on a comparative basis the fatty acid profile of SCOs obtained from the isolated strains.

Materials and Methods

Collection of soil

Soil sample was collected from rice bran oil industry situated in Alamgunj, Burdwan, West Bengal, India, and used for isolation of oleaginous fungi. Sampling was done in December 2018. Soil was taken from a depth of 5-10 cm below the surface using sterile spatula and kept in sterile plastic container and stored at 4°C under refrigeration immediately until use.

Materials

Black mustard seeds were bought from local market of Shibpur, Howrah. All other chemicals and solvents used were purchased from Merck Millipore, India and were of analytical grade.

Fungal isolation

Oleaginous fungi were isolated from the collected soil sample by serial dilution and plating method. 1 g of soil was diluted with 9 mL of 0.9% (*w/v*) sterile saline water added in 100 mL conical flask and shaken vigorously for five mints. Serial 10-fold dilutions of the original suspension ranging from 10^{-1} to 10^{-6} were prepared. 0.1 mL from each dilution was spread onto the Petri plates made with potato dextrose agar (PDA) medium containing 20% (*w/v*) extract sliced potato, 2% (*w/v*) dextrose and 2% (*w/v*) agar (pH 6.0). Streptomycin was added to the medium (at a concentration of 10000 U/mL) to prevent bacterial growth. Each dilution was plated in triplicate. The screening plates were incubated at 28°C for 2-4 days and those containing isolated colonies were used for further study.

Isolated fungal colonies were transferred repeatedly to fresh PDA plates until pure cultures were confirmed.

Screening for oleaginous fungi

The isolated colonies of different fungal strains were further screened for their lipid producing abilities by qualitative analysis with the Sudan Black B staining technique[17] and observed under microscope on oil immersion for the presence of blue or grayish colored fat globules within the cell. The potential pure oleaginous fungal isolates were maintained on PDA slants at 28°C and subcultured once every months.

Preparation of oilseed meal

25 g of black Mustard seeds were collected from the market. Seeds were dried at 50°C for 24 h to reduce the moisture content. Dried seeds were crushed in a grinder and the powder was put into a thimble and subjected to solvent extraction of oil by Soxhlet using hexane (1:6 *w/v* ratio) as solvent. After 7 cycles, the thimble was taken out and dried in the oven for total evaporation of residual solvent.

Proximate composition of mustard meal (de oiled seed)

Proximate composition of the de-oiled mustard meal, *i.e*., total carbohydrate, crude protein, moisture content, ash, and fiber content was measured after total extraction of oil from Black Mustard seed, brought from the local market. Proximate analysis was done by standard AOAC methods (2000)[18]. As the mustard seeds were completely defatted, lipid content was not measured.

Fungal inoculation and fermentation of oleaginous fungi

The seed cultures of oleaginous fungi were initially streaked and activated on PDA slants for 3 days, then they were transferred to 100 mL Erlenmeyer flask separately containing preculture medium consisted of (in g/L): glucose 30 g, peptone 5 g, yeast extract 5 g with initial pH of 5.0 and grown at 28°C for 2-3 days. 10% of precultures (seed) broth were added to 100 mL of fermentation medium containing (in g/L): Glucose-50 g, $(NH_4)_2SO_4$- 3 g, KH_2PO_4-0.8 g, K_2HPO_4- 0.2 g, $MgSO_4$, $7H_2O$- 0.5 g (pH 5.5) in 250 mL Erlenmeyer flask, and then incubated at 28°C for 7 days. This medium was used as control. Another three media designated as Medium I, II and III were prepared by addition of 0.1% (*w/v*), 0.5% (*w/v*) and 1% (*w/v*) deoiled mustard meal respectively to the control medium. Duplicate samples were analyzed in order to determine dry biomass and percentage of lipid.

Fungal dry mass determination

After 7 days of fermentation, the fungal biomasses from the culture broth were harvested by filtering and washed twice with distilled water. The harvested biomasses werethen freeze dried (Eyela FDU-1110, Japan) to constant mass and weighted dry cell weight.

Extraction of lipid compounds

The dried fungal biomasses were subjected to lipid extraction according to Bligh and Dyer method, 1959[19] with methanol, water and chloroform in a ratio of 2:1:1 (*v/v/v*). The lower chloroform layer containing lipid was collected and lipid content was measured by evaporating the solvent.

The productivity of oil produced was also calculated according to the following equation:

$$\text{Lipid content} = \frac{\text{Lipid yield}\,(L_{max})\,\text{g/L}}{\text{Biomass production}\,(X)\,(\text{g/L})} \times 100$$

Every fungal oil sample was methyl-esterified to evaluate the content and composition of fatty acids by gas chromatography (GC). Fatty Acid Methyl Ester (FAME) was prepared by the method described by Metcalfe and Schmitz, 1961[20]. Triacylglycerides (TAGs) were converted to their methyl esters by adding 0.5 (N) Methanolic KOH shaken vigorously for 10 min. To neutralize the alkali, 1 ml of 1 (N) HCL was added into the sample solution and shaken for a while. FAME was then extracted with petroleum ether (boiling point 40–60°C). FAME will be analyzed by Gas Chromatography immediately.

Fatty acid composition determination

FAMEs were analyzed by an Agilent 6890 N; J & W Scientific, computerized Gas Chromatograph (network GC system – G 1530 N), fitted with a DB-Wax capillary column (30 m × 0.32 mM × 0.25 mM) and a flame ionization detector. N_2, H_2 and airflow rate were maintained at 1, 30 and 300 mL/min, respectively. Inlet and detector temperatures were kept at 250°C and the oven temperature was programmed to increase from 150 to 190°C at a rate of 58°C/min, then to hold for 5 min, and then to increase to 230°C at a rate of 48°C/min, and then again to hold for 10 min.

Fatty acids were identified against standard methyl ester (Supelco-37 component FAME mix C4-C24, 18919-1AMP) of corresponding fatty acids.

Results and Discussion

Screening and characterization of oleaginous fungi

Sudan Black B staining which gave only partial information on the ability of lipid accumulation of filamentous fungi identified 3 fungal colonies as potential producers of lipid biomass. This staining technique could not give precise insight into cellular lipid content, as it stained only the lipidic matter surrounding the cytoplasmic membrane, presence of blue-black colored fat globules within the cell denoted that the isolated fungal strains were oleaginous in nature[21].

These 3 isolates (designated as F-$RB_1$1,F-$RB_1$2 and F-$RB_1$3) which were able to convert glucose into lipids were then further characterized for different parameters such as biomass production and single-cell

Table 1 — Dry biomass production, lipid yield and productivity of oil produced by isolated fungi

Fungus culture	Biomass production (X) g/L	Lipid yield (L_{max}) g/L	SCO productivity ($Y_{L/X}$) g/g	Lipid content (%)
F-$RB_1$1	7.90±1.00	1.80±0.30	2.27±0.10	22.78±1.20
F-$RB_1$2	10.30±2.10	1.70±0.20	1.65±0.20	16.50±0.90
F-$RB_1$3	9.50±1.70	1.10±0.00	1.15±0.10	11.50±0.50

Sample evaluation is done in triplicate. Values are calculated as Mean± SD (n=3).

oil productivity and the results were shown in (Table 1).

The lipid accumulation process in cellular storage of oleaginous microorganisms generally requires the exhaustion of essential nutrients particularly nitrogen or phosphorus usually when carbon remains in excess which allows excess carbon to be incorporated into lipids[5].

It was observed that in the control medium, the values of glucose supported biomass growth X (g/L) were in the range of 7.9 to 10.3 g biomass yield/L medium in 5% glucose medium. Results clearly indicated that all the 3 tested fungal strains had the ability to accumulate lipids in different amounts ranging from 11.5 to 22.78% of their dry weight. However, the isolate F-RB_1 1 cells gave the highest amount of lipid accumulation in biomass (22.78%) followed by F-RB_1 2 (16.5%) and F-RB_1 3 (11.5%).

Among the 3 isolates screened for lipid production, only one isolate F-RB_1 1 was able to convert glucose into lipids with the lipid content in biomasses greater than 20% of its dry cell mass within 7 days of cultivation. Therefore, this isolate was designated as oleaginous. Hence, further work was carried out with this isolate only. Qingwei Yao *et al.*,[9] screened and isolated 19 oleaginous fungal isolates from soil with the genus *Mortierella* and one strain (SL-4) accumulated maximum lipid content up to 32% of its biomass weight using glucose as the sole carbon source. In another study, RS Yehia *et al.*[22], among the 14 filamentous fungi isolated from soil, 3 fungi were able to accumulate lipid greater than 20% of their dry cell mass (3.6-6.8 g/L).

Fatty acid analysis of the extracted fungal lipids (SCOs)

In the present work, the methyl ester of SCO of the oleaginous fungal isolate was subjected to GC for fatty acid analysis and results were presented in (Table 2). The FAME profile showed that the isolate F-$RB_1$1 appeared to produce considerable amount of PUFA (34.73%), LCFA (24.9%) and MUFA (33.8%). The fatty acids in the decreasing order of abundance were linoleic (C18:2, 26.88%), palmitic (C16:0, 17.3%), oleic (C18:1, 15.6%), stearic (C18:0, 9.15%), erucic (C22:1, 8.60%), DHA (2.03%), EPA (C20:5, 1.84%) and ARA (C20:4, 1.17%).

Table 2 — Fatty acid composition of oil produced from isolated fungus F-RB_1 1

Fatty acid (%) w/w as FAME	F-RB_1 1
14:0	1.00±0.0
16:0	17.30±2.10
16:1	1.32±0.10
18:0	9.15±1.20
18:1	15.61±1.90
18:2	26.88±2.70
18:3	GLA(1.71±0.0) ALA(1.10±0.0)
20:1	8.27±0.90
20:4	1.17±0.0
20:5	1.84±0.10
22:0	-
22:1	8.61±0.60
22:6	2.03±0.10
24:0	3.34±0.10

Sample evaluation is done in triplicate. Values are calculated as Mean± SD (n=3).

The FAME profile indicated that the isolate F-$RB_1$1 appeared to produce long-chain PUFAs like ALA, GLA, ARA, EPA and DHA in small amounts, which are important n-6 and n-3 fatty acids, normally absent in vegetable oils. EPA is a precursor of a series of eicosanoids and due to its anti thrombic effect, it is important in protecting against heart attack. ARA has unique biological activities as it has a structural role in the brain, used as a substrate for the synthesis of eicosanoids and also has roles in cell signaling. DHA is essential for visual, neural, and behavioral development of infants. Along with EPA, it affects the blood lipids and lipoproteins, vascular function, blood pressure, blood flow, and inflammation, lowering the risk of CVD[2].

Proximate analysis of mustard meal (de-oiled seed)

Mustard seed meal contains a considerable amount of carbohydrate and protein, which helped in the biomass growth and oil production of the oleaginous fungus. Percentages of these compositions are illustrated in (Table 3).

It was observed that de-oiled mustard meal contained high protein (31.46%) and fiber (9.8%). higher amount of protein (38.17%) and fiber (12.17%) along with higher moister (9.20%) and lower ash (1.93%) content was reported by Sarkar *et al.*[23], who

Table 3 — Proximate composition of (de-oiled seed) mustard meal

Seed meal	Carbohydrate (%)	Protein (%)	Moisture (%)	Ash (%)	Fiber (%)
De oiled Mustard seed meal	18.39±1.80	31.46±3.40	7.20±1.10	8.60±1.80	9.80±1.20

Sample evaluation is done in triplicate. Values are calculated as Mean± SD (n=3).

Table 4 — Dry biomass production, lipid yield and productivity of oil produced in mustard meal supplemented media from F-RB_1 1

Medium	Biomass production (X) g/L	Lipid yield (L_{max}) g/L	SCO productivity ($Y_{L/X}$) g/g	Lipid content (%)
Medium I	3.20±0.40	2.00±0.10	6.25±0.90	62.50±5.10
Medium II	5.30±0.80	3.20±0.10	6.03±0.80	60.37±4.90
Medium III	4.10±0.50	2.10±0.10	5.12±0.30	51.21±4.20

Sample evaluation is done in triplicate. Values are calculated as Mean± SD (n=3).
Medium I, II and III contained 0.1%, 0.5% and 1% (*w/v*) de-oiled mustard meal respectively.

analyzed proximate composition of black mustard meal collected from local oil industry of Bangladesh. Another study reported much higher protein (41.03%), carbohydrate (32.73%) and ash (10.23%) content than our study, but the moister (8.07%) and fiber (10.63%) contents were more or less similar to our result, which were found to be 7.2% and 9.8%, respectively[24]. The data obtained in our study revealed that de-oiled mustard meal contained significant amount of protein, soluble carbohydrate along with insoluble fiber and ash, which could be used as a source of carbon, nitrogen and minerals in the oil production media. Hence, mustard meal can be used as a good supplement for SCO production by fungi.

Effect of mustard meal (de-oiled seed) on SCO production

Various concentrations of mustard meal (0.1% *w/v*, 0.5% *w/v* and 1% *w/v*) were added as a supplement to the fermentation media. It was observed that when the fungus F-$RB_1$1 was grown in a medium containing de-oiled mustard meal, both the biomass production and lipid content were increased compared to those observed in control medium. The maximum increase in biomass production was observed when the isolate was grown in medium II (5.30 g/L) containing 0.5% de-oiled mustard meal and the highest SCO productivity was reached in the medium I (62.5%) containing 0.1% de-oiled mustard meal. It was previously mentioned that the de-oiled mustard meal contained a significant amount of protein which was found to be 31.46%. Our results indicated that nitrogen present in the de-oiled mustard meal as protein has an effect on microbial oil production from the fungus F-$RB_1$1. Table 4 clearly indicated that, as nitrogen content in the fermentation medium increased, lipid content and oil productivity decreased. Hence, the highest amount of de-oiled mustard meal (1%) produced the lowest amount of lipid (51.21%).

Alok Patel *et al*.[25], used the hydrolysates obtained from the organo-solvent pretreated Brewers' spent grain as substrate for the cultivation of *Rhodosporidium toruloides* for SCO production. They observed that the dry cell weight (18.44 g/L) and lipid content (10.41 g/L) with total lipid concentration (56.45%) of this oleaginous yeast were higher compared to the results obtained when grown on synthetic media containing glucose, xylose or mixture of glucose and xylose. This is in agreement with the results of our work. The lipid profile of *Rhodosporidium toruloides* exhibited similar fatty acid contents to vegetable oil. Similar observation was carried out for the strain *Aspergillus sp*. ATHUM 3482 when grown on waste cooking olive oil added in the growth medium at 15 g/L[26]. It was reported that high biomass quantity up to 0.65 g/L was produced and *Aspergillus sp*. ATHUM 3482 accumulated lipid up to 64% (*w/w*) in dry fungal mass in the oil supplemented medium. Similar findings were also reported by Matsakas *et al*., 2017[7] who evaluated the fungus *Fusarium oxysporum* for its potential to accumulate microbial lipids when grown on synthetic media containing glucose (42.2%), fructose (25.5%), sucrose (48.7%) or mixture of these sugars (52.9%) and natural media consisted of saccharified sweet sorghum stalks. They found that biomass production was increased greatly from 5.07 g/L when grown on synthetic media (sucrose) than 10.99 g/L in natural medium. They demonstrated that proteins present in saccharified sweet sorghum stalks supported fungal growth and lipid accumulation without external nitrogen addition which provided a positive and important impact on process economy[7]. In another study rapeseed meal hydrolysate (RMH) which is another important agro-industrial by product was used as fermentation feedstock for growth and lipid production of microalgae *Crypthecodinium cohni* that produce DHA. They reported that *Crypthecodinium*

cohni is commonly fermented on glucose-based medium composed of glucose, yeast extract and sea salts. The biomass and DHA yield were greatly enhanced on the addition of waste molasses as a supplementary carbon source. RMH-molasses medium composed of diluted RMH (7%) and waste molasses (1-9%) exhibited the highest biomass concentration and DHA yield reached to 3.43 g/L and 8.72 g/L[14].

In another study Chaudhury *et al.*[16], found that when *Mortiella elongate* SC-208 isolated from mustard seed extraction plant grown on defatted mustard meal, the dry mycelium yield (27.2 g/L) was to some extent higher than the control medium (21.5 g/L) (PD broth without mustard meal).

Effect of mustard meal (de-oiled seed) on fatty acid composition of SCO

There was a marked change in the fatty acid composition of the SCO produced by the isolate F-RB$_1$1in de-oiled mustard meal supplemented fermentation media (Table 5) in comparison with control medium (without mustard meal). Oil produced from media containing mustard meal resulted in an oil of exceptional fatty acid profile. It contained considerable amount of long-chain saturated fatty acids like, palmitic (16:0), stearic (18:0), arachidic (20:0), behenic (22:0) and lignoceric (24:0) acid along with a high amount of MCFAs like caproic (6:0) caprylic (C8:0) and capric (C10:0) acids. The FAME profile of oil obtained from the isolate F-RB$_1$ 1when grown in medium I comprised 24.4% C6:0, 10.4% C8:0 and 8.7% C10:0, when grown in medium II had 12.6% C6:0, 10.97% C8:0 and 9.75% C10:0, and when grown in medium III had 20.47%, 8.19% and 7.37% of C6:0, C8:0 and C10:0 respectively. The oil produced from supplemented medium contained various amounts of long-chain fatty acids as well. Oil obtained from F-RB$_1$ when grown on medium I contained 9% C16:0, 4.5% C18:0 and only 1.5% C22:0. Medium II contained lower amount of palmitic (7.31%) and stearic (1%) along with higher amount of behenic acid (9.26%). Oil obtained from medium III has palmitic and stearic acid content 7.37% and 4.91% respectively, with the behenic acid content of 7.37%. SCOs extracted from the isolate F-RB$_1$ 1 after supplementation contained PUFAs in various amounts in all the supplemented mediums. Linoleic (18:2) acid ranged from 9.0-16.58% and linolenic (18:3) acid ranged from 2.54-5.12%. It was found that MUFA ranged from 4.09-13.65%. Other fatty acids like C12:0, C14:0 were produced in little amounts. Similarly, an improvement in lipid production accounting for up to 62% of the total fatty acid profile followed by palmitic (16%) and linolenic (8%) acid along with other medium and long-chain fatty acids was observed by Ali *et al.*[27], when an oleaginous fungi *Penicillium brevicompactum* was grown in a medium containing sunflower oil cake.

Table 5 — Fatty acid composition of oil produced in mustard meal supplemented media from F-RB$_1$ 1

Fatty acid (%) *w/w* as FAME	Medium I	Medium II	Medium III
6:0	24.40±3.20	12.6±2.10	20.47±2.30
8:0	10.40±1.50	10.97±1.90	8.19±0.90
10:0	8.70±0.90	9.75±0.90	7.37±0.60
12:0	6.90±0.70	4.87±0.50	4.91±0.30
14:0	-	-	2.45±0.10
16:0	9.00±0.80	7.31±0.80	7.37±0.60
16:1	-	-	3.27±0.10
18:0	4.50±0.30	1.0±0.0	4.91±0.30
18:1	5.30±0.30	13.65±1.70	4.09±0.60
18:2	9.00±0.80	16.58±1.90	14.74±1.70
18:3	-	5.12±0.70	2.45±0.10
20:0	9.00±0.70	0.97±0.0	8.91±0.90
20:1	6.81±0.50	-	2.45±0.10
20:2	-	6.34±0.50	-
22:0	1.50±0.10	9.26±0.90	7.37±0.50
22:1	4.00±0.30	-	0.81±0.0

Sample evaluation is done in triplicate. Values are calculated as Mean± SD (n=3).

Medium I, II and III contained 0.1%, 0.5% and 1% (*w/v*) de oiled mustard meal, respectively.

The highest MCFA (C6:0-C12:0) production (50.4%) was observed with medium I, while very long-chain saturated fatty acids (C20:0, C22:0) was highest (15.56%) in medium III. It was observed that as the mustard meal concentration increased, MCFA production decreased along with unsaturated fatty acid production. Erucic acid (C22:1), from residual mustard oil of the meal was converted to saturated behenic acid (C22:0) as mustard meal content increased. Very long-chain saturated fatty acids have higher melting point and easily form insoluble calcium salts in body. Therefore, long-chain SFAs are relatively less absorbable than unsaturated fatty acids. Hence they have relatively lesser calorie than other. Attaching long and short or long and medium-chain fatty acid into one glycerol molecule as TAG gives low-calorie structured lipid with better stability. This fatty acid composition has various importances in the food industry. For this kind of valuable fatty acid production, increased cost of substrate does not matter substantially.

The altered fatty acid composition of SCOs obtained from F-RB$_1$ 1grown on defatted mustard

meal made this strain suitable for production of the structured lipid Caprenin. Caprenin has anti-obese and anti-cholesteromic properties. Cholesterol lowering effect is a very crucial functional property, as it helps to lower the risk of various metabolic disorders, obesity and cardio vascular disease[28]. It is a suitable cocoa-butter and other confectionery fat replacement composed of MCFAs such as C8:0, C10:0 and very-long-chain C22:0 fatty acids. It contained about 4 kcal/g energy and thus designed for lowering the calorie content of food. This calorie reduction is due to incomplete absorption of very-long-chain fatty acid and the thermogenic effect of short-chain fatty acids present in caprenin[29]. Nabisco Food Group developed low-calorie SL known as Salatrium which consists of SCFA and C18:0[10]. The abundance of MCFAs produced from plant sources (coconut oil and palm kernel oil) has been found insufficient to cope up with industrial demand. Thus there is a strong research interest to acquire the desired elevated amounts of MCFAs by searching new promising MCFAs producing microorganisms and by adopting diverse strategies to improve MCFA biosynthesis from metabolically engineered oleaginous strains. Hussain *et al.*[12], boosted de novo MCFAs production by expressing acyl carrier protein (ACP) thioesterase (TE) encoding genes in *Mucor circinelloides* 65. This recombinant strain elevated de novo MCFA biosynthesis within the range from 28 to 46% of total cell lipid. In another study Rigouin *et al.*[30], promoted MCFAs accumulation in oleaginous yeast *Yarrowia lipolytica* by over expressing a plant diglyceride acyl transferase specific for MCFA, reached up to 45% of total lipid content. In our study 50.4% MCFA was produced by using defatted mustard meal as a supplementation for growth along with the synthetic media. Mustard meal contained considerable amounts of thioglucoside glucohydroxylase (E.C.3.2.3.1) or myrosinase enzyme, which may have some influence on the synthesis of MCFAs in an analogous manner to that of the thioesterase by gene cloning. In a study carried out by Voelker *et al.*[31], it was found that undomesticated California bay contained a medium chain acyl-carrier protein thioesterase (ACP TE) BTE, which hydrolyze growing acyl thioesters and can produce capric (C10:0) and lauric acid (C12:0). They reported that, expression of the complementary DNA (formed by cloning of the BTE sequence) into the seeds of *Arabidopsis thaliana* (Brassica rape seed), elevated the activity of 12:0-ACP TE by up to 70 folds and 14:0-ACP TE up to 7 folds, suppressing the activity of endogenous 18:1-ACP TE, resulted in production of high amount of C12:0.

Analysis of fatty acid composition of fresh mycelia of anaerobic rumen fungi belonging to the genera *Neocallimastix*, *Caecomyces*, *Orpinomyces* and *Anaeromyces* grown on M 10 medium enriched by 30% rumen fluid with glucose (4 g/L) as carbon source was found to comprise carbon chain of length ranging from 12 to 24 carbons. Most common fatty acids were palmitic (1.38-3.07%), stearic (6.02-8.32%), oleic (1.78-4.12%), arachidic (6.86-8.58%), behenic (5.06-7.35%) and traces of linoleic and linolenic acid[32]. Thioesterase is likely to produce more saturated fatty acids. Mustard meal thioesterase is a possible explanation for high content of (1.5-12.1%) behenic acid in SCOs.

The thioglucoside content of defatted mustard meal may have an influence on the fungus for this kind of fatty acid profile. Higher concentration of this component may have some inhibitory effects on the fungus, having excess thioesterase enzyme or sulphur compounds, resulted in decrease of MCFA content proportionately. The capacity of the fungus for utilization of these components is ranged to some extent. Higher concentration than capacity may reduce the quantity of some beneficial fatty acids such as behenic acid.

Conclusion

The present study evaluated the capacity of the isolated fungal strain, as an oleaginous fungus to grow on synthetic medium and accumulate lipids with essential PUFAs under submerged fermentation condition. Defatted mustard meal was found better nutrient source for cultivation of this fungus compared to commercial glucose indicating potential of defatted mustard meal as a substrate for oleaginous microorganisms. The FAMEs derived from this fungus grown on de oiled mustard meal have unique fatty acid composition composed of MCFAs, LCFAs and some PUFAs having enormous scope of structured lipid and nutraceutical application. As evident from biomass production and the fatty acid profiling of SCO obtained from the aforementioned fungus grown on defatted mustard meal suggested that the fungus not only utilized de oiled mustard meal for its growth and lipid production but also altered its fatty acid composition making it more suitable for the production of value added fatty acids. Defatted mustard meal is an underdeveloped agricultural by product. An alternative utilisation

of this low cost, easily available waste as substrate for SCO production is significantly important as it offered value addition to mustard meal. In addition, direct utilization of this agro-industrial by product by the fungus without any conventional pre treatment method made the SCO production as an environmentally favourable process. Further investigation is needed to know the mechanism involved in the synthesis of both MCFAs and LCFAs fatty acids along with some PUFAs while using mustard meal in the medium.

Conflict of interest

All authors declare no conflict of interest.

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