SHORT COMMUNICATION

Effect of Mustard Meal on the Production of Arachidonic Acid by *Mortierella elongata* SC-208

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ABSTRACT: A strain of *Mortierella elongata* SC-208 that was isolated from soil of a mustardseed extraction plant can produce arachidonic acid in significant amounts, and it was grown in three different media, one of which contained 0.5% deoiled mustard meal. The arachidonic acid content in the lipid part of dry mycelium (23.2 g/L) was as high as 33% w/w from the medium that contained mustard meal, and the overall yield of arachidonic acid was 0.49 g/L. The arachidonic acid contents in the phospholipid fraction and the triglyceride fraction were 39.5 and 30.2%, respectively.

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KEY WORDS: Arachidonic acid, *Mortierella elongata,* mustard meal.

The importance of arachidonic acid (AA) (*cis*-5,8,11,14-eicosatetraenoic acid) has increased tremendously because of its unique biological activities (1–3). The available lipid sources rich in arachidonic acid are animal tissues and algal cells (4). Several workers have tried to produce arachidonic acid (AA) and other polyunsaturated fatty acids (PUFA) from microorganisms by fermentation technology in a suitable medium.

In a shake-flask *Mortierella elongata* IS-5 produced 16.4–30.1% AA of the total fatty acid content (5). *Mortierella* sp. S-17 produced 0.96 g/L of AA at an optimal C/N ratio 20 (6). *Mortierella alpina* ATCC-16266 produced 2.1 g/L of AA in a medium that contained 10% glucose, and the highest percentage of AA in the lipid was 43.3% at a glucose concentration of 2% (7). *Mortierella alpina* ATCC-32222 could produce 3.73 g/L AA at a glucose concentration of 100 g/L (8). There are also several patented processes for the production of AA (9–11). A strain of *M. alpina* has produced 5.3 g/L AA in soy flour- supplemented medium, and an extract with 72.5% w/w AA was prepared from the recovered mycelium (12). Totani and Oba (13) cultivated *M. alpina* IFO 8568 in a potato paste and dextrose medium at 20°C for 20 d and produced AA at 11.8 g/kg medium. Another *Mortierella* sp. can produce 3.6 g/L of AA in 7 d of incubation (14). *Mortierella alpina* CBS-210.32 produced 0.86–0.96 g/L of AA in 5–7 d (15), and *M. isabellina* 224 could produce 0.505 g/L of AA in a malt extract medium (16).

Recently, Kawashima *et al.* (17) have shown that *M. alpina* S-14 produced 1.6 g/L AA (26% of total cellular fatty acids) in a 5-L jar fermenter on the eighth day. The cellular lipids of the S-14 strain comprised 75.8% triacylglycerol (TG), 6.7% diacylglycerol (DG), and 13.3% phospholipid (PL). The percentage of AA was higher in PL than in TG and highest in phosphatidylcholine (32.6%). Chang *et al.* (18) have optimized AA production by *M. alpina* Wiji-4 isolate. It was capable of producing 0.504 g/L of AA in the screening medium. The AA yield was raised to 1.817 g/L by a step-by-step approach.

We have screened different PUFA-producing microorganisms from soil (19), which was collected from a mustardseed extraction plant. Among the microorganisms, only *M. elongata* SC-208 and *M. parvispora* SC-156 can accumulate AA in the mycelium. Several workers have already studied extensively the effects of carbon source, carbon/nitrogen ratio, effect of aging, temperature, etc. on AA production by different strains. Our intention was to study whether there is any effect of mustard meal on the production of AA in the above two *Mortierella* sp. that occur in the soil from a mustardseed extraction plant.

The present study investigates the growth of the *Mortierella* strains in three different media in a shake flask with or without mustard meal to ascertain the effect on the PUFA level, particularly the AA content of the strains.

MATERIALS AND METHODS

Several soil samples were collected from the different mustard oil extraction plants of West Bengal, India, for the isolation of fungi, which were grown on agar plates. Strains of *M. elongata* SC-208 and *M. parvispora* SC-156 were isolated, which can accumulate AA. The purified cultures were then grown in different media (60 mL medium in 250-mL conical flask) for 5 d at 28°C in an incubator with shaking (120 strokes/min). The composition in g/L of different media was: Medium I—Extract of potato, 200 g (prepared by boiling 200 g of sliced potato in distilled water for 25 min, followed by filtration through a filter cloth that retained the pulp; the volume of the filtrate broth was maintained at 400 mL), and dextrose, 50 g.

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Medium-II—Urea, 4 g; glucose, 200 g; ammonium sulfate, 2.25 g; potassium dihydrogen phosphate, 7 g; magnesium sulfate, 1 g; sodium chloride, 0.3 g; malt extract, 0.6 g; yeast extract, 0.3 g; peptone, 0.3 g; minerals, 3.0 mL (mg/mL: $FeSO_4 \cdot 7H_2O$, 10.0; $CaCl_2 \cdot 2H_2O$, 1.2; $CuSO_4 \cdot 5H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 1.0; $MnCl_3 \cdot 7H_2O$, 1.0).

Medium-III—Extract from potato, 150 g; mustard meal (deoiled by hexane in a Soxhlet apparatus), 5 g.

After growth for 5 d, the fungal mycelium was harvested and dried at 50°C in a vacuum oven for 5 h. Then, it was subjected to solvent extraction in a Soxhlet apparatus with chloroform/methanol (1:1, vol/vol) in the dark. After extraction, the solvent was removed under the stream of nitrogen, and finally the extract was dried under vacuum.

The isolated lipid was mainly a mixture of PL and TAG, which was confirmed on a thin-layer chromatographic (TLC) plate (silicagel G; solvent system, hexane/diethyl ether, 70:20 vol/vol) with phosphate statin (21). The PL and TAG were separated on a preparative TLC plate (20 cm × 20 cm) with the above solvent system, and the individual fractions were extracted with diethyl ether.

The total fatty acid compositions of the fungal lipid and of the isolated TAG and PL were determined by a gas-liquid chromatography (GLC) method after convertion into methyl esters (22). The HP-5890A GLC (Hewlett-Packard, Palo Alto, CA) was connected with a HP-3390A data integrator. The GLC was fitted with a glass column (6′ × 1/8″ i.d.), packed with 10% diethyleneglycol succinate (DEGS) supported on Chromosorb-WHP (100/200 mesh), of HP make. The oven, injector, and detector block temperatures were maintained at 190, 230, and 240°C, respectively. IOLAR-2 nitrogen (Indian Oxygen Limited, Calcutta, India) was used as the carrier gas (flow rate 30 mL/min). The fatty acid ester peaks were identified and calibrated with standard methyl esters, supplied by Sigma Chemical Company (St. Louis, MO). Data are averages of three determinations.

RESULTS & DISCUSSION

Mortierella elongata SC-208 and *M. parvispora* SC-156 are two AA-producing organisms, as reported previously (19). These two strains have been grown in shake flasks on three different media in a definite culture condition. Medium I, which is mainly prepared from potato dextrose, is employed for our screening medium. The yield of AA has also been compared with the recommended medium for *Mortierella* fungi (20). The dry mycelia yield, lipid content, and total fatty acid composition are shown in Table 1. In Medium I, which is prepared from potato extract and dextrose, SC-208 accumulated 72 mg of lipid per g of dry biomass (dry biomass yield, 21.5 g/L). The lipid contained 9.8% AA, along with 11.6% docosahexaenoic acid (DHA). On the other hand, SC-156 produced 48.0 mg of lipid per g of dry biomass (dry biomass yield, 27.2 g/L). The lipid contained 8.1% AA, along with 3.4% DHA. In Medium II, which is recommended by Suzuki (20) as a potential medium for the growth of *Mortierella* species for the production of PUFA, the dry mycelium yield (27.3 g/L) was to some extent higher than in Medium I, but the AA yield in the lipid was only 4.2%, and the essential fatty acid content was only 31%. SC-156 was also grown in Medium II, but the AA in the lipid was only 1.5%.

In Medium III, 0.5% mustard meal was added, and we observed that the biomass yield (23.2 g/L) increased for SC-208, compared to that grown in Medium-I, but its lipid content was

TABLE 1
Biomass Yield, Lipid Content, and Fatty Acid Composition of the Total Lipid from *Mortierella elongata* SC-208 and *M. parvispora* SC-156 in Different Media[a]

Fatty acids (% w/w)	Medium I		Medium II		Medium III	
	SC-208	SC-156	SC-208	SC-156	SC-208	SC-156
$C_{16:0}$	13.7	22.4	14.6	16.1	8.5	18.6
$C_{16:1}$	—	—	1.0	—	—	—
$C_{18:0}$	6.6	9.1	13.0	13.1	4.3	8.5
$C_{18:1}$	27.5	20.2	26.7	15.4	15.5	23.4
$C_{18:2}$	12.6	24.2	24.1	12.3	15.4	22.7
$C_{20:0}$	—	—	2.0	1.2	4.6	1.1
$C_{18:3}$	6.8	2.4	2.0	18.8	7.2	4.2
$C_{20:1}$	—	—	—	—	4.4	1.2
$C_{20:4}$	9.8	8.1	4.2	1.5	33.2	7.4
$C_{20:5}$	—	—	—	4.1	1.1	—
$C_{22:5}$	3.7	5.5	4.9	2.1	Trace	3.4
$C_{22:6}$	11.6	3.4	—	10.1	1.1	6.2
$C_{24:1}$	—	—	4.9	—	1.7	—
Others	7.7	4.7	4.6	5.3	3.0	3.3
Dry biomass (g/L)	21.5	27.2	27.3	34.1	23.2	28.1
Lipid content (mg/g dry biomass)	72.0	48.0	92.5	130.0	63.7	62.3
$C_{20:4}$ (AA) yield (g/L)	0.15	0.105	0.1	0.06	0.49	0.13

[a]AA, arachidonic acid.

TABLE 2
Fatty Acid Composition of the Triacylglycerol (TG) and Phospholipid (PL) Fractions of the Lipid from *Mortierella elongata* SC-208 on Medium-III

Fatty acids (% w/w)	TG	PL
$C_{16:0}$	8.6	7.8
$C_{18:0}$	3.6	8.0
$C_{18:1}$	16.6	9.7
$C_{18:2}$	16.0	9.5
$C_{20:0}$	4.4	5.5
$C_{18:3}$	7.9	3.1
$C_{20:1}$	3.8	7.7
$C_{20:4}$	31.2	39.5
$C_{20:5}$	1.0	1.2
$C_{22:6}$	1.1	1.3
$C_{24:1}$	1.8	1.5
Others	4.0	5.2

lower (63.7 mg/g of dry mass) than for the biomass of the other two media. The lipid, however, contained 33.2% AA, which is higher than the reported values of IS-5 (5). AA yield by SC-208 was 0.5 g/L in a medium that contained mustard meal, which is comparable with *M. isabellina* (16). The strain SC-156 was also grown in Medium III with mustard meal, but there was little change in AA content in comparison with medium I.

The fatty acid composition of the TG and L parts of the lipid, produced in Medium III by SC-208, is shown in Table 2. AA in the PL fraction is 39.5%, and in the TG fraction it is 30.2%. So, in the PL fraction, AA accumulation is higher than the TG fraction. Kawashima *et al.* (17) have also observed a higher amount of AA in the PL fraction than in TG for the IS-4 strain.

In conclusion, *M. elongata* SC-208 can be used as a potential source of AA, in comparison with other reported strains, and the use of deoiled mustard meal in a medium can effectively increase the AA yield. Preliminary observation has shown that the strain SC-208 is specific enough to increase AA synthesis in the medium with mustard meal, while the strain SC-156 in the same medium does not increase AA in the lipid. Further investigation is needed to know the mechanism involved in the synthesis of AA or other PUFA in enhanced quantities while using mustard meal in the medium.

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