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RESEARCH ARTICLE

Lipid profile from *Absidia spp.*

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Abstract

Polyunsaturated fatty acids includesOmega-6 fatty acids which are a vital part of our diet and perform a wide range of**f**unctions in the body**.** This fatty acid is uncommon, but is found in certain seed oils,such as evening primrose, borage and blackcurrant of which evening primrose has been most widely used and expensive. **O**leaginous microorganisms like *Mucor*, *Absidia*and*Cunninghamella* spp. were screened for microbial production ofGLA. Production of GLA by the use of waste material was attempted. 11 cultures of *Absidiasp.* were screened. Media composition, growth conditions like pH, temperature, carbon source etc, were optimized for selected cultures for higher biomass & lipid content. Among the different species tested, *A. corymbifera* MTCC-1549 and MTCC - 3132 produced 42.60g /l of biomass and 27.5 % fatty acid at room temperature, in a simple glucose media.

INTRODUCTION

The term oleaginous was used to describe all these microorganisms that have the ability to accumulate more than 20% w/w lipid in their biomass (Ratledge,1991).Fungal source are also gaining potential source of PUFA. GLA is found in human milk and in small amounts in a wide variety of common foods and meats (Horrobin 1990). Essential Fatty Acids (EFAs) are essential to human health but cannot be synthesided in the body.It has to be provided by dietary source**.**Over the past decade, consumers have begun to recognize the importance ofthese unsaturated fatty acids within their diets. Of the omega 6 oils, Gamma Linolenic acid (GLA; C18: 3n-6) and arachidonic acid (ARA; C20: 4n-6) constitute the largest number of beneficial health benefits(Gill. *et al*., 1997).Jangbua, *et al* (2009),had reported that the value-added fermented product containing GLA can be at least incorporated into food as additive. It is an expensiveessential oil, traditionally extracted from evening Primrose and Borage seeds.While evening primrose is the most established source of GLA in the market, borage is becoming the preferred source due to its high seed oil content and high proportion of GLA.

Borage oil contains the highest level of GLA (22-25%); evening primrose oil the lowest(8-10%); and black currant oil values are intermediate (≈15%).GLA was used for human health, treatment of eczema, rheumatoid arthritis, diabetic neuropathy and menstrual syndrome in women. Yang *et al*(1998) had reported that dietary approaches has been the possible prophylactic role of dietary γ-linolenic acid (GLA) in treating various chronic disease states. Frances,*et al*,experimented and reported that GLA with tamoxifen as primary therapy in breast cancer(Rajendra,*et al*.,2007).

Material and methods:

Organisms were obtained from:MTCC-Institute of Microbial Technology (IMTECH), Chandigarh, India.
Culture media : Potato dextrose broth
Culture condition: The fermentation was carried out by submerged culture in a medium containing g/l Glucose(83.2),Yeast extract(5.0),KH_2PO4

Carbon sources : Glucose, Sucrose, Sugarcane juice and Tapioca were tried.
Method: Flasks containing media were inoculated with 1 ml of the fragment mycelia. Theculture were cultivated at 28±1^0 Con an orbital shaker at 200 rpm.

1. Extraction of crude Gamma Linolenic Acid (GLA) – from *Absidia*,11 cultures procured from IMTECH, Chandigarh as shown in the Table 1wereused in the study (Absidia*glauca.*Absidia*fusca,* Absidia*orchidis, A*bsidia*repens).*
2. Production of GLA by the use of waste material was attempted. 11 cultures of *Absidiasp.* were screened.
3. Media composition, growth conditions like pH, temperature, carbon source etc, were optimized for selected cultures for higher biomass & lipid content.
   Extraction of lipids from the biomass was performed according to the Folchextraction method *(Folch et al., 1957).*
4. Extraction also done by Blight and Dyer (1959) methodusing chloroform/methanol as shown in the table-3.
5. Fatty acid profiles were determined by GC.

RESULTS :

Carbon sources like Glucose, Sucrose, Sugarcane juice and Tapioca were tried to get good biomass and lipid content.Eleven cultures were grown in synthetic medium (glucose) in baffled flasks. The sugar utilization and biomass yield were determined. *Absidiacorymbifera* was found to produce 37 g / L biomass, in a simple glucose medium at ambient temperature.In the case of media containing ,Sucrose, Sugarcane juice and Tapioca were given good biomass, but no lipid content.

Cultures were harvested at maximum growth 5-6 days and filtered through Waterman No 1, filter paper anddried in a lyophilizer (Helo,Germany).

Estimation of Biomass and lipid content from different spsof*Absidia*:
Extraction of lipid content: The dried biomes (felt) was ground to a fine powder in a blend. Samples were prepared as shown in the Fig-4.
Biomass ranged from 23.5 to 50.5 g / liter. Lipid contents varied from 22.3 % to 27.6 % and fatty acid contents were determined. Among the different species tested, *A. corymbifera* MTCC-1549 and MTCC - 3132 produced 42.60.g /l of biomass and 27.5 % fatty acid. Other group of workers conducted toxicological studies and reported that it is as good as olive oil.
11 species were giving >30% lipid. *Absidiacorymbifera* was found to produce 37 g / lt biomass, in a simple glucose medium at ambient temperature. Optimum condition for maximum PUFA, production is arbon source 60:1with ammonium nitrate,pH around 6.0-6.5, and incubation in shaker incubator for 5 to 6 days.

Table.1,Stnadard culture used in the study.

Sl no	Standard culture
1	*A. corymbifera*MTCC 1619
2	*A. corymbifera* MTCC 379
3	*A. corymbifera*MTCC 390
4	*A.glauca*MTCC 928
5	*Absidiasp*MTCC 148
6	*A.blakeshleana*MTCC 148
7	*A. Repenses*MTCC 1327
8	*A. pseducylinrophora*MTCC 550
9	*A.corymbifera*MTCC 1549
10	*A.corymbifera*MTCC–3132
11	*A.corymbiferaMTCC 1327*

Table:2 Fat extraction by Standard method

4 gm of dry Biomass

↓

10 gm of 1 N HCl, Autoclave, steaming for 30 min

↓

Filter with paper (No. 1) wash with water

↓

Filtered biomass along with the paper is transferred to 250 ml of Iodine flask & add 150 ml of chloroform + methanol mixture (2:1)

↓

Keep it in shaker for 30 min

↓

Filter the mixture and transfer the filtrate to a 250 ml separate funnel

↓

Add 0.4% calcium chloride (30 ml) to remove moisture

↓

Shake and keep it in cold room for over night

↓

Separate out the bottom layer in a flat bottom flask

↓

Concentrate the fat by flask evaporator & take the weight of the fat

Table.3-Extraction PUFA from dried biomass

Dried ground biomass (felt) 4 g

↓

1 N Hcl (100) ml

↓

Steamed for 30 minis

↓

Filtered through waterman No 1 filter paper

↓

Washed the hydrolyzed felt with water three times with intermittent sanction under vocum.

Table .4, Different *Absidiasp* with various biomass and total lipids

	Standard culture	Biomass g/l	Total lipids %
1	*A. corymbifera* MTCC 1619	24.5	33.6
2	*A. corymbifera* MTCC 379	26.6	32.9
3	*A. corymbifera* MTCC 390	26.9	36.4
4	*A.glauca* MTCC 928	27.0	08.5
5	*Absidiasp* MTCC 148	28.6	31.7
6	*A.blakeshleana* MTCC 148	19.4	31.3
7	*A. repenses* MTCC 1327	28.6	51.4
8	*A. pseudocylindrophora* MTCC 550	20.6	29.3
9	*A.corymbifera* MTCC 1549	42.60	27.5
10	*A.corymbifera*MTCC–3132	42.60	27.5
11	*A.corymbifera*MTCC 1327	22.2	36.1

CONCLUSION:–omega 6 fatty acids play a key role in human metabolism but, because of various factors common in the Western societies, many people may be suffering from some degree of functional deficiency. There is therefore a case for some form of supplementation with oils containing GLA, which can alleviate this deficiency. Such supplements have been available and successful for many years in the health food market and are well understood by consumers. The time may therefore be ripe for their use to be extended into a wider range of 'enhanced' foodstuffs.The GLA production fromspsof*Absidia* could be enhanced by optimizingthe agricultural by-product substrates and culture condition, i.e.,C:N ratio by 60:1 %.

However, if kept fresh, polyunsaturated fats can provide many health benefits. They have little cholesterol and can decrease heart disease risk if eaten in place of saturated fats. They also carry essential fats that our body cannot produce but must consume, such as fatty acids omega-6 and the DHA-containing omega-3.

Significance and Impact of the Study: Low cost GLA production process wasachieved, and fermented product containing GLA can be incorporated into foodas additives without further expensive plant oils.

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