Production of γ-Linolenic Acid by *Rhizopus nigricans* SSSD-8

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Abstract : A gamma linolenic acid (GLA, cis-6,9,12-Octadecatrienoic acid) producing fungus was isolated and identified for the first time as *Rhizopus nigricans*, labeled as SSSD-8.

The alteration of carbon and nitrogen sources on the production of GLA by *Rhizopus nigricans* SSSD-8 was examined in flask cultures for two separate media. The cultivation conditions were optimised for GLA production by appropriate selection of carbon and nitrogen sources and their concentrations, temperature, time of incubation, and pH.

On an identical weight basis, soluble starch is the best carbon source and urea the most preferred nitrogen source for GLA yield irrespective of the media composition. A maximum GLA yield of about 1640 mg/L was obtained for *Rhizopus nigricans* SSSD-8 when incubated at 30°C and at pH 5.5 in the medium containing 15% (w/v) potato extract, 15% (w/v) soluble starch, 0.5% (w/v) yeast extract, and 0.2% (w/v) of urea.

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Key words : Gamma Linolenic acid, *Rhizopus nigricans* SSSD-8, starch, urea and GLA yield

1 Introduction

γ- linolenic acid (Gamma Linolenic Acid) i.e. all cis-6,9,12-octadecatrienoic acid, is an intermediate in the transformation of linoleic acid into prostaglandins(PG), which play very important roles in the body. In a variety of diseases which include diabetes, virus infections, atopic eczema, premenstrual syndrome and aging, the activity of the Δ-6 desaturase enzyme that converts linoleic acid into endogenous GLA, the rate determining step of the PG synthesis system, is low or impaired. From this point of view, GLA has wide therapeutic, pharmaceutical, medicinal as well as dietary applications.

GLA, or a lipid containing it, is conventionally obtained by extraction of the seeds of plants, e.g. evening primrose, borage, black current, gooseberry, and red current (1). Microbial production of a lipid rich in GLA has been attempted widely and several strains of *Mortierella* (2,3), *Mucor* (4), *Cunninghamella* (5), *Rhizopus* (6), *Thamnidium* (7), and *Absidia* (8) genera could serve the purpose.

Influence of carbon sources on growth, lipid content and fatty acid composition in four strains of Mucorales (9) such as *Mucor mucedo*, *Mucor plumbeus*, *Mortierella ramanniana* and *Rhizopus arrhizus* were studied. Glucose was converted into GLA well by all the strains of *Mucorales* investigated. *M. mucedo* 1384 grew well on glycerol and produced 0.58 g GLA/100 g of glycerol. For all Mucorales the GLA yield on lactose was very low. Soluble starch acted as an excellent carbon source for *Mortierella ramanniana* 1022 and *Rhizopus arrhizus* VUPL 23. The highest amount of GLA (26% of total fatty acids) was found in starch grown cells. Another strain, *Cunninghamella echinulata* CCRC 31840 (5) produced GLA to the extent of 964 mg/L after 5 days of cultivation at 20°C in a medium containing 10% soluble starch as the carbon source.

Apart from the expensive yeast extract as a complex nitrogen source, simple nitrogen sources for microbial production of lipids have also received attention from numerous workers (10,11). Urea as the most effective

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nitrogen source for GLA production has been observed in *Cunninghamella echinulata* CCRC 31840 (5) and in several other microbes.

In the present study a particular fungal strain capable of synthesising GLA has been isolated and identified. The effects of various carbon and nitrogen sources on the GLA production and the optimisation of culture conditions for GLA production by the fungus have been reported.

2 Experimental

2·1 Microorganism

Several samples of soil were collected from the vegetable oil industry of Calcutta, West Bengal, India. Different fungi were isolated from the soil by serial dilution and purified on Potato-dextrose-yeast (PDY) agar slants at 30°C. The PDY medium contained 10 ppm of tetracycline to eliminate bacterial growth.

Of all the fungal isolates, a particular fungal strain was found to contain GLA to a considerable extent. The fungus was identified. The fungal cultures were prepared and mounted on slides for observation under microscope. The reproductive parts like conidia, conidiophore and vesicles were measured and compared with the specifications given in manuals (12).

2·2 Cultivation of Fungus

The fungal culture was grown in two different media (60 ml in a 250 ml conical flask) for 6 days at 30°C in an incubator with shaking (120 strokes/min). The composition per litre of the two basal inoculum media consisted of the following :

Medium I : Dextrose, 100 g ; yeast extract, 5.0 g ; KH_2PO4, 1.0 g ; NaCl, 2.0 g ; $MgSO4.7H_2O$, 0.25 g.

Medium II : Potato extract 200 g, (prepared by boiling 200 g of sliced potato in distilled water for 25 min, followed by filtration through a filter cloth that maintained the pulp ; the volume of the filtrate broth was completed to 1000 ml) ; dextrose, 100 g ; yeast extract, 5.0 g.

A few culture sets of the two media were initially maintained at 30°C for 2 days followed by the incubation of the culture flasks at 5°, 10°, 15°, 20°, 30°, 32°, 35° and 40°C for four days.

Culture flasks of the two media, medium I and medium II, were maintained at different pH of 4-8.

A culture age study was made by incubating culture flasks for 2, 3, 4, 5, 6, 7, 10, 15, and 20 days.

The fungus was then inoculated in several sets of media I and II where dextrose was substituted with other carbon sources such as sucrose, soluble starch, glycerol and lactose, and also grown in various concentrations of carbon sources as 20, 50, 100, 150, 170 and 200 g per litre of medium.

The effect of nitrogen sources at 0.2% (w/v of medium) level as of NH_4NO_3, NH_4Cl, Urea ($CO(NH_2)_2$), $(NH_4)_2SO_4$, and KNO_3 in the media I and II (dextrose being substituted with soluble starch) at the most favourable medium conditions of temperature, pH and culture was examined.

2·3 Biomass and Lipid Content Determination

After fermentation, the fungal mycelium was harvested by filtration, washed three times with 50 ml distilled water, and gently dried at 30°C in a vacuum oven for 5 hours. The dry fungal mass was weighed (moisture content of biomass being determined). Then the cell mass was subjected to solvent extraction in a Soxhlet apparatus with chloroform : methanol (1:1). After extraction the solvent was removed under a stream of nitrogen, the extract was dried under vacuum and finally weighed to determine the lipid content.

2·4 Fatty Acid Analysis

The fatty acids of the lipids were methylated (13) and the composition of the fatty acids was then determined by gas-liquid chromatography (GLC) of their methyl esters. The double-bond index (DBI, (Δ/mol), or degree of lipid unsaturation was calculated (14). Data are averages of three determinations.

The isolated fungal lipid was mainly a mixture of phospholipid and triglyceride which was confirmed on a TLC plate (Silica gel-G, solvent system- hexane : diethyl ether-70:30) using phosphate staining. The phospholipids and triglycerides 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 fatty acid composition of the triglycerides and phospholipids were also determined.

3 Results and Discussion

The fungus was identified as *Rhizopus nigricans* and labeled SSSD-8 (**Fig. 1**).

When grown on Potato-Dextrose-Yeast (PDY) medium the fungus showed a fatty acid composition containing 25.6% palmitic acid, 35.7% oleic acid,

22.9% linoleic acid, and 15.8% GLA.

3·1 Effect of Temperature on GLA Production by *Rhizopus nigricans* SSSD-8

The influence of temperature on biomass, lipid content of biomass, GLA content of lipid, and GLA yield of SSSD-8 isolate was studied at 5°C to 40°C at an interval of 5°C in the medium containing 150 g/L soluble starch. Initially, to allow minimum growth and lipid synthesis of the fungus, the culture flasks were all kept at 30°C for two days, and they were then incubated at the stipulated temperatures. As shown in **Table 1**, the biomass, lipid in biomass and GLA yield of the fungus SSSD-8 increased gradually from 5° to30°C and decreased thereafter. The optimum temperature for growing the fungus *R. nigricans* SSSD-8 in order to obtain the maximum GLA yield is 30°C. The observation is quite comparable with that recorded for *Mucor* sp. M-595 (FERM-9680) (15) and *Mucor circinelloides* I.M.I. 307741 (16) showing the highest GLA production at 25-30°C.

3·2 Effect of Initial Medium pH on GLA Yield by *Rhizopus nigricans* SSSD-8

The data in **Table 2** reveal that the cell mass, lipid concentration and GLA yield were maximum at pH 5.5 for the media used.

The observations are, however, contrary to *Mucor circinelloides* I.M.I. 307741 (16) and *Rhizopus arrhizus* (6) where maximum GLA content in lipids were obtained at pH 4.0 and pH 7.0, respectively. The fungus *R. nigricans* SSSD-8 synthesised the maximum GLA yield in its lipid at pH 5.5.

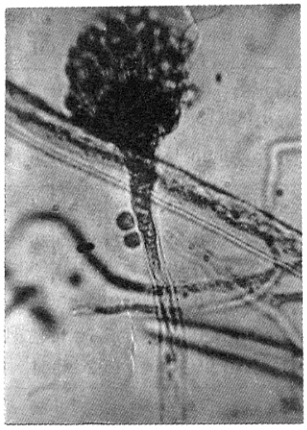

Fig. 1 Photograph of *Rhizopus nigricans* SSSD-8 as Observed under the Microscope.

3·3 Effect of Culture Age on GLA Yield by *Rhizopus nigricans* SSSD-8

The period of cultivation has a positive effect on the biomass production, lipid accumulation in biomass and the incorporation/enhancement of GLA yield per litre of medium as shown in **Table 3**. The lipid accumulation in the biomass continued to a maximum till 6 days beyond which both declined significantly. These results are quite similar to the reports of *CunninghamelIa echinulata* CCRC 31840 (5) where the GLA yield in the medium went along with lipid accumulation, and the maximum yield of 964 mg/L was obtained after 5 to 6 days of fungal growth.

3·4 Effect of Carbon Source on GLA Production by *Rhizopus nigricans* SSSD-8

The effects of various carbon sources (2%, w/v) on the biomass, lipid content, GLA content and GLA

Table 1 Effect of Incubation Temperature on Growth, Lipid Content and GLA Yield by *Rhizopus nigricans* SSSD-8, Medium I and II.

Temp. (oC)	Biomass (g/L)		Lipid in Biomass (%, w/w)		GLA in lipid (%, w/w)		GLA yield (mg/L)		DBI (Δ/mol)	
	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II
5	6.6±0.36	8.2±0.22	9.9±0.22	12.4±0.02	9.5±0.07	7.2±0.09	62.1±0.94	3.2±0.04	1.03±0.00	1.47±0.01
10	7.2±0.23	8.9±0.22	10.2±0.56	12.8±0.07	9.9±0.08	7.4±0.05	72.7±1.18	84.3±0.22	1.05±0.00	1.53±0.02
15	10.8±0.43	12.8±0.38	18.9±0.38	16.3±0.14	10.2±0.17	9.5±0.02	208.2±1.25	98.2±0.38	1.20±0.01	1.61±0.01
20	16.9±0.42	18.6±0.19	20.2±0.52	8.4±0.37	11.0±0.01	1.1±0.03	375.6±0.74	379.9±0.45	1.28±0.05	1.36±0.01
25	18.6±0.19	22.4±0.21	21.3±0.26	21.3±0.53	11.1±0.03	1.8±0.00	439.8±0.67	688.8±1.01	1.31±0.01	1.51±0.02
30	21.7±0.21	27.8±0.12	27.9±0.22	26.1±0.02	14.2±0.04	13.8±0.00	859.7±0.51	979.5±0.03	1.53±0.01	1.58±0.02
32	21.6±0.19	27.0±0.08	27.6±0.35	25.9±0.01	14.2±0.04	13.8±0.00	846.5±0.62	965.0±0.07	1.53±0.01	1.58±0.01
35	21.5±0.67	26.9±0.07	27.4±0.13	26.0±0.01	14.2±0.53	13.6±0.00	836.5±3.15	951.2±0.07	1.47±0.01	1.49±0.01
40	17.1±0.24	23.0±0.04	19.2±0.45	21.0±0.69	11.1±0.01	12.6±0.01	364.4±0.84	608.6±0.09	1.30±0.04	1.39±0.01

Table 2 Effect of Initial Medium pH on Growth, Lipid Content and GLA Yield by *Rhizopus nigricans* SSSD-8, Medium I and II.

Initial medium pH	Biomass (g/L)		Lipid in Biomass (%, w/w)		GLA in lipid (%, w/w)		GLA yield (mg/L)		DBI (Δ/mol)	
	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II
4	9.5±0.49	12.3±0.80	14.6±0.54	14.6±0.87	8.1±0.03	8.2±0.02	112.3±0.89	147.3±0.66	1.16±0.01	1.51±0.01
5	19.5±0.20	16.4±0.71	21.9±0.28	19.5±0.41	13.9±0.04	11.9±0.01	593.6±0.21	380.6±0.32	1.39±0.01	1.70±0.00
5.5	21.6±0.20	27.8±0.53	27.8±0.22	26.2±0.00	14.4±0.03	13.7±0.00	864.7±0.32	997.9±0.06	1.53±0.01	1.48±0.00
6	18.5±0.19	24.8±0.13	19.9±0.22	26.0±0.00	21.6±0.02	13.5±0.00	795.2±0.34	870.5±0.21	1.45±0.01	1.52±0.00
7	17.3±0.21	23.0±0.29	19.7±0.41	21.2±0.23	15.6±0.08	11.8±0.03	531.7±0.81	575.4±0.51	1.38±0.04	1.41±0.01
8	10.2±0.41	12.0±0.72	15.2±0.41	15.6±0.29	12.3±0.01	9.3±0.07	190.7±1.61	174.1±0.23	1.22±0.01	1.69±0.01

Table 3 Time Course of Biomass, Lipid Concentration and GLA Production by *Rhizopus nigricans* SSSD-8, Medium I and Medium II.

Culture age (days)	Biomass (g/L)		Lipid in Biomass (%, w/w)		GLA in lipid (%, w/w)		GLA yield (mg/L)		DBI (Δ/mol)	
	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II
1	2.6±0.82	3.1±1.91	6.2±0.94	9.2±0.04	5.3±0.00	2.2±0.00	8.54±0.78	62.7±1.36	1.0 ±0.00	1.10±0.00
2	6.5±0.21	8.3±1.75	9.0±0.99	12.4±0.21	7.9±0.00	7.1±0.00	46.2 ±0.31	73.1±1.61	1.22±0.00	1.20±0.00
3	17.5±0.22	12.3±0.99	12.3±1.8	14.2±0.91	10.9±0.00	9.2±0.01	234.6 ±0.08	160.7±0.81	1.31±0.00	1.12±0.00
4	19.1±0.01	15.4±1.45	18.5±2.01	16.2±1.41	12.4±0.00	11.7±0.00	438.2 ±1.94	291.9±1.96	1.3 ±0.00	1.30±0.01
5	20.7±0.03	26.8±1.00	23.9±0.92	24.6±0.32	13.2±0.00	13.3±0.01	653.0 ±0.27	876.8±1.42	1.51±0.00	1.25±0.01
6	21.6±0.00	27.9±0.58	27.9±0.05	25.9±0.33	14.3±0.00	13.8±0.00	861.8 ±0.05	997.2±0.54	1.53±0.00	1.23±0.00
7	21.0±0.00	26.7±0.43	27.0±0.87	21.2±0.45	11.3±0.00	13.0±0.00	640.4 ±0.36	735.9±1.61	1.37±0.00	1.20±0.00
10	20.5±0.01	26.7±0.42	23.8±0.76	19.3±0.77	10.9±0.00	11.3±0.00	531.8 ±0.81	582.3±2.9	1.25±0.00	1.15±0.00
15	20.5±0.00	26.2±1.79	21.7±0.00	16.1±0.15	10.4±0.00	10.0±0.00	462.6 ±0.01	421.8±1.82	1.35±0.00	1.17±0.00
20	20.5±0.00	26.0±0.96	21.0±0.01	15.2±0.28	10.0±0.00	9.1±0.00	430.5 ±0.06	359.6±1.81	1.37±0.00	1.12±0.00

Table 4 Effect of Different Carbon Sources (2%, w/v) on Biomass, Lipid Content and GLA Yield by *Rhizopus nigricans* SSSD-8, Medium I and II.

C-Source	Biomass (g/L)		Lipid in Biomass (%, w/w)		GLA in lipid (%, w/w)		GLA yield (mg/L)		DBI (Δ/mol)	
	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II
Dextrose	6.9±2.57	7.5±1.00	14.9±0.08	15.6±0.94	15.8±0.00	16.1±0.00	162.4±2.18	188.4±1.04	1.51±0.00	1.39±0.00
Sucrose	7.0±1.97	8.7±1.01	13.8±0.77	14.8±0.79	13.6±0.00	15.7±0.00	131.4±1.63	202.2±1.83	1.23±0.00	1.32±0.01
Lactose	1.9±1.24	2.1±0.97	3.9±0.25	4.0±1.21	14.7±0.00	15.0±0.00	10.9±1.43	12.6±1.91	1.14±0.00	1.21±0.00
Glycerol	6.5±0.95	7.3±1.41	7.3±1.31	8.5±1.01	12.9±0.00	13.2±0.00	61.2±1.27	81.9±1.43	1.21±0.00	1.27±0.00
Soluble Starch	7.2±0.23	9.2±0.13	15.5±0.44	16.3±0.86	16.8±0.00	16.6±0.00	187.5±0.81	248.9±0.84	1.40±0.00	1.18±0.00

yield of *R. nigricans* SSSD-8 are included in **Table 4**. It can be noted that soluble starch is the best carbon source for GLA production by the fungus. The results are in close agreement with the earlier reports on *Mortierella ramanniana* 1022 and *Rhizopus arrhizus* VUPL 23 (9) where soluble starch served as an excellent carbon source for GLA production. However, the results are contrary to the recent report on *Cunninghamella echinulata* CCRC 31840 (5), in which higher biomass, lipid contents and GLA yield were obtained with starch grown cells, but the GLA content in lipid was lower. *Rhizopus nigricans* SSSD-8 when grown on an easily assimulatable substrate like dextrose favoured GLA yield and sucrose yields with almost identical amounts of biomass and lipid in biomass, but the GLA content and GLA yield differed. On the other hand, starch promoted biomass, lipid in biomass, and GLA yield, and the growth of this strain on lactose and glycerol resulted in a poor GLA yield. The most unsaturated lipid as indicated by DBI values was produced with dextrose as the carbon source.

The reason that soluble starch is favoured is that perhaps a gradual formation at a comparatively lower concentration of glucose from starch has avoided enzyme inhibition and favoured instead the biochemical pathways for conversion of carbohydrate to lipid and to GLA synthesis, thus causing enhanced GLA accumulation in the starch medium.

3·5 Effect of Soluble Starch Concentration on *Rhizopus nigricans* SSSD-8

Upon altering the concentration of soluble starch from 2 to 20% in the medium (2, 5, 10, 15, 17 and 20%) while keeping the concentration of nitrogen source fixed, it was seen in **Table 5** that cell mass, lipid content and GLA content of the fungus increased with a rise in starch concentration from 2 to 15% (w/v) and decreased thereafter. This behaviour is quite close to that of *Mucor circinelloides* HUT 1121 (17) where the carbon source at a concentration of 150 g/L in the medium resulted in the highest amount of GLA production by the fungus.

3·6 Effect of Nitrogen Source on GLA Production by *Rhizopus nigricans* SSSD-8

The influence of different nitrogen sources, ammonium nitrate, ammonium chloride, urea, potassium

Table 5 Effect of Starch Concentration on Biomass, Lipid Content and GLA Yield by *Rhizopus nigricans* SSSD-8, Medium I and II.

Starch (%, w/w)	Biomass (g/L)		Lipid in Biomass (%, w/w)		GLA in lipid (%, w/w)		GLA yield (mg/L)		DBI (Δ/mol)	
	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	MediumII
2.0	7.2±1.12	9.2±1.39	15.5±1.35	16.3±1.23	16.6±0.00	16.6±0.00	185.3±1.42	248.9±3.81	1.41±0.00	1.18±0.00
5.0	12.1±1.91	12.9±1.84	16.4±2.72	20.7±3.11	15.3±0.00	15.3±0.00	303.6±3.82	408.6±4.16	1.41±0.00	1.05±0.00
10.0	20.1±0.31	18.4±1.01	23.3±2.53	22.1±1.97	14.9±0.00	14.2±0.00	697.8±2.14	585.3±3.18	1.39±0.00	1.01±0.00
15.0	21.6±0.26	27.8±0.06	27.9±1.11	25.9±1.11	14.3±0.00	13.8±0.00	861.8±1.01	993.6±1.01	1.51±0.00	1.23±0.00
17.0	24.5±0.71	23.2±0.29	22.1±1.21	21.1±1.22	9.1±0.00	13.0±0.00	492.7±2.41	636.4±1.54	1.30±0.00	1.22±0.00
20.0	16.1±2.51	22.8±1.78	16.1±2.18	20.2±2.18	8.0±0.00	12.0±0.00	217.7±4.61	552.7±3.16	1.16±0.00	1.19±0.00

Table 6 Effect of Different Nitrogen (N)Sources on Biomass, Lipid Content and GLA Yield by *Rhizopus nigricans* SSSD-8, Medium I and II.

N-Source	Biomass (g/L)		Lipid in Biomass (%, w/w)		GLA in lipid (%, w/w)		GLA yield (mg/L)		DBI (Δ/mol)	
	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	Medium II	Medium I	MediumII
NH_4NO_3	20.6±0.35	22.4±0.24	13.8±0.47	14.1±0.17	18.2±0.00	17.4±0.00	517.4±1.20	543.6±0.35	1.42±0.00	1.40±0.01
NH_4Cl	21.6±0.24	30.8±0.31	27.9±0.01	25.7±0.23	14.3±0.00	13.2±0.00	861.8±0.34	963.5±0.62	1.31±0.01	1.32±0.00
Urea	20.2±0.29	27.1±0.09	17.6±0.01	25.0±0.03	26.9±0.00	24.2±0.00	956.3±0.30	1638.7±0.23	1.51±0.01	1.40±0.02
KNO_3	20.7±0.21	23.8±0.12	6.87±0.12	8.4±0.09	16.8±0.00	15.0±0.00	196.2±0.32	259.9±0.07	1.39±0.01	1.37±0.00
$(NH_4)_2SO_4$	20.1±0.01	20.4±0.10	10.1±0.11	10.8±0.18	9.2±0.00	8.5±0.00	186.7±0.21	187.3±0.21	1.24±0.01	1.18±0.01

nitrate, and ammonium sulphate on the biomass, lipid content, GLA production, and DBI values by *R. nigricans* SSSD-8 were compared on the same weight (2 g/L) basis as shown in **Table 6**. All of the five nitrogen sources provided good growth to the fungus. The poorest lipid contents (6.87%±0.12 in medium I and 8.4%±0.09 in medium II) and richest lipid contents (27.9%±0.01 in medium I and 25.7%±0.23 in medium II) were found from the media containing KNO_3 and NH_4CI as nitrogen sources, respectively. This observation is just opposite to that of Hansson and Dostalek (18), who found that the lipid content of *M. ramanniana* was 67% higher on KNO_3 than on NH_4CI. The GLA content in the lipid of *R. nigricans* SSSD-8 when grown in NH_4CI was only 14.3% for medium I and 13.2% in medium II, but that on KNO_3 was 16.8% for medium I and 15.0% in medium II. This result suggests that the nitrogen source, which favours lipid accumulation, may not be the best choice for GLA production. This observation is in agreement with that observed for *Cunninghamella echinlata* CCRC 31840 (5), where potassium nitrate provided a far better lipid yield than urea, though the latter caused a much higher GLA concentration in lipid compared to the former.

But amongst all of the N-sources used, urea was found to be the best in influencing GLA production for *R. nigricans* SSSD-8. The GLA contents in the lipid (26.9% in medium I and 24.2% in medium II) and the GLA yield (956.3±0.30 mg/L in medium I and 1638.7±0.23 mg/L in medium II) with urea were the highest. The DBI value was the highest for urea as an N-source for *R. nigricans* SSSD-8, just as *Cunninghamella echinulata* CCRC 31840 (5).

The probable reason that can be suggested in favour of urea is that a very high nitrogen percentage (47% of the total molecular weight) as compared to the other nitrogen sources has created an inert atmosphere that has prevented the oxidation of unsaturated lipids.

Table 7 Effect of Urea Concentration of the Medium on GLA Content of *Rhizopus nigricans* SSSD-8, Medium I and II.

Urea (%, w/v)	GLA in lipid (%, w/w)	
	Medium I	Medium II
0.10	21.7±3.42	23.0±0.46
0.15	23.2±1.45	24.0±0.98
0.20	26.9±0.09	24.2±0.07
0.25	26.3±1.57	24.2±0.76
0.30	26.0±0.53	24.0±2.89

Table 8 Fatty Acid Composition of the Triglyceride and Non-Triglyceride Fractions of the Lipid from *Rhizopus nigricans* SSSD-8, Medium I and II.

Fatty acid	Fatty Acid (%, w/w)			
	Triglyceride		Phospholipid	
	Medium I	Medium II	Medium I	Medium II
$C_{16:0}$	31.7±0.34	27.2±0.96	6.2±0.37	11.0±0.71
$C_{18:0}$	4.1±0.97	5.7±0.41	0.5±0.02	4.7±0.81
$C_{18:1}$	40.6±0.33	39.1±0.47	31.6±0.51	20.3±0.24
$C_{18:2}$	17.2±0.17	19.2±0.14	35.7±0.21	40.9±0.37
$C_{18:3(\gamma)}$	6.4±0.21	8.8±0.31	26.0±0.75	23.1±0.11

3·7 Effect of Urea Concentration on GLA Yield by *Rhizopus nigricans* SSSD-8

By varying the concentration of urea from 1 g/L to 3 g/L it is observed as shown in **Table 7** that the GLA content and the GLA yield of the fungus is highest at 2 g/L (0.2%, w/v) of urea as the N-source of the medium.

On the basis of the above observations it can be stated that after 6 days of incubation in a medium containing 150 g soluble starch, 100 g crushed potato extract, 50 g yeast extract, and 20 g urea at pH 5.5 and at 30°C, *Rhizopus nigricans* SSSD-8 is capable of yielding GLA to the extent of 1638.7 mg/L±0.23. Thus the Potato-dextrose-yeast (PDY) growth medium proved to be better for GLA production by *Rhizopus nigricans* SSSD-8 as compared to medium I, which is conventionally used for the growth and poly unsaturated fatty acid production of fungus.

3·8 Fatty Acid Composition of the Triglyceride (TG) and Phospholipid Fractions of the Lipid from *Rhizopus nigricans* SSSD-8

Column chromatographic separation of the lipids showed that the triglyceride formed 70-75% and the phospholipid formed 16-20% of the total lipid.

The fatty acid composition of the TG part and phospholipid part of the lipid produced in medium I and II by *Rhizopus nigricans* SSSD-8 is shown in **Table 8**. The GLA content in the TG part is 6.4%±0.21 in medium I and 8.8%±0.31 in medium II and in the phospholipid part it is 26.0%±0.75 in medium I and 23.1%±0.11 in medium II. Thus it is evident that GLA accumulation is higher in the phospholipid fraction. This observation is quite close to that of Kawashima *et al.* (19) where a higher amount of

PUFA accumulated in the phospholipid in the case of IS-4 compared to the TG portion.

The poly unsaturated fatty acids form an integral part of the microbial membrane and since phospholipids constitute the major part of membrane lipids the concentration of GLA in phospholipids is higher.

4 Conclusion

Rhizopus nigricans SSSD-8 has an enormous potentiality for exploitation in producing gamma linolenic acid (GLA) as evident from the yield of GLA when compared with the cultures of other microbial species provided in the literature.

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