SUMMARY & CONCLUSION
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Single cell oil (SCO) in the recent past, has become an accepted biotechnologically important product, fulfilling major roles in human health. They are also become very essential for nutrition of infants as well as geriatrics for overall maintenance. SCO are the edible oils extracted from microorganisms. As the single celled entities they are all at the bottom of the food chain (Zui and Ratledge 2005). Yeasts, fungi and several algae are able to produce high levels of nutritionally and pharmaceutically important SCO, rich in PUFAs (Cohen & Ratledge 2005; Waltermann et al. 2009).

In this study, *M. rouxii* CFR-G15, a zygomycetous fungi producing high amount of gamma linolenic acid was isolated from soil and has been subjected various experimental conditions to produce maximum lipid and GLA in its biomass.

In this section an overall conclusions from each study have been described:

In order to obtain native isolate for GLA production, 250 soil samples from various habitats (river banks, humus soil, forest agricultural land, Zoo zone, garden and pond) were collected and screened for oleaginous fungi with special reference to *Mucor* spp. Normally, *Mucor, Rhizopus, Aspergiluus and penicillium species* were observed from the above soil samples. Higher percentage (40%) of *Mucor* species were noticed among the fungal population. All *Mucor* spp. were saprophytic and proteolytic in nature and they grew faster on the nutrient rich medium when compared to other fungi.

Cottony hairy with white or grey coloured growth was the morphological character of *Mucor* sp. Later these *Mucor* spp. were identified with standard culture obtained from MTCC Bank, confirmation was also performed using Gilman manual of soil fungi and further confirmed by molecular methods (Fig. 4.1.1) using rRNA analysis and Δ^6^-desaturase gene cloning and sequencing analyses.
All *Mucor* species isolated through screening methods were observed for their oleagenecity by qualitative method using Sudan B black stain and quantitatively by gravimetric method, respectively.

Mycelia with highly stained fat globules inside were selected for further study. About 20 *Mucor* isolates along with the standard cultures were cultivated in fat production medium by submerged fermentation to find out the growth characteristics, total lipid production and GLA content. Among all the 20 isolates, CFR-G15 showed higher biomass production (8.82±0.93 g/L) on dry basis, lipid percentage (30±1.32%) and GLA (14.42±0.74 as % of fatty acids) content.

Optimization of cultural conditions for maximum biomass, lipid and GLA content of *Mucor rouxii* CFR-G15 (screened and selected as potent culture for GLA production) was carried out. The cultivation conditions for GLA production were optimized by appropriate selection of pH, temperature, aeration, effect of inoculum size/concentration, media composition, various carbon and nitrogen sources in the cultivation media, C:N ratio, incorporation of certain vegetable oils in the growth media and certain minerals with different concentrations. From the above experiments on optimization parameters a pH of 5.5-6.0, temperature at 28 ±2°C for good biomass buildup and 15°C for GLA formation in mycelial structure were found to be optimum.

Effect of aeration on the culture with different volumes of medium in the cultivation flask was tested. Our result concluded that 100 ml working volume in 500 ml capacity culture flasks gave maximum biomass, lipid and GLA production in *M. rouxii* CFR-G15.

Evaluation of various carbon sources for maximizing the GLA production was carried out. Carbon sources like glucose, fructose, sucrose, starch, galactose, maltose, and lactose were tested for the above purpose. The result indicated that, when glucose and soluble starch were used as carbon source individually, a good biomass build up (8.44±0.34 and 8.12±0.81 g/L) was found. Sucrose and lactose didn’t give any significant growth of *M. rouxii*
Slight variation was observed among the other carbon sources in growth of *M. rouxii* CFR-G15.

The effect of nitrogen on the production of GLA in *M. rouxii* CFR-G15 was studied by using different nitrogen compounds. They are yeast extract, peptone, ammonium sulphate, ammonium nitrate, potassium nitrate and urea. Our results concluded that the medium containing yeast extract and ammonium nitrate gave the highest cell biomass and total lipid yield and GLA content. Urea in the cultivation medium gave more biomass but very low lipid content.

Studies on C:N ratio by varying the carbon and nitrogen sources were carried out for optimal production of GLA in the fungus. Through this study, it was concluded that C:N ratio 40-80 gave maximum lipid and GLA production with *M. rouxii* CFR-G15.

Several studies indicated that addition of certain minerals such as Ca$^{2+}$, Mg$^{2+}$, Na$^{+}$, K$^{+}$, Zn$^{2+}$, Fe$^{2+}$, Cu$^{2+}$ and Mn$^{2+}$ ions at different concentrations had an effect on growth, lipid and GLA production. Our results indicated that Mg$^{2+}$, and Ca$^{2+}$ when added at 0.5 g/L in growth medium increased the concentration of GLA more than 10% in *M. rouxii* CFR-G15. Other minerals like Na$^{2+}$, K$^{+}$, Zn$^{2+}$, Fe$^{2+}$, Cu$^{2+}$ and Mn$^{2+}$ didn’t show any significant increases on these parameters.

Similarly inclusion of certain plant oils also had an effect on biomass, lipid and GLA production. Coconut, palm, sunflower, ground nut, niger, gingelly and mustard oils were added to cultivation medium. The results concluded that niger seed oil showed increase in biomass, lipid content and GLA content in the *M. rouxii* CFR-G15. When growth medium was supplemented with coconut oil and palm oil, the GLA content was found to be very low when compared to basal medium, because these oils very low linoleic acid content in their lipid. Hence we conclude that oil sources containing saturated fatty acids didn’t favour the production of GLA. It is confirmed again from our experiment that linoleic acid is the precursors for the formation of GLA and other PUFAs. Niger seed oil contains good amount of linoleic acid.
thus incorporation of this oil in the growth medium favoured the GLA production in *M. rouxii* CFR-G15.

Response surface methodology (RSM) was used with the aim of optimizing the levels of carbon and nitrogen source to maximize the GLA production in *M. rouxii* CFR-G15. Various trial experiments revealed that glucose, yeast extract and ammonium nitrate played a major role in GLA production in this fungus. The optimization study was carried out with CCR design (CCRD) with these variables. 20 experiments were carried out and central point was experimented 6 times. The results indicated that the maximum yield of biomass (12.2 g/L), lipid (39.9 %) and GLA (18.89%) observed with glucose 65 g/L, yeast extract 3.5 g/L and ammonium nitrate 0.5 g/L were used.

Strain improvement is an important criterion for microorganisms to improve the product performance. This fungus, *M. rouxii* CFR-G15, was subjected to mutation (UV, EMS and NTG) and the mutants were selected based on the auxotrophic growth requirements. Lysine (lys⁻), alanine (ala⁻), isoleusine (isoleu⁻) and methionine (met⁻) auxotrophs were selected as markers for protoplast fusion studies. Though we could produce a few auxotrophic markers, many of them have reverted back to original genetic status due to DNA autorepair mechanism. A double mutation on this fungus, have resulted in getting met⁻ auxotroph permanently. This marker was used as selection criterion for hybrid selection.

Intrastrain/intraspecific protoplast fusion (hybridization) experiment was carried out to obtain hybrids with high lipid and GLA content. PEG 6000 at 30% level was used as fusogenic agent. By using standard protocol a few hybrids were selected. Quantitative analysis revealed that, out of 15 hybrids, CFR-HyG9 and CFR-HyG12 had more GLA content when compared to parent and met⁻ auxotroph mutant cultures of *M. rouxii* CFR-G15.
This study on GLA production using Musor sp. (Mucor rouxii CFR-G15) indicates a promising alternative/additional source for industrial scale production from conventional sources like evening primrose, borage seed oil and other potential microbial sources. Screening studies are useful identifying organisms for PUFAs production. Further study can be carried out to identify potential PUFAs producers especially GLA. Exploring the native isolate for the production of the speciality lipid GLA using biotechnological approaches is an important step. The present work enabled the identification of new fungal strains for their potential GLA production through manipulation of growth conditions, mutation and hybridization.

The genes and genes products involved in the biosynthesis of PUFAs is being identified and characterized all over the world for higher production of PUFAs from microbial sources. Additionally, gene encoding Δ6-desaturase enzyme involved in GLA biosynthesis in M. rouxii CFR-G15 was also identified. The present research work provides useful information for further work on PUFA metabolic pathways and gene engineering about GLA production from microbial sources. A potential strain was reported that could be used for the development of an economical process in industrial GLA production.
OUTCOME OF THIS RESEARCH WORK

PAPERS

Mamatha, S. S., Ravi, R. and Venkateswaran, G. “Medium optimization of Gamma Linolenic Acid production in *Mucor rouxii* CFR-G15 by RSM”. Food and bioprocess technology An International journal (Published)

Mamatha, S. S. and Venkateswaran, G. “Differential temperature effect on Gamma linolenic acid production in *Mucor rouxii* CFR-G15. Indian journal of Microbiology (Accepted)

Mamatha S S, Prakash M Halami and Venkateswaran G. “Identification and characterization of omega 6 fatty acids producing *Mucor rouxii* native isolate CFR-G15” .European Journal of lipid science and technology (Accepted)

Mamatha S S, Muthukumar S P and Venkateswaran G. “Safety evaluation of *Mucor rouxii* CFR-G15 biomass containing ω-6 fatty acids in rats”. Regulatory toxicology and pharmacology (communicated)

Papers presented in National/International Conferences/Symposia


Mamatha S. S. and G.Venkateswaran (2005) Studies on Gamma Linolenic Acid (GLA) of *Mucor* sps: Isolation and screening from soil sources. 17th Indian Convention of Food Scientists and Technologists organized by AFSTI (I), CFTRI and DFRL held between 9th and 10th December 2005 at NIMHANS, Bangalore. Best poster Award in the area of Microbiology and Biotechnology

Mamatha, S. S. and Venkateswaran, G. “Effect of extraction methods on lipid yield with special reference to γ-linolenic acid from *Mucor rouxii* CFR-G15” at the 17th ICFOST jointly organized by CFTRI, and
AFST (I) held between 16th and 17th of Nov-2006 at Agricultural university, Hyderabad Also awarded **Best poster award in the area of Biotechnology and Microbiology**

**Mamatha, S. S.** and Venkateswaran, G. “Influence of C:N ratio on the production of lipid and γ-linolenic acid by *Mucor rouxii* CFR-G15” 47th Annual Conference of AMI held at Biotechnology and bioinformatics department, Barkatulla University, in the month of Dec-2006

**Mamatha, S.S., Ravi, R. and Venkateswaran, G.** “Response Surface Optimization Of Media Components For Gamma Linolenic Acid Production By *Mucor Rouxii* CFR- G15” at the 75th Annual Conference of SBC(I) held at life sciences department, Jawaharlal Nehru University, in the month of Dec-2006


**Mamatha S S, Prakash M Halami and Venkateswaran G** (2008) “Molecular characterization of omega 6 fatty acids producing *Mucor rouxii* native isolate CFR-G15” IFCON-2008 organized by AFST(I) held at CFTRI, Mysore

**Mamatha S S, MuthuKumar S P† and Venkateswaran G** (2008) “Safety evaluation of omega 6 fatty acids containing *Mucor rouxii* CFR-G15 biomass in albino rats for food formulation” IFCON-2008 organized by AFST(I) held at CFTRI, Mysore