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BIOMASS PRODUCTION AND UNSATURATED FATTY ACID SYNTHESIS BY *NITZSCHIA* SP.

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ABSTRACT: Diatoms are the finest alternative to the natural feed stock of fuel and energy rich molecules. They are abundant in ecosystem and contains lipid as their primary reserved food material hence turns out to be a promising source of industrial fatty acids and bioactive compounds. Present study investigates potential applications of native diatom *Nitzschia sp.* isolated from Karnatak University Campus, Dharwad, further employed for the synthesis of lipids and high value fatty acids. This newly isolated diatom *Nitzschia sp.* cultivated by photoautotrophic cultivation method using f/2 growth media supplemented with $NaNO_3$, trace metals and vitamin solution. Nitrogen concentration in the media gradually lowered to enhance lipid synthesis though batch culture. Nitrogen deficient culture showed increase in lipid productivity by *Nitzschia sp*. and total lipid yield obtained is 05.751mg/L. The GC-MS analysis indicates n-hexane lipid fraction of test diatom mainly composed of decosahexaenoic acid C22:6 and myristic acid C14:0 as major fatty acids also presence of palmitic acid C16:0, steric acid C18:0, and oleic acid C18:1. These fatty acids have economic importance and applied health benefits being used as bioactive therapeutic natural compounds.

KEYWORDS: *Nitzschia sp.,* Photoautotrophic cultivation, GC-MS, Decosahexaenoic acid.

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1. INTRODUCTION

Since depletion of natural resources, uprising energy crisis, and food scarcity remain major global concern and demands to develop sustainable renewable feedstock for the production of energy and food [1]. In a present scenario microalgal research has gain much interest in microalgae based

biorefinaries in the co-production of biofuels and value added substances [2]. Some photosynthetic diatoms are promising microalgal biorefinaries for biosynthesis of energy rich molecules, biofuel as well as unique co-products rarely found in other algae such as biomaterials and biopolymers or monomers for neutraceutical and biomedical applications [3]. Despite of numerous benefits critical point of concern is large scale production of these important molecules from microalgae becomes challenging task facing various problems. Typically wide range of species sourced from a number of culture collections are difficult to adopt in outdoor environments compared to the species grown under controlled conditions unlike native strains. This may be attributes to strains acclimating to optimal conditions in the laboratory and their inability to thrive under variable outdoor conditions [4]. In contrast, native bio-prospected strains are often well adapted to the local environmental conditions in addition selection of native microalgal strains from local environments reduces bio-security risks as posed by introduction of new strains [5]**.** In developing countries bioprospecting of algal biorefinaries does not fulfill the economic feasibility of overwhelming capital investment and sustainable development of unit operations [6]. However the large scale production of microalgal biofuels faces several difficulties which make the current growth and development of the biorefinaries industry still economically unviable. Improving the economy of microalgae biorefinaries is possible by exploration and selection of native strains for scale up and development of process parameters. Present work aimed to promote sustainability of biorefinary process by evaluating native diatom species for production of unsaturated fatty acids having applications as bioactive compounds in various therapeutic treatments. Numerous studies have demonstrated that the quantity and quality of lipid can be improved by varying growth parameters such as nutrients, the pH, temperature, CO_2 concentrations and photosynthetically active radiation [7]. In the case of nutrients, nitrogen limitation is well associated with increase in lipid content in numerous species, followed by other nutrients such as iron and phosphate [8].

2. MATERIALS AND METHODS

a. Microalgae culture selection and identification

Algal samples are collected from five different ponds of KU campus Dharwad, Karnataka. Isolation of unialgal strain performed by using serial dilution technique and frequent sub culturing for each isolate [9]. Morphological observation carried out for primary identification, green microalgae *Chlorella sorokoniana, Nanochloropsis, Scenedesmus* and diatoms *Navicula, Amphora* and *Nitzschia* are observed and identified based on morphology. Out of these isolates *Nitzschia sp.* selected for the further experiments for lipid production via photoautotrophic cultivation. Pure non axenic clonal culture of the *Nitzschia sp.* is maitained on f/2 growth media [10] at 25°C temperature, followed by 16:18light and dark cycle.

b. Photoautotrophic cultivation process

Inoculum preparation

For inoculum preparation three Erlenmeyer flask of 2L capacity containing 1500mL of growth medium seeded with 10% previously grown culture of diatom. In next step 30% of this growth media used to develop the inoculum of cell density 10^{-5} to10^{-6} cells ml^{-1} for biomass production [11].

c. Biomass production under photoautotrophic conditions

Photoautotrophic cultivation continued with *Nitzschia sp*. Grown in f/2 nutrient media. Growth media supplemented with vitamin complex .Continuous nitrogen depletion strategy used where concentration of $NaNO_3$ was depleted from 0.0651g/L (650 µM) $NaNO_3$ to 0.176 g/L (1µM) this concentration depletion found to enhance lipid production [12]. Photoautotrophic cultivation continued with transferring the inoculum to the 25L PVC pet jar provided continuous aeration with filtered ambient air at 40Lmin^{-1} using line regulator valve, maintained constant temperature 25°C, light intensity of 350-400µmol$m^{-2}s^{-2}$using 15wt LED lamp [13] the 14:16 light and dark photo cycle. Each replicate flask was sampled every 48hrs for next 22 days. All the experiments are carried out in triplicates.

d. Analytical methods

Determination of ash free dry weight (AFDW) of biomass

The dry biomass obtained photoautrophic cultivation method was placed in the furnace at 500°C followed by cooling in desiccator. The weight difference of cell placing before and after in furnace is considered to be AFDW of biomass [14].

Determination of lipid

Total lipid extraction was carried out by homogenizing one gram of the biomass with 20 ml of chloroform: methanol 2:1(v/v) mixture. Cell debris then removed by filtration using Wattman No.1filter paper. Homogenate and collected cell debris washed and the phase separation brought by solvent addition as methanol: chloroform: water (10:10:8 v/v/v) followed by addition of 0.73% Nacl solution after vortexing the mixture was centrifuged at low speed (2000rpm) to separate the two phases further organic phases collected and kept overnight for solvent evaporation [15].

Fatty acid analysis

Fatty acids were analyzed by gas-liquid chromatography after fatty acid extraction from lipid by transesterification method. Fatty acid extraction begin with addition of the solvent mixture methanol:hydrochloric acid:chloroform (10:1:1: v/v/v) to 1000mg of lyophilized test diatom sample. Cells suspended by gentle vortexing and immediately heated at the temperature 90°C for 60 min. Sample then cooled to room temperature followed by addition of water 1ml per tube. Fatty acid methyl ester extracted by addition of solvent hexane:chloroform (4:1v/v 3×2ml) to the transesterification tube. Sample is then diluted with chloroform as internal injection standard and

injected into the chromatogram [16].

3. RESULTS AND DISCUSSION

Biomass and lipid productivity under phototrophic cultivation

The growth of *Nitzschia sp.* was measured with respect to cell yield under the influence of nutrient, temperature and light intensity [17]. The total dry weight of biomass obtained through phototrophic cultivation is 6.9545g/L. The growth media supplemented with trace metals and vitamin complex found to support the growth of newly isolated diatom. The nitrogen deficient media showed increase in the lipid production by *Nitzschia sp.* after lipid extraction total yield of lipid obtained is 305.751mg/L.

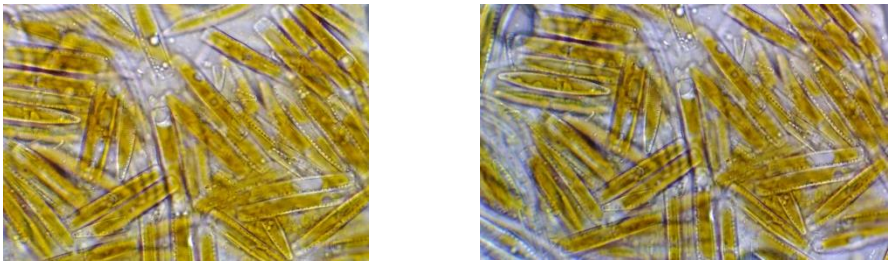

Fig 1: Light microscopic view of *Nitzschia sp.* isolated from Karnataka University Campus, Dharwad

Fatty acid analysis by GCMS

Fatty acids analysis by gas-liquid chromatography showed that n-hexane extract of the *Nitzschia sp.* contains C22:6 decosahexanoic, (wt. %+41.11) , C14:0(29.24%) myristic acid as major fatty acids along with C16:0 palmitic acid, Steric acid C18:0 and oleic acid C18:1 contribute considerably to fatty acid composition with wt% of 9.0% and 8.26% respectively.

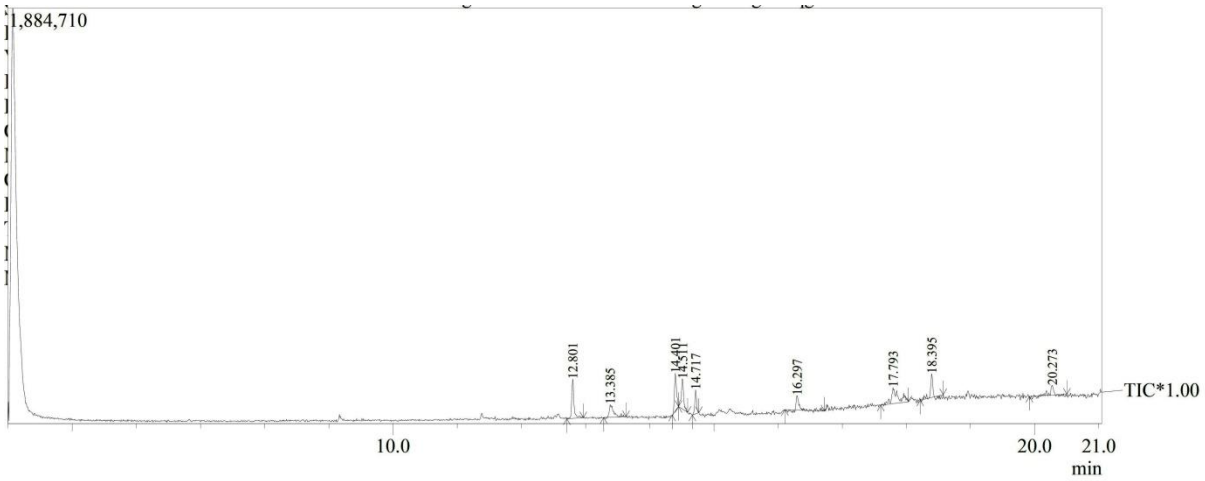

Fig 2: GC-MS chromatogram for fatty acid analysis of n-hexane fraction of lipid extracted from *Nitzschia sp.* Isolated from Karnataka university campus, Dharwad.

The diatoms are rich source of important metabolites and their extensive use in food and neutraceuticals is mainly influenced by intra cellular composition of the lipids [18]. In present work the results of morphological study shows isolate microalgae from Karnatak University Campus,

Dharwad is a pennate diatom belongs to class Bacillariophyceae and genus *Nitzschia.* The molecular study of the isolate with respect to 18S rRNA sequencing may aid species identification in detail [19]. Previous investigations on *Nitzschia* reports that ,several species have shown effective production of omega fatty acids under optimized growth conditions [20]. Several investigations on microalgae report that different strains of each species desire different culture conditions and influences enhanced production of LC-PUFA than other strains [21]. Results of nitrogen deficient photoautotrophic cultivation indicates that decrease in concentration of $NaNO_3$ from 650µM to 1µM increased biomass and lipid production by *Nitzschia sp.* total lipid yield is 05.751mg/L and dry biomass is 6.974g/L these obtained values are significant compared to earlier reports [22]. Results of the GC-MS analysis indicate that n-hexane extract of *Nitzschia sp.* contain omega fatty acid decosahexanoic acid and myristic acid as major fatty acids along with palmitic acid, steric acid and oleic acid [23] and the fatty acid composition found typical for the test diatom. The isolated species produces sufficient amount of DHA by photoautotrophic cultivation, DHA is having numerous health benefits as dietary supplement, biological activity like cardio protective and neuroprotective activity [24]. Fatty acid profile also resulted in confirming presence of myristic acid and other LC-PUFA which are convertible into biodiesel via transesterification [25]. Hence experimental results clearly indicates newly isolated diatom produces increased amount of biomass and is a rich source of polyunsaturated fatty acids, optimization of growth media by altered nutrient composition and light intensity and photoperiod for photoautotrophic cultivation helps in acclimatization study of the native strain in the outdoor environment [26]. Test diatom is suitable candidate in bioprospecting of microalgae in production of industrially important fatty acids, and serves best alternative feedstock [27].

4. CONCLUSION

This study represents the first attempt in bioprospecting for lipid containing fresh water diatom from Dharwad region. The strain isolated *Nitzschia sp.* shown significant biomass and lipid content when grown in optimized nutrient media (f/2). Nitrogen deficient strategy would work best for increase in lipid production with this strain. Among fatty acid DHA was present in highest concentration hence turn out to be promising rich source of DHA. The Isolated strain is effective alternative feed stock as renewable resources for algal based biorefinary industries.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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