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STUDY ON MARINE CYANOBACTERIUM PLACTONEMA SPECIES IN OSSEIN EFFLUENT AND THEIR GROWTH IN LIPID AND BIODIESEL PRODUCTION

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ABSTRACT

Cyanobacteria are more amenable microbial forms to genetic manipulation for installing biodiesel producing chemical pathways. These organisms have been shown to be highly tolerant to the introduction of foreign genes. Microalgae which include algal strains, diatoms, and cyanobacteria have been found to contain high levels of lipids - over 30% and upto 75%. Due to the high lipid content, these microalgal strains are of great interest in the search for sustainable sources for the production of biodiesel. The potential of this cyanobacterial fuel is the primary advantage of biodiesel that it is one of the most renewable fuels and non-toxic and biodegradable. It is powerful alternative transportation fuels as it can not only be used in existing diesel engines without modification, but also highly suitable for blending in at any ratio with petroleum diesel.

KEYWORDS: Cynabacteria, Plactonema, Oscillatoria, Ossein effluent, lipid, biodiesel.

INTRODUCTION

Biodiesel has evolved several generations since 1960's. First generation biofuels are derived from edible biomass, primarily corn and soybeans in the United States, and sugarcane in Brazil. Second generation biofuels are made from cellulosic biomass. Sources include wood residues like sawdust and other cellulosic sources. The advantage of second generation biofuels is that they are abundant and do not interfere with the production of food. Third generation biofuel includes fuel produced from algae and cyanobacteria. Biofuel produced from algae is an intriguing option. Microalgae contain lipids and fatty acids as membrane components, metabolites, storage products, and sources of energy. Microalgae which include algal strains, diatoms, and cyanobacteria have been found to contain high levels of lipids -

over 30% and upto 75%. Due to the high lipid content, these microalgal strains are of great interest in the search for sustainable sources for the production of biodiesel. The potential of this cyanobacterial fuel explored in the present study.

An appealing fourth generation biofuel is based on the conversion of algal oil to biodiesel by synthetic biology and recombinant technology. Cyanobacteria are more amenable microbial forms to genetic manipulation for installing biodiesel producing chemical pathways. These organisms have been shown to be highly tolerant to the introduction of foreign genes. Chemically biodiesel is the alkyl ester of long chain fatty acids. (Fischer *et al*., 2001). The world total biodiesel production was estimated to be around 1.8 billion liters (Fulton 2004). Although there was no increase in biodiesel production between 1996 and 1998, a sharp increase in biodiesel production was observed in the past several years. It is speculated that the production of biodiesel will be further tremendously increased because of increasing demand for fuels and ‘‘cleaner” energy globally the production of biodiesel has recently received much attention worldwide The primary advantage of biodiesel is that it is one of the most renewable fuels and non-toxic and biodegradable (Van gerpen, 2005). It has advantages as a powerful alternative transportation fuels as it can not only be used in existing diesel engines without modification, but also highly suitable for blending in at any ratio with petroleum diesel. Microalgae and cyanobacteria are potential to be used as a raw material for biodiesel production as it meets all of the aforesaid requirements. They possess high growth rate and provide high lipid fraction for biodiesel production (Song *et al*., 2008).

The Biomass productivity, lipid cell content, nutrient optimization and overall lipid productivity are some of the key parameters affecting the economic feasibility of algae oil for biodiesel production. High lipid content is produced by cell under stress, typically nutrient limitation (Li *et al*., 2008). Among the different components of the culture medium, the source and concentration of nitrogen can provoke important changes in the growth and biochemical compositions of microalgae species (Kaplan *et al*., 1986). Hence an attempt was made to grow marine cyanobacteria in Ossein effluent collected from gelatin producing industry. Ossein is the chief organic substance of the animal bone tissue that remains as a residue after removal of the mineral contents from the degreased bone by dilute acids used for gelatin preparation. Effluents were collected from Ossein production industry at three different stages of clarification and used for the study. The selected high lipid producing marine cyanobacterium was also used to remediate the Ossein effluent and explored further for biodiesel production. The study aims to find out a marine cyanobacterium that can grow in cheaper marine media and industrial effluents with high lipid production potentials.

Cyanobacteria are oxygenic photosynthetic microorganisms that have significant roles in global biological carbon sequestration, oxygen production and the nitrogen cycle. Cyanobacteria can be developed as an excellent microbial cell factory that can harvest solar energy and convert atmospheric CO_2 to useful products. Fossil traces of cyanobacteria are claimed to have been found from around 3.5 billion years ago, and most probably played a key role in the formation of atmospheric oxygen, and are thought to have evolved into present-day chloroplasts of algae and green plants (Tamagnini *et al*., 2007). Cyanobacteria and microalgae are the only organisms known so far that are capable of both oxygenic photosynthesis and hydrogen production.

MATERIALS AND METHODS

Source of Samples

Twelve marine cyanobacterial strains obtained from the Repository of National Facility for Marine Cyanobacteria, Tiruchirappalli, Tamil Nadu, India for the present study. The selected samples (strains) were observed under the microscope to identify the morphological features of cyanobacteria. The marine cyanobacterial strains chosen represent filamentous non-heterocystous types.

Culture Conditions

Extraction and Estimation of Total Protein: (Lowry *et al.*, 1951)

Marine Nutrient Medium

PH Measurement

Screening of Cyanobacteria

Estimation of Pigments

Chlorophyll- (Mackinney, 1941)

Exraction of Lipids (Folch, 1957)

Fatty acid Methyl Ester Conversion (Transesterification)

1. Saponification

Triglyceride + 3 KOH → Glycerol + fatty acids.

2. Acidification

K Salt of Fatty Acid + HCl → free fatty acid + KCl

3. Methyl Ester Conversion

Free acid (non volatile) → Methyl ester (volatile)

Optimization Studies

Optimization of physical conditions

Optimization of chemical conditions

Doubling Time

Growth of Marine Cyanobacteria in Ossein Effluents

RESULTS AND DISCUSSION

Exploring marine cyanobacteria for biodiesel production thus open a new arena in the field of cyanobacterial biotechnology to meet the mounting energy hassles of the near future. In this view, the present study has endeavoured to screen the lipid production of twelve marine cyanobacteria from the repository of National Facility for Marine cyanobacteria for biodiesel production and optimization for different nitrogen and phosphate source for enhanced lipid productivity in Ossein effluent as an approach to bio remediate. The study has looked into lipid content of twelve fast growing filamentous non- heterocystous organisms. The chosen strains were observed morphologically under inverted automated microscope (Leica DMI3000B) and found to be under the genera, *Oscillatoria* and *Spirulina* according to Cyanophyta (Desikachary, 1969)

Morphological Features at Genus Level

The following morphological features were observed for selected strains under the microscope.

Oscillatoria species

Trichome single or forming a flat or spongy free-swimming thallus, sheath absent, rarely with a more or less very delicate sheath, motile, mostly by a creeping movement causing rotation on the longitudinal axis; end of trichome distinctly marked, pointed, bent like a sickle or coiled more or less like a screw. Homogones formed by the division of the trichome. (Plate – 1 & 2).

Phormidium species

Filaments many forming a gelatinous or leathery stratum, thallus attached by the lower side, or floating in water with torn margins; sheath present, more or less firm, sometimes agglutinated, sometimes partly diffluent, thin, colourless; trichomes cylindrical, in some constricted at the joints, apices often attenuated, straight or bent, never regularly spirally coiled or non-capitates apical cell in many species with a calypta.

Phormidium corium

Thallus expanded, membrane, leathery, blackish to brownish green; filaments long, more or less flexuous, densely entangled; sheath thin, gelatining or diffluent, coloured violet by chlor-zinc-iodine; trichome blue green, not constricted at the cross-walls, ends straight, briefly attenuated, not capitable, 3-4.5µm broad; cell nearly quadrate, up to twice as long as broad, 3.4-8µm long, not granulated at the cross- walls; ends obtuse conical, calyptras absent.

Phormidium valderianum

Thallus lubricous, expanded, up to 3 cm. high, lamellate, outer surface dull green, inner surface colourless; trichrome flexous, densely entangled, not constricted at the cross- walls ends not attenutated not curved 2-2.5µm broad, blue green; sheath thin, firm, finally becoming more or less diffluent, coloured violet by chlor - zinc- iodide; cells longer than broad, 3.5-6.7µm long cross- walls with 1 or 2 granules on either side; end – cell rounded, calyptras absent.

Plectonema species Trichrome variously bent, with a thin, firm sheath; false branched, branches single or geminate; heterocytes absent; homogonous present; spores not known.

Morphologyphotos

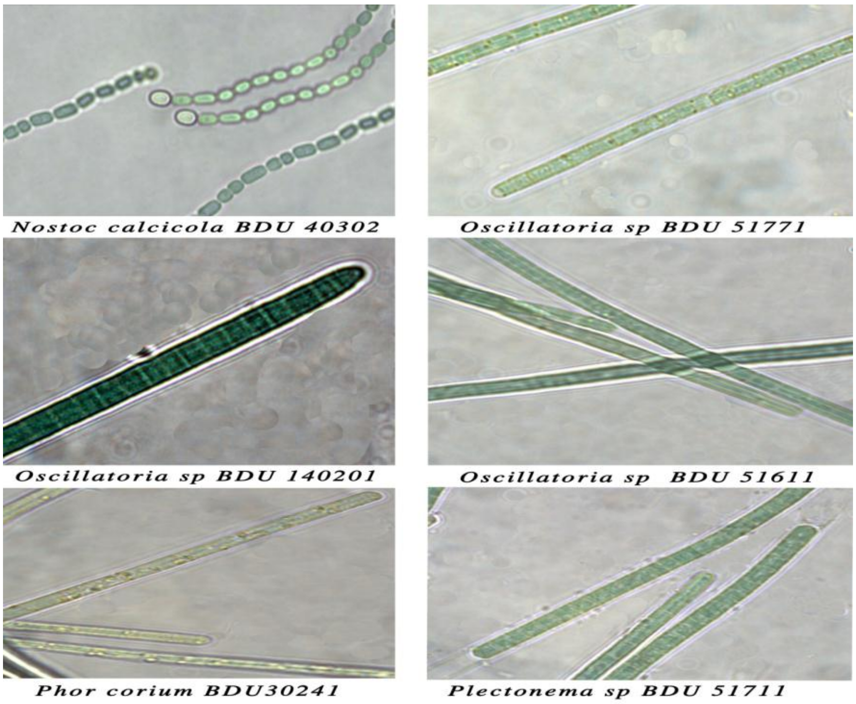


Plate-1

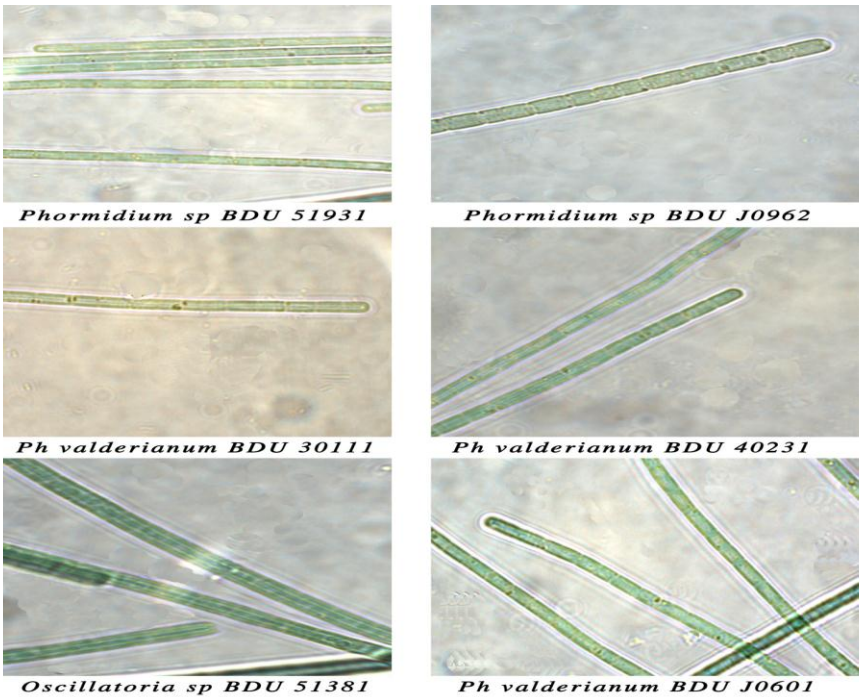


Plate-2

Lipid Productivity of the Selected Strains

The objective of the project is to choose the best lipid producing organism by gravimetric method. Each extraction was carried out in duplicates and the lipid samples were dried in

Savant Speed vac plus SC210A concentrator and flushed with nitrogen before storage in order to avoid rancidity and oxidation. The dry weight of the lipid was expressed as % lipid Gm^{-1} of dry weight. The initial result of our study came out with *Plectonema species* as the highest lipid producing marine cyanobacterial strain among the strains screened.

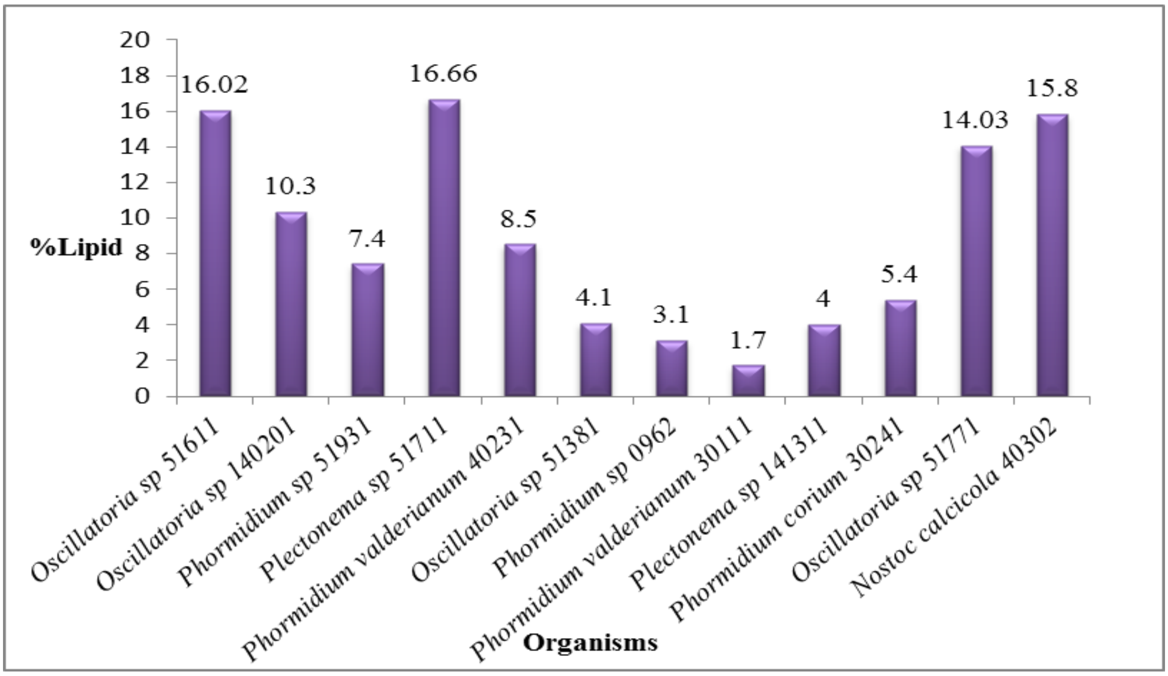
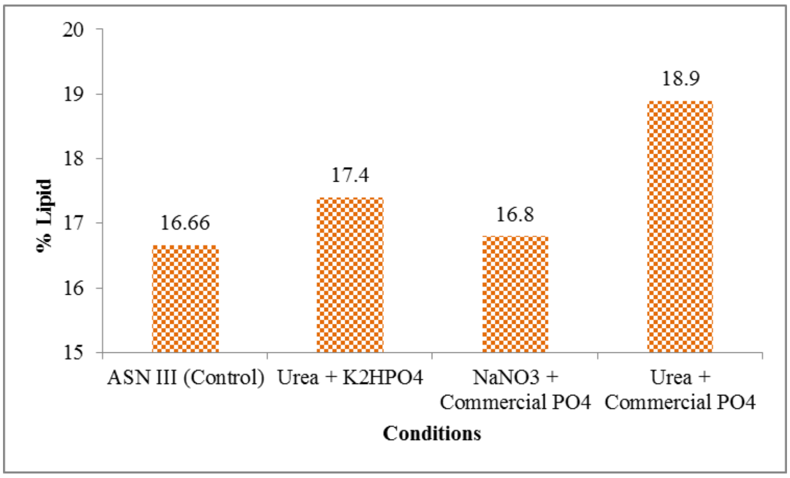


Fig. 2: Lipid content of selected Marine Cyanobacteria (% Lipid Gm-1).

From the results the organisms were categorized into three classes (Table 1) and three potent strains were identified that were capable of producing high lipid content of more than 15% and above. *Plectonema species* showed a maximum lipid production of 16.66% Gm^{-1} of dry weight, followed by *Oscillatoria species* with 16.02% Gm^{-1} of dry weight and *Nostoc calcicola* yielded 15.8% of lipid. Thus based on the yield of lipid the strains were categorized into three classes' high yielders, moderate yielders and low yielders of lipid and tabulated (Table 1). The least lipid producer of the study was reported to be *Phormidium valderianum* which showed a yield of 1.7% Gm^{-1} of dry weight. The promising strain *Plectonema species* was chosen for further studies on optimization in sea water medium and effluent based medium to explore its biodiesel potential.

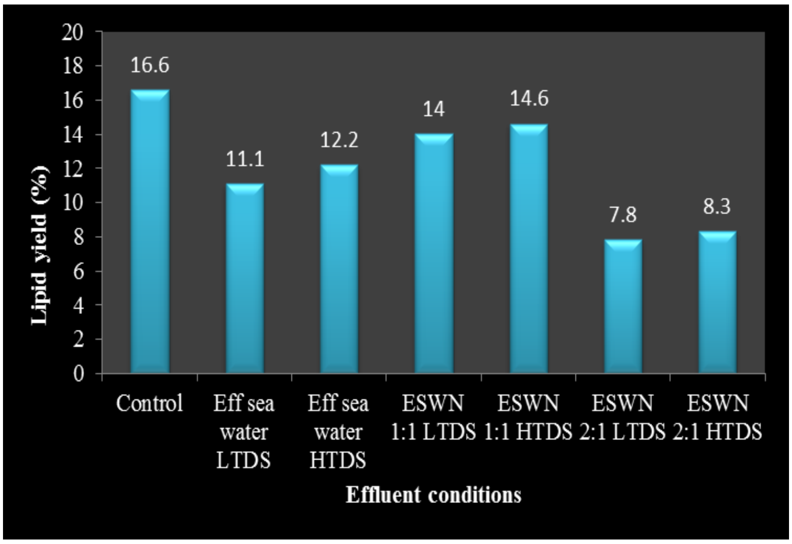
Growth of marine cyanobacterium *Plectonema sp.* in Ossein effluent for Biodiesel

Commercial phosphate (16.8%). Thus from the growth and lipid yield results, Urea and commercial PO4 was chosen as nutrient source for the formulation of effluent based seawater medium (Fig.6).



Lipid yield of *Plectonema species* in selected effluent and sea water based media

Based upon the growth profile of the selected strain in effluent and sea water based media, the combinations namely EFF- Sea water HTDS and LTDS, Effluent sea water nutrient (ESWN) 1:1 and 2:1 concentrations, with HTDS and LTDS were chosen. The organism was adapted to these effluent conditions under optimized laboratory conditions and the lipid yield was studied on the 7th day of the adapted culture. From the result the best growth and lipid accumulation condition of the *Plectonema species* in Ossein effluent identified was biodiesel production (Fig.10).



Thus our study which began with screening of marine cyanobacteria for biodiesel production found two promising strains for the same. *Plectonema species* and *Oscillatoria species* were taken for nutrient optimization experiments for enhanced biodiesel and growth studies in Ossein effluent. These findings are in laboratory and they have to be scaled up to be tried in field to reach the level of commercial application. It is a stepping stone for the upcoming technology, development of lipid production and conversion of algal biomass into fuel, turning out to be an alternative to depleting fossil fuel. Hence it suggests that cyanobacteria can be potential feedstock for biodiesel in near future.

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