

Research Article

Isolation of Microalgae Strains from Pond Water and their Medium Standardization for Lipid Production

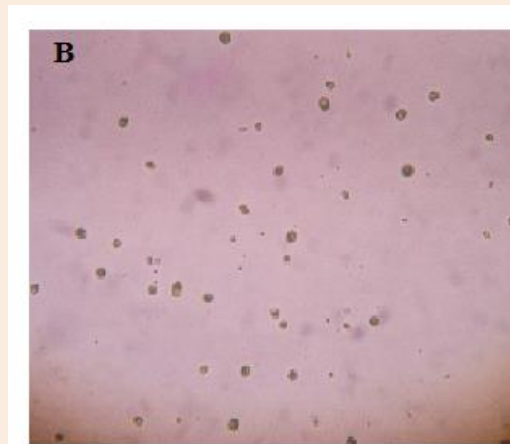
Vidyadharani Gopalakrishnan, Dhandapani Ramamurthy*

Department of Microbiology, Periyar University, Salem-11

Abstract

In the present study, microalgae were isolated from fresh pond water samples collected from Mamangam near Omalur Taluk, Salem. After performing the isolation procedure three microalgae isolates were successfully isolated. The synthetic medium namely B old Basal Medium (BBM), Tris acetate Phosphate (TAP), Bristol Medium were supplemented to all the three strains, further their biomass and lipid productivity are analysed. As a result one of the isolate namely *Chlorella* MS3 strain grown in Bristol medium found with higher percentage of total lipid. In particular that *Chlorella* MS3 strain grown in Bristol was able to store neutral lipid proportion than other two strains. It was confirmed by Nile Red imaging studies. The *Chlorella* MS3 strain was selected for lipid enhancement procedures for biodiesel feed stock preparation.

Keywords: Microalgae, fresh water, BBM, Bristol, Nile Red, Lipid

***Correspondence**

Dhandapani Ramamurthy,
Email: danpani@gmail.com

Introduction

At present the global energy need is mostly faced by traditional energy resources such as coal, petroleum based fuel and natural gas. Due to the limitation in oil reserves in certain regions of the world. According to the assumptions based on the consumption and exhaustion of oil reserves, fossil based fuel system will be exhausted in less than 50 years. This gives an extra credit to renewable energy resources.[10]

The only way to maintain the present global economic development is by implementing alternative fuels in transportation and energy sector. The alternative fuel should be technically feasible, economic and ecosystem friendly, also sustainable in nature. Among other biofuel, biodiesel possess great potential and many benefits such as reduced green house gas emission, sustainability, does not lead to food crisis and so on. The biodiesel usage will help to maintain a balance between environment and economic development. Nevertheless, the cost effective biodiesel production is still a major obstacle and it can be overcome by optimising the microalgae strain for increased lipid production.

Microalgae comprise diverse group of prokaryotic and eukaryotic organisms with great ecological importance. Based on numerous biochemical and cellular differences, two major groups of green microalgae are recognized: Chlorophyta and Conjugophyta. Microalgae contain about 50% of global organic carbon fixation. Many species are source of natural products such as pigments, enzymes, unique fatty acids, vitamins 12 and continue to be used for biotechnological applications.

Microalgae are photosynthetic miniatures, present in most of the aquatic ecosystems such as fresh, marine water, sewage to stagnant water, soil, wood and so on. They represent a promising new source of feedstock for the

production of biodiesel. While the mechanisms of photosynthesis in microalgae is similar to that of higher plants, they are often more efficient converters of solar energy to useful biochemical products like oil because of their simple cellular structure. Because the cells grow in aqueous suspension, they have more efficient access to water, CO₂ and other nutrients. In addition, numerous algal strains have capable of producing more than 50% of their biomass as lipids, sometimes even up to 80% [9]. For these reasons, microalgae are capable of producing much higher amount oil per unit area of land, compared to many terrestrial oilseed crops, such as soybean, coconut and palm.

The present work was aimed to isolate natural microalgae strains and its medium standardization for analyzing the lipid producing ability of the strains.

Experimental

Materials and Reagents

Isolation of microalgae

Initially water samples was collected from Mamangam fresh water pond, Omalur taluk, Salem district(11° 7333" latitude and 78° 06667"). The water samples were filtered through 120 micron planktonic mesh and inoculated in sterile 200 ml BBM and incubated at 25° C under 2000lux fluorescent light intensity for 21 days. The centrifugation and plating method was performed for the isolation of unialgal strains[8]. Each single colony is sub cultured and checked for its purity by subsequent microscopic examinations.

Medium standardization

All the isolates were subjected to three different medium namely BBM, Bristol, Tris Acetate Phosphate medium and incubated at the same growth condition mentioned above for the period of 21 days.

Biomass production rate

10ml of 15 days grown culture were taken and centrifuged at 5000 rpm for 10minutes and the supernatant was discarded. Total wet weight was measured initially. Then the tubes were kept at 60° C in hot air oven for half an hour. [1]

Lipid Extraction

From 5 ml of grown algal culture lipid samples were extracted using Bligh and Dyer method 1959[2].

Nile Red staining

0.5 ml of grown culture was treated with Nile Red stain in 1:100 (v/v) ratios. [5]. The processed sample were analyzed through fluorescent microscopy and the images were captured.

Results and Discussion

Isolation of Microalgae

In algal biodiversity the searching for new natural isolate with high lipid productivity was considered to be the prime task. In this study among four natural isolate, three were selected based on their monoculture state, purity and growth rates. Microscopic observation under 400 X magnification of the algal isolates revealed its colonial existence and purity[**Figure 1**]. After performing centrifugation and streak plating method, three unialgal strains were isolated and designated as MS1, MS2, MS3 and one mixed type culture named MS4. Every two days interval the purity of the strains were checked and stored in BBM agar slant for prolonged usage.

Medium Standardisation

Selection of medium for the particular strain was considered to be an important step for their implementation in industrial oriented application. At present, all the three isolates were initially screened in three different medium

BBM, Bristol, TAP. After incubation period MS1 found to grow well in TAP medium and MS3 was growing well in BBM medium[Figure 3]. It was confirmed with biomass and lipid producing capability. Other MS2 culture was contaminated with bacterial and fungal contamination. MS2 strain was found to adapt only BBM medium for 4 to 7 days[Figure 2], due to the contamination the strain was not able to grow well in any of the medium.

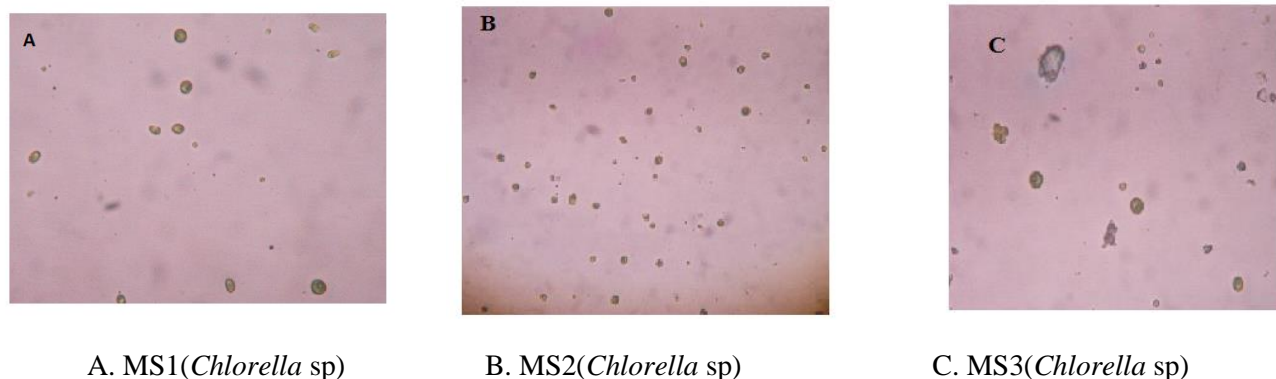


Figure1: Figure showing the microscopic appearance of natural isolates.A-MS1,B-MS2,C-MS3

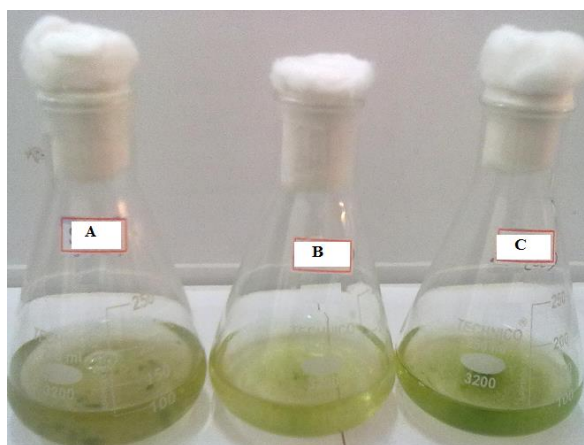


Figure 2 Figure showing microalgae isolates after 7 days of inoculation.
A-MS1 in TAP, B-MS2 in BBM, C-MS3 in BBM

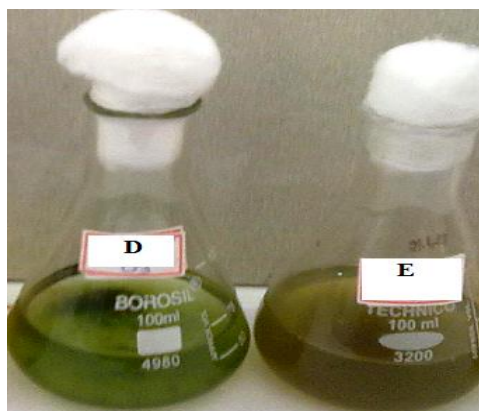


Figure 3 Figure showing the growth of MS1 and MS3 strains.
D-MS3 in BBM medium (after 21 days) E-MS1 in TAP medium (after 21 days)

Biomass production rate

After incubation period the grown cultures were taken for biomass estimation studies. Total biomass estimation results were denoted that MS3 strain in BBM can give maximum biomass up to 129g/litre in wet weight, 112g/litre in dry weight. Else in TAP medium MS3 strain has given only 23g/litre of wet biomass and in bristol 09g/litre of wet biomass these are negligible when going for large scale procedures.

Likewise MS1 strain in TAP medium also gave biomass up to 89g/litre in wet weight , 82g/litre in dry weight[Table-2]. In Bristol medium did not show any change after inoculation and in BBM medium it was found with low amount of biomass in wet weight up to 15g/litre[Table-1].As a result for MS1 strain TAP medium was selected, for MS3 strain BBM medium was selected for further lipid production.

Table 1 Table showing the biomass production rate of isolates in supplemented medium

S.No	Sample name	Total biomass in wet weight(g/litre)		
		BBM medium	TAP medium	Bristol medium
1.	MS1	15	89	0.5
2.	MS2	0.1	6	17
3.	MS3	129	23	09

After selecting the specific medium for MS1 and M S3 isolates total biomass in dry weight were measured. Lipid extraction was performed by following Bligh and Dyer method[2]. The total lipid was estimated[Table] for both the selected isolates, MS1 was found to have 0.148g/litre and MS3 was having 0.3g/litre total lipid in their biomass.

Table 2 Total biomass and total lipid production of natural and reference strain(dry and wet weight (g/litre))

S.No	Sample name	Suitable Medium	Wet weight (g/litre)	Dry weight (g/litre)	Total lipid(g/litre)
1.	MS1	TAP	89	82	0.148
3.	MS3	BBM	129	112	0.3

Discussion

Algae are a diverse group of aquatic and marine organisms. They are mainly classified into two types namely macroalgae and microalgae. Macroalgae are larger species including seaweed, are known as macro algae, and can be grown at sea or in tanks. Microalgae are versatile in the earth ecosystems, found in aquatic to terrestrial land, also adapted to wide variety of environmental situations. Approximate estimation on the existence of microalgae species exceeds than 50,000 species, only up to 30,000 were reported previously [10]. They are Single celled algae are usually grown in open ponds or in enclosed systems known as photo bioreactors. Like plants they can carry out photosynthesis using sunlight to fix carbon dioxide either in the form of carbohydrates, oils and proteins [11]. Microalgae are consists of both prokaryotic and eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Examples of prokaryotic

microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae such as green algae (Chlorophyta) and diatoms (Bacillariophyta).

Nile Red staining

Nile red imaging was performed to confirm the presence of *in vivo* neutral lipid content in microalgae cells [Figure 4]. It was used as a screening step for selecting the natural isolate for further optimization procedures.

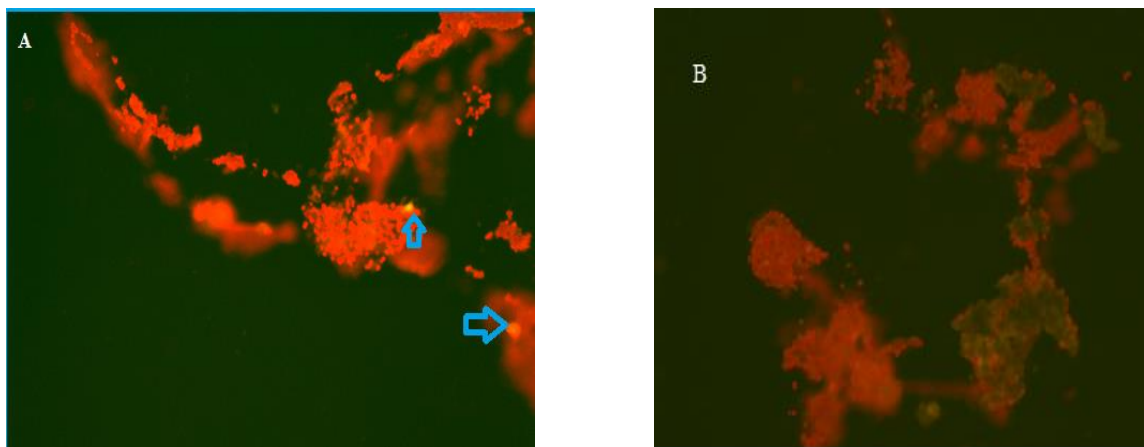


Figure 4: Nile red fluorescence images, A- MS3 strain in BBM, B-MS1 strain in TAP

Microalgae are emerging to be one of the most promising long-term sustainable sources of biomass they utilize the nutrients from wastewater and produce commercial valuable products such as, oil for fuel, nutritional supplements for human and animals, aquatic and poultry feeds, Cosmetic products, High valuable content, like Fatty acid and pigment. The first use of microalgae by humans dates back 2000 years to the Chinese, who used *Nostoc* to survive during famine. Nowadays, there are numerous commercial applications of microalgae. For example, microalgae can be used to enhance the nutritional value of food and animal feed owing to their liquid fuels from algae show many advantages [3].

They are nominated as beneficial feed stock because of their advantages including photosynthetic efficiency, doubling time, high biomass production and growth rate than any other traditional energy crops.[6] Also many microalgae store their lipid substance in the form of triacylglycerol (TAG) during photo-oxidative stress or other adverse environmental conditions. TAG is considered to be desired form of lipid for biodiesel production and they can accumulate 20-50% in their dry cell weight. Species selection also an important task, the reason is depends on the species the form of lipids and other complex oil can be changed. [7]

Among four natural isolates (MS1,MS2, MS3 and MS4), only three isolates were selected due to the unialgal state namely MS1, MS2 and MS3. Those three isolates were screened in three different medium (BBM. TAP.Bristol). This was considered to be an important step before going for industrial scale production.

Conclusion

Microalgae were mainly focused to produce lipid for their application in bio-energy sector. The biomass results suggested that both the MS1 and MS3 strains were capable to produce high amount of biomass. Also the lipid content of MS3 was higher than MS1. Further confirmation of the lipid type is essential. The preferable feed stock for biodiesel was triglycerides that were belonging to the class of neutral lipid. This could be verified with Nile Red fluorescence imaging. As outcome of the present study among three isolates initially MS1 grown in TAP medium and MS3 grown in BBM were selected based on their biomass productivity. Also lipid producing capacity was analyzed.

Then the Nile red fluorescence imaging suggested that the MS3 strain grow in BBM could produce more amount of neutral lipid than MS1 strain can grow in TAP. The selected MS3 strain that could grow well in BBM medium was the best candidate for lipid production.

References

- [1] APHA, American Public Health Association. 1999. Standard Methods for the Examination of Water and Wastewater.
- [2] Bligh ,E.G and Dyer,W.J. 1959. A rapid method for total lipid extraction and purification. Can J Biochem Physiol.37:911-91
- [3] Borowitzka, M. A.: Microalgae as sources of pharmaceuticals and other biologically active compounds. J. Appl. Phycol., 7, 3–15 (1995).
- [4] Chisti, Y., 2007. Biodiesel from microalgae, Biotechnol. Adv. 25, 294–306.
- [5] Chen W, Zhang C, Song L, Sommerfield M, Hu Q.2009."A high throughput Nile red method of quantitative measurement of neutral lipids in microalgae". J. Microbiol. Methods 77.41-47.
- [6] Lin Q, Lin J. Effects of nitrogen source and concentration on biomass and oil production of a Scenedesmus rubescens like microalga. Bioresour. Technol. 2011,102, 1615-1621.
- [7] Metzger P, Largeau C, Botryococcus braunii: a rich source for hydrocarbons and related ether lipids. Appl Microbiol Biotechnol 2005, 66, 486–96.
- [8] Parvin,M., Zannat, M.N and Habib, M A B.; Two Important Techniques for Isolation of Microalgae. Asian Fisheries Science. 2007.20:117-124.
- [9] Richmond A, Handbook of Microalgal Culture: Biotechnology and Applied Phycology, Science Ltd. Blackwell. Pp 2004, 104–105.
- [10] Sheehan J, Dunahay T, Benemann J, Roessler P, A Look Back at the U.S. Department of Energy's Aquatic Species Program-Biodiesel from Algae. U.S.Report NREL/TP-580-24190. National Renewable Energy Laboratory 1998, Golden, CO.
- [11] Yang,J , Xu,M, Zhang,X, Hu, Q, Sommerfeld,M, Chen, Y Life-cycle analysis on biodiesel production from microalgae:Water footprint and nutrients balance. Bioresour Technol 2011, 102, 159-165.

© 2014, by the Authors. The articles published from this journal are distributed to the public under "Creative Commons Attribution License" (<http://creativecommons.org/licenses/by/3.0/>). Therefore, upon proper citation of the original work, all the articles can be used without any restriction or can be distributed in any medium in any form.

Publication History

Received 18th April 2014
Revised 12th May 2014
Accepted 13th May 2014
Online 29th May 2014