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Assessment of biomass production of indigenously isolated microalgae *Tetradesmas obliquus* in three different culture media

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Keywords

Microalgae,
DNA barcoding analysis,
Biomass Production,
Three different media.

Abstract

Microalgae are microscopic, photosynthetic organism found in fresh water and marine environment. They are capable of converting sunlight, carbon dioxide and nutrients through photosynthesis. Microalgal isolates were isolated from water sample of Ketti, Ooty The Nilgiris of Tamil Nadu, and were identified as *Tetradesmus obliquus* sp**. by DNA barcoding analysis using Nucleopin plant IIkit (Macherey Nagel) (Rajiv Gandhi Center for Biotechnology).** In addition, the assessment of biomass production in microalgae is essential for optimizing their application in biofuel, Nutraceutical, and waste water treatment industries. In this study, indigenous *Tetradesmus obliquus* were isolated from local freshwater habitats and cultivated in three different culture media: Bold's Basal Medium(BBM), Bristol Medium, and Diatom Medium. Optical density were recorded to assess growth performance. Among the three media, Bold's Basal Medium supported the highest biomass yield, followed by Diatom medium and Bristol medium.

Introduction

Biomass production is the rate of which living oraganisms, especially autotrophs like microalgae or higher plants, accumulate organic matter in a given area and time (Chisti, Y.2007). Micro algae are single celled microorganism that lack complex organelles yet perform photosynthesis efficiently (Varman 2023). Microalgal isolates were isolated from water sample of Ketti, Ooty The Nilgiris of Tamil Nadu, and were identified as *Tetradesmus obliquus*(Turpin) M.J Wynne, 2016 by DNA barcoding analysis using Nucleopin plantkit (Macherey Nagel) The growth and biomass productivity of microalgae are influenced by various environmental and nutritional factors,

including the composition of the culture medium. This study focuses on the assessment of biomass production of indigenously isolated microalgal in three different culture media- Bold's Basal Medium (BBM) Bischoff,H.W and Bold(1963), Bristol Medium,H.C Bold(1949) and Diatom Medium Guillard,R.R.L.,and Ryther(1962). By comparing the growth rates and biomass yields in thesemedia, the study aims to identify the most suitable culture conditions for maximizing algal productivity. The findings could contribute to developing efficient algal cultivation systems for industrial and environmental application.

Materials and Methods

Study area and sample collection

The area selected for the present work in Ketti. It located in the Nilgiris district of Tamilnadu. Revenue village of Coonoor taluk. The collected sample tube was labelled and brought to laboratory for further study. One part of sample was transferred in nutrient medium (Bold's basal medium) for microalgal growth and enrichment. The algal isolates were purified by enrichment technique and were subjected to purification by serial dilution followed by plating. The individual colonies were isolated and inoculated into a liquid medium. The purity of culture was ensured by repeated plating and by regular observation under the light microscope.

Molecular identification of the isolated microalgae

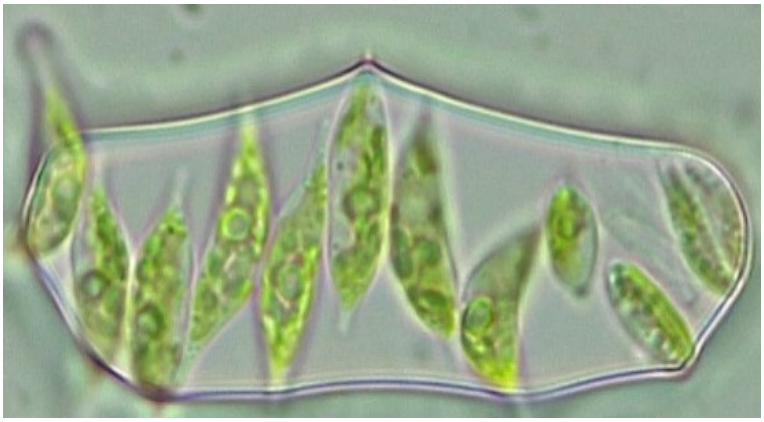
The experiment was performed by DNA isolation using Nucleopin plantII kit (Macherey Nagel) RbclCFForwardAAAGATGATGAAAACGTGA ACTRbclM1390R.ReverseCTTTCCAWAYTTC ACAAGCAGCAG to perform a polymer chain reaction (PCR)98^{o}C-5 sec,54^{o}C- 10 sec, 72^{o}C ,-15 sec, 72 ^{o}Sec -2 min, 4^{o}C - ∞40 cycles.The PCR products were checked in 1.2% agarose gels prepared in 0.5X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6X loading dye was mixed with 4 µl of PCR products and was loaded and electrophoresis was performed at 75V power supply with 0.5X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad). The obtained sequences were then analysed using the Basic Local Alignment Search Tool (BLAST) on the server of the National Center for Biotechnology information to identify the closely related sequences. Further, the nucleotide sequence was submitted to NCBI and got an accession number: Bankit 3009797 SR5159-RBCLCFF-E05-ab1PX438747.SUB 15672936 SR5159-A-18sF-GO3.ab1 PX442035.

Analytical techniques: Optical density (OD)

Portion of algal culture is transferred into a cuvette and the optical density was measured using spectrophotometer using wavelength of 680 nm (Lee *et al*., 2013).

Results

Microalgal isolates were isolated from water sample of ketti, Ooty, The Nilgiris of Tamilnadu, and were identified as *Tetradesmus obliquus* sp., within the family scenedesmaceae, part of the class chlorophyceae by DNA barcoding analysis using Nucleopin plant II kit (Macherey Nagel).Further, the nucleotide sequence was submitted to NCBI got an **accession number: Bankit 3009797 SR5159-RBCLCFF-E05-ab1 PX438747.SUB 15672936 SR5159-A-18sF-GO3.ab1 PX442035.** Inaddition, the assessment of biomass production in microalgae is essential for optimizing their application in biofuel, Nutraceutical, and waste water treatment industries. In this study, indigenous microalgal were isolated from local freshwater habitats and cultivated in three different culture media: **Bold's Basal Medium (BBM), Bristol Medium, and Diatom Medium.** Periodic measurements of optical density were recorded to assess growth performance. Among the three media, **Bold's Basal Medium** supported the highest biomass yield, followed by Diatom medium and Bristol medium.



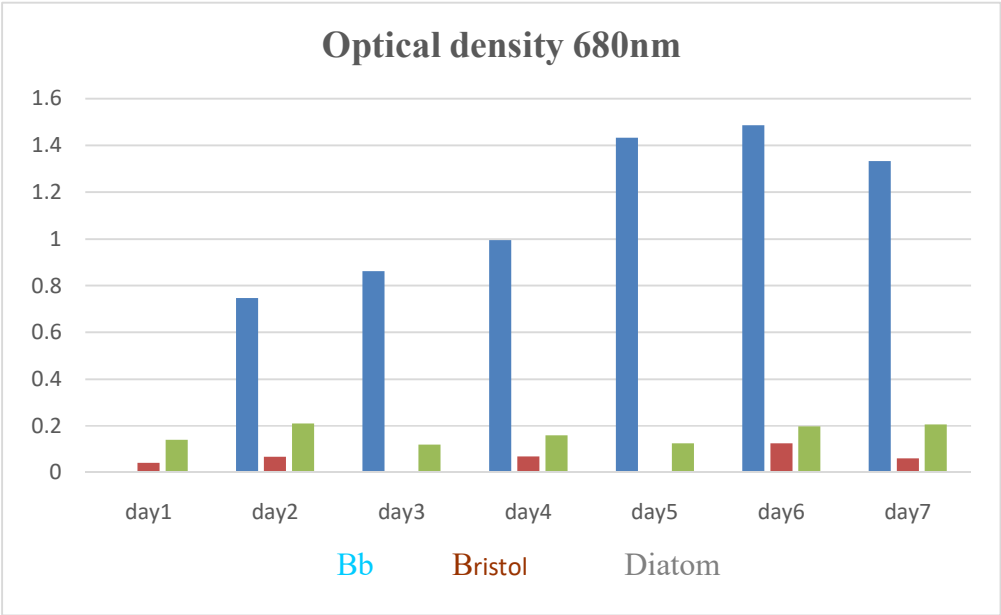
Images of *Tetradesmus obliquus*

Classification

Kingdom	**:**	**Plantae**
Sub kingdom	**:**	**Viridiplantae**
Infrakingdom	**:**	**Chlorophyta**
Phylum	**:**	**Chlorophyta**
Sub phylum	**:**	**Chlorophytina**
Class	**:**	**Chlorophyceae**
Order	**:**	**Sphaeropleales**
Family	**:**	**Scenedesmaceae**
Genus	**:**	**Tetradesmus**

Nomenclature

1. ***Achanthus obliqua*** **(Turpin)**
2. ***Scenedesmus acutus*** **(Meyin)**
3. ***Scenedesmus bijugatus*** **(Kutzing)**
4. ***Scenedesmus obliquus*** **(Kutzing)**
5. ***Scenedesmus basilensis*** **(Chodat)**
6. ***Acutodesmus obliquus*** **(Hegewald)**
7. ***Tetradesmus obliquus*** **(Wyne)**



Graph 1 showing periodical measurements with (optical density) in three different media.

Discussion

Microalgal cultures were isolated from different fresh water source of Tamilnadu. The occurrence of green algae in freshwater bodies has been earlier reported by several workers (Banerjee *et.al.*,2002:Metzger and Largeau. 2005). Barsanti and Gualtieri 2006) Isolated cyanophyceae microalgae from freshwater by using the BG11 medium. As previously noted recent studies showed that the potential of Tetradesmus obliquus for large scale biomass production has not been exploited yet. Potential of *Tetradesmus obliquus* to grow in nitrogen and phosphorous rich waste water has also been proven in several studies(Martinez et.al 2000: Hodaifa et.al 2019). The objective of present work that aimed at selecting the best medium for *Tetradesmus obliquus*. Three well known culture media were adopted for growth of *Tetradesmus obliquus* in this study. The obsorbance of the sample was measure using a spectrophotometer. It can be seen graph 1that *Tetradesmus obliquus* growing faster (in 7 days) in BBM as compared to other media.BBM, while Bristol, DM came at the final as arranged. The bar diagram did not show lag phase and it demonstrates that there was a quick adaptation of *Tetradesmus obliquus* to all media. Morphological identification of the microalgae was conducted by using standard manuals. (G. Mahendra perumal and N. Anand, Jose John. M.S Francis) based on these microscopic observations, the isolated microalgae tentitevely classified as belonging to the genus *Tetradesmus obliquus* within the family scenedesmaceae, part of the class chlorophyceae.

Conclusion

The freshwater microalgae were collected, isolated and molecularily identified from ketti the Nilgiris. Isolated microalgae successfully molecularly identified as *Tetradesmus obliquus* using rbcl gene marker and 18s rRNA gene marker.

Acknowledgments

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