International Journal of Applied Science
ISSN (Online): 2208-2182
Volume 12 Issue 01 January 2026

DOI: https://doi.org/10.53555/8za5j306

HARNESSING MICROALGAL DIVERSITY FROM DHOSI HILL, MAHENDERGARH, HARYANA, INDIA FOR RENEWABLE BIODIESEL APPLICATIONS

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Abstract
Microalgae are recognized as hopeful sustainable feedstocks for biodiesel production due to their fast growth rate, high photosynthetic efficiency, and ability to accumulate considerable lipid content. The present study focuses on the isolation, purification, and molecular identification of freshwater microalgae from Shiv Kund Dhosi Hill, Mahendergarh, Haryana, India, and the evaluation of their biodiesel production potential. From the water source one algae was isolated and named as MKI01, it was successfully established in sterile culture. Biodiesel extraction using a solvent-based method revealed that MKI01 yielded a higher lipid-derived biodiesel output, producing approximately 10.0 ± 0.1 mL from 20 g of dried biomass. Molecular identification using 18S rRNA gene sequencing confirmed MKI01 was Chlorella sp. showing 100% similarity with reference sequences in the NCBI database. Phylogenetic analysis further supported the taxonomic placement. The results focous on Chlorella sp. from the Dhosi Hill region as a promising candidate for biodiesel production.

Keywords: *Microalgae, 18s rRNA, molecular identification*

1.Introduction

Microalgae involve wide range of unicellular, microscopic, photosynthetic organisms, including eukaryotic algae and cyanobacteria which inhabit approximately every ecological niches. They are fast growing organisms that convert carbon dioxide and nutrients into biomass and reproduce more rapidly compared to terrestrial plants (Marousak *et al*., 2023). Their growth rate and photosynthetic activity are affected by environmental characteristics, namely temperature, light, pH, nutrient and aeration. Generally, most cultured algae bloom at pH 7-9, light wavelength 400 to 700nm (Kombe *et al*., 2023) and temperature 25-450ºC, though growth conditions may vary among species. More than 50,000 species are discovered and up to 30,000 are studied (Osnai *et al*.,2017). Cultivation of microalgae for the production of metabolites, such as carotenoids, chlorophyll, long-chain polyunsaturated fatty acids, antioxidants, phycobiliproteins and enzymes, has received researchers' interest (Hasnain *et al*., 2023). Similarly, they are utilized as food for humans, animal feed, bio-fertilizer and cosmetics among others. The primary step towards the successful investigation of microalgae is optimal methods for sampling environments and the isolation, purification and maintenance of strains (Shivanandi *et al*., 2022). One of the challenges in culturing microalgae is the high susceptibility of cultures to contamination by algal species, bacteria, viruses and fungi, which may interfere with cell structure, biomass yield and the erroneous identification of monocultures for molecular genetic analysis (Surya *et al*., 2024).

Over the past few decades, unicellular microalgae have been extensively advocated as a sustainable and green bioenergy resource and could serve as an alternative to traditional fuels (petroleum oil, coal, and natural gas) (woljko et al., 2023). Microalgae offer numerous benefits for biodiesel production compared to land-based crops and plant materials, including greater photosynthetic efficiency, quicker nutrient absorption rates, shorter life cycles, elevated lipid content, increased biomass productivity, and adaptable cultivation environments (zare et al., 2022). The industrial-scale cultivation of microalgae demands a significant quantity of nutrients and freshwater (Zayadan *et al*., 2023). Water is an essential resource that should be utilized wisely, and employing non-potable water for the cultivation of microalgae can reduce the consumption of freshwater. In fact, the majority of the freshwater used is released as wastewater, which is nutrient-dense and presents a significant threat of eutrophication (Kumar et al., 2020).

Despite having greater levels of neutral lipids, fatty acids, polar lipids and growing quickly in their natural environment, microalgae are still largely unexploited for manufacture of biodiesel. Isolation and characterization of microalgal strains having enhanced oil-producing potential therefore continue to be key areas of research. In the present study, naturally isolated microalgal species were analised, and the 18S rRNA gene of isolates has been sequenced to ensure their taxonomic identities.

2. Materials and methods

2.1 Analysis of Dhosi hill water

Biological oxygen demand (BOD), Ammonical nitrogen (NH3-N), total nitrogen (TN), chemical oxygen demand (COD), total phosphate (TP) of water sample have been evaluated through following standard processes, ISO 5815-1, ISO 6060, ISO 6878, ISO 13878, ISO 23695 respectively. The pH of water sample has been estimated utilizing the instrument, pH meter (Alasady *et al*., 2021).

2.2 Sample collection, purification and isolation

From Shiv kund, Dhosi Hill, one microalgal sample has been collected and named as MKIO1 (latitude 28.052758º N, longitude 76.028563º E) located in Haryana, India.

Fig 1. Shiv kund, Dhosi hill, Mahendergarh, Haryana, India.

For eliminating contaminants and potential microalgal predators such as protozoa and rotifers, the samples were immediately filtered through sieves having mesh sizes of 60 along with 120µm (Nutiyal *et al*., 2014). Meanwhile microalgal samples attained from natural environments are typically contaminated with various microorganisms, collected samples have been purified three times at 5000 rpm for 5 minutes by centrifugation. Biomass pellets have been produced and then reconstituted in growth media that contained mixture of 0.22µm filter-sterilized antibiotic solutions that contained 500mg/mL cefotaxime, 200mg/mL kanamycin, 50 mg/mL nystatin. The use of a combination of broad-spectrum antibiotics was essential, as different bacterial species may exhibit varying sensitivities to individual antibiotics (Kumar *et al*.,2019).

2.3 Genomic DNA extraction

From algal tissue, genomic DNA has been extracted utilizing HiPurA™ Fungal DNA Purification Kit (Cat. No. MB543, HiMedia Laboratories, India) following manufacturer's instructions. Briefly, around 100 to 150mg of algal tissue has been ground as well as mixed with 400 µl of Lysis Buffer (PL), afterward incubation at 65°C for 10 minutes with intermittent mixing (Adhoni *et al*.,2016). Following incubation, mixture has been incubated on ice for 5 minutes prior to 130µl of Precipitation Buffer (PS) was added. After being moved to a HiShredder column, lysate has been centrifuged for 120 seconds at 13,000 rpm (Pandey *et al*.,2019).

Flow-through has been collected and mixed with 1.5 volumes of diluted Binding Buffer (BB). The mixture has been loaded onto a HiElute Miniprep Spin Column in two successive steps, each followed by centrifugation at 6000 × g (≈8000 rpm) for 60 seconds. Later column has been washed twice with 500µl of diluted Wash Buffer (WSP), first at 6000 × g (≈8000rpm) and then at 13,000 rpm. Column has been centrifuged at 13,000 rpm for extra 2 minutes to eliminate any residual wash buffer (Jagadevan *et al*.,2019). Finally, genomic DNA has been eluted through integrating 50µl of Elution Buffer (ET) to column then centrifugation. Purified DNA was quantified as well as kept at –20°C for downstream utilizations (Banerjee *et al*., 2021).

2.4 PCR amplification and purification

The 18S rRNA gene has been amplified utilizing 2 oligonucleotide primers: 194F (5′-CCATGCATGTCTAAGTNTAA-3′) and 195R (5′-CAATTGTTCCTCGTTAAG-3′). Purified genomic DNA served as the template for PCR amplification of a fragment approximately 700 bp in length (Sabu *et al*.,2017).

20µl reaction mixture including 1µl of DNA template, 10×PCR buffer, 0.2mM of every dNTP, 0.5µM of every primer, 2 U/ml of Taq DNA polymerase has been used for every PCR reaction (*TaKaRa Taq*™, Cat. No. R001C). 32 cycles of denaturation at 95°C for half a minute, annealing at 45°C for 40sec, extension at 72°C for 40sec, followed by final extension at 72°C for 5min, comprised amplification process (Hawary *et al*.,2024). PCR products have undergone electrophoresis on 1.5% agarose gel in Tris-acetate-EDTA (TAE) buffer, stained utilizing 0.5µg/ml of ethidium bromide and examined under UV illumination (Jagadevan *et al*., 2021). Positive amplicons were excised and purified utilizing the FavorPrep™ GEL/PCR Purification Kit (Cat. No. FAGCK001, Favorgen Biotech Corp., Taiwan) as per protocol of manufacturer (Abou *et al*.,2024).

2.5 Sequencing and phylogenetic analysis

The purified PCR product was sequenced using the Sanger method on an ABI 3500XL Genetic Analyzer. The obtained 18S rRNA sequences were visually inspected and manually edited in BioEdit (version 7.2.5) to remove non-overlapping regions at the 5′ and 3′ ends. Molecular identification of the microalgal isolates has been carried out by applying BLAST (Basic Local Alignment Search Tool) method to compare modified sequences with pre-existing sequences in National Center for Biotechnology Information (NCBI) database. Resulting sequence has been submitted to NCBI GenBank database under accession number MN365023.1 (Khan *et al*.,2017).

2.6 Biodiesel production

Biodiesel was produced using the solvent extraction method. The dried microalgal biomass was ground into a fine powder using a laboratory grinder to increase surface area. The powdered algal biomass was then mixed with a solvent mixture of *n*-hexane and methanol to extract lipids (Neofotis *et al*.,2016). The mixture was thoroughly agitated to ensure efficient extraction and then allowed to settle for phase separation. After settling, two distinct layers were observed the upper organic layer containing biodiesel and the lower layer containing residual biomass and solvent impurities. The upper layer of biodiesel was carefully separated and collected (Sirohi et al.,2023)**.**

3. Results and discussion

3.1 Physiochemical characterizations of Dhosi Hill water

The physicochemical characteristics of water sample collected from Shiv kund, Dhosi Hill, Mahendergarh, Haryana, India are presented in table 1. The pH was found to be slightly alkaline 7.07 and 7.14. The COD of water was 118.08 mg/ml and 98.4mg/ml. The BOD OF water was 20.4 mg/ml and 17.8 mg/ml. The concentration of total phosphate in water was found to be 0.71 mg/ml and 0.69 mg/ml. The concentration of total nitrogen in water was found to be 9.8 mg/ml and 8.4 mg/ml. The ammonical nitrogen concentration of water was 3.9 mg/ml and 4.2 mg/ml.

Table 1. Physiochemical characteristics of Dhosi Hill water

Sr no.	Parameters	Sample 1 (mg/l)	Sample 2 (mg/l)
1	pH	7.07	7.14
2	COD	118.08	98.4
3	BOD	20.4	17.8
4	Phosphate as P	0.71	0.69
5	Total nitrogen	9.8	8.4
6	Ammonical nitrogen	3.9	4.2

3.2 Isolation and biodiesel production

One algal culture MKI01 was successfully isolated from a Shiv kund, Dhosi Hill, Mahendergarh Haryana, India. MKI01 was tested for biodiesel production and it was observed that MKI01 produce high amount of biodiesel. Approximately **10.0 ± 0.1 mL** of biodiesel was obtained from **20 g** of dried MKI01 algal biomass using the solvent extraction method. So MKI01 was selected for further study.

3.3 18s rRNA coding region amplification, sequencing and identification

18S rRNA gene fragment (~100bp) was identified by clear single band on agarose gel electrophoresis, which was result of effective PCR amplification of genomic DNA from algal isolates using reverse primers along with universal forward (Rukhmana *et al*.,2021). 18S rRNA gene, which encodes small subunit of eukaryotic ribosomes, contains both conserved and variable regions (V1–V9, excluding the V6 region). The conserved regions are widely used to determine phylogenetic relationships among species (Ahmad *et al*.,2021).

Sequence analysis revealed that the MKI01 strain shared 100% sequence similarity with *Chlorella spp*, confirming its close phylogenetic relationship to this species. The obtained 18S rRNA sequences were deposited in NCBI GenBank database, corresponding accession numbers, sequence lengths, and closest homologous species are listed in Table 2. The identification of the isolate was further validated by phylogenetic analysis of the 18S rRNA sequences, which supported its clustering with *Chlorella spp*. A phylogenetic tree was constructed using the bootstrap method.

Table 2. Comparing the amplicon produced by sequencing 18s rRNA genomic area in the NCBI database allows for the molecular identification of isolated microalgae. Information on freshwater isolated algae sequences, including accession numbers, amplicon length in base pairs, similarities among amplified sequences.

Microalgal strain identified	Accession number	Length (nt)	Precent similarity
Chlorella sp.	AB970452	570BP	100%

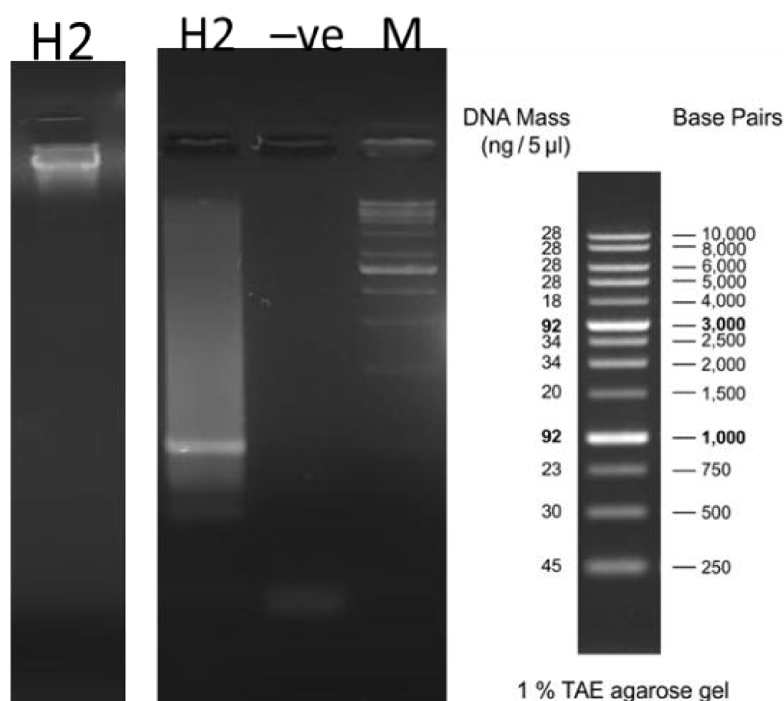

Figure a: Isolated genomic DNA Figure b: Quality check of PCR subjected to 1% agarose gel. Product Subjected to 1.5% Agarose gel electrophoresis. M is 1000 bp marker.

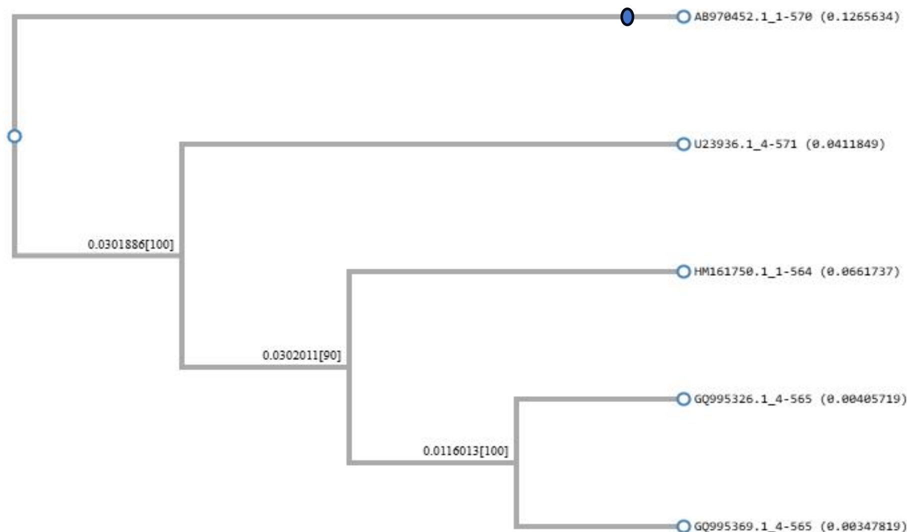

Figure 2. Blast result of *chlorella sp.* showing the phylogeny of the organism with other algal species.

4.Conclusion

The present study successfully isolated and molecularly identified one freshwater microalgal strain from the Shiv kund Dhosi Hill in Mahendergarh, Haryana, India. Using 18S rRNA gene sequencing, the isolate was genetically characterized and identified as *Chlorella* sp. Physicochemical analysis of the pond water indicated favourable conditions for algal growth, with slightly alkaline pH and moderate nutrient concentrations.The identification of *Chlorella* species from a natural freshwater habitat highlights the biodiversity of microalgae in the Dhosi Hill region and their potential for various biotechnological applications such as biofuel production, bioremediation, and nutrient recycling. Future research should focus on optimizing growth conditions and exploring the biochemical composition of these isolates to assess their suitability for commercial and environmental use.

REFERENCE

1. Adhoni, S. A., Shivasharana, C., & Kaliwal, B. (2016). Identification and characterisation of Chlorella vulgaris for biodiesel production. *International Journal of Scientific Research and Engineering Studies*, *3*(1), 7-15.
2. Al-Shahery, Yousef J., and Isrra N. Alasady. "Identification of saturated and unsaturated fatty acids produced by Chlorella vulgaris as a potential candidate for biodiesel production." (2021): 238-242.
3. Arunachalam Sivagurulingam, A. P., Sivanandi, P., & Pandian, S. (2022). Isolation, mass cultivation, and biodiesel production potential of marine microalgae identified from Bay of Bengal. *Environmental Science and Pollution Research*, *29*(5), 6646-6655.
4. Balouch, H., Zayadan, B. K., Sadvakasova, A. K., Kossalbayev, B. D., Bolatkhan, K., Gencer, D., ... & Allakhverdiev, S. I. (2023). Prospecting the biofuel potential of new microalgae isolates. *International Journal of Hydrogen Energy*, *48*(50), 19060-19073.
5. Elsharaihy, K. K., Al-Hawary, I. I., Assas, M., Saleh, A., Abou Zaid, A. A., Abualreesh, M. H., & Elbialy, Z. I. (2024). Isolation and identification of marine microalgae from Egyptian coasts as potential source of biodiesel: morphology and sequence-based screening. *Marine Biology Research*, *20*(7-8), 304-313.
6. Ermavitalini, D., Rukhmana, S. Y., Meidina, T., Baskoro, L. P. D. C., Saputro, T. B., Ni'matuzahroh, N. M., & Purnobasuki, H. (2021). Astaxanthin-producing microalgae identification using 18S rRNA: isolates from Bangkalan mangrove waters and Sowan Tuban northern waters, East Java, Indonesia. *Journal of Tropical Biodiversity and Biotechnology*, *6*(3), 64882.
7. Haghighi, M., Zare, L. B., & Ghiasi, M. (2022). Biodiesel production from Spirulina algae oil over [Cu (H2PDC)(H2O) 2] complex using transesterification reaction: Experimental study and DFT approach. *Chemical Engineering Journal*, *430*, 132777.
8. Hasnain, M., Munir, N., Abideen, Z., Macdonald, H., Hamid, M., Abbas, Z., ... & Radicetti, E. (2023). Prospects for biodiesel production from emerging algal resource: Process optimization and characterization of biodiesel properties. *Agriculture*, *13*(2), 407.
9. Hoang, A. T., Sirohi, R., Pandey, A., Nižetić, S., Lam, S. S., Chen, W. H., ... & Pham, V. V. (2023). Biofuel production from microalgae: challenges and chances. *Phytochemistry Reviews*, *22*(4), 1089-1126.
10. Jabłońska-Trypuć, A., Wołejko, E., Ernazarovna, M. D., Głowacka, A., Sokołowska, G., & Wydro, U. (2023). Using algae for biofuel production: a review. *Energies*, *16*(4), 1758.
11. Khan, S., Siddique, R., Sajjad, W., Nabi, G., Hayat, K. M., Duan, P., & Yao, L. (2017). Biodiesel production from algae to overcome the energy crisis. *HAYATI Journal of Biosciences*, *24*(4), 163-167.

12. Kombe, G. G. (2023). An overview of algae for biodiesel production using bibliometric indicators. *International Journal of Energy Research*, *2023*(1), 9596398.
13. Kumar, N., Banerjee, C., & Jagadevan, S. (2021). Identification, characterization, and lipid profiling of microalgae Scenedesmus sp. NC1, isolated from coal mine effluent with potential for biofuel production. *Biotechnology Reports*, *30*, e00621.
14. Kumar, N., Banerjee, C., Kumar, N., & Jagadevan, S. (2019). A novel non-starch based cationic polymer as flocculant for harvesting microalgae. *Bioresource Technology*, *271*, 383-390.
15. Kumar, V., Al Momin, S., Kumar, V. V., Ahmed, J., Al-Musallam, L., Shajan, A. B., ... & Al-Zakri, W. M. (2021). Distribution and diversity of eukaryotic microalgae in Kuwait waters assessed using 18S rRNA gene sequencing. *PLoS One*, *16*(4), e0250645.
16. Mafat, I. H., Palla, S., & Surya, D. V. (2024). Machine learning and artificial intelligence for algal cultivation, harvesting techniques, wastewater treatment, nutrient recovery, and biofuel production and optimization. In *Value added products from bioalgae based biorefineries: opportunities and challenges* (pp. 463-487). Singapore: Springer Nature Singapore.
17. Maroušek, J., Gavurová, B., Strunecký, O., Maroušková, A., Sekar, M., & Marek, V. (2023). Techno-economic identification of production factors threatening the competitiveness of algae biodiesel. *Fuel*, *344*, 128056.
18. Nautiyal, P., Subramanian, K. A., & Dastidar, M. G. (2014). Production and characterization of biodiesel from algae. *Fuel Processing Technology*, *120*, 79-88.
19. Neofotis, P., Huang, A., Sury, K., Chang, W., Joseph, F., Gabr, A., ... & Polle, J. E. (2016). Characterization and classification of highly productive microalgae strains discovered for biofuel and bioproduct generation. *Algal Research*, *15*, 164-178.
20. Osanai, T., Park, Y. I., & Nakamura, Y. (2017). Biotechnology of microalgae, based on molecular biology and biochemistry of eukaryotic algae and cyanobacteria. *Frontiers in Microbiology*, *8*, 118.
21. Pandey, A., Srivastava, S., & Kumar, S. (2019). Isolation, screening and comprehensive characterization of candidate microalgae for biofuel feedstock production and dairy effluent treatment: a sustainable approach. *Bioresource technology*, *293*, 121998.
22. Sabu, S., Bright Singh, I. S., & Joseph, V. (2017). Molecular identification and comparative evaluation of tropical marine microalgae for biodiesel production. *Marine Biotechnology*, *19*(4), 328-344.
23. Sigamani, S., Ramamurthy, D., & Natarajan, H. (2016). A review on potential biotechnological applications of microalgae. *Journal of Applied Pharmaceutical Science*, *6*(8), 179-18