MIKAILALSYS
Journal of Multidisciplinary Sciences

e-ISSN : 2987-2286
p-ISSN : 2987-3924

Index : Dimensions, Scilit, Crossref, Garuda, Semantic, Lens,Google Scholar,Base, etc

https://doi.org/10.58578/mikailalsys.v2i3.3851

Isolation and Molecular Characterisation of *Chlorogonium sp.* from Industrial Wastewater

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Sep 1, 2024	Sep 14, 2024	Sep 17, 2024	Sep 20, 2024

Abstract

Microalgae are photosynthetic, unicellular microorganisms also known as phytoplankton. They are small plant-like entities. In this research, the sample were collected from cement factory in a sterilised 20L container wrapped with foil paper and were transported down to Federal University, Wukari where it was kept in refrigerator at biochemistry laboratory. Blue-Green media (BG-11) was prepared. Wastewater containing Microalgae obtained from cement wastewater pond were cultivated in BG-11 medium to determine the growth of the organism. BG-11 medium contained macronutrients, trace metals and some vitamins which aid the growth of the organism. The wastewater sample containing with suspected microalgae was inoculated (inoculum ratio = 25%) and incubated under atmospheric CO_2 at room temperature (30±2°C) in our laboratory for two to three weeks during the incubation period. In order to purify the isolates, the upper growth layer was first decanted into a freshly produced medium and then plated on BG-11 media that had been solidified with 1% agar-agar. For several of the cultures, growth on the agar plates continued for around three weeks. Following repeated sub-culturing, the emerging greenish colonies were re-emerged into a sterile BG-11 agar medium.

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In isolation of organism from the industrial cement waste water, the isolate was identified by morphological and molecular identification by extracting the DNA, run the electrophoretic analysis and PCR using primers for 18S rRNA eukaryotic microalgal and then run the sequence analysis. The results of this study obtained, indicated that, the electrophoretic result show the band has 1800-2000base pair and the organism isolated from the industrial cement waste water were *chlorogonium sp*. with a percent similarity of 78.65% and accession number of OR886595 based on data Gene Bank blast results.

Keywords: Microalgae, Isolation, Morphological, Molecular, Wastewater

INTRODUCTION

Microalgae are photosynthetic, unicellular microorganisms with basic reproductive structures ranging from 0.2 to 2 μm to filamentous forms with diameters of 100 μm or more. Because they are prone to dehydration, they must develop in wet conditions where they can carry out oxygenic photosynthesis (Mooij *et al*., 2011, Gerardo *et al*., 2015). These living things are single-celled and can grow rapidly in freshwater, saltwater, and wastewater environments. Prokaryotic (Cyanophyceae) and eukaryotic (Chlorophyta) species are included in them. A few chosen species of microalgae, such *Scenedesmus* and *Chlorella*, can thrive in the harshest kinds of conditions (Maity *et al*., 2014).

Microalgae are important constituents of the food chain that may be found in both fresh and salt water (Workers *et al*., 2011). There are hundreds of thousands of species. Most organisms have chlorophyll, rely on sunlight for energy, and convert CO_2 into biomass. There are two types of algae: macro algae (seaweed) and microalgae (phytoplankton). According to Graham and Wilcox (2000), microalgae are classified into nine phyla based on their molecular sequences. They are characterised by rapid grow and can live in a harsh condition. Microalgae are normally invisible to the unaided eye, but in eutrophic water, large-scale algal blooms can turn the water a translucent green, brown, blue, or orange mass. However, unlike higher plants, microalgae do not require a vascular system for nutrient delivery since each and every one of their cells is photoautotrophic and able to absorb nutrients directly. Microalgae cells are solar-powered factories that may generate critical bioactive substances like docosahexaenoic acid, animal feed ingredients, and vital products like bio-hydrogen, biodiesel, and bioethanol from carbon dioxide as a raw material (Milledge, 2011). Wastewater from other industrial processes may be utilized to

fulfill microalgae's high CO_2 and nutrient requirements, resulting in environmental advantages and decreased biomass production costs.

MATERIALS AND METHODS

Sample Collection

The samples were collected from the wastewater ponds located inside the Dangote Cement Factory headquarter Obajanna. The samples were collected into a sterile 20L container wrapped with aluminum foil paper to prevent sunlight which may aid microorganism activity. Collections were carried out from the top and bottom layer of water with the aim of obtaining the dominant microalgae species present in that particular area. The container was transported to Federal University Wukari and maintained in refrigerator at biochemistry laboratory.

Isolation and Culture Condition

Wastewater containing Microalgae obtained from cement wastewater plant Obajanna were cultivated in BG-11 medium to determine the growth rate of the organism. The medium comprises 1.5 g of $NaNO_3$, 0.04 g of $K_2HPO_4.3H_2O$, 0.2 g of $KH_2PO_4.3H_2O$, 0.001 g of disodium EDTA, 0.001 g of Fe ammonium citrate, 0.006 g of citric acid, 0.02 g of Na_2CO_3, and 1 ml of trace metal solution per litre at pH 7.3. Per liter, the trace metal solution comprises 2.85 g of H_3BO_3, 1.8 g of $MnCl_2.4H_2O$, 0.02 g of $ZnSO_4.7H_2O$, 0.08 g of $CuSO_4.5H_2O$, 0.08 g of $CoCl_2.6H_2O$, and 0.05 g of Na_2MoO_4. Each isolate was grown in a 75 mL sterile medium housed in a 200 mL sterile translucent Roux bottle topped with urethane foam. We infected industrial water with microalgae (inoculum ratio = 25%) and incubated it near windows in our laboratory at room temperature (30±2°C) with atmospheric CO_2. The incubation period ranged between two and three weeks. The isolates were purified by decanting the top growth layer into a freshly produced medium, which was then plated on the BG-11 medium solidified with 1% agar-agar. Growth on the agar plates lasted around 3 weeks for some of the cultures. The emerging colonies were re-inoculated into a sterile BG-11 agar medium and sub cultured repeatedly. The colonies were then moved to a new, sterile BG-11 media.

Morphological Identification of Microalgal

Morphological Identification of microalga which has been isolated is done microscopically. Observations were carried out regularly under the microscope to make sure it had learned a single cell. The morphological characteristics of the algae were matched to identification codes and the algal compendium present in the freshwater algae of the British Isles (John *et al*., 2002).

Molecular Identification

From the isolated microalgae as much as 10 ml were centrifuge at 10,000 rpm for 30 minutes and the DNA were extracted. Each extract of DNA samples obtained were processed by electrophoresis. The 18S rRNA gene were PCR-amplified using universal primers, Sequencing and bioinformatics analysis were carried out for molecular identification of the isolate. The results obtained were amplified by PCR Using ALGAE PRIMER as forward primer forward primer (SS3 5'-GATCCTTCCGCAGGTTCACCTACGGAAACC - 3') and reverse primer (SS5 5' – GGTGATCCTGCCAGTAGTCATATGCTTG – 3') with about 1800-2000 bp PCR product. And it was set at the temperature of 95^0C for 5 minutes and 94^0C, 54^0C, 72^0C all for 30 seconds for pre-denaturation and denaturation, annealing, extension respectively. The process occurred for about 35 times cycle to achieve the four process of PCR. At the end, the final extension was occurred for 5 minutes at 72^0C. PCR products were electrophoresed with 1.5% agarose, a voltage of 100 volts and a time of 1 hour 30 minutes. Direct sequencing was carried out directly against DNA electrophoresis results. Sequences data obtained were Blast in NCBI data base.

RESULTS

The initial division of microalgal cultures and contaminations was done by morphological analysis of colonies grown on the nutrient medium. The shape of each isolate's individual cells under a microscope was used to identify the isolates.

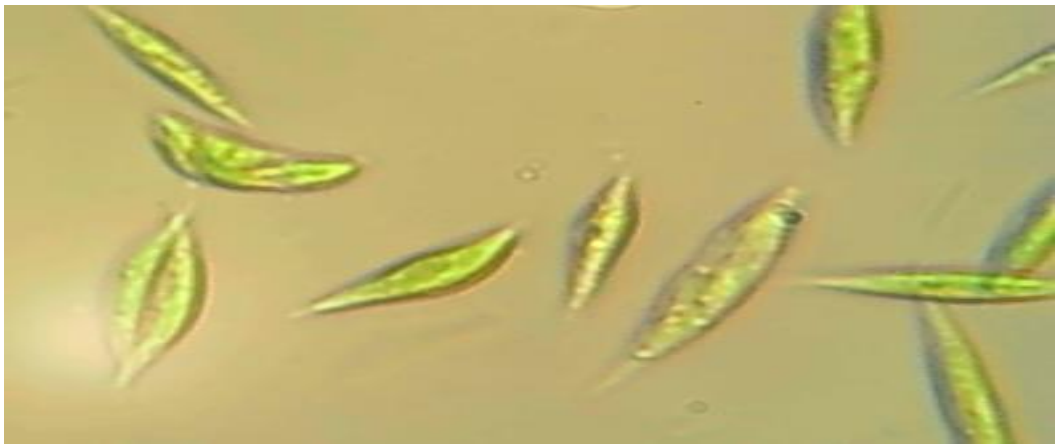


Figure 1: microscopic view of the Isolate

Table 1: Morphological Description of Microalgae Isolated From the Industrial Wastewater

These table 1 shows the morphological assumption of the organism when view under microscope base on morphological characteristics.

S/no	**Microscopic Description**	**TENTATIVE NAMES**
I.	Spherical or oval single-cell green-algae, small round spherical in shape and is without flagella	*Chlorella* sp.
II.	Green algae, unicellular, cell biellipsoidal, oval spindle shape. Spherical, dark green colony and motile.	*Characium* sp.
III.	Unicellular greenish yellow algae, spherical cell in shape of variable sizes (10-30 microns) similar to little tadpole	*Chlorogonium* sp.
IV	Chloroplasts than older ones which have numerous peripheral chloroplasts and many nuclei.	*Chlorogonium* sp or *Micrasterias* sp.
V	Unicellular singled celled flagella green algae.	*Euglene* sp.

Based on the results of amplification and sequencing of 18s ribosomal DNA gene fragments are 1800-2000 bp partial sequence of nucleotides

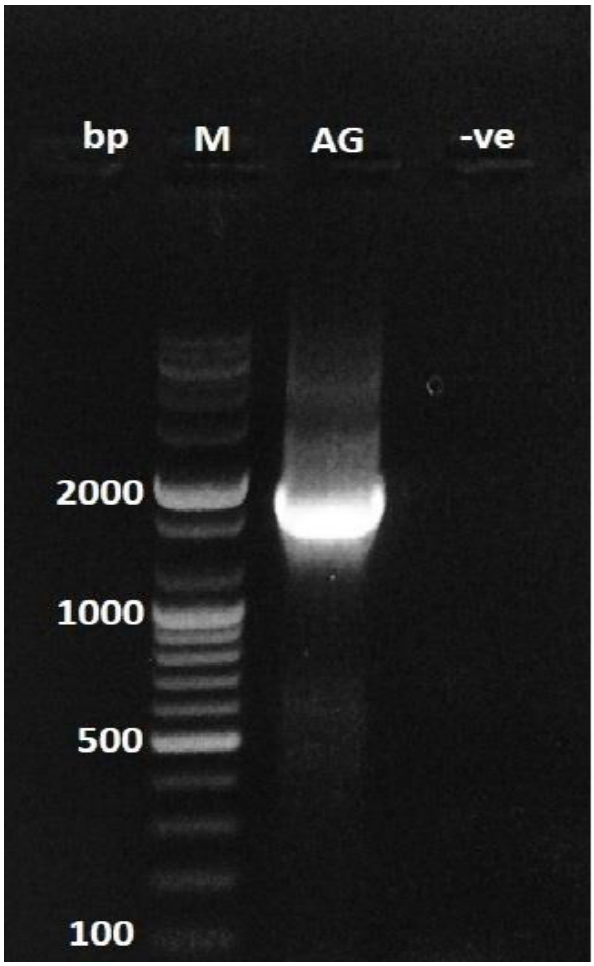


Figure 2: Electrophoretic result of 18S rRNA PCR of isolate

Table 2: Molecular Analysis: 18S rRNA sequence of microalgae isolate

Organism	**Percentage Score**	**Sequence**	**Accession number**
Chlorogonium sp	78.65%	AAGGGCTTGGACGTATCCACCA CGATGACTCTTACTACCTTAAGA TTCCTCGTGAAGACAATAATCT GCAATAATCTATCCCCTCACGAT GCAGTTTCAAAGATACCCGGAC CTTCGCGGATCGCGATCGACGC GGCGGCCCAAACTCTCCGGGCA TCACAGACCTGTTATTGCCTCAC ACTTCCATCTGGCCTTA	OR886595

BLAST analysis of the DNA sequences of isolates is shown in below. The sequence search revealed partial sequence of a small subunit ribosomal RNA gene from *Chlorogonium* sp. AJD-A with 100 % similarity score and E-value of $2e^{-99}$. The sequence has been submitted to NCBI with the accession number OR886595 (Ajiduku *et al*., 2023).

DISCUSSION

The microalga identified in this investigation is *chlorogonium* sp. thus; many microalgal species grow in wastewater due to the abundance of carbon, nitrogen, and phosphorus, which serve as nutrients for the algae ((Dahiya, 2015). The isolated specie is in agreement with the species isolated by Ifeanyi *et al*., (2016) and Stoermer *et al*., (1999). And also Shankar (2013) claimed to have isolated different microalgae from fresh water. According to Aullon (2010), a significant number of isolated microalgae have been associated with the production of biodiesel because of their high lipid oil storage capacity. When compared to other energy crops, algae have been suggested as superior choices for fuel generation because of their higher photosynthetic efficiency, biomass, and quicker growth (Christi, 2007).

The microalga was cultured on BG-11 nutrient agar. According to Khaw *et al.* (2020), the most regularly used media for microalgae production are Blue-Green media (BG-11) and Bold's Basal media (BBM), which are widely utilised to identify and maintain species diversity across a wide variety of species. Zainul (2016) further stated that BG-11, BBM, and Modified Medium (MM) were more suited for growing and enriching the maximum diversity from an environmental sample than Murashige and Skoog (MS) media.

Molecular examination of conserved DNA sections, such as Ribulose Bisphosphate Carboxylase, can also be used to identify microalgae. Large subunit gene (rbcL), 16S and 18S ribosomal RNA (rRNA) genes are employed to profile prokaryotic and eukaryotic microalgae (Wang *et al*., 2016; Ballesteros *et al*., 2021).

The PCR amplicon was purified and sequenced, and then subjected to a sequence search at NCBI using nucleotide BLASTn. The sequence search revealed partial sequence of a small subunit ribosomal RNA gene from *Chlorogonium* sp. AJD-A with 100% similarity score and E-value of $2e^{-99}$. The sequence has been submitted to NCBI with the accession number OR886595.

The molecular identification had confirmed the earlier morphological inferences that the isolated microalga was indeed *Chlorogonium* sp. known for robust application in bioremediation and potentially biodiesel production.

CONCLUSION

From the research that has been done it can be concluded that the, a thick band in electrophoretic result showing 1800-2000bp and the sequence was BLAST in NCBI data base showing the organisms to be *chlorogonium* sp. with a percentage score of 100%, E-value of $2e^{-99}$. and query value of 75% and assigned an accession number OR886595. Algae are found almost everywhere. Both freezing mountain streams and hot inland ponds and marshes with both fresh and salt water are ideal habitats for them. Almost every water environment may support the growth of algae. They use light, water, and carbon dioxide to make biomass and oxygen during photosynthesis. Algal biomass is required to produce many bioproducts such as bioethanol, biofertilizers, biofuel, cosmetics pharmaceutical drugs and many others. As a result of this work, microalgae from fresh water have been identified, separated, cultivated, and collected; these algae might be a useful source for bioremediation and the production of biofuel.

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