



Isolation of *Chlorella vulgaris* from water sample of Bhagva Lake (Kala Talao)

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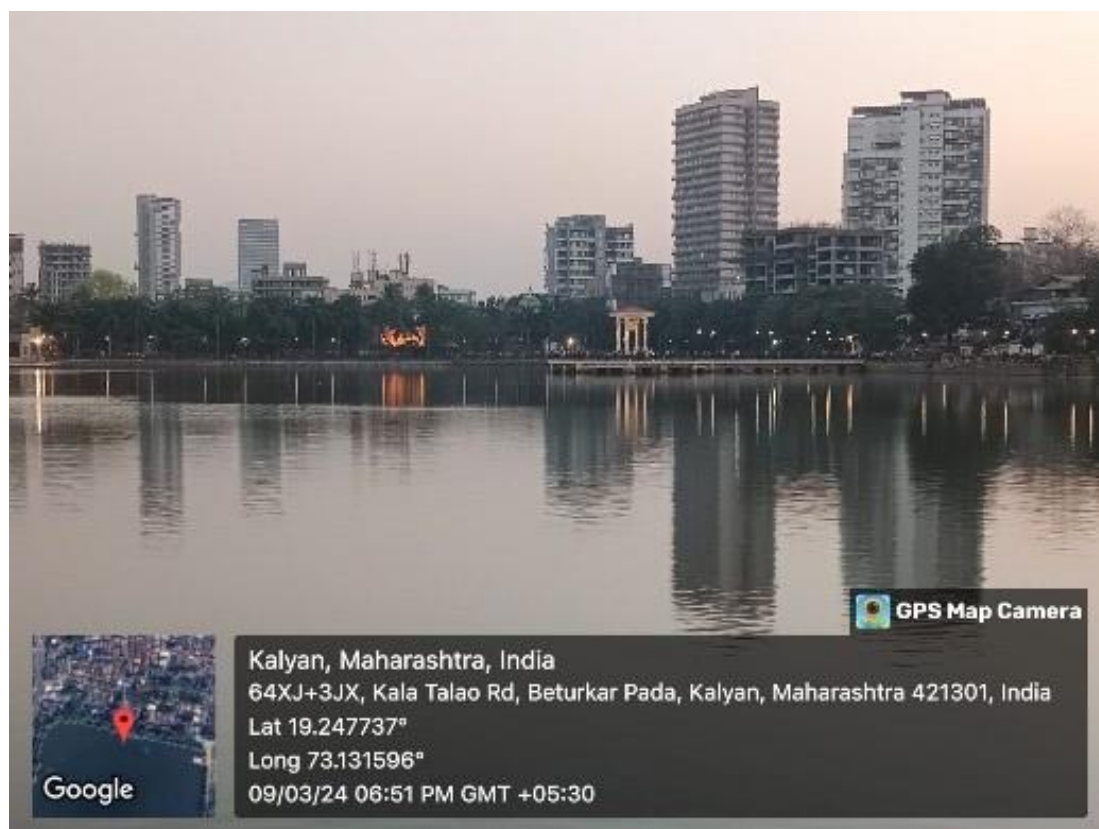
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Abstract: *Chlorella vulgaris*, a unicellular green alga, holds significant promise in various biotechnological applications owing to its robust growth characteristics and rich nutritional content. This study aimed to isolate and characterize *Chlorella vulgaris* strains from Bhagva Lake (Kala Talao), [Kalyan, Maharashtra] India an ecologically diverse freshwater body. The *Chlorella vulgaris* strains were recovered from water samples obtained from Bhagva Lake by a combination of microscopic inspection and various culturing procedures. Light microscopy was used for morphological characterisation. According to preliminary findings, *Chlorella vulgaris* was successfully isolated and identified. *Chlorella* species characteristics were revealed by morphological study. The isolated strain has potential use in biofuel production, wastewater remediation, and food and feed supplements. This study provides a foundation for further exploration of *Chlorella vulgaris* strain from Bhagva Lake, offering insights into their ecological significance and biotechnological potential. Future research endeavours may focus on optimizing cultivation conditions, enhancing biomass production, and exploring the diverse applications of *Chlorella vulgaris* in sustainable bioprocessing industries.

IndexTerms - *Chlorella vulgaris*, Bhagva lake, Isolation, Culturing media, Freshwater alga.

1. Introduction:

Chlorella vulgaris, a unicellular green microalga, is recognized for its significant potential in various fields including biotechnology, environmental remediation, and human nutrition. with its rapid growth rate, ability to photosynthesize, and high content of proteins, lipids, and vitamins, *Chlorella vulgaris* holds promise for sustainable solutions in food production, biofuel generation, and wastewater treatment.



Bhagva Lake, situated in [Kalyan, Maharashtra] India, presents an intriguing environment for the exploration and isolation of microorganisms due to its unique ecological characteristics. The lake's ecosystem, influenced by factors such as water quality, temperature, and nutrient availability, offers an ideal habitat for the proliferation of microalga like *Chlorella vulgaris*. With the purpose of establishing the foundation for future investigation into its possible uses, this pilot project was to isolate and identify *Chlorella vulgaris* strain from Bhagva Lake. Through our investigation of this specific microalga and its environment, we hope to further our knowledge of the diversity of microbes in freshwater environments and investigate the potential of *Chlorella vulgaris* in biotechnological research.

2. Materials and Methods:

1. Collection of Sample:

- Water samples were obtained from Bhagva Lake using plastic containers.
- Samples from different areas of the lake were collected to ensure diversity.

2. Centrifugation:

- Water samples were centrifuged at 3700 rpm for 10-15 minutes.
- This step helps to concentrate the algae cells at the bottom of the centrifuge tube.

3. Preparation of Bold's Basal Media:

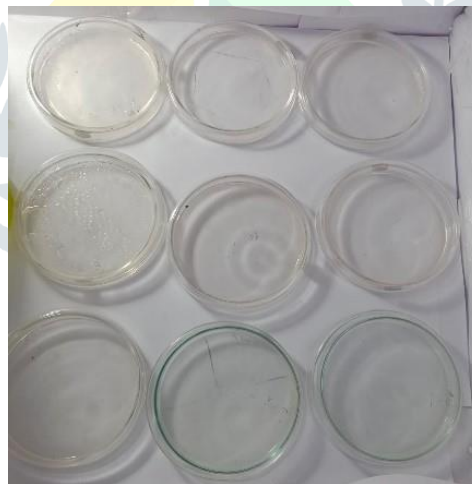
- Bold's Basal media was prepared with slight modifications
- pH of the media was adjusted to the optimal range for algae growth (usually around pH 7).

4. Culturing algal sample:

- Concentrated algae cells from the centrifuged sample were inoculated into Bold's Basal Media in sterile culture flasks.
- The flasks were kept in a self-made incubator set to the appropriate temperature (usually around 25-30°C) with a 12-hour light-dark cycle.

5. Bold's Basal media agar plating for Isolation of alga:

- Agar plates were prepared using Bold's Basal Media supplemented with agar (1.5%).



Bold's Basal media agar plates

- 1-2 ml of algal culture was suspended onto the surface of the agar plates using a sterile spreader.
- The agar plates were incubated in the same conditions as the liquid cultures.

6. Colony Culturing:

- After incubation, the agar plates were observed for the formation of colonies.
- Well-separated colonies were selected, picked, and were inoculated in the fresh liquid media in culture flasks.

- Incubate the liquid cultures under the same conditions as before.



Day1

Day 12

7. Sub-culturing by Serial Dilution:

- Once the liquid cultures reach the stationary phase, perform serial dilution by transferring a small volume of the culture into fresh media.
- Repeat the process multiple times with increasing dilutions to isolate pure cultures of *Chlorella vulgaris*.

8. Maintenance:

- Regularly sub-culture and maintain the isolated *Chlorella vulgaris* strains to prevent contamination and ensure their viability for further studies.

3. Observations:

Observation 1:

Initial Sample Collection:

After collecting samples from Bhagva Lake and subjecting them to centrifugation at 3700 rpm, subsequent observation under a compound microscope at 40x magnification revealed the presence of 16 distinct species of microalgae.

Species of micro algae observed before culturing	
<i>Scenedesmus quadricauda</i> (Turp.)	<i>Chlorella vulgaris</i>
<i>Scenedesmus dimorphus</i> (Turpin)	<i>Cosmarium granatum</i>
<i>Arthrodesmus gibberulus</i>	<i>Euastrum insulare</i> (Wittrock)
<i>Ankistrodesmus falcatus</i> (Corda)	<i>Tetraedron tumidulum</i> (Reinsch)
<i>Schroederia indica</i>	<i>Coelastrum microporum</i>
<i>Dicloster acuatus</i>	<i>Ankistrodesmus convolutus</i>
<i>Chlorococcum humicola</i>	<i>Gloeocapsa nigrescens</i>
<i>Synechocystis crassa</i>	<i>Pediastrum tetras</i>

Observation 2:

Observation after Centrifugation and Culturing in Bold's Basal Media:

After collecting and identifying the algae present in the sample, centrifugation at 3700 rpm was conducted to isolate them. Following deposition, 16 distinct species were cultured in Bold's basal media for 7-8 days within a self-constructed bio-reactor. Subsequent observation under a compound microscope at 40X magnification revealed the presence of 9 species, while the remaining species that failed to adapt were not observed.

Species of micro algae observed (after culturing in B.B.M)	
<i>Scenedesmus quadricauda</i> (Turp.)	<i>Chlorella vulgaris</i>
<i>Arthrodesmus gibberulus</i>	<i>Cosmarium granatum</i>
<i>Schroederia indica</i>	<i>Coelastrum microporum</i>
<i>Chlorococcum humicola</i>	<i>Pediastrum tetras</i>
<i>Tetraedron tumidulum</i> (Reinsch)	

Observation 3:

Agar Plating and Culturing:



Day 1

Day 8

The algae specimens were cultured in Bold's basal media for a duration of 10 days, following which the nine species were utilized in agar plating exclusively with Bold's basal media for 8 days. This method was employed to isolate distinct colonies, aiming for subsequent culturing to specifically isolate *Chlorella vulgaris*.

Observation 4:

Microscopic Examination of Isolated Colonies:

After a 10-day incubation period, isolated colonies emerged on the agar plates. These colonies were meticulously extracted from the plates to undergo further subculturing in Bold's basal media within a self-constructed bioreactor. Following a subsequent

cultivation period of 10-15 days, microscopic examination at 40X magnification revealed the presence of 5 distinct micro-algae species within the sample.

Species of micro algae observed.
(Isolated Colonies in agar plates)
<i>Chlorococcum humicola</i>
<i>Chlorella vulgaris</i>
<i>Cosmarium granatum</i>
<i>Coelastrum microporum</i>
<i>Pediastrum tetras</i>

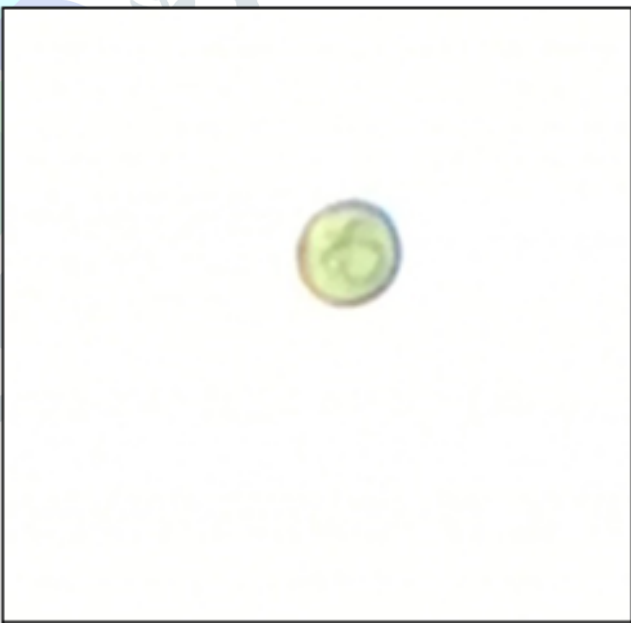
Observation 5:

Isolation of *Chlorella Vulgaris*:

To isolate *Chlorella Vulgaris*, a serial dilution of 10^{-5} was performed on the sample containing a mixture of five algal species obtained in the preceding step. Subsequently, this serially diluted sample underwent culturing on Bold's basal media for a duration of 11 days. *Chlorella Vulgaris* was successfully isolated and identified via morphological characterization, facilitated by careful observation under a compound microscope at 40x magnification after conducting a number of observations.



Observation under 10x



Magnified image of isolated *Chlorella vulgaris*

4. Results:

1. Initially 16 species were identified.
2. 9 algal species were observed after culturing in Bold's Basal Media. Unsuccessful adaptation noted in some species.
3. Microscopic examination revealed 5 distinct micro-algae species colony after serial dilution and agar plating.
4. *Chlorella vulgaris* colony was Isolated and picked from agar plates of Serial dilution (10^{-5}) inoculum.
5. *Chlorella vulgaris* was successfully isolated and identified via morphological characterization under a compound microscope at 40x magnification.

These results demonstrate the effectiveness of various isolation and culturing techniques in identifying and isolating *Chlorella vulgaris* from a mixed sample of microalgae.

5. Conclusion:

The study conducted aimed at isolating *Chlorella vulgaris* from Bhagva Lake (Kala Talao) Maharashtra, India, in pursuit of exploring their potential applications in various biotechnological endeavours. Through a series of meticulous methodologies encompassing sample collection, centrifugation, culturing, agar plating, and serial dilution techniques, the study successfully achieved its objectives and yielded valuable insights into the ecological significance and biotechnological potential of *Chlorella vulgaris*.

6. Key Findings and Implications:

Diverse Microalgal Population: Initial observation following sample collection and centrifugation revealed the presence of 16 distinct species of microalgae in Bhagva Lake, highlighting its ecological richness and diversity.

Effective Culturing Techniques: Culturing of the isolated microalgae strains in Bold's basal media showcased the adaptability of certain species, with 9 species successfully adapting to laboratory conditions. This observation underscores the importance of appropriate culturing techniques in preserving microalgal diversity.

Isolation of *Chlorella vulgaris*: Through agar plating and subsequent culturing, *Chlorella vulgaris* strains were successfully isolated from the diverse microalgal population present in Bhagva Lake. This isolation process serves as a crucial step towards further exploration and utilization of *Chlorella vulgaris* in various biotechnological applications.

Future Research Directions: The study lays the groundwork for future research endeavours aimed at optimizing cultivation conditions, enhancing biomass production, and exploring novel applications of *Chlorella vulgaris* in sustainable bioprocessing industries. Further studies may focus on elucidating the genetic and metabolic mechanisms underlying the unique properties of *Chlorella vulgaris* strains from Bhagva Lake, thereby unlocking their full biotechnological potential.

In conclusion, this preliminary study provides valuable insights into the isolation and characterization of *Chlorella vulgaris* strains from Bhagva Lake, offering a platform for further exploration of their ecological significance and biotechnological applications. The findings contribute to the growing body of knowledge surrounding microalgal diversity and underscore the importance of microalgae such as *Chlorella vulgaris* in sustainable biotechnological innovations.

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