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ORIGINAL ARTICLE

Isolation and Identification of Microalgal Isolates from Contaminated Lagoon

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ABSTRACT
Pollution is a man- made phenomenon, arising either when the concentration of naturally occurring substances are increased or when non-natural synthetic compounds (xenobiotics) are released into the environment as a result of domestic, agricultural and industrial activities. Aquatic pollution became a major concern of all other pollution because that causes adverse effects to aquatic flora and fauna. However aquatic pollution can be very well addressed by algal organisms, which are able to thrive of at wider range of habitat and have the potential of scavenging and sequestering many different toxic chemicals. Therefore, the present study was undertaken on isolation and identification of potential microalgal isolates from contaminated lagoon. The aquatic sample used for the study was collected from a lagoon near Dindigul Town, Tamil Nadu and enriched with four different culture medium viz., BG11, FOGG'S,CHU10 and ZARROUK. About 21 microalgal isolates were isolated from the lagoon sample and identified based on the microscopic cultural characteristics and authenticated by key manual.
Keywords: Aquatic pollution, Xenobiotics, Sequestration, Lagoon, Microalgae, Flora and Fauna.

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INTRODUCTION

Human activities directly or indirectly affect the environment adversely. In the developing countries, activities like construction, transportation and manufacturing not only deplete the natural resources but also produce large amount of wastes that leads to environmental pollution [1]. Improper release of untreated or improperly treated industrial wastes become a major source of water pollution that causes ill health in aquatic animals and human beings. Hence, an intervention in most required to minimize and or control toxic contaminants from polluted aquatic and surrounding ecosystems. The toxic chemical substance found in waste water can be removed through several methods viz., physical, chemical and biological processes. Recently, bioremediation process has been recognized as efficient, cost effective, and suitable alternative to conventional methods for removing contaminants from polluted environment [2]. Bioremediation processes are depends on the utilization of living organisms (microbes, plants and animals) and their components viz., enzymes, plant materials etc.

Algae such a potential source of organism widely used for bioremediation processes. Algae are a group of organisms that have been generally described as photoautotrophic, simple microscopic or macroscopic, unicellular to multicellular organism [3]. Algal biomass has historically served as fertilizer; as food source for both humans and animals and also has important role in bioremediation of waste water. Microalgae have a rapid growth rate and its cultivation consumes less water than land crops. The microalgae have the same process with higher plants to reduce the greenhouse effect with capture of carbon dioxide through photosynthetic reaction using energy from light, so that it is environmental friendly [4].

Algae are the major health indicator of oceans in which 71% of the earth surface is covered by algal population. Algae are the original source of fossil carbon found in the crude oil and in natural gas. Microalgae is found all over the world and occupies almost 75% algae species, contribute approximately 40% of the oxygen in the atmosphere [5]. They are mainly distributed in the waters, but are also found on

the surface of all type of soils. Although they are free-living, a certain microalgae live in symbiotic association with a variety of other organisms. Microalgae consist of a wide range of autotrophic organisms and have comparable photosynthetic efficiency to higher plants, rapid growth rate, and notable adaptability. Carbon, nitrogen, and phosphorus are essential elements for microalgal growth and can be effectively used via different metabolic pathways [6]. The pH value for optimum growth of algae ranges between 7 to 12. Every algal species has a different optimum salinity range. Microalgae have a great biological resource and have high growth and tolerance for varying environmental conditions including organic and inorganic polluted environment. In general the pollutants are of various types viz., heavy metals (e.g. lead, cadmium, arsenic, mercury), chemical fertilizers and pesticides, petroleum hydrocarbons (crude oil) and are introduced into the environment through industrial discharges, agricultural uses and improper waste disposal practices. All these toxic chemical contaminants may be immobilized and accumulated in water bodies as sediments or may be subject to transformation and activation processes [7]. The persistence of these chemicals in the aquatic environment poses a chronic threat to the health and safety of aquatic flora and fauna and also for human and wildlife [8].
The main intension of the present study is to isolate and identify the microalgae from contaminated lagoon and compare the growth performance of potential microalgal isolates using different culture medium.

MATERIALS AND METHODS

Sample collection : The aquatic sample was collected from the contaminated lagoon near Dindigul Town, Tamil Nadu, India which is lie in the latitude of 10°.34'55.58" N in the longitude of 77°.94'91.9"E (Fig 1). The aquatic sample was transported to the laboratory, Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Gandhigram, Tamil Nadu, for further analysis.

Fig.1. GPS map of sample site

Enumeration of microalgae using single cell isolation technique: The serial dilution method was used to reduce the algal cell population using multi well plate method [9] and this procedure increases the probability of single-cell isolation. Single algal strain was isolated through spread and streak plate methods using BG 11 medium which contains stock solution 1:NaMg.EDTA – 0.1g, Ferric ammonium citrate – 0.6, Citric acid. $1H_2O$ – 0.6g, $CaCl_2.2H_2O$ – 3.6g; stock solution 2: $MgSO_4.7H_2O$ – 7.5g; stock solution 3: $K_2HPO_4.3H_2O$ – 4.0g, K_2HPO_4 – 3.05g; Microelements: H_3BO_3 – 2.86g, $MnCl_2.4H_2O$ – 1.81g, $ZnSO_4.7H_2O$ – 0.222g, $CuSO_4.5H_2O$ – 0.079g, $COCl_2.6H_2O$ – 0.050g, $NaMoO_4.2H_2O$ – 0.391g and MoO_4 (85%) – 0.018g. Final medium was prepared by mixing of different proportion from various stock solutions and adjusted to pH – 7.1.
In Spread plate technique, the diluted samples were pipette out on to the centre of the sterile BG11 agar plate and spread the sample on the agar surface. In streak plate technique, the diluted microalgal culture was streaked across the agar surface using sterile inoculation loop. All the inoculated plates were incubated at 28°C with light intensity of 3000 lux for 7 days. A well isolated algal colony was taken and transferred to BG 11 broth to maintain the pure culture suspension for further studies.
Identification of microalgal isolates: All the 21 microalgal strains isolated from lagoon were identified based on their microscopic cultural characteristics [10]. A drop of unialgal culture suspension was transferred to a clean glass slide and directly observed under light microscope (Motica light microscope) to reveal the morphology and organization of individual microalgal strains. Based on the morphological characteristics, the selected isolates were authenticated using the key manual [11].
Evaluation study on growth performance of microalgal isolates in various enrichment medium: The enrichment process provides a suitable environment for the growth and reproduction of a special group of microalgae while being inhibitory or lethal for non-target organisms. Different enrichment medium being used for the algal cultures viz., BG11, CHU10, FOGG's and ZARROUK Fig 2. The composition

of BG 11 medium is described in method II. CHU 10 medium contains Ca $(NO_3)_2$ - 0.04 g; K_2HPO_4 - 0.01 g; $MgSO_4$ - 0.025 g; Na_2CO_3 - 0.2 g; Na_2SiO_3 - 0.025 g; FeCl3 - 0.8 g; distilled water 1000 ml; pH 6.5-7.0. FOGG's medium contains K_2 HPO 0.2 g; $MgSO_4.7H_2O$ - 0.2 g; $CaCl_2.2H_2O$ - 0.1 g; Fe-EDTA -1.0 ml; A5 micronutrient solution (Dissolved 1.81 g MnCl2 , 0.0177 g MoO3, 0.222 g ZnSO4.4H2O, 0.079 g CuSO4. 5H2O, 2.86 g H3BO3 in 1000 ml distilled water) - 1.0 ml; distilled water 1000 ml ; pH 6.5- 7.0. The ZARROUK medium contains NaCl $_2$ – 1.0g; $CaCl_2.2H_2O$ – 0.04g; KNO_3 – 2.5g; $NaNO_3$ – 2.5g; $FeSO_4.7H_2O$ – 0.01g; EDTA (Na) – 0.08g; K_2SO_4 – 1.0g; $MgSO_4$. $7H_2O$ – 0.2g; $NaHCO_3$ - 16.8g; K_2HPO_4 - 0.5g; A5 solution - 1.0ml; pH 6.5-7.0. All the microalgal isolates were inoculated in the respective medium and the algal culture setup was incubated at 28°C with a light intensity of 3000 lux for 15days. The comparative growth performances of different algal isolates were observed at the end of 15 days.

RESULTS AND DISCUSSION

In the present study, the aquatic sample was obtained from contaminated logoon near Dindigul town (Fig 1). The sample was inoculated in to the BG 11 medium for the enrichment of the culture, and then it was serially diluted in the multi well plates for reducing the non-targeted organisms. The culture was maintained as a unialgal condition by spread plate and streak plate methods (Fig 2).

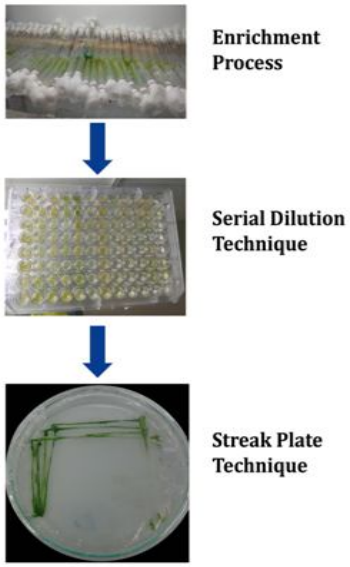

Fig 2 Single cell isolation steps

The enrichment process provide, suitable environment for growth and reproduction of a special group of microalgae. The initial step towards single-cell isolation by adding nutrients to facilitating the growth of microalgae [12]. The isolated colonies were identified based on the colony morphology observed on the agar plates. Formulating a medium, it may be important to decide whether it is likely to promote microalgal growth. Richly organic media should be avoided unless the algae being cultivated are axenic [13]. In this study, algal isolates were grown in four enrichment culture medium viz., BG11, FOGG'S, CHU10 and ZARROUK. The optimization of growth promoting ability of the selected medium was demonstrated by [14].

Table 1: List of twenty one microalgal strains isolated from lagoon sample.

Strain No	Microalgal Strains	Family	Common Classification	Microscopic observation
S1	*Lyngbya* sp.,	Cyanophyceae	Blue green algae	Long unbranched filaments
S2	*Oscillatoria* sp.,	Cyanophyceae	Blue green algae	Filamentous cyanobacterium
S3	*Microcystis* sp.,	Cyanophyceae	Blue green algae	Presence of gas filled vesiciles
S4	*Phormidium* sp.,	Cyanophyceae	Blue green algae	Filament
S5	*Anabaena* sp.,	Cyanophyceae	Blue green algae	Heterocyst forming photoautotrophic filament
S6	*Nostoc* sp.,	Cyanophyceae	Blue green algae	Presence of gelatinous sheath
S7	*Gloeocapsa* sp.,	Cyanophyceae	Blue green algae	Spherical cells arranged in pairs
S8	*Chlorella* sp.,	Chlorophyceae	Green algae	Single round cells
S9	*Spirogyra* sp.,	Chlorophyceae	Green algae	Filaments with zig zag arrangement
S10	*Oedogonium* sp.,	Chlorophyceae	Green algae	Unbranched filament

S11	*Gonium* sp.,	Chlorophyceae	Green algae	Presence of uniform 16 cells
S12	*Scenedesmes* sp.,	Chlorophyceae	Green algae	Presence of spindle shaped cells
S13	*Eudorina* sp.,	Chlorophyceae	Green algae	Cells with extra cellular matrix
S14	*Chlorococcum* sp.,	Chlorophyceae	Green algae	Unicellular cells
S15	*Sphaerocystis* sp.,	Chlorophyceae	Green algae	Spherical aggregated cells
S16	*Neochloris* sp.,	Chlorophyceae	Green algae	Presence of spherical cells
S17	*Cladophora* sp.,	Chlorophyceae	Green algae	Presence of reticulated filament
S18	*Microspora* sp.,	Chlorophyceae	Green algae	Long Cells with enormous segments
S19	*Cosmarium* sp.,	Chlorophyceae	Green algae	Presence of bilobed cells
S20	*Ulothrix* sp.,	Chlorophyceae	Green algae	Unbranched filamentous structure
S21	*Tribonema* sp.,	Xanthophyceae	Yellow green algae	Filamentous in shape

Among the 21 microalgal isolates, only four isolates viz., *Phormidium sp, Chlorococcum sp, Chlorella sp* and *Neochloris sp,* shown favourable growth in all four enrichment medium. However these four microalgal isolates were exhibit excellent growth in BG 11 medium (Table 2)

Table 2: Comparative growth performance of various microalgal isolates in different culture medium

S. No	Microalgal species	BG 11 [18]	CHU 10 [19]	FOGG'S [20]	ZARROK [21]
1.	*Lyngbya sp*	+	_	_	_
2	*Oscillatoria sp*	+	_	_	+
3	*Microcystis sp*	+	_	_	_
4	*Phormidium sp*	+ +	+	+	+
5	*Anabaena sp*	+	_	_	_
6	*Nostoc sp.*	+	_	_	+
7	*Gloeocapsa sp*	+	_	+	_
8	*Chlorella sp*	+ +	+	+	+
9	*Spirogyra sp*	+	+	_	_
10	*Oedogonium sp*	+	_	_	_
11	*Gonium sp*	+	_	_	_
12	*Scenedesmus sp*	+	+	_	_
13	*Eudorina sp*	+	_	_	_
14	*Chlorococcum sp*	+ +	+	+	+
15	*Sphaerocystis sp*	+	_	_	_
16	*Neochloris sp*	+ +	+	+	+
17	*Cladophora sp*	+	_	+	_
18	*Microspora sp*	+	_	_	_
19	*Cosmarium sp*	+	_	_	+
20	*Ulothrix sp*	+	_	_	_
21	*Tribonema sp*	+	_	+	+

(+) presence of growth, (++) maximum growth, (-) no growth

Different culture media tested in this study had different effects on growth of microalgae. Among the four culture media tested for this study, BG 11 media was recorded as efficient in encouraging the growth of algae when compared with other culture media. The culture media also played an important role in completing the duration of growth cycle.

Based on the effectiveness of BG11, it was selected for maintenance of the microalgal cultures. In this study the microalgal strains were allowed to grown in a continuous illumination (3000 lux) with proper temperature (28-34^{0}C) and with BG 11 medium in short period. The growth performance of microalgae mainly depends on light intensity, growth temperature and the culture medium Fig 3. Light intensity take part in the major role, but the requirements greatly vary with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture. Too high light intensity (e.g., direct sunlight, small container close to artificial light) may result in photoinhibition [15]. Most often employed light intensities range between100 and 200 µE sec-1 m-2, which correspond to about 5–10% of full daylight (2000 µE sec-1 m-2). Moreover, overheating due to both natural and artificial illumination should be avoided. Light may be natural or supplied by fluorescent tubes emitting either in the blue or the red light spectrum, as these are the most active

portions of the light spectrum for photosynthesis. Thus it is depicted that light exposure, intensity and penetration are important factor for algal cultivation [16].

Most commonly cultured species of microalgae showed the optimum temperature range between 16°C and 27°C, and vary with the species, and with strain cultured. An intermediate value of 18–20°C is most often employed. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species. In another study Scientists have found that the optimal temperature for growing for some species of algae range between 20°C to 30°C. In optimum temperature will encourage the maximum growth of the selected microalgal [17] and the temperature fluctuation may variate the size and number of microalgae.

Therefore this study highlighted that, among 21 microalgal isolates, four strains *Phormidium sp, Chlorococcum sp, Chlorella sp* and *Neochloris sp* can with stand for long period and with better growth performance. In the midst of four enrichment microalgal culture medium BG11, FOGG'S, CHU10 and ZARROUK, BG 11 proved as best growth medium for selected microalgal strains.

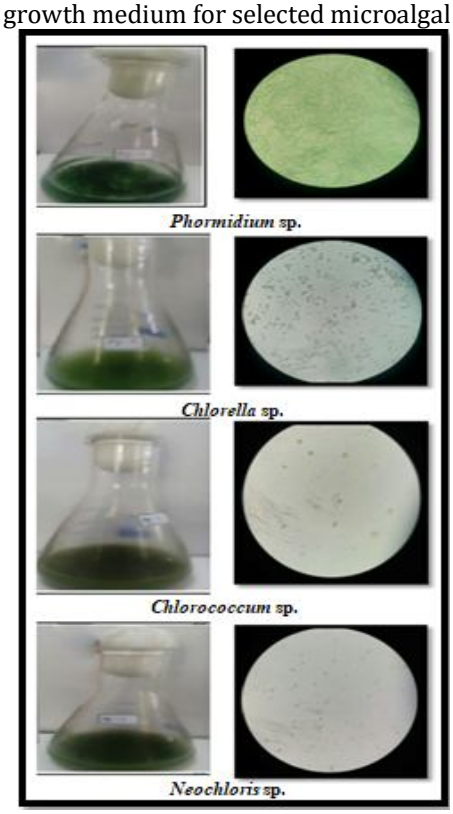

Fig 3: Growth and microscopic observations of four selected microalgal strains in BG 11 medium

CONCLUSION

Contaminated lagoon sample had abundance microalgal population due to rich presence of both organic and inorganic nutrients. In this study, 21 microalgal strains were recorded in the lagoon sample. Along with 21 microalgal isolates, four strains viz., *Phormidium sp, Chlorococcum sp, Chlorella sp* and *Neochloris sp.* showed better growth rate in four different enrichment culture medium viz., BG11, CHU10, FOGG'S and ZARROUK. Based on the comparative growth performance BG 11medium optimized as best medium for cultivation of microalgal isolates. From this study, it is concluded that the four microalgal isolates would mass cultured and explored for various application including waste water treatment, biofertilizer industry and single cell protein industry etc.

FUTURE SCOPE

Microalgal bioremediation is evolved as one of the best sustainable way to remove heavy metals present in the environment. The main advantage of selection of microalgae is the best heavy metal accumulating, low cast, easy developing and eco-friendly process. Further scope of the study focuses on isolation of efficient metal tolerant microalgae from various sources.

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Conflict of interest. None.

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