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Application of NaCl for Cultivation of Isolated *Botryococcus Braunii* Strains

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Abstract

One of the possible sources of renewable biofuel is the colonial green micro alga Botryococcus braunii, which is rich in hydrocarbons and lipids. The outdoor biomass production of the alga is severely affected by ingestion of growing alga by various types of mosquito. In an attempt to control the growth of mosquito larvae by using NaCl, tolerance range of two strains of B. braunii, one isolated from Udaisagar Lake, Udaipur, Rajasthan, India and other from Loktak Lake, Assam, India, and the Anopheles mosquito larvae to the NaCl was identified. When 2 days old mosquito larvae were incubated along with two strains of B. braunii separately in Chu-13 medium supplemented with different concentrations of NaCl under controlled conditions of light and temperature, 0.5 M NaCl caused the killing of larvae on 9^{th} day without much affecting the algal growth. During outdoor cultivation of the two strains in plastic trays, the mosquito larvae did not appear when nutrient medium was supplemented with 0.5 M NaCl. Also, 0.5 M NaCl in Chu-13 medium exhibited a marked positive impact on hydrocarbon and oil content (produced more than double) in both the strains of the alga. This finding suggests the exploitation of NaCl and possibility of salt rich sea water for outdoor commercial cultivation of B. braunii for biomass and bio-energy production.

Keywords: Biofuels, NaCl, Anopheles mosquito, Botryococcus braunii, hydrocarbon.

1. Introduction

The limited Life span of conventional fuels has led to shifting the focus on to renewable, environment friendly, non-conventional fuel sources. Algal Biofuel technology offers the opportunity to utilize land unsuitable for any other use without stressing additional demand on limited agricultural land. The colonial green micro alga *Botryococcus braunii* is a rich source of hydrocarbons and lipids amounting to 30-70% of its dry weight under different conditions of growth (Dayanand *et al*. 2007; Pal *et al*. 1998; Papa *et al*. 2008; Wolf 1983; Yamaguchi *et al*. 1987). It has been recognized as one of the possible sources of renewable liquid hydrocarbons (Ranga Rao *et al.* 2007); producing more of the right kinds of natural hydrocarbons needed to biodiesels and has become the exclusive focus of the research. It is widely distributed in freshwater, brackish and saline lakes, reservoirs or small pools situated in temperate, tropical and continental zones (Metzger and Largeau 2005; Qin, 2005). Various strains of *B. braunii* found in different types of natural water bodies differ in the type of hydrocarbons they synthesize and accumulate. These include *n*-alkadienes and trienes, triterpenoid botryococcenes, methylated squalenes, or the tetraterpenoid, lycopadiene. They were also found out to produce ether lipids closely related to hydrocarbons (Metzger and Largeau 2005). In the present study the exploitation of NaCl and possibility of salt rich sea water for outdoor commercial cultivation of *B. braunii* for biomass and bio-energy production has been done. The effect of NaCl on controlling larvae growth has also been studied. Growth of two strains of *B. braunii* in outdoor trays during different months in different mediums was done. The research also targets at leading to the development of technologies that can have large-scale and economical production of the algal biomass and oils where there could be potential use of sea water as observed from significant stimulation of oil and hydrocarbon production by NaCl.

2. Materials and Methods

2.1 Test Organisms and Growth Conditions

One of the two strains of *Botryococcus braunii* was isolated from Udaisagar Lake, Udaipur, Rajasthan and the other from Loktak Lake, Assam by serial dilution method followed by plating on solidified Chu-13 nutrient medium (Chu 1942; Yamaguchi *et al*. 1987). The individual colonies were isolated and inoculated into liquid medium. For isolation, growth and hydrocarbon experiments, cultures were incubated at 27 ± 1ºC under 1.2 ± 0.2 klux light intensity with 16:8 hrs light:dark cycle. The purity of the culture was ensured by repeated plating and by regular observation under the microscope.

2.2 Biomass Estimation

For biomass measurement in terms of dry weight (gL^{-1}), the cultures were harvested by centrifugation and the cells were washed twice with distilled water. The pellet was freeze dried and the dry weight of algal biomass was determined gravimetrically.

2.3 Extraction and Estimation of Total Chlorophyll

Time course growth experiments with *B. braunii* were carried out in Erlenmeyer flasks of 250 ml capacity, containing 100 ml medium for a period of 3 weeks. Cultures were harvested and total chlorophyll was estimated at different time intervals in 90 % methanol using Lichtenthaler equations (Lichtenthaler 1987).

2.4 Estimation of Survival of Mosquito Larvae

Two-days old mosquito larvae collected from outdoor trays filled with tap water, washed with sterile distilled water thrice and incubated with the alga in Chu-13 medium supplemented with different levels of sodium chloride (0.25-1.75 M) in the vessel covered with mosquito net in the incubator and growth of both the alga and larvae was observed at different time intervals.

2.5 Extraction and Gravimetric Estimation of Hydrocarbon

The dry biomass was homogenised in mortar and pestle with *n*-hexane for 15 minutes and centrifuged. The extraction process was repeated twice and supernatant was transferred to pre-weighed glass vial and evaporated under the stream of nitrogen to complete dryness. The quantity of residue was measured gravimetrically and expressed as dry weight percentage (Dayananda *et al.*, 2005).

2.6 Extraction and Gravimetric Estimation of Oil

Algal oil extract was prepared by homogenizing the dried algal powder in the solvent (0.1 g/ml) chloroform-methanol (2:1) and collecting the supernatant. Quantitative analysis of lipids extracted was performed gravimetrically.

2.7 Statistical Analysis

All the experiments were repeated at least three times with consistently the same results. Mean values and standard deviation were determined from three replicates for each treatment.

3. Results and Discussion

Growth of isolated strains was checked in Chu-13 medium. Figure 1 shows a comparative account of growth at different time periods of the isolated two strains of *B. braunii* in Chu-13 medium. It is evident that Loktak strain has better growth pattern than the Udaisagar strain. Figure 2 displays growth of two strains of *B. braunii* in Chu-13 medium supplemented with different concentrations of NaCl on at different days (upto 18^{th} day). Loktak lake strain showed better growth compared to Udaisagar lake strain. Hydrocarbon and oil production by *B. braunii* in Chu-13 medium supplemented with 0.5 M NaCl on 18^{th} day was studied. It is clear from figure 3 that both the oil and hydrocarbon production increased with the addition of NaCl without affecting the growth. This finding suggests the possible exploitation of sea water for outdoor commercial cultivation of *B. braunii* for biomass and bio-energy production.

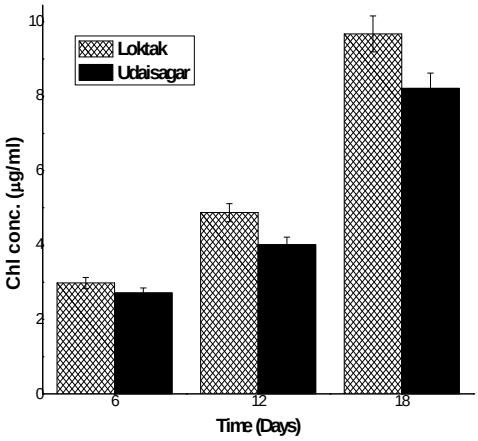

Fig. 1: Growth pattern of B. braunii strains isolated from Udaisagar and Loktak lakes in Chu-13 medium.

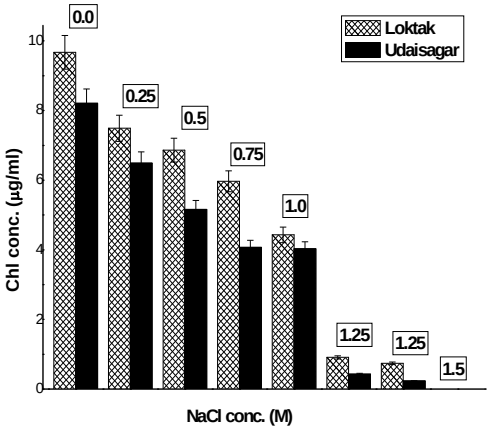

Fig. 2: Growth of two strains of B. braunii isolated from Loktak and Udaisagar lakes in Chu-13 medium supplemented with different concentrations of NaCl on 18th day.

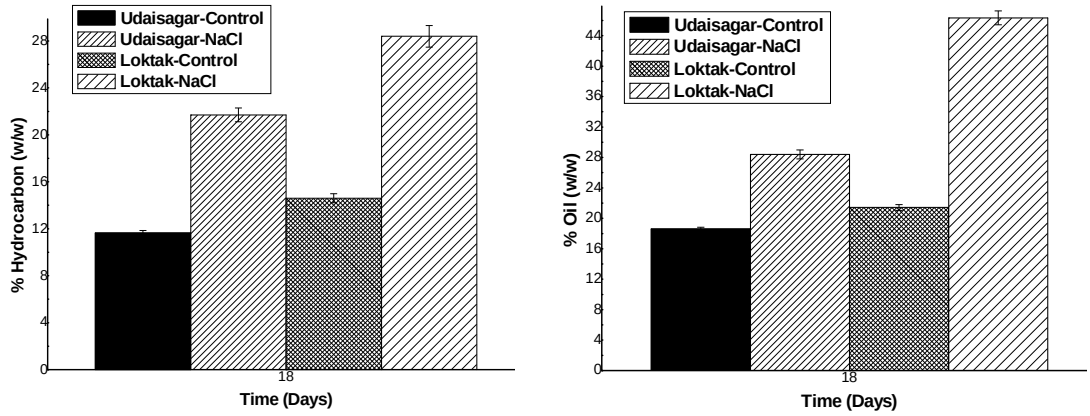

Fig. 3: Hydrocarbon and oil production by B. braunii in Chu-13 medium supplemented with 0.5 M NaCl on 18th day (Udaisagar-Control: % hydrocarbon/oil in Chu-13 medium for Udaisagar strain, Udaisagar-NaCl: % hydrocarbon/oil in Chu-13 medium with 0.5 M NaCl for Udaisagar strain, Loktak-Control: % hydrocarbon/oil in Chu-13 medium for Loktak strain, Loktak-NaCl: % hydrocarbon/oil in Chu-13 medium with 0.5 M NaCl for Loktak strain).

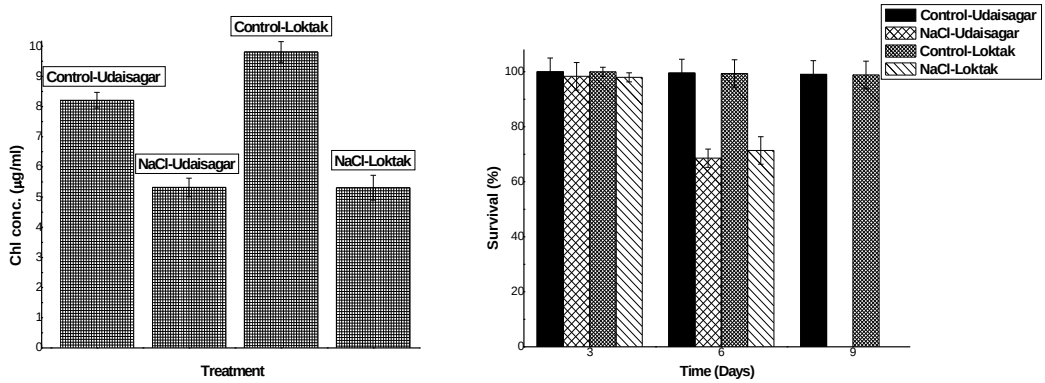

Fig. 4: Growth of Udaisagar and Loktak strains of B. braunii on 18th day and survival of mosquito larvae with the two strains of the alga under controlled light and temperature in Chu-13 medium with 0.5 M NaCl (NaCl) and control (Chu-13). Larvae were transformed into adults on 9th day, if alive.

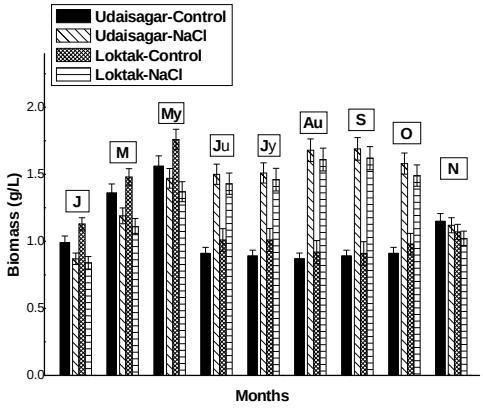

Fig. 5: Growth of two strains of B. braunii in outdoor trays during different months in economic medium (Control) and the medium supplemented with 0.5 M NaCl (NaCl) on 18th day. (J/Jan: January, M/Mar: March, My: May, Ju: June, Jy: July, Au: August, S/Sep: September, O: October, N/Nov: November, Control: Growth in economic medium in tap water under controlled conditions)

Growth patterns of Udaisagar and Loktak strains of *B. braunii* on 18th day and survival of mosquito larvae with the two strains of the alga under controlled light and temperature in Chu-13 medium was studied with 0.5 M NaCl (NaCl) and control (Chu-13). Larvae were transformed into adults on 9th day, if alive. Figure 4 exhibits that controlled conditions exhibited better results. Figure 5 demonstrates the growth of two strains of *B. braunii* in outdoor trays during different months of the year in economic medium (Control) and the medium supplemented with 0.5 M NaCl (NaCl) on 18th day.

The results of the study reveal that knowledge of tolerance of the algal strains to environmental factors may help in selecting the appropriate strains according to the prevailing environmental conditions of a place and for successful commercial cultivation throughout the year.

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