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Salinity stress on growth and lipid accumulation in *Anabaena circinalis*: a preliminary study

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Abstract

Growth and lipid accumulation in the blue-green alga *Anabaena circinalis* in response to different concentrations of NaCl (0.4-0.8 M) was undertaken in the present study. Elevated levels of NaCl concentration resulted in enhancement of lipid content and reduced growth rate in the studied alga. The highest amount of lipid content (8.33 ± 0.32%) was found in the test organism growing in 0.8 M NaCl stress culture.

Keywords: *Anabaena circinalis*, growth, lipid content, salinity

Introduction

Biofuels derived from photosynthetic microorganisms such as cyanobacteria and microalgae have received considerable interest in recent years (Tan and Lee, 2016). Microalgae utilize solar energy, water and CO_2 which are assimilated into the reserve storage components of carbohydrates, lipids and proteins, of which lipids have the highest energy content (Quintana *et al*., 2011). Microalgae have already reached upto 300 times more oil productivity for biodiesel production than traditional crops on an area basis (Hempel *et al*., 2012). Furthermore, some microalgae are capable of accumulating large amounts of lipids (upto 70% w/w) and can recycle water and nutrients from effluent streams, and do not directly compete with food production (Subramanian *et al*., 2013). They can also be grown in brackish and wastewater (Guccione *et al*., 2014).

Though microalgae is considered as one of the most promising sustainable sources for biofuel production (Fan *et al*., 2014), the amount of accumulation of lipids was recorded to be different under different growth conditions. Till now a number of factors are known to influence the lipid content of microalgae, such as nitrogen (Illman *et al.,* 2000) and silicon deficiency (Lynn *et al.,* 2000) and phosphate limitation (Reitan *et al.,* 1994). Salt stress is another factor which intervenes in lipid accumulation in algae (Rao *et al.,* 2007; Alvensleben *et al*., 2016).

Anabaena circinalis is photosynthetic, heterocystous, filamentous and nitrogen-fixing Blue-green Algae of the family Nostocaceae. It has been reported that it can produce bioactive metabolites and hydrolytic enzymes. *A. circinalis* is highly abundant in the polluted water bodies of Assam which harbours 1.18 ± 0.29% amount of lipid in natural condition and 1.67 ± 0.21% in laboratory condition. Percentage increase of lipid accumulation in laboratory condition makes us encouraged to select *A. circinalis* for further study. The present endeavor was therefore undertaken to study the NaCl induced changes on the lipid accumulation of *A. circinalis*.

Materials and methods

Microorganism and culture conditions

The axenic strain of *A. circinalis* was obtained from the culture collection of microalgae, Ecology Laboratory, Department of Botany, Gauhati University, Assam, India. Sterilization of all the glasswares and media were carried out prior to cultivation. The alga was cultured in 250 ml Erlenmeyer flask with 100 ml nitrogen-free BG-11 growth medium (pH 7.5). The cultures were incubated at a temperature of 27 ± 1 ^{o}C and an irradiance of 140 µmol photons $m^{-2}s^{-1}$ provided by cool white fluorescent lamps with a 16:8 h light:dark photoperiod. The cultures were agitated twice daily in order to ward off the cells from clumping and to maintain the organism in homogenous state. All inoculums and experimental cultures were carried out under aseptic laboratory conditions.

Treatment

To investigate the effects of salt on lipid production, *A. circinalis* was batch cultured in a 250 ml Erlenmeyer flask containing 100 ml BG-11 medium and 5 ml each of 0.4 M, 0.5 M, 0.6 M, 0.7 M and 0.8 M NaCl salt solution. The

culture without NaCl was referred as control unless otherwise stated. Cultures were maintained aseptically as mentioned above.

Growth measurement

Optical density was used as a parameter for algal growth. Growth of the test organism was monitored by taking absorbance of the homogenized cell suspension at 750 nm with a UV-Visible Spectrophotometer 119 (SYSTRONICS). For examining cell growth, cultures were harvested at 3 days of intervals upto 15 days of incubation.

Lipid content

The lipid content was estimated using chloroform-methanol mixture as described by Folch *et al*., (1957). Known amount of the algal sample was homogenized in 5 ml of extraction solvent Chloroform: methanol (2:1) (v/v). The extracts were filtered through Whatman No.1 filter paper and 3 ml of 1% NaCl was added to the filtrate. The resulting solution was transferred to a separating funnel to initiate phase separation. The lower organic phase containing the lipid components was collected in a small pre-weighed beaker. It was kept overnight in a dessicator at room temperature in dark place. The beaker containing the dried extracts was reweighed and total lipids were estimated by subtracting the initial weight from the final weight.

Results

Growth

Growth measurements of *A. circinalis* cultured under control conditions and different salt concentrations were reported in Fig 1. Growth rate was observed to decrease with increasing saline concentration. Highest growth rate of the microalga was obtained under control conditions at the end of the test i.e. after 15th days of incubation. Culture with 0.4 and 0.5 M NaCl solution showed moderate growth, while culture with 0.8 M NaCl solution showed the least growth of the test organism.

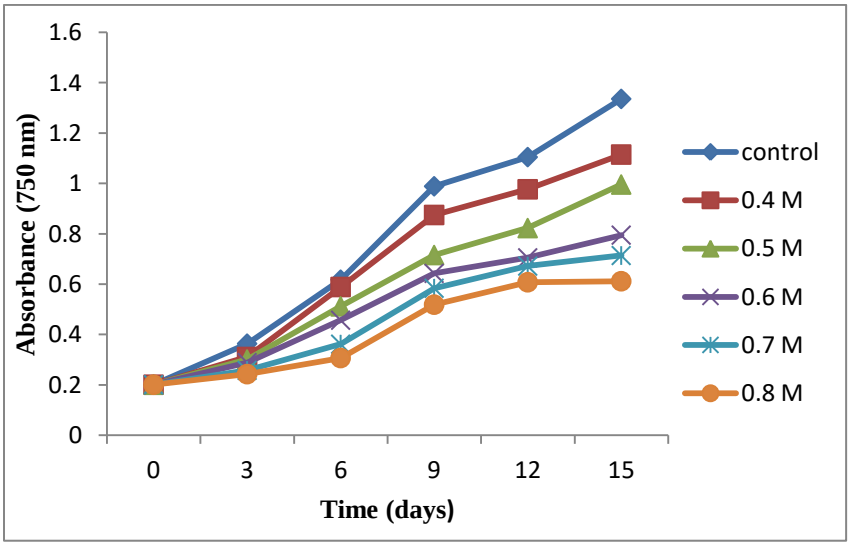

Fig. 1 Growth of *A. circinalis* in culture conditions with different concentrations of NaCl

Lipid content

Lipids are known to play an important role in the protection of Blue-green algae in salt stresses (Gorham *et al*., 1985; Ritter and Yopp, 1993). Though the growth of the test organism was reduced with the increasing concentrations of NaCl, the lipid accumulation was found to be enhanced in response to saline stress. The highest amount of lipid content (8.33 ± 0.32%) was found in the test organism growing in 0.8 M NaCl stress culture followed by 7.73 ± 0.2% and 6.36 ± 0.15% in 0.7 M and 0.6 M NaCl stress culture respectively. The total lipid was found to be lowest in the control set (1.67 ± 0.21%).

Table 1 Lipid contents in the different NaCl concentrations

NaCl concentration	Lipids (%)
Control (Without NaCl)	1.67 ± 0.21
0.4 M	2.46 ± 0.11
0.5 M	4.26 ± 0.15
0.6 M	6.36 ± 0.15
0.7 M	7.73 ± 0.20
0.8 M	8.33 ± 0.32

Values indicate the mean of three replicates ± S.D, n=3

Discussion

The result revealed in the present study showed that *A. circinalis* can tolerate NaCl salinity to a great extent. Hifney (2013) though stated the tolerance level of *A. circinalis* was 0.5 M of NaCl stress, our study revealed that *A. circinalis* can tolerate NaCl salinity to the level of 0.8 M which is in concomitance with the result found by Csonka and Hanson (1991). High concentration of salt reduced the growth rate of the organism; this is due to the inhibition of photosynthetic and respiratory system after exposed to the high concentration (Moradi and Ismail, 2007) of NaCl.

The highest lipid production achieved in this study was 8.33 ± 0.32% at 0.8 M NaCl stress condition which was much more than that of the lipid content recorded in the control set (1.67 ± 0.21%). The increase in lipid content at higher NaCl concentration may be due to adaptation under stress conditions which help in accumulation of lipid content and these results are in accordance with the finding of Takagi *et al*. (2006) in *Dunaliella* cells. Battah *et al*. (2014) also reported that high salinity is directly related to minimum growth and over production of lipids.

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