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Salt tolerance and biochemical responses of *Anabaena sphaerica* isolated from a semi-arid wasteland

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Abstract
Tolerance of *Anabaena sphaerica* isolated from semi- arid wastelands was studied in response to salinity ranging from EC 5 to 15 dS/m, in terms of biomass and concentrations of chlorophyll, carotenoids, phycobilins, lipids and proteins in the cells. Major photosynthetic pigment chlorophyll was not significantly affected by salinity, though other pigments declined in concentration. Biomass and proteins decreased, whereas total sugars increased many folds and lipid concentration was also enhanced with increasing salinity indicating salt tolerance response of the cyanobacterium.

Keywords: *Anabaena*, cyanobacteria, salinity, chlorophyll, sugars, proteins, lipids

1. Introduction

Cyanobacteria, also called blue-green algae are prokaryotic oxygenic autotrophs, which are ubiquitous in nature and found in all inevitable places on earth including desert soils [1] Heterocystous cyanobacteria, known for their ability to fix atmospheric nitrogen are helpful in improving the productivity of various crops, mainly in the tropical rice fields and hence need to be explored for their tolerance to various types of stress conditions [2]. Use of cyanobacteria has been reported to increase the nitrogen content of soil at an average rate of 25 kg/ha [3]. There are reports of occurrence and isolation of heterocystous cyanobacteria from saline-sodic soils and their role in improving the growth of salt stressed seedlings [4]. In India, nearly 7 million hectares of land is salt affected, hence there is a need to explore and exploit indigenous salt - tolerant strains of cyanobacteria [5] that are likely to have inherent capability of salt –tolerance and hence would be more effective when used as biofertilizers in salt-affected solis.

In the present study therefore, *Anabaena sphaerica*, a heterocystous cyanobacerium isolated from a wasteland was examined for its salt tolerance potential and biochemical response in terms of biomass, chlorophyll content, carotenoids, carbohydrate and protein content with an objective to test its suitability as potential biofertilizer for salt- affected soils.

2. Materials and Methods

The study site from where the cyanobacterial strain was isolated was an uncultivated undisturbed wasteland with a natural cover of native plants species dominated by *Salsola baryosma.* The site located at Rohtak – Haryana (28º55' N, 76º43' E) lies 219.84 m above main sea level. The climate of the region is semi-arid subtropical. The soil had sandy loam texture and had formation of salt crust at places during the dry season.

Soil EC and pH were determined using 1:2 soil water suspension using conductivity meter and pH meter, respectively. Electrical conductivity of the waste-land soil was found to vary widely from 0.37 dSm^{-1} to 26 dSm^{-1}, while soil pH was in the range of 8.0 to 8.6.

Anabaena sphaerica was isolated from the wasteland soil using standard isolation and culturing techniques. Pure culture was obtained by agar plate spreading, serial dilution, streaking and purification techniques [6] using Fogg's medium [7]. Identification was done with the help of key given by Desikachary [8]. Pure culture of the cyanobacterium was maintained at 27 ± 3ºC under a continuous illumination using cool white fluorescent light. Tolerance range of alga to varying salt concentration was studied in nutrient media at EC 5, EC 10 and EC 15dS/m prepared by using NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ in a ratio of 13:7:4:1 which is general soil composition of the region [9] and maintained at pH 7.5. Culture medium without salts served as control, which had an electrical conductivity EC 0.2 dS/m.

Growth in terms of absorbance was measured following turbidity technique [10]. 10 ml each of nutrient broth of EC 5, 10, 15 dsm^{-1} along with a control (EC 0.2) was taken in tubes in triplicate. 1 ml of exponentially growing inoculum of *Anabaena sphaerica* was inoculated into each test tube and incubated at 27± 3ºC in continuous light. For each parameter 96 test tubes were used.

For biomass estimation, 10 ml. of algal suspension was taken after thorough shaking and centrifuged at 5000 rpm for 10 minutes. Supernatant was discarded and algal pellet was washed repeatedly with distilled water to remove the salts. Then pellet was dried to constant weight at 80^0 C for 6 hrs and weighed using a pre-weighed filter paper.

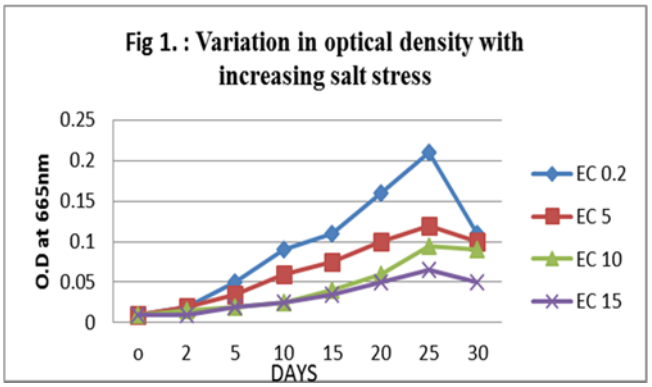
Chlorophyll, carotenoids and phycobilins were estimated according to McKiney [11], Jensen [12] and Bennet & Bogorad [13]. Total sugars were estimated using anthrone method [14].

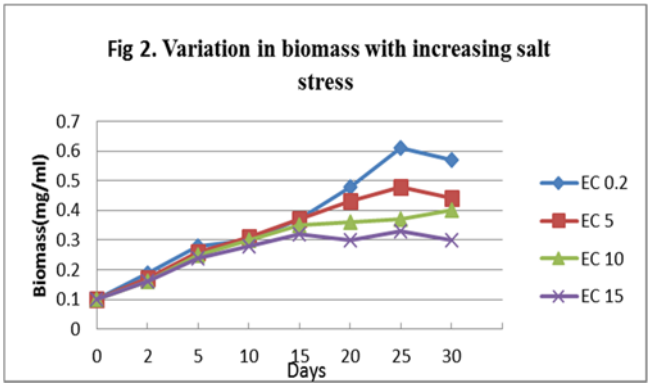
Lipids and proteins were estimated in the exponentially growing culture on 20^{th} day of growth. For lipids, extraction was done using chloroform-methanol (2:1v/v) solution and taking absorbance at 350 nm [15]. Concentration of lipids was calculated from standard curve prepared by taking graded concentrations of palmitic acid in chloroform. Proteins were estimated by using folin-ciocalteau reagent [16].

3. Results and Discussion

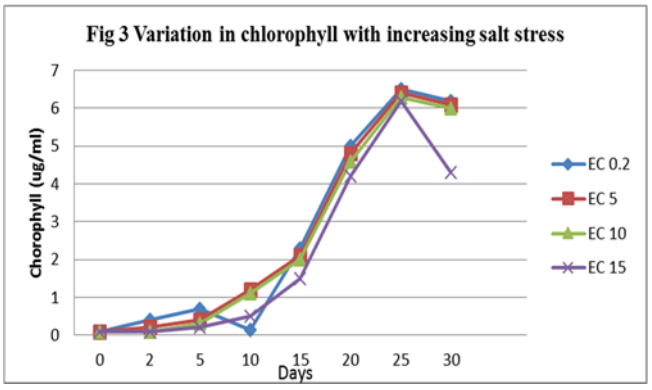
Absorbance pattern of cell suspension showed that *Anabaena sphaerica* isolate used in the present study was able to tolerate salinity stress up to EC15dS/m, though its growth was better in non-saline medium. About 35-40% decrease in absorbance of cell suspension was observed at EC 10 and 15 at peak growth

period, indicating that it tolerated stress by maintaining the growth at a retarded rate (Fig. 1). Similarly, dry weight also decreased (0.32- 0.34 mg/ml) as compared to 0.6 mg/ml in control (EC 0.2) on 25th day (Fig.2). This shows that at higher salinity, the cyanobacterium grows at about 50% of its normal growth that it has in the absence of salinity stress. Similar retardation of algal growth under salinity has also been reported earlier [17].

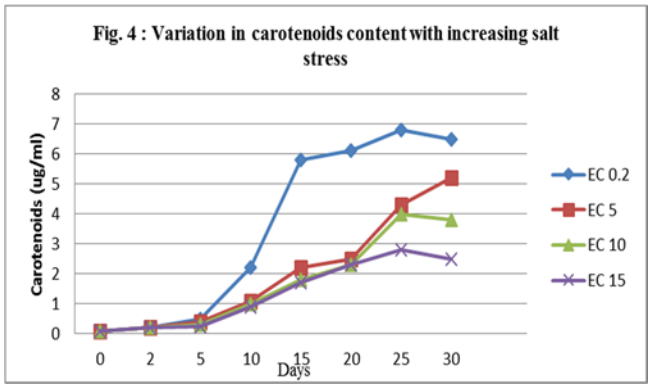




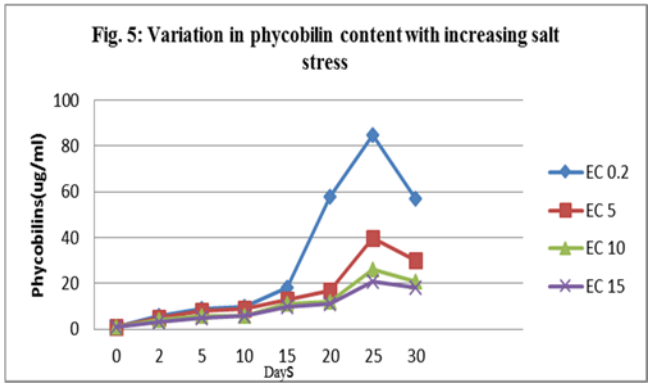
Interestingly, photosynthetic pigment chlorophyll did not show any significant variations in response to salt stress up to 25 days when peak growth was achieved. However, after that the pigment concentration declined drastically by 50% at EC 15 (Fig. 3), indicating earlier setting of cell death under high salinity. Several earlier researchers while working on different algal species from diverse areas reported a decrease in chlorophyll content, photosynthetic rate and proteins [18]. The present isolate that was isolated from wasteland soils with varying salt concentrations in upper soil profile seems to have exposed the species to salt stress for a long time in natural conditions and thus imparted it better salt tolerance potential.



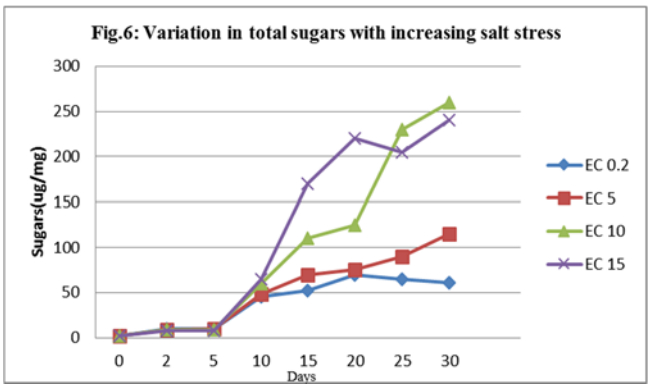
Carotenoid content of the cells, however, decreased on exposure to increasing salt stress (Fig. 4). The concentrations started increasing exponentially on 5th day in control whereas in the presence of salts exponential growth of carotenoids was seen after 15 days indicating retardation of certain growth parameters under salt stress.



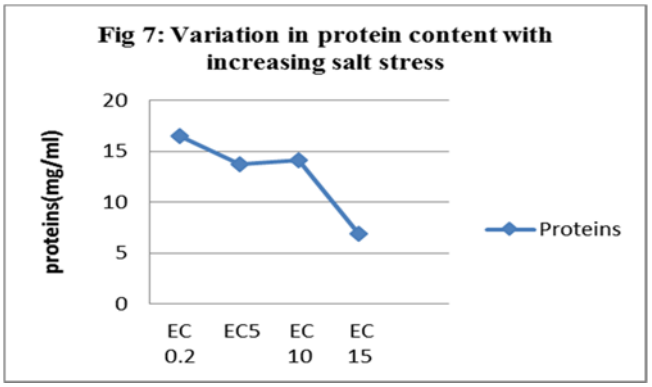
Similar types of observations were found for phycobilins (Fig. 5), the decline however, was more pronounced here, being 55-70% at higher salinity concentrations. Thus, though the primary photosynthetic pigment chlorophyll was not significantly affected due to salt stress, but the accessory pigments carotenoid and phycobilins concentrations reduced when salt concentration in the medium increased that resulted in reduced biomass of the cyanobacterium. There are, however, reports that some cyanobacteria show increased concentrations of accessory pigments when given stress of metals, dyes and salts as adaptive response to oxidative stress[19] [20]. In the present cyanobacterium, there was only salt stress and some other ways of salt tolerance seem to have developed.



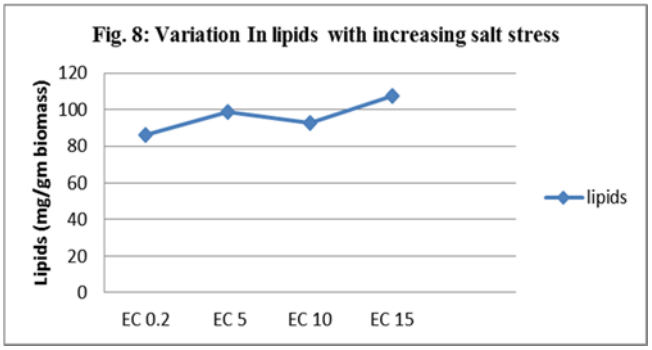
Sugars in cells of cyanobacterium can play a role in imparting osmotic tolerance to the organism. Changes in total sugars with increasing salt stress over a period of 30 days are depicted in Fig.5. Concentrations of total sugars increased distinctively with increasing time as well as salinity. On the 20th day of observation sugar in control (EC 0.2) was 77 µg/ mg dry wt. and under salt stress at EC 15 was about three folds (220 microgram). A strong positive correlation was observed between salinity and sugar ($p<0.01$). This increase in sugar may be an adaptive measure under salt stress as reported [21]. In earlier studies it has also reported that sugars play an important role in osmotic regulation of cells during reproduction and stress conditions [22]. Increasing sugar concentration may be generating an osmotic pressure adequate to counteract the osmotic pressure outside the cell.



Protein concentration indicates good growth and metabolism of an organism, hence protein content at peak growth was determined, which is shown in Fig. 7. Protein content declined with increasing salt stress and the decrease was statistically significant ($p<0.01$) due to salt stress at EC 15. This type of reduced protein synthesis under salt stress has been observed in earlier studies [23], which could be due to more amino acid accumulation in stressed plants for increased osmo-tolerance, thereby reducing protein content.



Another adaptive feature in *A. sphaerica* in response to salts was found to be through enhanced lipid accumulation, as lipid content increased from 86.36 mg/g in control to 98.48mg/g at EC 5, and 107.6 mg/g at EC 15 (Fig. 8). Role of lipids in salt tolerance has been emphasized in cyanobacterium *Synechococcus* [24, 25].



The present study shows that the native isolate of *A. sphaerica* from the saline – alkali soil has developed certain biochemical adaptations to deal with enhanced salinity stress by way of accumulating total sugars and lipids. It shows appreciable protection of the major photosynthetic pigment chlorophyll under salt stress. However, overall growth, biomass and protein content are reduced under high salinity in this cyanobacterium, which indicates that the species is maintaining itself under high salinity at reduced growth rate. Salt tolerance of this native strain is likely to make it a potential biofertilizer for saline soils.

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