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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (13): 2528-2530, 2006
ISSN 1028-8880

Effect of NaCl on Growth and Physiological Traits of *Anabena cylindrica* L.

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Abstract: *Anabena cylindrica* L. is an important nitrogen fixing cyanobacterium native to paddy soils. In order to study the effects of salinity which occurs in flooded paddy soils, caused by high level of NaCl, on the growth and physiological parameters, Anabena was exposed to six levels of NaCl stress ranging from 50-300 mM besides control. The study revealed that all the growth parameters showed a declining trend with increased NaCl stress upto 200 mM beyond which there was complete degeneration. Similar trend was found in case of various pigmentation attributes, protein and sugar content except in case of total sugar content for which there was initial increase with increasing NaCl concentration probably due to an adaptive mechanism employed by *Anabena* under NaCl stress.

Key words: Cyanobacteria, *Anabena cylindrical*, NaCl, growth parameters

INTRODUCTION

Cyanobacteria (blue green algae) are ancient prokaryotes capable of photolysis-mediated oxygen evolving photosynthesis and nitrogen fixation. They are unicellular, filamentous, microscopic and have similar organization of plant body and complete absence of sexual reproduction. Cyanobacteria exhibit great diversity in occurrence and are capable of surviving in extreme environments such as high temperature (upto 85°C) and low temperature (upto-196°C). One such system of considerable importance is *Azolla*-*Anabena* complex, which flourishes in certain rice fields. The *Anabena* inhabits the cavities in the leaves of floating fern *Azolla* and fix quantities of nitrogen comparable to those of Legume-*Rhizobium* association.

Soil salinity is a serious global problem. Nearly 950 million hectares of land are affected globally, including one third of irrigated land. A large number of studies have been undertaken to asses the effect of various factors on growth and physiology of cyanobacteria such as *Anabena* (Crammer *et al*., 1990; Blackburn *et al*., 1996; Rai and Tiwari, 1990). From its practical economic importance point of view it has been established that algalisation of rice fields substantially increase grain yield and quantity parameters such as crude protein, lipid and total amylase content (Tiwari *et al*., 2001). Therefore the present study was undertaken to study the effect of Nacl on various growth and physiological traits in Anabena.

MATERIALS AND METHODS

The experimental organism used in present study i.e., Anabena cylindrical is a filamentous, diazotrpic, freshwater Cyanobacteria capable of heterocytes differentiation and is native to paddy fields and was obtained from Deptartment of Biochemistry Allahbad University Allahbad India. All the glassware was cleaned by immersing in chromic acid for 24 h followed by washing by cleaning water. The glassware containing nutrient medium (BG11) was sterilized by wet heat in an autoclave at 121°C for 20 min. The cyanobacteria *Anabena cylindrica* L. was maintained in an autoclaved BGII medium (Table 1) without any source of nitrogen. The phosphate and citrate were also autoclaved and added aseptically to cold sterile medium. The pH of nutrient solution was adjusted to 7.5 before autoclaving. The maintaince was done in the laboratory at 30+2°C in batch culture under continuous illumination (70 ME m^{-2} Sec^{-1}).

The cultures were stressed with 6 levels of NaCl concentrations viz. 50, 100, 150, 200, 250 and 300 mM besides the control i.e., no NaCl. The various growth and physiological parameters studied were growth, dry weight, filament length, specific growth rate, heterocyst frequency and generation time. Besides parameters such as pigmentation (Chlorophyll, phycocyanin and carotenoid) and protein and total sugar content were estimated to establish the effect of NaCl on such parameters.

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The data recorded during the study was subjected to RBD analysis. The significant and non-significant effects were judged with help of F-test. The significant differences between means were judged on the basis of CD at 5% level.

RESULTS

The analysis of variance for various growth and physiological traits (Table 1) revealed significant mean squares due to treatment effects for all parameters indicating significant difference in the treatments. The effect of NaCl on various growth parameters (Table 2) reveled that there was decline in growth parameters estimates from control upto 200 mM after which there was no survival of the experimental organism. Consequently the generation time increased from 0-200 mM.

As far as the pigmentation traits (Table 3) are concerned there was gradual decline in total chlorophyll and phycocyanin content from 0-200 mM but carotenoid content gradually increased from 0-100 mM after which it declined again. Similarly in case of protein content there was gradual decrease in protein content with increased NaCl stress while as total sugar content increased upto 100 mM after which it also declined. From 250 mM NaCl upwards there was no survival of Anabena.

DISCUSSION

The cyanobacteria response to salinity involves several physiological and biochemical process such as nucleic acid synthesis, carbohydrates and protein metabolism, photosynthesis and respiration. In the present study, the general evidences were that salinity significantly inhibited growth of *Anabena cylindrica* L. In fact the cyanobacteria cells grown at height salinity levels such as 250 and 300 mM failed to survive beyond two days and degenerated completely. The decrease in growth rate at lower salinity may be an adaptation of cyanobacteria cells to NaCl stress. In fact Rai and Tawari (1990) concluded that growth is the most sensitive parameter of plants exposed to increased concentrations of NaCl in a concentration dependent manner. Similar decline was found in case of heterocytes frequency. Niven *et al.* (1987) proposed that heterocysts were more susceptible to salt than vegetative cells.

The degradation of chlorophyll and phycocyanin may also be one of the reasons for decline in growth. Thus reduced growth at lower salinity may be due to increased carotenoids observed in present study. Schubert *et al.* (1993) also reported similar results in *Synechococus* exposed to salinity. The decline in proteins content under salinity stress is in agreement with the

Table 1: Analysis of variance for growth and physiological traits as affected by NaCl stress in *Anabena cylindrica*

Source of variation	df	Dry weight (mg)	Specific growth rate (M)	Generation time length	Heterocyst frequency (%)	Total chlorophyll (mg mL^{-1})	Phycocyanin mg mL^{-1}	Carotenoid (mg mL^{-1})	Average filament length	Protein content (mgmL^{-1})	Total sugars (mg mL^{-1})
Replication	2	0.1863	0.0233	0.1167**	0.4666	0.0070	0.7136**	0.0752**	0.0533	0.0343	0.0033
Treatment	4	71.3738**	359.4287**	69.8489**	215.6572**	0.0791**	0.14966**	0.1402**	122.2897**	2.3618**	0.3120**
Error	14	0.6438	0.0366	0.0512	0.5933	0.0197	0.0831	0.0135	0.3655	0.0857	0.0061

Table 2: Effect of various concentrations of NaCl on growth parameters of *Anabena cylindrical*

Concentration of NaCl (mM)	Dry weight (mg)	Specific growth rate (M)	Generation time	Average filament length (micrometer)	Heterocyst frequency (%)
0	0.095±0.001	0.084±0.0024	2.70±0.026	23.70±0.115	12.21±0.296
50	0.074±0.001	0.065±0.0017	4.09±0.029	22.33±0.285	10.55±0.133
100	0.063±0.002	0.040±0.0008	8.07±0.023	22.30±0.120	8.05±0.015
150	0.045±0.002	0.008±0.0005	32.33±0.086	21.60±0.145	5.92±0.020
200	0.0025±0.001	0.006±0.0005	52.17±0.028	20.60±0.152	3.91±0.010
250	Ns	NS	NS	NS	NS
300	Ns	NS	NS	NS	NS

NS = Not Significant

Table 3: Effect of various NaCl concentrations on pigmentation, protein and sugar content of *Anabena cylindrica*

Concentration of NaCl (mM)	Chlorophyll (mg mL^{-1})	Phycocyanin (mg mL^{-1})	Carotenoid (g mL^{-1})	Protein content (mg mL^{-1})	Total sugar (mg L^{-1})
0	0.017±0.001	0.013±0.0005	0.14±0.005	0.94±0.014	0.4±0.017
50	0.014±0.001	0.011±0.0003	0.16±0.012	0.71±0.011	0.63±0.017
100	0.008±0.005	0.007±0.0005	0.22±0.008	0.54±0.025	0.84±0.017
150	0.005±0.001	0.005±0.0057	0.05±0.008	0.14±0.018	0.26±0.012
200	0.004±0.005	0.002±0.0057	0.04±0.003	0.20±0.008	0.25±0.008
250	NS	NS	NS	NS	NS
300	NS	NS	NS	NS	NS

NS = Not Significant

existing report. In fact Hagemann *et al*. (1990) found almost complete blockage of protein synthesis in cyanobacteria. Strikingly carbohydrates content increased from 0-100 mM. The increase in sugar content may be an adaptive measure aimed at survival under saline conditions.

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