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Impact of Salinity on the Physiological and Biochemical Traits of *Chlorella vulgaris* Beijerinck.

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ABSTRACT: A study was conducted to investigate the impact of NaCl on the physiological and biochemical traits of *Chlorella vulgaris* Beijerinck. In order to determine the impact of NaCl, *Chlorella* was exposed to different concentrations of NaCl ranging from 0.1-0.4M besides control over a period of 30 days. It was found that total chlorophyll contents stimulated at lower concentrations of NaCl (0.1 and 0.2M) but reduced at higher (0.3 and 0.4M) concentrations. The total protein contents inhibited at all concentrations of NaCl . While β-carotene and carbohydrate contents increased upto 0.3M and thereafter declined. It is interesting to note that the proline content increased with increase in NaCl concentrations. However, glycine betaine was noticeably increased upto 0.3 M and thereafter declined. The results indicated that *Chlorella vulgaris* showed diverse response to NaCl stress.

Key Words: *Chlorella*, NaCl, β-Carotene, Proline, Glycine betaine

INTRODUCTION

Salinity is a serious agro-economical problem which leads to metabolic alterations and graded reduction in the plant growth in terms of all the growth parameters. Microalgae are rich sources of proteins, carbohydrates and fatty acids. Unicellular eukaryotic microalgae have special importance due to the simplicity of their structures showing all metabolic activities and having similarities with higher plants. Microalgae differ in their adaptability to salinity and other stress conditions. The ability of cells to survive and flourish in saline environment under the influence of osmotic stress has received considerable attention. Cells develop many adaptive strategies in response to different abiotic stresses such as salinity, dehydration, cold and excessive osmotic pressure. Against these stresses, cells adapt themselves by undergoing different mechanisms including changes in morphological and developmental pattern as well as physiological and bio-chemical processes (Bohnert et al. 1995). Adaptation to stress is associated with metabolic adjustments which lead to the accumulation of several organic solutes and osmolytes. Compatible osmolytes such as proline, glycine betaine, sugars, polyols and amino acids are synthesized in response to stress. In higher plants, these are well studied (Hasegawa et al 2000; Hoque et al 2007).These osmotic adjustments protect sub-cellular structures and reduce oxidative damage caused by free radicals, produced in response to high salinity (Hare et al 1998; Hong et al 1992). In the present study attention has been given to investigate the impact of NaCl on physiological and biochemical attributes of *Chlorella vulgaris.*

MATERIALS AND METHODS

The organism used in the present study i.e., *Chlorella vulgaris* Beijerinck was isolated from the garden soil of Gulbarga University Gulbarga, Karnataka. De’s modified Beneck’s medium was found best suited for the growth of the alga in the laboratory. The cultures were maintained in the culture room at temperature of 26 ± 2°C.

Further to study the impact of NaCl, the experiments were carried out in 250ml conical flasks, contained 100ml of Bold’s basal medium. The exponentially growing algal suspension was centrifuged and inoculated in the flasks containing different concentrations of NaCl such as 0.1, 0.2, 0.3 and 0.4 M besides control and kept for observation to 30 days.

The samples were drawn periodically during growth (10^{th}, 20^{th} and 30^{th} day) from control and different concentrations of NaCl and were subjected for the analysis of physiological and biochemical parameters. The total chlorophyll contents were measured according to the method of Arnon (1949), total proteins by the method of Lowry et al. (1951), β-Carotene accumulation was estimated according to the method of Jensen (1978), total carbohydrates were determined according to Yemm and Willis (1954), proline was determined according to the method of Bates et al. (1973) and Glycine betaine was estimated by the method of Barak and Tuma (1981). All the experimental results were analyzed and compared by Anova using SPSS Package version 10.

RESULTS AND DISCUSSION

The results indicated that, the total chlorophyll contents stimulated at lower concentrations (0.1 and 0.2M) of NaCl when compared to control but it was reduced at higher (0.3 and 0.4M) concentrations for all the cultures studied (Table1). According to Moradi and Ismail (2007), reduced chlorophyll contents at higher salinities are due to decrease in photosynthetic rate because of salt osmotic and toxic ionic stress. Many previous studies reported that the cultivation with higher saline concentrations had lower chlorophyll and protein contents (Vonshak et al. 1996). It has also been reported that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting reduced photosynthesis and reduced growth (Rai 1990; Rai and Abraham 1993). *Chlorella* exhibited decline in the total protein contents in all the concentrations of NaCl and cultures studied (Table 1).

Table-1: Effect of Various Concentrations of NaCl on total Chlorophyll, total proteins and β-Carotene contents of *Chlorella vulgaris* Beijerinck.

Cellular Metabolites	**Con. of NaCl (M)**	**10th day**	**20th day**	**30th day**
Total chlorophyll (μg/gFW)	Control	46.0 ± 0.03	55.0 ± 0.03	72.0 ± 0.02
	0.1 M	58.0 ± 0.04	67.0 ± 0.02	89.0 ± 0.03
	0.2 M	72.0 ± 0.02	88.0 ± 0.01	101.0± 0.03
	0.3 M	31.0 ± 0.02	42.0 ± 0.02	56.0 ± 0.04
	0.4 M	19.0 ± 0.02	28.0 ± 0.04	30.0 ± 0.01
Total Proteins (μg/gFW)	Control	73.0 ± 0.04	90.0 ± 0.02	104.0 ± 0.02
	0.1 M	58.0 ± 0.02	70.0 ± 0.04	81.0 ± 0.03
	0.2 M	41.0 ± 0.02	52.0 ± 0.02	61.0 ± 0.01
	0.3 M	30.0 ± 0.04	38.0 ± 0.01	29.0 ± 0.02
	0.4 M	12.0 ± 0.02	20.0 ± 0.02	18.0 ± 0.03
β-Carotene (μg/gFW)	Control	50.0 ± 0.01	71.0 + 0.02	90.0 ± 0.01
	0.1 M	71.0 ± 0.02	92.0 ± 0.01	107.0 ± 0.02
	0.2 M	89.0 ± 0.04	103.0 ± 0.03	118.0 ± 0.03
	0.3 M	101.0 ± 0.02	116.0 ± 0.01	126.0 ± 0.02
	0.4 M	38.0 ± 0.01	51.0 ± 0.04	45.0 ± 0.01

Each value is expressed as mean ± S. D. (n=3) X Statistically significant at $P < 0.05$.

FW=Fresh Weight

The present results are in agreement with the results of sheik et al. (2006). Hageman et al. (1990) found complete blockage of protein synthesis in cyanobacteria. Many previous studies reported that stress cells have lower protein synthesis capacity increasing lipid and carbohydrate metabolism (warr et al. 1985; Tomaselli et al. 1987). β-Carotene is an important source of provitamin-A is common among cyanobacterial strains. It was increased in all the concentrations of NaCl except 0.4 M for all the cultures studied (Table 1). Similar observations were made by Reddy et al. (2003).

Carbohydrate contents increased in all the concentrations of NaCl except 0.4 M for all the cultures studied (Table 2). Many previous studies reported that carbohydrates synthesis was stimulated by stress conditions (warr et al. 1985; Tomaselli et al., 1987). Gill et al. (2002) made an observation that soluble sugars play an important role in the osmotic regulation of cells during reproduction and stress conditions. Among different solutes accumulating in response to stress sugar may play a key role to maintain the osmotic regulation of cells. The increase in the sugar content may be an adaptive measures under saline conditions. The most interesting observation made during the study was drastic increase in the proline content in all the concentrations of NaCl and the cultures studied (Table 2). Szekely G (2004) made an observation that in higher plants proline is considered to play an important role in defense mechanism of stressed cells providing carbon, nitrogen and energy source after stress by degradation. According to Hong et al. (2000) increased resistance to oxidative stress is due to accumulation of proline and other metabolites. He also made an observation that proline increased salt tolerance of microorganisms. Therefore in microalgae and other plants proline acts as a free radical scavenging and increases salt tolerance of microorganisms. It is likely that proline accumulation may be one of the major mechanism of salinity tolerance by the alga. Glycine betaine is the principle solute in highly salt tolerant halophilic forms. It was also increased with increase in NaCl concentrations upto 0.3 M and thereafter declined (Table 2)

Table-2: Effect of Various Concentrations of NaCl on total Carbohydrates, Proline and Glycine-betaine contents of *Chlorella vulgaris* Beijerinck.

Cellular Metabolites	Con. of NaCl (M)	10th day	20th day	30th day
Total Carbohydrates (µg/gFW)	Control	40.0 ± 0.03	53.0 ± 0.01	62.0 ± 0.04
	0.1 M	53.0 ± 0.04	68.0 ± 0.02	79.0 ± 0.02
	0.2 M	66.0 ± 0.02	77.0 ± 0.01	83.0 ± 0.03
	0.3 M	85.0 ± 0.01	92.0 ± 0.03	104.0 ± 0.01
	0.4 M	24.0 ± 0.03	43.0 ± 0.04	50.0 ± 0.04
Proline (µg/gFW)	Control	30.0 ± 0.02	46.0 ± 0.01	62.0 ± 0.02
	0.1 M	58.0 ± 0.04	69.0 ± 0.01	96.0 ± 0.04
	0.2 M	79.0 ± 0.01	98.0 ± 0.02	120.0 ± 0.02
	0.3 M	98.0 ± 0.02	119.0 ± 0.03	138.0 ± 0.03
	0.4 M	115.0 ± 0.01	131.0 ± 0.02	150.0 ± 0.03
Glycine-betaine (µg/gFW)	Control	69.0 ± 0.02	78.0 ± 0.01	99.0 ± 0.01
	0.1 M	98.0 ± 0.01	110.0 ± 0.02	130.0 ± 0.02
	0.2 M	122.0 ± 0.02	135.0 ± 0.01	146.0 ± 0.03
	0.3 M	141.0 ± 0.03	149.0 ± 0.02	151.0 ± 0.01
	0.4 M	102.0 ± 0.02	110.0 ± 0.03	121.0 ± 0.02

Each value is expressed as mean ± S. D. (n=3) X Statistically significant at $p < 0.05$; FW=Fresh Weight

Record et al. (1998) made an observation that glycine betaine and proline increase the cytoplasmic volume and free water content and permit cell proliferation under unfavorable conditions. However glycine betaine preserves thylakoid and plasma membrane integrity when exposed to saline conditions (Rhodes and Hanson 1993). Several species of marine algae have been reported to contain glycine betaine as a stabilizing osmolyte.

CONCLUSION

The effect of various concentrations of NaCl on *Chlorella vulgaris* showed, reduced chlorophyll content mainly at 0.3 and 0.4 M and reduced protein content at all concentrations of NaCl. While β- carotene, carbohydrates, and glycine betaine increased upto 0.3 M where as significant increase in the production of proline was observed at all concentrations of NaCl. These beneficial properties indicated that, adaptation of the alga to salinity was characterized by the accumulation of osmolytes like carbohydrates, proline and glycine betaine.

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REFERENCES

Arnon, D.I. 1949. Copper enzymes in isolated chloroplast polyphenoloxidase in *Beta vulgaris*, Plant Physiol. 24: 1-15.

Barak, A.J. and Tuma, D.J. 1981. Determination of choline, phosphorylcholine and betaine. In: J.M. Lowstein (ed.), methods of Enzymology, Academic Press, New York, 72: 287-292.

Bates, L.S., Waldeen, R.P. and Teare, I.D. 1973. Rapid estimation of free proline for water stress studies. Plant Soil, 39: 205-208.

Gill, P.K., Sharma, A.D., Singh, P. and Bhullar, S.S., 2002. Osmotic stress induced changes in germination, growth and soluble sugar contents of *Sorghum bicolor* (L.) Moench seeds under various abiotic stresses. Plant Physiol. 128: 12 – 25.

Hong, Z., K. Lakkineni, Z. Zhang and D.P.S. Verma, 2000 Removal of feedback inhibition of A1 – pyrroline -

5- carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. Plant Physiol., 122: 1129 – 1136.

Hageman, M.L. Wolfel and Kruger, B., 1990. Alterations of protein synthesis in *Syncechocytes* Sp. PCC 6803 after salt shock. J. Gen. Microbiol., 136: 1390-1399.

Hare PD, Cress WA, Van-Staden J (1998) dissecting the roles of osmolyte accumulation during stress. Plant Cell Environ 21:535-554.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular response to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463-499.

Hong B, Barg R, Ho TH (1992) Development organ specific expression of an ABA- and stress indused protein in Barley Plant Mol Biol 18:663-874.

Hoque MA Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007) Exogenous proline mitigates the detrimental effects of salt stress more than the betaine by increasing antioxidant enzyme activities . J Plant Physiol 164:553-561.

Moradi, M and A.M. Ismail, 2007. Responses of Photosynthesis, chlorophyll fluorescence and ROS – Scavenging systems to salt stress. During seedling and reproductive stages of Rice. Ann. Botany 99:1161-1173.

Record, M.T. Jr., Couternay, E.S., Cayley, S. and Guttman, J.H. 1998. Biophysical compensation mechanisms buffering *E. coli* proteins, nucleic acid interactions against changing environment. Trend Biochem. Sci. 23:190-194.

Reddy O.V., Dolly Wattal-Dhar, Radha Prasanna and Singh, P.K. 2003. The influence of sodium stress on selected cyanobacterial isolates. Indian J. Plant Physiol. 332-338.

Rhoades, D. and Hanson, A.D. 1993. Quaternary ammonium and tertiary sulphonium compounds in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 44: 357-384.

Rai, A.K. (1990) Biochemical characteristics of photosynthetic response to various external salinities in halotolerant and fresh-water cyanobacteria. FEMS Microbiology Letters 69, 177-180.

Rai, A.K. and Abraham, G. (1993) Salinity tolerance and growth analysis of the cyanobacterium *Anabaena doliolum*. Bulletin of Environmental contamination and Toxicology 51, 724-731.

Sheikh, T.A. Baba, Z.A. and Parvez Sofi, 2006. Effect of NaCl on growth and physiological traits of *Anabaena cylindrica* L. Pakistan Journal of Biological Sciences 9(13) : 2528-2530.

Szekely G 2004. The role of proline in *Arabidopsis thaliana* osmotic stress response. Acta Biol Szeged 48 : 81.

Tomaselli, L., G. Torzillo, L.Giovanetti, F.Bocci, M.R. Tredici, B. Pusharaj .T. Pupuazzo, W. Balloni and R. Meterassi, 1987. Recent research of *Spirulina* in Itali. Hydrobiol., 151: 79-82.

Vonshak, A.N., Kancharaksa, B. Bunang and M. Tanticharoen, 1996. Role of Right and Photosynthesis on the acclimation process of the cyanobacteria *Spirulina platensis* to salinity stress. J. Appl. Phycol., 8: 119-124.

Warr, S.R.C., Reed R.H., Chudek, J.A., Foster, R. and Stewart, W.D.P., 1985, Osmotic adjustment in *Spirulina platensis*, Planta, 163: 424 - 429.

Yemm, E.W. and Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57: 508-514.