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INTRA CELLULAR MINERAL CONTENT OF THE CYANOBACTERIUM ANABAENA ORYZAE IN RESPONSE TO SALINE MEDIA

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Summary

Anabaena oryzae (Egyptian strain) grows over the external salinity range from 50 to 400 mM NaCl in nutrient media. Cultural biomass, heterocyst frequency, intracellular mineral and total carbohydrate content have been determined.

Cultural biomass did not decrease by increasing NaCl and showed only clear reduction at 350 and 400 mM NaCl. Heterocyst frequency was diminished at higher salinity.

The alga responded to increased osmolarity in the medium by an elevation of the internal concentration of carbohydrate content.

The most pronounced effect of NaCl was a trend of increasing the intracellular concentration of Na with increasing extracellular salt which reached 387 fold at 350 and 400 mM NaCl over their corresponding control.

Intracellular P, K, Ca, Mg, Mn and Zn contents were diminished throughout the tested salinity range, while Fe and Cu showed minor changes.

The sensitivity of the N2-fixing system of the alga comprised a gradual decrease in concentration of intra and extracellular nitrogen in response to increasing salinity associated with the increased trend in carbohydrates.

Introduction

The wide distribution and bloom-forming disposal of cyanobacteria have been attributed in part to the fact that they include many species which can fix atmospheric nitrogen. Nitrogen-fixation is commonly limited by certain conditions including salinity. Many species of cyanobacteria thrive in saline environments and were found to exhibit certain degree of adaptability (Fogg et al, 1973; Tel-Or, 1980 a,b; Melamed-Harel and Tel-Or, 1981). The potential mechanisms employed by cyanobacteria for salt adaptation are yet uncompletely clear. Significant ion accumulation has been reported for several micro-algae as a function of increasing salinity (Ahmad and Hellebust, 1984; Ehrenfeld and Coussin, 1984; Gyinzburg, 1981, a,b) which may lead to inhibition of growth and metabolism.

Hellebust, 1985 suggested that, the role of inorganic ions in osmoregulation of micro-algae is uncertain, since technical difficulties in determining ion content in cells in highly saline media have yielded conflicting values (Ahmad and Hellebust, 1984; Ehrenfeld and Coussin, 1984; Gimmler and Shirling, 1978; Ginzburg, 1981 and Munns et al, 1983). Other authors ascertain the important role of inorganic ions in osmoregulation (Brown & Borowitzka, 1979; Brown Hellebust, 1980; Kauss, 1978 and Setter & Greenway, 1983).

However, more recent studies of osmotic adjustment in cyanobacteria have demonstrated that organic osmotic rather than inorganic ions, may play a major role in the maintainance of positive turgor under conditions of salt-stress (Borowitzka et al, 1980; Blumwald and Tel-Or, 1982 a,b; Reed and Stewart, 1983; Reed et al, 1984).

The aim of the present study is to estimate the internal concentration of inorganic mineral and total carbohydrates of the cyanobacterium Anabaena oryzae (isolated from Egyptian soil) over a range of external salt concentration (50 to 400 mM NaCl.) Biomass accumulation and heterocyst frequency were also

determined. Residual macro and microelements were detected by direct assay of the culture supernatant as a result of the algal growth.

Material and Methods

Organism: Anabaena oryzae, Egyptian strain was provided from the Agriculture Research Centre, Giza, Egypt.

Culture media and conditions: Batch cultures were grown photoautotrophically under continuous fluoresent illumination (20 Watt/m^2) at 28°C in (Watanabe et al, 1959) medium. Inocula were grown to mid-exponential-phase. Aliquots of (5 ml) cell suspensions were inoculated ints 500 ml Erlenmeyer flasks containing 200 ml of medium, 5-10 flasks were used for each treatment, sodium chloride, was added to the flasks to the desired concentrations. Experiments were repeated at least twice.

Growth: Cultures were harvested in the late-logarithmic phase of growth The organisms were separated from the medium by centrifugation at 10000 g. for 10 min. at 4°C, the harvested cells were washed twice with distilled water.

Growth was determined in terms of culture biomass estimates (mg dry cells/ml culture) following drying for 24 h. at 70°C (Layzell et al, 1985).

Heterocyst frequency: Cells were fixed in 2.5% (V/V) gluteraldehyde in phosphate buffer, pH 7.2; at beast 1000 cells were counted under light microscope for each treatment and the frequencies of differentiated cells were expressed as a percentage of the total vegetative cell population (Sharma, 1984).

Carbohydrate concentrations: Carbohydrates were estimated by the phenol sulphuric acid test relative to standard solution of glucose. (Dubois et al, 1956).

Analysis of intracellular mineral content: Intracullular ion concentrations were analyzed from the dried algal cells which were powdered and digested in nitric-perchloric-sulphuric acid. Phosphorus was analyzed using the vanadatomolybdate colorimetric method (Chapman and Prott, 1978).

Ca, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectrophotometry. K and Na were estimated by flame photometry. (Jackson, 1967).

Nitrogen was measured by kjeldahl method (Jackson, 1967).

Residual ions of the culture suprenatant were directly assayed.

Results and Discussion

Response of Anabaena oryzae cells to saline media:

The growth of A.oryzae was tested as a function of external salinities with (0.05 to 0.4 M. NaCl) in the culture media. It is interesting to note from Table 1 that, cell growth was reduced by 0.35 M and 0.4 M. NaCl while cultures were adapted to 0.05-0.3 M NaCl and showed tolerance to this condition and were similar to the control, which indicates the algal capacity to osmoregulation. This is in line with the data presented by Mahmoud et al, (1980) and Alla-El-Din et al, (1980), who suggested that, growth of Nostoc calcicola, N.muscorum, Anabaena variabilis and A.oryzae were stimulated by the addition of up to 9000 pp.m. salt.

Table 1 illustrates the microscopic examination of algal filaments and shows that, the proportion of heterocysts was greatest with the low NaCl concentration (0.05 to 0.2 M)amounted to 10.7 to 8.1% of the cells, while heterocysts proportion amounted to 5.5 to 4.1% with 0.25 to 0.4 μ NaCl. This indicates the relationship between salinity and heterocyst frequency (Fig. 1).

However, since heterocysts are the sole site of aerobic N_2-fixation in filamentous cyanobacteria

(Haselkorn, 1978, Stewart, 1980 and Wolk, 1982), a change in number or quality of heterocysts is bound to affect nitrogenase activity.

Nitrogen and carbohydrate content in relation to NaCl concentration:

Since A.oryzae can grow and adapt to NaCl concentration ranging from 0.05 to 0.4 M, we checked its adaptation to different NaCl concentration in regard to intracellular nitrogen, carbohydrates and mineral content.

Table 2, shows that, salt-grown cells contained much lower nitrogen content amounting to 6.1 - 55% of the control cells, nitrogen decreased linearly with increased salt concentration. The observed decrease in nitrogen content under such saline condition is explained to be mainly due to reduction in nitrogenase activity as compared with control cultures (Table 2) It is obvious that, this change is parallel to the pattern of heterocysts differentiation response to salinity as shown in table 1. It has been postulated that, appearance of nitrogenase activity is concomitant with the development of heterocysts (Bradley and Carr, 1976; Fleming and Haselkorn, 1974; Murry and Benemann, 1979; Neilson et al, 1971)

Total carbohydrate content increased with increasing NaCl concentration (Table 2). In 400 mM NaCl, total carbohydrate amounted to 176.6 mg/g. dry wt. compared with 109.8 mg/g.dry wt. for the control cells. Carbohydrate accumulation during salt-stress suggests that, carbohydrate may play a role in osmotic regulation in Anabaena oryzae cells. Blumwald and Tel-Or (1982b) adaptation to environmental stress requires a larger investment of energy for biosynthesis and reorganization by Nostoc muscorum. Probably A.oryzae cells meet these requirements, by enhanced photosynthetic activity which is an immediate response to salt-stress and resulted in higher carbohydrate content.

Intracellular mineral content:

Elemental analysis of the dried cells revealed that the alga exhibited changes in intracellular elements with increase of external salt concentration (Table 3). Addition of NaCl to the growth media decreased intracellular P, K, Mg and Ca content of A.oryzae and increased the residual minerals in the culture supernatant (Table 4), Accumulation of Mn and Cu in response to various NaCl concentrations was lesser than the previously mentioned set of elements. Moreover, Fe, Zn did not show clear dependance on NaCl concentration.

A most interesting observation was the drastic increase in intracellular Na content of cells over the full range of salinity, increasing with increasing extracellular salt concentration and reached 387 fold at 350 mM and 400 mM NaCl over their corresponding control cells.
The steep increase in Na content per gram dry cells is expected to play an essential role in the osmoregulatory mechanism of this alga.

These results lead us to believe that Na^+ ions are responsible for the adaptation of the cells to the saline medium and have specific ionic effect beside osmotic stress. Studies of Blumwald and TelOr, 1982 a, b and Watad, 1983, support these findings. Reed et al, 1985, mentioned that Na^+ exporting system is the limiting process for salt resistance of cyanobacteria from hyper saline environments. Other cyanobacteria responsed to changes in external water status by adjusting intracellular K^+ concentration (Miller et al, 1976; Yopp et al, 1978; Reed et al, 1984). Recently a variety of osmoregulants, employed by cyanobacteria, have been reported, e.g. significant ion accumulation (Ahmad and Hellebust, 1984; Blumwald et al, 1983; Ehrenfeld and Coussin, 1984; Ginzburg, 1981, Reed et al, 1985), synthesis of sucrose (Erdmann, 1983, Blumwald

et al, 1983), accumulation of osmoprotective compounds: glucosylglycerol (Hagemann et al, 1987); glycerol (Hagemann et al, 1987); glycerol derivatives (Borowizka et al, 1980; Tel-Or et al, 1986) and increasing concentration of prolein content (Ahmad and Hellebust, 1984).

From the results of Tables 3 and 4 it is clear that A.oryzae possesses a marked capacity to take up and accumulate ions with different rates from the external medium. This accumulation of ions appears to be almost sufficient to counterbalance the external salt concentration in cells grown at different salinity.

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Table 1. Cultural biomass and heterocyst frequency in batch cultures of Anabaena oryzae grown at a variety of NaCl concentrations in the media

NaCl Concentration	Culture biomass (mg dry cells/ ml culture)	Heterocyst frequency (% Heterocysts/ veg. Cells)
Control	1.06 ± 0.04	12.8 ± 2.0
50 mM	1.05 ± 0.06	10.7 ± 1.8
100 mM	1.11 ± 0.20	11.1 ± 1.2
150 mM	1.18 ± 0.08	9.7 ± 1.8
200 mM	1.17 ± 0.31	8.1 ± 0.7
250 mM	1.16 ± 0.23	5.5 ± 1.0
300 mM	1.14 ± 0.09	5.3 ± 0.8
350 mM	0.78 ± 0.11	5.2 ± 0.6
400 mM	0.48 ± 0.05	4.1 ± 0.7

Table 2. Effect of NaCl on the intracellular carbohydrate and nitrogen content of Anabaena oryzae. Cultures were grown with and without NaCl until late logarthmic phase of growth.

NaCl content mM/l	Total carbohydrates mg/g. dry wt.	Total nitrogen mg/g.dry wt.
Control	109.8	60.4
50 mM	115.3	50.7
100 mM	122.8	51.1
150 mM	151.2	54.8
200 mM	146.3	51.3
250 mM	159.6	44.8
300 mM	158.3	42.7
350 mM	169.5	23.9
400 mM	176.6	27.0

Table 3: Effect of increasing NaCl concentration on the accumulation of intracellular macro and micro elements in Anabaena oryzae. Each value is a mean of at least duplicate determinations

Concentration of NaCl	mg/g dry weight					µg/g dry wt.			
	P		Na	Ca	Mg	Fe	Mn	Zn	Cu
Control	5.4	3.0	0.1	8.8	3.1	172.40	64.80	57.00	19.57
50 mM	6.2	2.0	5.4	2.0	3.0	132.37	20.88	86.26	14.20
100 mM	5.4	1.7	7.7	2.0	2.6	113.99	13.68	95.76	11.52
150 mM	5.1	1.3	8.1	2.2	2.4	125.29	13.68	43.94	10.24
200 mM	4.5	1.7	12.6	1.7	1.8	233.75	12.96	55.72	12.8
250 mM	2.7	1.1	18.0	0.7	0.9	104.72	9.36	32.68	6.04
300 mM	2.1	1.2	30.7	1.0	0.1	207.57	14.4	66.26	11.52
350 mM	1.0	1.2	38.7	1.1	0.4	134.64	17.28	27.74	6.4
400 mM	1.2	1.2	38.7	1.0	0.3	269.28	33.12	60.42	10.24

Table 4 : Changes in macro and microelements concentration of the culture supernatant as a result of growth of Anabaena oryzae.

NaCl Concentration	N	P	K	Na	Ca	Mg	Fe	Mn	Zn	Cu
			µg/ml					p.p. m		
Watanabe medium	Zero	49.92	201.86	0.72	16.0	20.98	0.57	0.56	0.05	0.05
Control	0.042	21.12	187.44	3.6	12.0	5.95	0.27	0.23	0.02	0.05
50 mM NaCl	0.012	18.56	201.86	1020.3	28.0	7.73	0.27	0.24	0.02	0.05
100 mM NaCl	0.003	24.96	201.86	1637.85	40.0	11.47	0.38	0.29	0.02	0.04
150 mM NaCl	0.003	25.60	187.44	2040.6	44.0	11.66	0.30	0.30	0.02	0.05
200 mM NaCl	Zero	28.16	187.44	2309.1	48.0	13.15	0.19	0.33	0.05	0.03
250 mM NaCl	0.006	32.64	187.44	2685.0	58.0	16.90	0.11	0.36	0.05	0.04
300 mM NaCl	0.001	35.84	187.44	2738.7	58.0	18.24	0.19	0.41	0.03	0.03
350 mM NaCl	0.001	40.96	187.44	3275.7	62.0	20.40	0.23	0.36	0.05	0.03
400 mM NaCl	0.003	42.88	187.44	3714.6	66.0	21.07	0.11	0.39	0.05	0.03

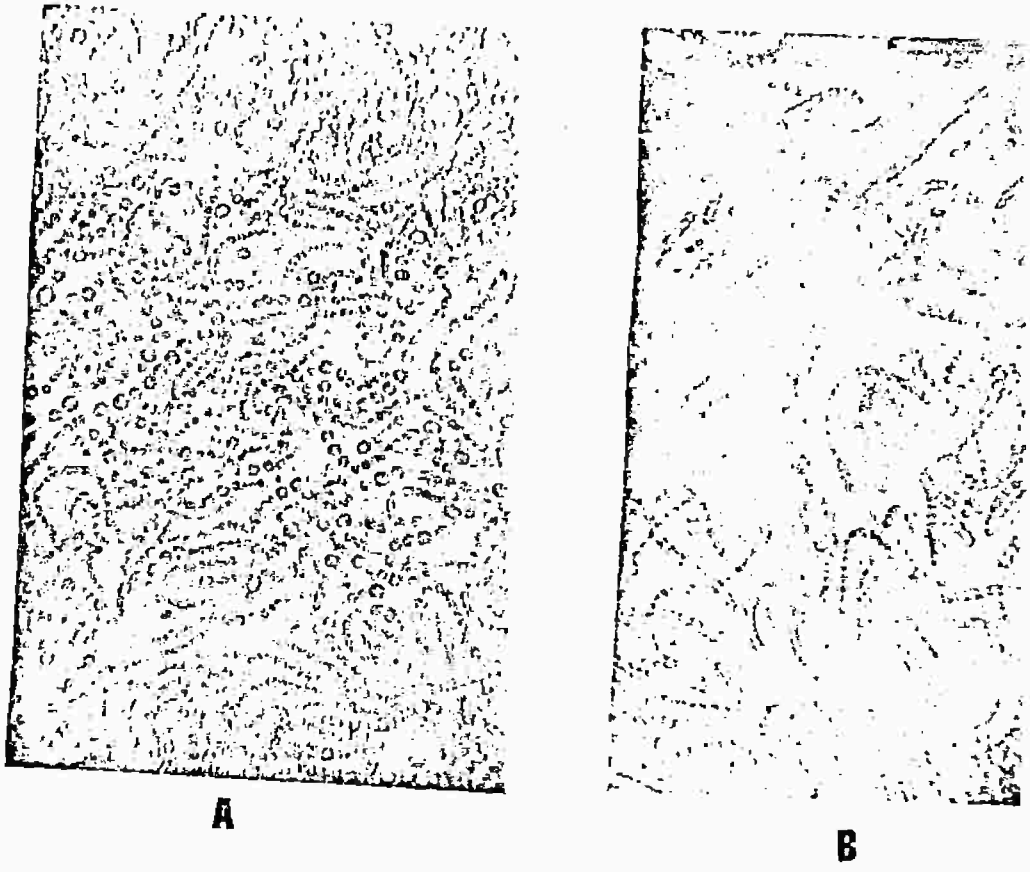

Fig. 1: Anabaena oryzae filaments
A control
B 400 mM NaCl
showing reduction in heterocyst frequency and change in colour