JABU JOURNAL OF ALGAL BIOMASS UTILIZATION
JABU - Copyright © 2009 PHYCOSPECTRUM

Impact of graded concentration of NaCl on the growth and heterocyst Frequency of parent and mutant strain of *Anabaena variabilis* RDU-1

Mridul Singh[1], Jai Prakash Pandey[1*], Amit Tiwari[1] and U. K. Chauhan[2]

1. **Department of Biotechnology, Govt. T.R.S. College, Rewa(M.P.)**

2. **Department of Biotechnology, A.P.S. University, Rewa(M.P.)**

Email: *jayse25@gmail.com

ABSTRACT

Cyanobacteria *Anabaena variabilis* as biofertilizer for rice cultivation has a beneficial effect on crop productivity and maintenance of soil fertility. In the present study, the parent and multiple herbicide resistent mutents of *Anabaena variabilis* , RDU-1 strains were taken for examining their relative response to salt stress under diazotrophic growth conditions. Mutant strains differentiated heterocyst with more or less similar frequency in all the concentration of NaCl ranging from 20 mM to 100 mM in comparision to parent strain decreased even in 20 mM to NaCl. Heterocyst differentiation is salt tolerant in the mutant strain and salt sensitive in parent strain.

Keywords: *Anabaena variabilis*, Heterocyst, Mutant strain and Parent strain.

INTRODUCTION

Cyanobacteria are oxygenic, photosynthetic prokaryotes growing and multiplying in a wide range of ecological habitats ranging from hot springs to Antarctic. Most of them are photoautotroph growing and multiplying diazotrophically at the expense of water, light and air. The diazotrophic cyanobacteria includes non-heterocystous, unicellular forms, filamentous and heterocystous forms. Heterocystous cyanobacteria are the most efficient aerobic N_2 – fixers under oxygenic photosynthetic conditions than nonheterocystous forms mainly because of

heterocyst (Ley, 1959). The cyanobacteria are a phototrophic group of eubacteria that carry out oxygenic photosynthesis. Most cyanobacteria can use nitrate and ammonium as nitrogen sources, and many are also able to fix atmospheric nitrogen. The enzyme complex that fixes dinitrogen, nitrogenase, is highly sensitive to oxygen. Nitrogen fixation and oxygenic photosynthesis are generally separated, either temporally or spatially, in cyanobacteria. (Flores, *et. al.* 1994)

Heterocystous nitrogen fixing cyanobacteria are the natural components and water logged rice field and tropical countries like India where they grow luxuriantly and fix nitrogen abundantly. Documents are available to show that nitrogen fixing cyanobacterium of rice are the two main heterological system for maintaining fertility of paddy fields year after year without any external provision of fixed nitrogen source like ammonia or urea. This realization has recently led to the development of technology for multiplying and supplying a mixer of diazotrophic cyanobacteria biofertilizer for rice cultivation. Indications are that cyanobacterial biofertilizer technology must be the future source of nitrogen supply to rice agriculture. (Singh, *et. al.* 1994)

In this present investigation, the parent and multiple herbicide resistant mutants of *Anabaena variabilis*, RDU-1 were taken for examining their relative response to salt stress under diaxotrophic growth conditions.

MATERIALS AND METHODS

Source of Organism, culture medium and cultivation

The cyanobacterium *Anabaena variabilis*, RDU-1 its multiple herbicide resistant mutant used in the present investigation was obtained resistant mutant used in the present investigation was obtained through Department of Biological Sciences, R. D. University, Jabalpur. The organism is a local isolate from, rice field of Jabalpur. The multiple herbicide

resistant mutant of *Anabaena variabilis*, RDU- 1 isolated through spontaneous mutation showed resistance to four commonly used rice field herbicides Arozin, Alachlor, Butachlor and 2. 4-D. The organism differentiates three kinds of cells i.e. vegetative cells, heterocysts and akinetes under nitrogen fixing condition.

In order to obtain clonal population of the cyanobacterium, the filaments were homogenized by vigorously using glass beads in a vortex mixer. 2-4 called trichomes thus produced were washed five times with sterile medium by centrifugation and streaked on agar plates. The plates were incubated under photoautotrophic growth conditions in a culture room maintained at 25± 1^0C. Algal colonies developed after 10 days of incubation were picked up and transferred to a liquid medium. The axenic status of the culture was tested from time to time by streaking cells on nutrient agar media. Bacteria free colonies were picked up with sterile glass capillaries under binocular microscope and incubated aseptically into sterile culture tubes containing 5 ml fresh N_2 medium.

BG-II medium without combined nitrogen source was used as basal medium (designated as N_2 medium) for routine cultivation of *Anabaena variabilis*. The multiple herbicide resistant mutant strain was maintained with growth, toxic level of the herbicides. The culture medium, glassware and chemicals were sterilized by autoclaving at pressure of 15 lb/inch^2 (121^0C) for 15 minutes. (Venkataraman, 1972)

Growth Conditions

The clonal culture of parent and mutant of *Anabaena variabilis* RDU-I was maintained in bacteria free state by routinely transferring (at interval of 6-7 days) aseptically, the mid exponential phase culture to 200 ml fresh medium contained in 500 ml conical flasks. The cultures were grown photoautotrophically in a culture room maintained at (25±2^0C) and illuminated with cool florescent light

at photon flux density of 2500 lux at the surface of the vessel for 16 hours a day.

Heterocyst Frequency

It was determined by counting the number of heterocyst in a given filament and expressed as % frequency

Impact of graded concentration NaCl on the growth and heterocyst frequency of parent and mutant strain of Anabaena variables RDU-1

Effect of increasing concentrations of NaCl of (0, 20, 40,60,80 and 100mM on the growth and heterocyst frequency of multiple herbicide Alachlor, Butachlor, Arozin, 2-4-D, resistant strain of *Anabaena variabilis*, RDU-1 was determined in N2 medium by monitoring in the concentration of heterocyst frequency at regular intervals for 12 days.

RESULTS AND DISCUSSION

Heterocystous diazotrophic cyanobacteria are known to grow and multiply luxuriantly in water logged rice field of tropical country, during rainy season during which rice crop is also growing. Heterocystous form or photosynthetic aerobic N_2 fixers with photosynthetic carbon assimilation taking place within vegetative cell and nitrogen fixation taking place in heterocyst (Singh, *et. al*. 1994). Heterocyst is the known site of the nitrogenase activity because of its role in activation of *nif* gene expression and in protection of O_2 toxicity. The known biochemistry and morphology associated with heterocyst differentiation from vegetative cell are the facts in support of the observed conclusion when a vegetative cell differentiates into a heterocyst fixing nitrogen.

Studies have revealed that under aerobic photosynthetic condition heterocyst remains the exclusive site for nitrogen fixation. No other diazotrophic microbes are known that can fix nitrogen aerobically under oxygenic photosynthetic condition. This attribute makes heterocystous diazotrophic cyanobacteria as the most potential source of biofertilizer

under field condition. The additional importance of heterocystous diazotrophic cyanobacterium is the fact that being photosynthetic they are never energy limited and against this background of knowledge studies have been carried out to construct biofertilizer strain of heterocystous form that are multiple herbicide resistant and salt tolerant. (Singh, *et. al.* 1997)

NaCl is a necessary ingredient in the growth medium of both diazotrophic and non-diazotrophic cyanobacteria. The physiology of sodium in cyanobacterium includes its role as sympatric for transport of nitrate and bicarbonate in addition it also required as a component of Na+/H+ antiporter in regulation of cytoplsmic pH. However a higher concentration of sodium is found toxic because of its general inhibitory role on enzyme activity. In comparison, Potassium is required as a physiological nutrient for maintenance of enzyme stability and activity as well as maintenance of cell osmolarity. NaCl at higher concentration causes salinity stress. (Reed, *et. al.*1986 and Singh, *et. al.* 2007)

The results suggests that ammonium nitrogen is an inhibitor for heterocyst differentiation and that heterocyst differentiation is a requirement for the diazotrophic growth. Both the strains were also compared for heterocyst frequency as shown in **(Table - 1)** parent produces heterocyst frequency of about 10 % and mutant a heterocyst frequency of about 6% . Thus the mutant strain differ from parent strain were also compared in respect of their response to heterocyst differentiation and frequency to varying concentration of NaCl.

Mutant strains differentiated heterocyst with more or less similar frequency in all the concentration of NaCl raging from 20 mM to 100mM in comparison heterocyst differentiation and frequency of parent strain decreased even in 20mM of NaCl and fail to occur in 60mM, 80mM and 100mM of salt concentration. Microscopic observation

reveal that salt concentration of 40mM and above induced fragmentation of filaments is parent culture, this effect of salt was comparatively much less in mutant strain. Thus to conclude heterocyst differentiation is salt tolerant in the mutant strain and salt sensitive in parent strain. Similar studies have also been done by Singh et. al., 1997 , Singh et. al., 2007.

Table 1 - Impact of graded concentration of NaCl on heterocyst frequency (%) of parent and multiple herbicide Al^r , But^r, Aro^r, 2-4-D resistant mutant of *Anabaena variabilis* RDU-1.

Days	Control		20mM		40mM		60mM		80mM		100mM	
	P	M	P	M	P	M	P	M	P	M	P	M
0	7.0	4.5	7.0	7.0	4.5	7.0	4.5	7.0	7.0	4.5	7.0	4.5
2	7.6	4.5	6.2	4.5	4.5	4.5	2.5	4.5	0	4.5	0	4.5
4	8.2	4.6	5.0	4.4	4.0	4.6	0	4.6	0	4.5	0	4.5
6	9.0	5.0	4.6	4.8	3.1	4.7	0	4.7	0	4.6	0	4.6
8	9.5	5.5	3.6	5.0	2.8	4.8	0	4.8	0	4.7	0	4.7
10	9.7	5.8	3.2	5.4	2.3	5.2	0	5.0	0	4.8	0	4.8
12	9.8	5.8	3.0	5.7	1.6	5.6	0	5.4	0	5.0	0	5.0

CONCLUSION

This paper has demonstrated that heterocyst differentiation is salt tolerant in the mutant strain and salt sensitive in parent strain. The resulting knowledge for the given study would provide basis to introduce salinity tolerant phenotype into the multiple herbicide resistant cyanobacterial strains for its ultimate use as an appropriate biofertilizer strains with potential for application in rice agriculture.

REFERENCES

Flores, E. and Herrero, A. 1994. Assimilatory nitrogen metabolism and its regulation. Kluwer Academic Publishers, Dordrecht, The Netherlands. 487–517.

Ley, S.H. 1959. The effect of nitrogen fixing blue green algae on the yields of rice plant. *Acta Hydrabiol*. Sinica.

Reed, R.H., Borowitzka, L. J., Mackay, M.A., Chudek, J. A., Fosler , R., Wars, S.R.C., Moore, D. J. and Stewart, W.D.P. 1986. Organic solute accumulation in asmotically stressed cyanobacteria. *FEMS microbiologica reviews*.506-546.

Singh, S. and Datta, P. 2007. Outdoor evaluation of herbicide resistant strains of Anabaena variabilis as biofertilizer for rice plants. *Journal of Basic Microbiology*. 296: 95-102

Singh, S., Negi, S., Bharti, N. and Singh, H.N. 1994. Common nitrogen control of Caesium uptake, caesium toxicity and ammonium (methylammonium) uptake in the cyanobacterium Nostoc muscorum. *FEMS Microbiology Letters*. 177:243-248

Singh, S., Singh, A.K., Chakravarthy, D., Singh, T.P.K. and Singh, H.N. 1997. Characterisitics of a Caesium-resistnat (Cs±R) multant of the N2-fixing cyanobacterium Nostoc muscorum : dependence on Cs+ or Rb+ for normal diazotrophy and osmoregulation. *New Phytol*. 136: 223-229.

Venkatraman, G.S. 1972. Algal Biofertilizer and Rice cultivation, Today and Tomorrow's publishers, New Delhi.455-468