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Comparative Osmoregulatory Studies of K^+ and Rb^+ in the Cyanobacterium *Nostoc muscorum*

Santosh Bhargava, Rajesh K. Kachouli and Vipin Kaithwas

Division of Microbiology, Department of Botany,
Government Motilal Science College, Bhopal 462003 (M.P.) India

Abstract: Rubidium (Rb^+) is an analog of potassium (K^+) and has been used as a tracer of potassium. In the present study we have analyzed its role as a nutrient as well as in osmoregulation in the cyanobacterium *Nostoc muscorum*. The filaments of *N. muscorum* will grow well in the medium in which K^+ is replaced by Rb^+. Salt and osmotic sensitivity of the Rb^+ grown cells increased in terms of percent survival, photosynthetic O_2 evolution and nitrogenase activity. Like K^+ grown cells exogenous proline provides no protection to the Rb^+ grown cells under salt and osmotic stresses. This suggested that intracellular accumulation of K^+ is a prerequisite for uptake and accumulation of proline. These results suggested that Rb^+ like that of K^+ is nutrient compatible, but could not act as an osmolyte.

Key words: Diazotrophy · *Nostoc muscorum* · Osmolyte · Potassium · Rubidium

INTRODUCTION

The extracellular environment plays a major role in determining relative concentrations of the inorganic elements within the cell. The amount and diversity of inorganic element in the external milieu and there impact on biological system has leads to the identification of major elements that commonly participates in metabolic processes within the diverse group of microorganisms [1]. In spite of the substantial fluctuation in the external environment all living cell maintained a constant internal ionic concentration.

In prokaryotes cytoplasmic K^+ concentration plays an important role in the regulation of turgor pressure. This observation is based on the fact that K^+ concentration of the cell varies with the medium osmolality [2]. The optimum K^+ concentration with in the cell is maintained either by high affinity or by low affinity K^+ transporters [2,3]. During the initial phase of stress K^+ is known to accumulated, followed by the accumulation/synthesis of low molecular weight organic compounds known as compatible solutes [4-6]

As rubidium is an analog of K^+ and has similar ionic radii (133 and 148 pm). The calculated sum of ionic radius is 314 and 329 for KCl and RbCl respectively, therefore; it is used as an analog tracer for K^+ [7,8].

Cyanobacteria are Gram negative, photoautotrophic prokaryotic microorganisms and are considered to be the model system for understanding the effect of various environmental factors. Cyanobacterial cell when expose to NaCl stress the internal ionic concentrations is maintained by Na^+ export and K^+ uptake [9]. Therefore, it is suggested that intracellular accumulation of K^+ acts as a primary osmolyte in cyanobacterial osmoregulation.

In this paper we reported that K^+ substitution by the Rb^+ in the growth medium, supports diazotrophic growth of the cyanobacterium *N. muscorum*, Further findings conform that in spite of having nutritive role like that of K^+, Rb^+ have no osmoregulatory role in the examined cyanobacterium.

MATERIALS AND METHODS

Organism and Growth Conditions: Axenic clonal culture of the wild type *Nostoc muscorum* (a fresh water cyanobacterium) was grown and maintained photoautotrophically with a light intensity at photon fluence rate of 50 µmol m^{-2} s^{-1} and temperature of 28 ± 2°C in Chu No. 10 medium [10]. The culture medium was buffered to pH 7.5 with 10 mM HEPES-NaOH.

Corresponding Author: Santosh Bhargava, Division of Microbiology, Department of Botany,
Government Motilal Science College, Bhopal 462003 (M.P.) India

Measurement of Growth: The Chu No.10 growth medium was modified to make it free from K^+ for the experiments examine the role of K^+/Rb^+ in the cyanobacterium diazotrophic growth. Growth was measured as optical density change at 663 nm. *N. muscorum* culture grow in a highly dispersed suspension form without forming clumps and, therefore, its direct use for measuring growth at 663 nm is as valid as growth of heterotrophic bacteria estimated by degree of culture turbidity.

Salinity and Osmotic Characteristic: The ionic osmoticum NaCl and the non-ionic osmoticum sucrose at graded concentration were used for a comparative study of the salt/osmo tolerance characteristics of the cyanobacterial strain under diazotrophic growth conditions. The inoculum size per nutrient plate was 5×10^2 CFUs. The salinity and osmotic survival was examined in 6-d old culture by counting CFUs appeared on the nutrient plates. The nutrient plate counting no NaCl and sucrose act as a control plate.

Chlorophyll *a* contents were measured by the method of Mackinney *et al.* [11], protein by the method of Lowry *et al.* [12], nitrogenase activity and photosynthetic O_2 evolution by the method as described by Bhargava [6].

RESULTS

The diazotrophic growth medium devoid of K^+/Rb^+ was used to analyze the role of K^+ and Rb^+ in the diazotrophic growth of the cyanobacterium. Wild type strain showed its maximum growth in the Chu No.10 medium. The same strains showing limited growth in the medium devoid of K^+, this suggested that K^+ plays an essential nutritive role in diazotrophic and autotrophic growth of the examined cyanobacterium. The growth rate of wild type in K^+-free diazotrophic growth medium restored when the growth medium supplemented with 2 mM RbCl. This indicates that Rb^+ like that of K^+ act as a nutrient for diazotrophic and autotrophic growth in the examined cyanobacterium (Table 1).

Fig. 1 shows percent survival of the wild type under graded concentration of NaCl and sucrose in the presence/absence of K^+. The cyanobacterium *N. muscurom* showed zero percent survival in the diazotrophic growth medium containing 100 mM NaCl or 250 mM sucrose. While the percent survival decreases under similar stress conditions in the medium lacking K^+. The addition of 2 mM RbCl in place of KCl in the diazotrophic growth medium could not able to restore salinity/osmotic survival characteristic of the wild type cells. Thus, it is suggested that Rb^+ like that of K^+ could not act as an osmolyte.

K^+ and Rb^+ grown cultures were further compared under NaCl and sucrose stresses with regards to diazotropy and oxygenic photosynthesis. Table 2 shows photosynthetic O_2 evolution and nitrogenase activity of K^+ and Rb^+ grown cells. The response of photosynthetic O_2 evolution and nitrogenase activity to salinity and osmotic stresses was significantly low in the Rb^+ grown cultures than that of K^+ grown cultures.

Table 1: showing diazotrophic characteristic of the wild type *N. muscorum* in the presence/absence of K^+ and in the presence of Rb^+ in terms of growth (OD change at 663nm), heterocyst frequency (HF%) and nitrogenase activity (m mol C_2H_4 formed g^{-1} Chl *a* h^{-1})

Parameters	Chu No. 10 medium	- K^+	- K^+ + Rb^+
Growth	0.86±0.04	0.41±0.02	0.82±0.04
HF%	7-8	5-6	7-8
Nitrogenase activity	12.3±2.1	544±44.5	7.7±1.9
Photosynthetic O_2 evolution	256±21.3	11.9±2.2	472±33.2

Non-heterocystous NH_4^+-grown cultures were used as inoculum for incubation in diazotrophic growth medium.
Each reading is an average (± SEM) of three independent experimental determinations.

Table 2: showing NaCl and sucrose tolerance characteristic of wild type *N. muscorum* in the presence of K^+/ Rb^+ in terms of nitrogenase activity (m mol C_2H_4 formed g^{-1} Chl *a* h^{-1}) and photosynthetic O_2 evolution (m mol O_2 evolved g^{-1} Chl *a*)

	+ K^+		+ Rb^+	
Parameters	N-activity	O_2 evolution	N-activity	O_2 evolution
Control	12.28±2.3	544±28.3	11.97±1.9	532±26.9
+30 mM NaCl	9.12±1.1	492±26.4	6.32±0.7	356±25.8
+60 mM NaCl	7.11±1.1	332±22.1	3.21±0.2	±22.3
+90 mM NaCl	1.23±0.1	156±11.5	0.0	0.0
+160 mM sucrose	9.87±1.0	486±25.5	6.52±0.6	±27.4
+200 mM sucrose	7.28±0.9	378±23.3	3.63±0.2	±25.2
+240 mM sucrose	2.11±0.2	187±12.6	0.0	0.0

Non-heterocystous NH_4^+-grown cultures were used as inoculum for incubation in diazotrophic growth medium.
Each reading is an average (± SEM) of three independent experimental determinations.

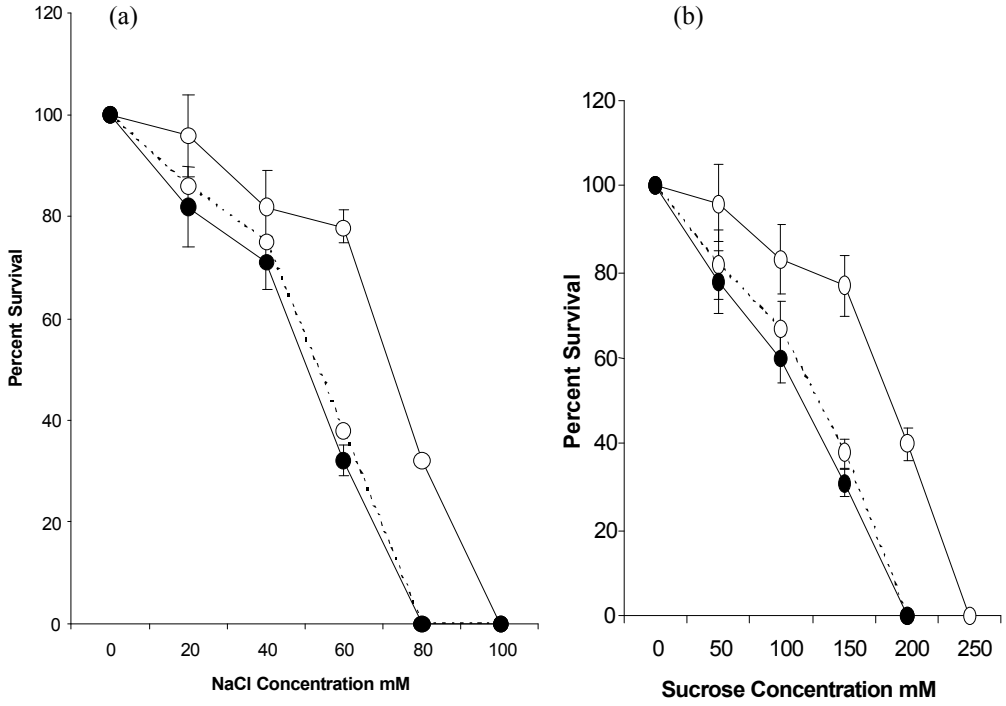

Fig. 1: (a) Percent survival of the wild type (WT) *N. muscorum* to increasing concentration of NaCl. WT with Rb^+ (●), WT without K^+ (---O---) and WT with K^+ (O). Each reading is an average (± SEM) of three independent experimental determinations.
(b) Percent survival of the wild type (WT) *N. muscorum* to increasing concentration of sucrose. WT with Rb^+ (●), WT without K^+ (---O---) and WT with K^+(O). Each reading is an average (± SEM) of three independent experimental determinations.

Table 3: showing NaCl and sucrose tolerance characteristic of wild type *N. muscorum* in terms of growth and percent survival in the medium containing K^+/Rb^+ in the presence of 1 mM proline

	+ K^+		+ Rb^+	
Parameters	Growth	% survival	Growth	% survival
Control	0.85±0.03	100	0.72±0.02	100
+30 mM NaCl + 1mM proline	0.82±0.03	100	0.62±0.03	66±5.6
+60 mM NaCl + 1mM proline	0.81±0.03	98±8.8	0.38±0.02	36±8.8
+90 mM NaCl + 1mM proline	0.79±0.02	96±7.9	0.18±0.02	06±7.9
+160 mM sucrose + 1mM proline	0.84±0.03	100	0.71±0.03	68±6.6
+200 mM sucrose + 1mM proline	0.82±0.03	99±9.1	0.40±0.02	39±3.3
+240 mM sucrose + 1mM proline	0.80±0.03	97±8.9	0.21±0.02	08±0.6

Non-heterocystous NH_4^+-grown cultures were used as inoculum for incubation in diazotrophic growth medium.
Each reading is an average (± SEM) of three independent experimental determinations.

Proline the well known compatible solute is utilized as a nitrogen source under normal growth conditions by the cyanobacterium *N. muscorum*. So we examine the effect of exogenously supplied proline on heterocyst formation and nitrogenase activity. It was observed that K^+ grown cells did not produce N_2-fixing heterocyst in the proline medium. In contrast Rb^+ grown cells produced normal frequency of heterocyst and showed nitrogenase activity (data not shown).

Intracellular concentration of K^+ acts as a primary osmolyte functioning in accumulation of secondary osmolyte i.e. proline. So, this investigation examined the role of exogenously supplied proline on salinity/osmotic survival in Rb^+ grown cultures. The results as shown in the table 3 suggested that coexistence of NaCl/sucrose + proline did not provide any protection to the Rb^+ grown cells.

DISCUSSION

There are number of K^+ transport systems *viz*. Trk, Kup and Ktr involved in K^+ and Rb^+ transport in various prokaryotic organisms [13-15]. These transport systems not only transport Rb^+ but also involved in osmoadaptation. In *Anabaena variabilis* it has been reported that K^+ transport systems also involved in Rb^+ transport and osmoadaptation [16].

The cyanobacterium *N. muscorum* is capable of growing on medium in which K^+ has been replaced by Rb^+, thus, it can be said that Rb^+ like that of K^+ act as an nutrient for *N. muscorum* diazotrophic growth. In *Synechococcus* similar nutritive role of Rb^+ has also been suggested [17,18]. In a halotolerant bacterium *Oceanomonas baumannii* (ATCC 700832) rubidium could substitute for potassium in alleviating growth inhibition due to potassium limitation [19].

In enterobacteria K^+ is reported to function as a primary intracellular signal for adaptation to salinity stress [20]. Like wise in cyanobacteria K^+ is essential for cyanobacteria growth, enzyme activator, membrane potential and as osmoregulatory element [6,21,22]. The Rb^+ grown WT cells do not show salinity and osmotic characteristics like that of K^+ grown cells. Therefore, it is suggested that Rb^+ could not act as a primary intracellular signal under salinity and osmotic stresses.

The cyanobacterium *N. muscorum* able to utilized proline as a nitrogen source under normal growth conditions [6]. Extracellular proline was also found to provide protection in response to salinity and osmotic stresses [6,23, 24]. The Rb^+ grown cells neither able to utilized proline as a nitrogen source nor extracellular proline provide any protection in response to NaCl and sucrose stresses. Therefore, it is concluded that Rb^+ like that of K^+ could not act as a primary osmolyte functioning in activating genes specific for accumulation/synthesis of secondary osmolyte.

When *N. muscorum* were cultured in the presence of graded concentration of NaCl and sucrose, distinct adverse effect were seen on nitrogenase activity and on photosynthetic O_2 evolution. Cyanobacterial nitrogenase activity and oxygenic photosynthesis were completely inhibited at 100 mM NaCl and 250 mM sucrose concentration, which is supported by the similar findings [25-27]. The Rb^+ grown cultures under similar stress conditions were found to show more sensitivity in respect to nitrogenase activity and photosynthetic O_2 evolution.

The present finding also demands more critical molecular biology studies to examine the role of Rb^+ as a substitute of K^+ in various physiological functions.

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REFERENCES

1. Wackett, L.P., A.G. Dodge and L.B.M. Ellis, 2004. Microbial genomics and the periodic table. Applied and Environmental Microbiology, 70: 647- 655.
2. Brown, A.D., 1990. Microbial Water Stress Physiology. Principles and Perspectives. John Wiley and Sons, Chichester, England.
3. Véry, A.A. and H. Sentenac, 2003. Molecular mechanism and regulation of K^+ transport in higher plants. Annual Review of Plant Biology, 54: 575-603.
4. Galinski, E.A. and R.M. Herzog, 1990. The role of trehalose as a substitute for nitrogen-containing compatible solutes (*Ectothiorhodospira halochloris*). Archives Microbiology, 153: 607-613.
5. Whatmore, A.M. and R.H. Reed, 1990. Determination of turgor pressure in *Bacillus subtilis*: a possible role for K^+ in turgor regulation. Journal of General Microbiology, 136: 2521-2526.
6. Bhargava, S., 2005. The role of potassium as an ionic signal in the regulation of cyanobacterium *Nostoc muscorum* response to salinity and osmotic stress. Journal of Basic Microbiology, 453: 171-181.
7. Ritchie, R.J., 1988. The ionic relations of *Ulva lactuca*. Journal of Plant Physiology, 133: 183-193.
8. Lide, D.R., (ed.), 1993. CRC Handbook of Chemistry and Physics, 73 rd edn., CRC Press, Boca Raton, EL, USA.
9. Marin, K., Y. Kanesaki, D.A. Los, N. Murata, I. Suzuki and M. Hagemann, 2004. Gene expression profiling reflects physiological process in salt acclimation of *Synechocystis* sp. strain PCC 6803. Plant Physiology, 136: 1-11.
10. Gerloff, G.C., G.P. Fittgerald and F. Skoog, 1950. The isolation, purification and culture of blue green algae. American Journal of Botany, 37: 216-218.
11. Mackinney, G., 1941. Absorption of chlorophyll solutions. Journal of Biological Chemistry, 140: 315-322.

12. Lowry, O.H., N.J. Rosebrough, A.L. Farm and R.H. Randall, 1951. Protein measurement with the folin-phenol reagent. Journal of Biological Chemistry, 193: 265-275.
13. Sprott, G.D., K.M. Shaw and K.F. Jarrell, 1985. Methanogenesis and the K^+ transport system are activated by divalent cations in ammonium-treated cells of *Methanospirillum hungatei*. Journal of Biological Chemistry, 260: 9244-9250.
14. Dosch, D.C., G.L. Helmer, S.H. Sutton, F.F. Salvacion and W. Epstein, 1991. Genetic analysis of potassium transport loci in *Escherchia coli*: evidence for three constitutive systems mediating uptake of potassium. Journal of Bacteriology, 173: 687-696.
15. Schlosser, A., A. Hamann, M. Schleyer and E.P. Bakker, 1992. The K^+ uptake systems TrkG and TrkH from *Escherchia coli*: a pair of unusual transport systems involve in osmoregulation, In molecular mechanisms of transport. E. Quagliariello and F. Palmieri (eds), Elsevier, Ansterdam, pp: 51-58.
16. Reed, R.H., P. Rowell and W.D.P. Stewart, 1981. Uptake of potassium and rubidium ions by the cyanobacterium *Anabaena variabilis*. FEMS Microbiology Letters, 11: 233-236.
17. Kumar, H.D. and S.S. Purohit, 1972. The effect of substituting rubidium for potassium on nutrition of the blue-green alga- *Anacystis nidulans*. Phykos, 11: 1-5.
18. Collier, J.L. and A.R. Grossman, 1992. Chlorosis induced by nutrient deprivation in *Synechococcus* sp. strain PCC 7942: not all bleaching is the same. Journal of Bacteriology, 174: 4718-4726.
19. Brown, A.D. and S.P. Cummings, 2001. Potassium uptake and retention by *Oceanomonas baumannii* at low water activity in the presence of phenol. FEMS Microbiology Letters, 205: 37-41.
20. Higgins, C.F.J., D.A. Cairney, L. Stirling, L. Sutherland and I.R. Booth, 1987. Osmotic regulation of gene expression: ionic strength as an intracellular signal? Trends in Biochemical Sciences, 12: 339-344.
21. Reed, R.H. and W.D.P. Stewart, 1985. Evidence for turgor sensitive K^+ influx in the cyanobacteria *Anabaena variabilis* ATCC 29413 and *Synechocystis* PCC 6714. Biochimica et Biophysica Acta, 812: 155-162.
22. Reed, R.H. and A.E. Walsby, 1985. Changes in turgor pressure in response to increase in external concentration in the gas-vacuolated cyanobacterium *Microcystis* sp. Archives Microbiology, 143: 290-296.
23. Singh, A.K., D. Chakravarthy, T.P.K. Singh and H.N. Singh, 1996. Evidence for a role of L-proline as a salinity protectant in the cyanobacterium *Nostoc muscorum*; Plant Cell and Environment, 19: 490-494.
24. Alia, E.A. and I.A. Gahiza, 2007. Accumulation of amino acids in *Anabaena oryzae* in response to sodium chloride salinity. Journal of Applied Science Research, 3: 263-266.
25. Fernandes, T.A. and S.K. Apte, 2000. Differental regulation of nitrogenase activity by ionic and osmotic stresses and permeable sugars in the cyanobacterium *Anabaena* sp. strain L- 31. Plant Science, 150: 181-189.
26. Moisander, P.H., E. McClinton and H.W. Pearl, 2002. Salinity effect on growth photosynthetic, parameters and nitrogenase activity in estuarine planktonic cyanobacteria. Microbial Ecology, 43: 432-422.
27. Okmen, G., G. Donmez and S. Donmez, 2007. Influence of osmotic and metal stresses on nitrogenase activity of cyanobacteria isolated from paddy field. African Journal of Biotechnology, 6: 1828-1832.