



# Journal of Biological Sciences

ISSN 1727-3048

**science**  
alert

**ANSI***net*  
an open access publisher  
<http://ansinet.com>

## Osmoregulatory Role of Potassium and Proline in the Cyanobacterium *Nostoc muscorum*

<sup>1</sup>S. Bhargava, <sup>1</sup>R.K. Kachouli, <sup>2</sup>R. Maithil, <sup>1</sup>S. Dubey and <sup>1</sup>V. Kaithwas

<sup>1</sup>Division of Microbiology, Department of Botany,  
Government Motilal Science College, Bhopal 462003 (M.P.), India

<sup>2</sup>School of Biotechnology, Rajiv Gandhi Technological University,  
Gandhi Nagar, Bhopal 462038 (M.P.), India

**Abstract:** The aim of this study was to investigate the role of K<sup>+</sup> and proline in the regulation of NaCl and sucrose caused stresses in the cyanobacterium *Nostoc muscorum*. Growth of *Nostoc muscorum* severely impaired under NaCl and sucrose caused stresses. Addition of 1 mM proline in the incubation mixture restores growth of the cyanobacterium under stress conditions. The growth is more pronounced in the presence of both K<sup>+</sup> and proline. The inhibitory effect of stress component also recovered in terms of photosynthetic O<sub>2</sub> evolution and nitrogenase activity in the presence of K<sup>+</sup> and proline. The present observations suggest that K<sup>+</sup> and proline in combination support not only growth but also oxygenic photosynthesis and nitrogenase activity under stress conditions. During the initial phase of stress condition uptake of K<sup>+</sup> taken place followed by the accumulation of proline. Thus, the accumulation of K<sup>+</sup> and proline in the cell cytoplasm act as a primary and a secondary osmolytes in the investigated organism.

**Key words:** *Nostoc muscorum*, potassium, proline, osmoregulation, oxygenic photosynthesis, nitrogenase activity

### INTRODUCTION

Many cyanobacterial strains are known to accumulate or synthesized various inorganic or organic molecules/compounds that provide protection to the organism when they exposed at high osmolality. In cyanobacteria it has been reported that during the initial up shock accumulation of potassium (K<sup>+</sup>) took place (Reed and Stewart, 1985; Matsuda and Uozumi, 2006). This is followed by the accumulation or synthesis of low molecular weight organic compounds i.e., compatible solutes. The known compatible solutes usually synthesized by cyanobacterial strains are sucrose, trehalose, proline, glucosyl-glycerol, glycine-betaine etc. (Higgins *et al.*, 1987; Warr *et al.*, 1988; Csonka, 1989; Singh *et al.*, 1996; Karandashova and Elanskaya, 2005; Roder *et al.*, 2006; Fatma *et al.*, 2007; Lu *et al.*, 2006).

In enterobacteria Trk and Kdp are the known low affinity and high affinity systems for K<sup>+</sup> transport (Rhoads and Epstein, 1978; Jung *et al.*, 2000; Ballal *et al.*, 2005). Similar K<sup>+</sup> transport systems have been present in the genome of the cyanobacterium *Synechocystis* sp., PCC 6803. The Trk/Ktr systems of the *Synechocystis* sp., PCC 6803 are known to respond to hyper osmotic shock (Matsuda *et al.*, 2004; Matsuda and Uozumi, 2006). The

subsequent studies on the role of K<sup>+</sup> in osmoregulation have shown that intracellularly accumulated K<sup>+</sup> is the primary osmolyte functioning in accumulation/production of secondary osmolyte i.e., compatible solutes (Csonka and Hanson, 1991; Bhargava *et al.*, 2006). In *E. coli* three transporters viz., PutP, ProP and ProU are involved in transport and accumulation of proline. The PutP system of *E. coli* is required for the catabolism of proline and the ProP and ProU systems are involved in the accumulation of proline (Grothe *et al.*, 1986; Schwan *et al.*, 2006).

In this study, we reported the role of intracellular K<sup>+</sup> and proline in the regulation of cyanobacterial response to salinity and osmotic stresses. The present observation indicates that K<sup>+</sup> transport systems are essential for the uptake and accumulation of proline.

### MATERIAL AND METHODS

**Organism and culture conditions:** The streptomycin resistant mutant clone of the cyanobacterium *N. muscorum* was used as an investigating organism in the present study. The Chu No. 10 culture media (Gerloff *et al.*, 1950) was used to grow the cultures. The cultures were incubated in the growth chamber with a light intensity of 50  $\mu\text{mol m}^{-2} \text{sec}^{-1}$  and temperature of 28±2°C.

**Estimation of K<sup>+</sup> and proline uptake:** The disappearance of K<sup>+</sup> contents in the 10 mM HEPES-NaOH buffer (pH 7.5) was determined with the help of Flame Photometer (Systronic 128). The incubation mixture was supplied with 0.1 mM KCl. The experimental samples (stressed and unstressed) were equilibrated for 30 min under the given growth conditions. The salinity stress and osmotic stress conditions were created by the addition of 100 mM NaCl and 250 mM sucrose to the buffer medium. The amount of K<sup>+</sup> in the growth medium was measured as function of incubation period and then subtracting these values from the control value.

**Estimation of intracellular proline:** The proline content in the growth medium was estimated by the method of Bates *et al.* (1973). The centrifuged 2 cm<sup>3</sup> of the media was reacted with 2 cm<sup>3</sup> of acid ninhydrin reagent (1.25 g ninhydrin + 30 cm<sup>3</sup> glacial acetic acid + 8 cm<sup>3</sup> orthophosphoric acid + 12 cm<sup>3</sup> DH<sub>2</sub>O) and 2 cm<sup>3</sup> of glacial acetic acid and the mixture was boiled for 1 h in a water bath. The reaction was terminated by dipping the reaction mixture in an ice bath. The 4 cm<sup>3</sup> of toluene is then added to the reaction mixture. The reactant chromophore extracted into toluene phase is then aspirated from the aqueous phase and its absorbance read at 520 nm.

Photosynthetic O<sub>2</sub> evolution and nitrogenase activity were measured as described previously by Bhargava *et al.* (2006).

Growth, K<sup>+</sup> uptake and intracellular proline contents were carried at the Department of Botany, Government Motilal Science College, Bhopal, while the photosynthetic O<sub>2</sub> evolution and nitrogenase activity were carried out at Department of Microbiology, Barkatullah University, Bhopal, during February 2009 to May 2009.

## RESULTS

The effect of NaCl and sucrose on the growth of *N. muscorum* was examined. NaCl at a concentration of 100 mM and sucrose at a concentration of 250 mM was found lethal to the diazotrophically grown cultures of the cyanobacterium (Fig. 1). Addition of 5 mM K<sup>+</sup> (as KCl) in the growth medium caused recovery in the growth during the initial phase and then no significant growth was observed. The damage caused by the stresses was almost completely recovered when incubation mixture supplied with proline. The growth was further enhanced when the incubation mixture supplied with both K<sup>+</sup> and proline (Fig. 2, 3). Therefore, it is suggested that K<sup>+</sup> and proline in combination completely restored the inhibitory effect of NaCl and sucrose.

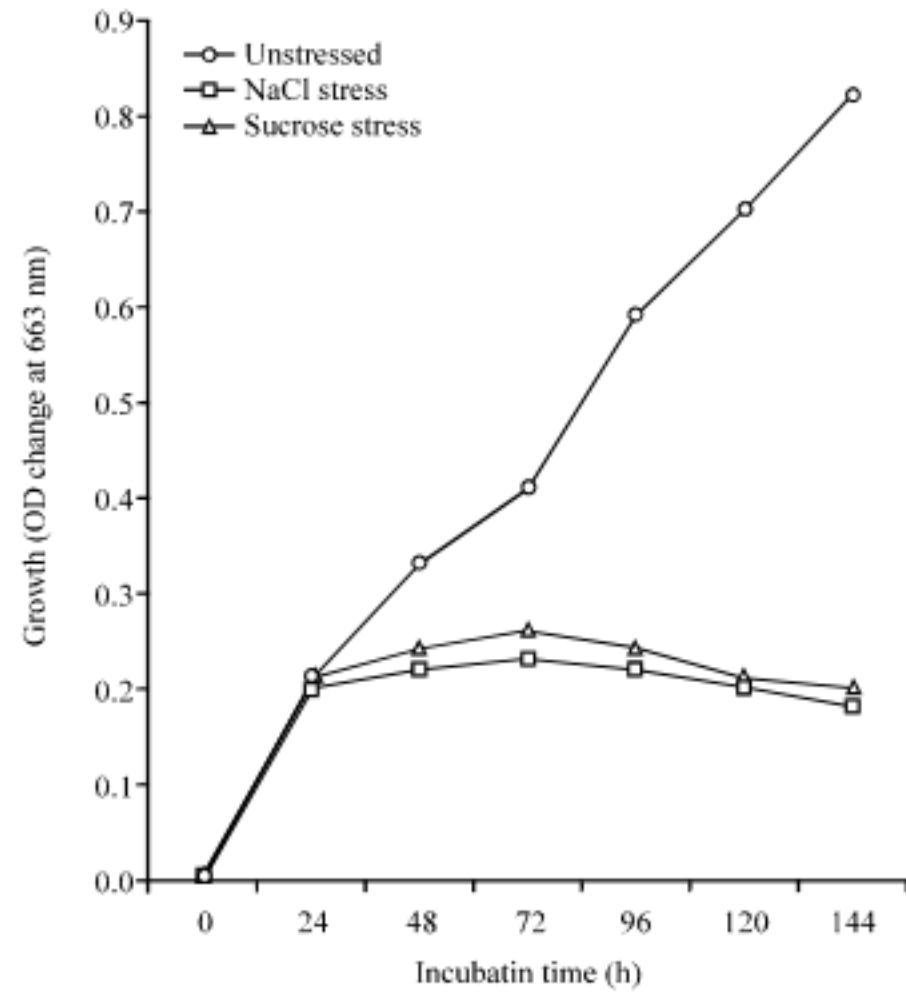


Fig. 1: The growth of *N. muscorum* under unstressed and NaCl stress and sucrose stress conditions. Each value is an average of three independent incubations

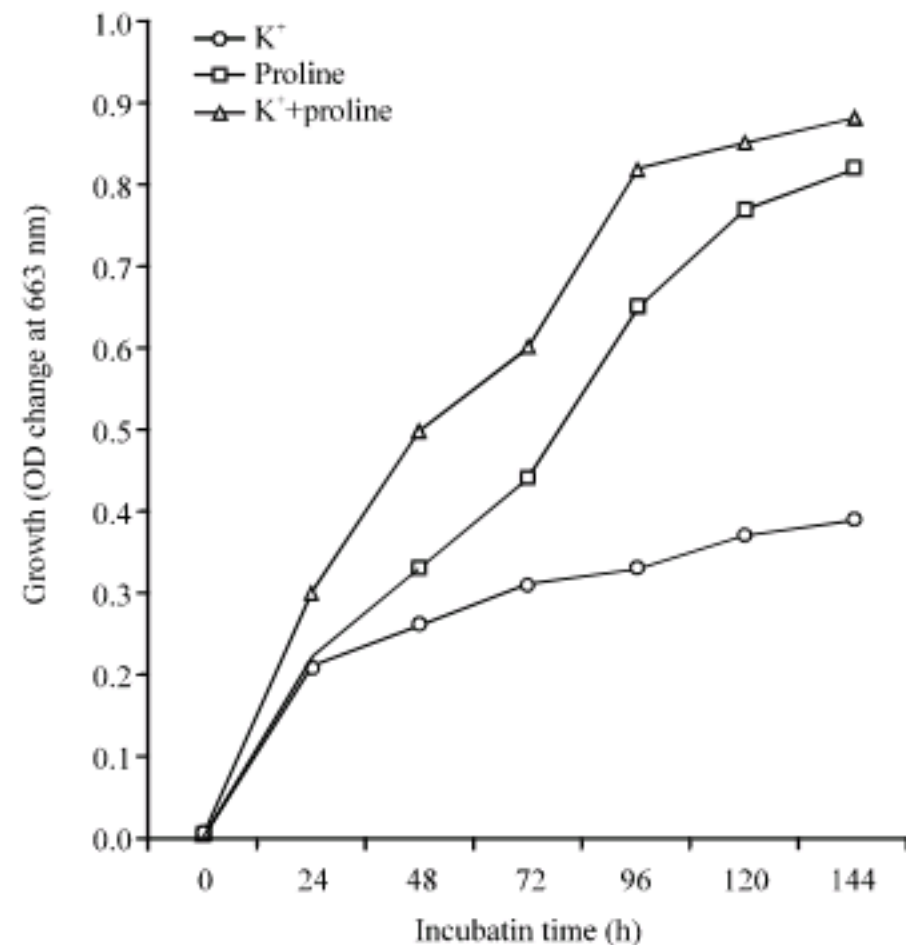


Fig. 2: Effect of K<sup>+</sup>, proline and K<sup>+</sup> + proline on the growth of *Nostoc muscorum* under NaCl stress condition. Each value is an average of three independent incubations

The cyanobacterial cells were starved for 72 h in K<sup>+</sup>-deficient medium. The K<sub>m</sub> value of the cultures grown under K<sup>+</sup> starved and proline deficient were low as

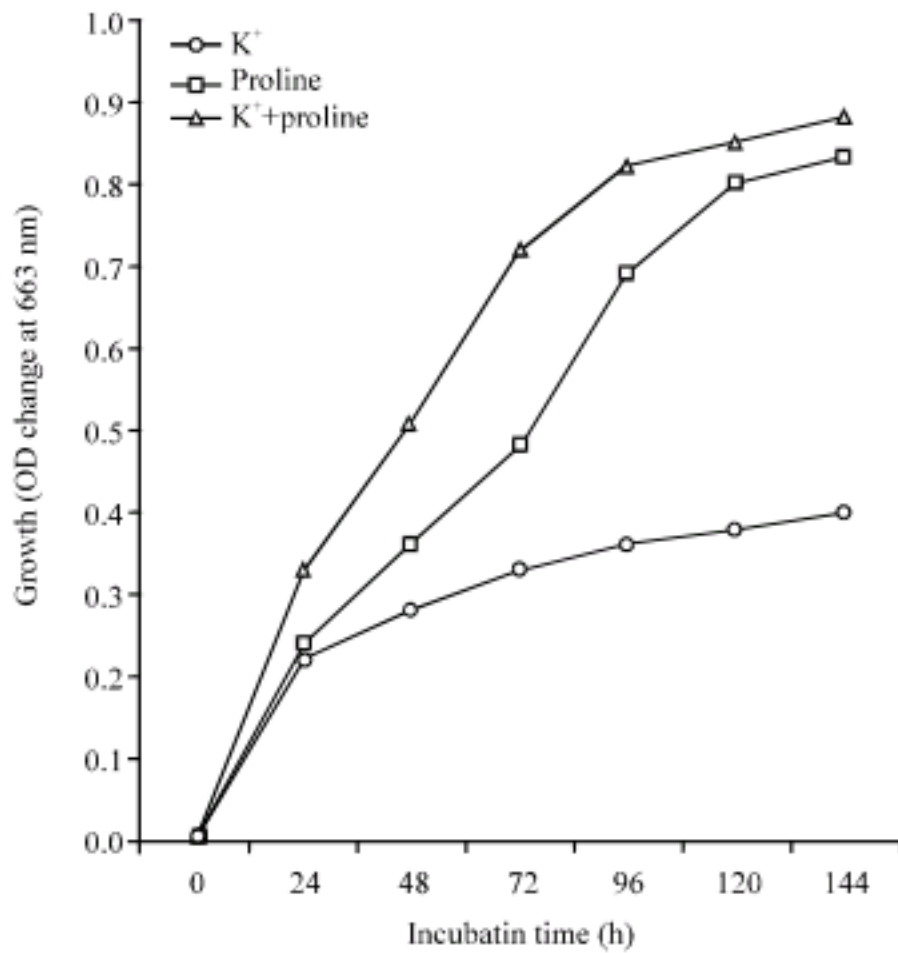


Fig. 3: Effect of K<sup>+</sup>, proline and K<sup>+</sup> + proline on the growth of *Nostoc muscorum* under sucrose stress condition. Each value is an average of three independent incubations

compared to the cultures grown in the medium containing K<sup>+</sup>/proline. Addition of K<sup>+</sup> and proline in the growth medium enhanced K<sub>m</sub> value for proline only (data not shown). This finding suggested that presence of K<sup>+</sup> in the growth medium accelerate proline uptake under stress conditions. The addition of CCCP in the incubation mixture abolished K<sup>+</sup> and proline uptake (data not shown), therefore it is suggested that both K<sup>+</sup> proline uptake were energy requiring processes.

The uptake of K<sup>+</sup> and proline as a function of NaCl and sucrose stresses were also examined. The addition of NaCl/sucrose in the incubation mixture initially caused no impact on K<sup>+</sup> uptake on the contrary Na<sup>+</sup> uptake was increases during the initial phase followed by K<sup>+</sup> uptake (data not shown). Further it has been reported that K<sup>+</sup> uptake was more under NaCl stress than sucrose (Fig. 4). The intracellular proline contents showed little variation under NaCl and sucrose stresses. On further examination it was found that intracellular proline contents were greatly enhanced when the similar stress cultures were supplied with 1 mM proline. The presence of K<sup>+</sup> in the growth medium further accelerate proline uptake (Fig. 5). This finding suggested that K<sup>+</sup> ions are necessary for the induction of secondary response under NaCl and sucrose stresses.

As *N. muscorum* is able to perform both oxygenic photosynthesis and oxygen sensitive nitrogenase activity, therefore, the osmolyte role of K<sup>+</sup> and proline on

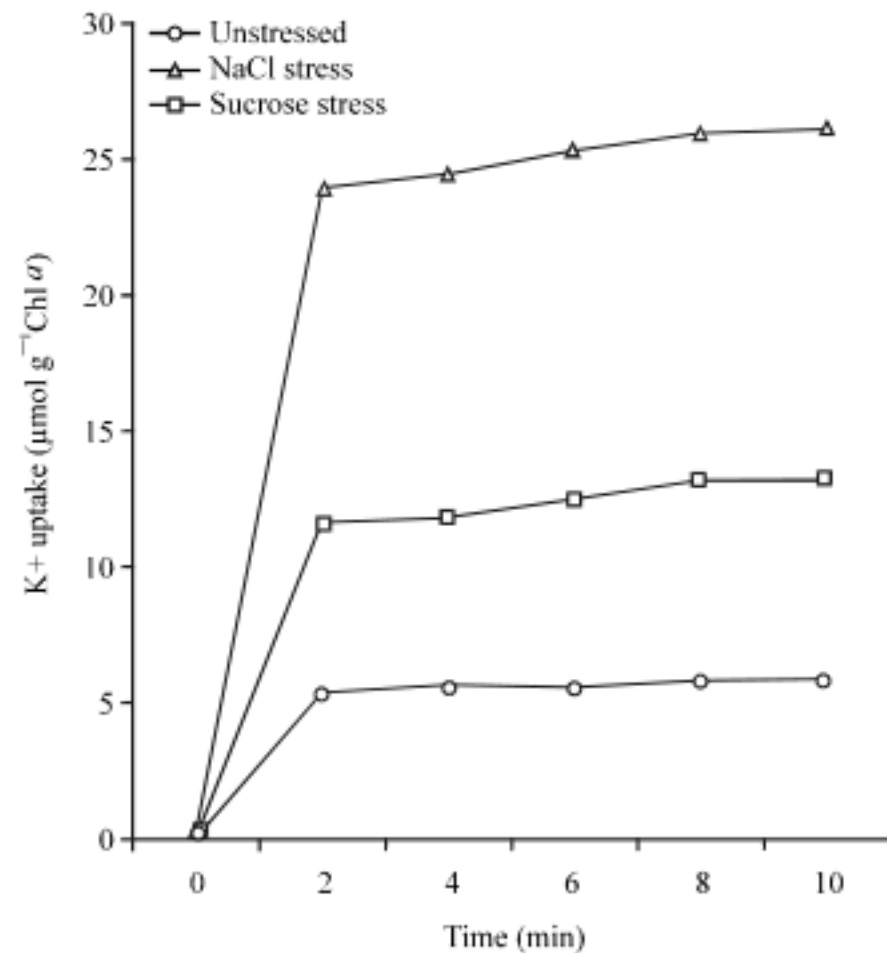


Fig. 4: K<sup>+</sup> uptake pattern in the *Nostoc muscorum* under unstressed condition, under NaCl stress and sucrose stress. Each value is an average of three independent incubations

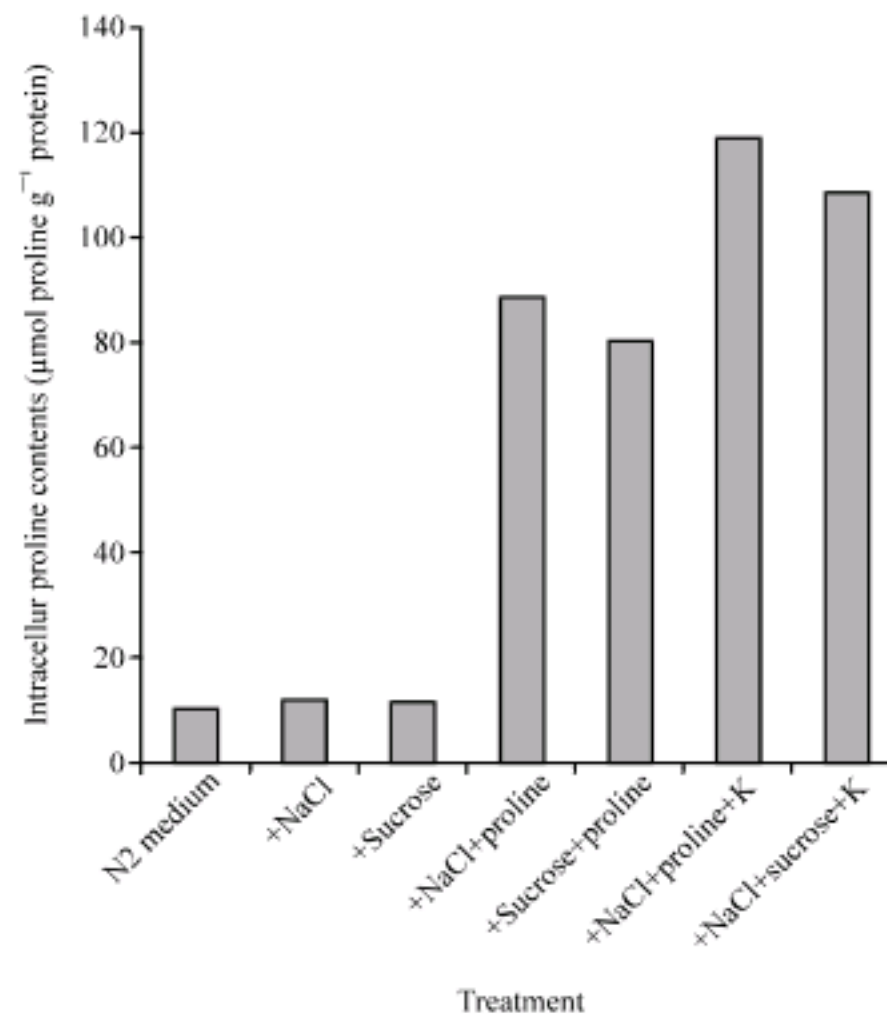


Fig. 5: Intracellular proline contents (µmol proline g<sup>-1</sup> protein) under unstressed and stressed conditions in the presence/absence of extracellular K<sup>+</sup>. Each value is an average of three independent incubations

these two vital processes was observed. The results as shown in the Table 1 and 2 indicate that the presence of

Table 1: Effect of NaCl and sucrose stresses on the photosynthetic O<sub>2</sub> evolution (mmol O<sub>2</sub> evolved g<sup>-1</sup> Chl *a* h<sup>-1</sup>) in the diazotrophic growth medium containing K<sup>+</sup> and proline

Medium/Incubation	Time (h)					
	24	48	72	96	120	144
N <sub>2</sub> -medium	212	256	312	386	421	522
+ 100 mM NaCl	202	238	216	184	162	112
+ 250 mM sucrose	208	264	220	190	177	143
+ 100 mM NaCl + K <sup>+</sup>	210	231	238	245	256	260
+ 250 mM sucrose + K <sup>+</sup>	212	242	251	263	271	277
+ 100 mM NaCl + proline	211	247	308	376	418	508
+ 250 mM sucrose + proline	213	250	311	380	425	510
+ 100 mM NaCl + K <sup>+</sup> + proline	210	255	315	382	468	516
+ 250 mM sucrose + K <sup>+</sup> + proline	212	259	321	388	472	520

Exponentially grown cultures were treated with NaCl and sucrose for 12 h and then examined for photosynthetic O<sub>2</sub> evolution. Each value is an average of three independent readings

Table 2: Effect of NaCl and sucrose stresses on the nitrogenase activity (mmol C<sub>2</sub>H<sub>4</sub> formed g<sup>-1</sup> Chl *a* h<sup>-1</sup>) in the diazotrophic growth medium containing K<sup>+</sup> and proline

Medium/Incubation	Time (h)					
	24	48	72	96	120	144
N <sub>2</sub> -medium	5.11	6.56	7.29	8.33	10.23	12.21
+ 100 mM NaCl	4.13	4.10	3.99	3.33	2.11	1.12
+ 250 mM sucrose	4.21	4.44	4.23	3.98	3.22	1.65
+ 100 mM NaCl + K <sup>+</sup>	4.42	4.55	4.88	5.12	5.22	5.32
+ 250 mM sucrose + K <sup>+</sup>	4.45	4.66	4.98	5.22	5.36	5.77
+ 100 mM NaCl + proline	4.88	5.11	5.87	6.72	7.88	10.21
+ 250 mM sucrose + proline	5.13	5.55	6.46	7.23	8.36	11.10
+ 100 mM NaCl + K <sup>+</sup> + proline	5.14	6.23	7.45	8.56	10.23	12.11
+ 250 mM sucrose + K <sup>+</sup> + proline	5.14	6.33	7.44	8.66	10.38	12.12

Exponentially grown cultures were treated with NaCl and sucrose for 12 h and then examined for nitrogenase activity. Each value is an average of three independent readings

K<sup>+</sup> alone in the growth medium has no role as an osmoprotectant. The addition of proline in the growth medium mitigates the adverse effect of NaCl and sucrose stress.

Therefore, it is suggested that existence of K<sup>+</sup> and proline in the growth medium repaired the inhibitory action of NaCl and sucrose.

## DISCUSSION

K<sup>+</sup> is a major essential nutrient for almost all the living forms. Living organisms for growth, development and in turgor maintenance require K<sup>+</sup>. In the cyanobacterium *Synechocystis* sp. PCC 6803 it has been reported that during the stress conditions three types of K<sup>+</sup> transport systems (Kdp, Kup, Trk) have been involved (Matsuda *et al.*, 2004). In the present study K<sup>+</sup> uptake is also regulated by the NaCl and sucrose caused stresses, suggesting a definite role of K<sup>+</sup> transporters. K<sup>+</sup> uptake was more under NaCl caused stress than sucrose stress suggested that autophosphorylation activity of KdpD (a primary stimulus for K<sup>+</sup> uptake) was more under NaCl stress. Studies on *E. coli* KdpD autophosphorylation also suggested its role in K<sup>+</sup> uptake (Jung *et al.*, 2000).

Present results suggested that the presence of K<sup>+</sup> in the growth medium enhanced proline uptake and simultaneously provides protection to the cyanobacterium under NaCl and sucrose stresses. In *E. coli* it has been reported that proline transporters i.e., ProP activity is stimulated by the presence of exogenously supplied K<sup>+</sup> and ultimately leads to higher accumulation of proline (MacMillan *et al.*, 1999). Thus the accumulation of intracellular K<sup>+</sup> is a prerequisite for the induction of proP genes in *E. coli* (Sutherland *et al.*, 1986). Our interpretation regarding the accumulation of proline is in agreement with the above findings. The addition of KCl resulted in an additive effect on the uptake of proline suggested that Na<sup>+</sup>/K<sup>+</sup> antiporter activity is involved in restoring the Na<sup>+</sup> gradient required for an enhanced proline uptake. Previous studies on potassium accumulation reported its loss from cells if compatible solutes either accumulated or synthesized (Welsh, 2000). The accumulation of proline under stressed conditions in the investigated cyanobacterium has no impact on potassium concentration. Therefore, it is suggested that even at high osmolarity potassium ions are required to accumulate proline. The extremely halophilic archaeobacteria accumulate large amount of Na<sup>+</sup> and K<sup>+</sup> in their cytoplasm to counter act high osmolarity. Indeed compatible solutes are neither synthesized nor accumulated by these microorganisms (Welsh, 2000). Present findings suggested that under high salinity/osmotic condition in the cyanobacterium *N. muscorum* initial upshock leading to accumulation of K<sup>+</sup> followed by accumulation of proline.

The cyanobacterial photosynthetic O<sub>2</sub> evolution and nitrogenase activity were severely impaired in the presence of NaCl and sucrose stresses. The presence of K<sup>+</sup> and proline in the incubation mixture recovered these two vital processes of the cyanobacterial metabolism. These findings further suggested that accumulation of compatible solute proline provides not only protection to the cyanobacterial photosynthesis and nitrogenase activity but also indispensable for survival under NaCl and sucrose stresses. This protective role of proline has also been documented in cyanobacteria (Singh *et al.*, 1996; Kachouli *et al.*, 2009). Similar protective role of proline for restoring various biochemical reactions has also been reported in *E. coli* (Nagata *et al.*, 2002). Further investigation at the molecular level will elucidate the interaction between primary and secondary osmolytes and its application for the generation of cyanobacterial biofertilizer strains for saline and user soils.

## ACKNOWLEDGMENTS

Authors are thankful to the MAPCOST, Bhopal (M.P.) for the financial support. Authors are thankful to the Director, RGTU, Bhopal (M.P.) for providing Laboratory facility and thankful to Department of Higher Education, Government of M.P. for providing fellowship.

## REFERENCES

- Ballal, A., M. Bramkamp, H. Rajaram, P. Zimmann, S.K. Apte and K. Altendorf, 2005. An atypical KdpD homologue from the cyanobacterium *Anabaena* sp., Strain L-31: Cloning, *in vivo* expression and interaction with *Escherichia coli* KdpD-CTD. J. Bacteriol., 187: 4921-4927.
- Bates, L.S., R.P. Walderren and I.D. Teare, 1973. Rapid determination of free proline for water-studies. Plant Soil, 39: 205-207.
- Bhargava, S., R. Chauhan, P.K. Patil and S.D. Singh, 2006. Auxotrophic mutant of the cyanobacterium *Nostoc muscorum* showing absolute requirement of Cs<sup>+</sup> or Rb<sup>+</sup> for diazotrophy and autotrophy. J. Basic Microbiol., 46: 255-262.
- Csonka, L.N., 1989. Physiological and genetic responses of bacteria to osmotic stress. Microbiol. Rev., 53: 121-147.
- Csonka, L.N. and A.D. Hanson, 1991. Prokaryotic osmoregulation: Genetics and physiology. Annu. Rev. Microbiol., 45: 569-606.
- Fatma, T., M.A. Khan and M. Choudhary, 2007. Impact of environmental pollution on cyanobacterial proline content. J. Applied Phyco., 19: 625-629.
- Gerloff, G.C., G.P. Fitzgerald and F. Skoog, 1950. The isolation, purification and culture of blue-green algae. Am. J. Bot., 37: 216-218.
- Grothe, S., R.L. Krogsrud, D.J. McClellan, J.L. Milner and J.M. Wood, 1986. Proline transport and osmotic stress response in *Escherichia coli* K-12. J. Bacteriol., 166: 253-259.
- Higgins, C.F.J., D.A. Cairney, L. Stirling, L. Sytherland and I.R. Booth, 1987. Osmotic regulation of gene expression: Ionic strength as an intracellular signal? Trends Biochem. Sci., 12: 339-344.
- Jung, K., M. Veen and K. Altendorf, 2000. K<sup>+</sup> and ionic strength directly influence the autophosphorylation activity of the putative turgor sensor KdpD of *Escherichia coli*. J. Biol. Chem., 275: 40142-40147.
- Kachouli, R.K., R. Maithil, S. Dubey, V. Kaithwas, V.K. Sethi and S. Bhargava, 2009. Osmotic stress in the cyanobacterium *Nostoc muscorum* overcome by the accumulation of proline. Can. J. Pure Applied Sci., 3: 669-673.
- Karandashova, I.V. and I.V. Elanskaya, 2005. Genetic control and mechanism of salt and hyperosmotic stress resistance in cyanobacteria. Russ. J. Gen., 41: 1311-1321.
- Lu, W.D., Z.M. Chi and C.D. Su, 2006. Identification of glycine betaine as compatible solute in *Synechococcus* sp. WH8102 and characterization of its N-methyltransferase genes involved in betaine synthesis. Arch. Microbiol., 186: 495-506.
- MacMillan, S.V., D.A. Alexander, D.E. Culham, H.J. Kunte, E.V. Marshall, D. Rochon and J.M. Wood, 1999. The ion coupling and organic substrate specificities of osmoregulatory transporter ProP in *Escherichia coli*. Biochem. Biophys. Acta., 1420: 30-44.
- Matsuda, N., H. Kobayashi, H. Katoh, T. Ogawa, L. Futatsugi, T. Nakamura, E.P. Bakker and N. Uozumi, 2004. a<sup>+</sup>-dependent K<sup>+</sup> uptake Ktr system from the cyanobacterium *Synechocystis* sp. PCC 6803 and its role in the early phases of cell adaptation to hyperosmotic shock. J. Biol. Chem., 279: 54952-54962.
- Matsuda, N. and N. Uozumi, 2006. Ktr-mediated potassium transport, a major pathway for potassium uptake, is coupled to a proton gradient across the membrane in *Synechocystis* sp. PCC 6803. Biosci. Biotechnol. Biochem., 70: 273-275.
- Nagata, S., Y. Maekawa, T. Ikeuchi, Y.B. Wang and A. Ishida, 2002. Effect of compatible solutes on the respiration activity and growth of *Escherichia coli* K-12 under NaCl stress. J. Biosci. Bioeng., 94: 384-389.
- Reed, R.H. and W.D.P. Stewart, 1985. Evidence for turgor-sensitive K<sup>+</sup> influx in the cyanobacteria *Anabaena variabilis* ATCC 29413 and *Synechocystis* PCC 6714. Biochim. Biophys. Acta., 812: 155-162.
- Rhoads, D.B. and W. Epstein, 1978. Cation transport in *E. coli*. IX. Regulation of K<sup>+</sup> transport. J. Gen. Physiol., 72: 283-295.
- Roder, A., E. Hoffmann, M. Hagemann and G. Berg 2006. Synthesis of the compatible solutes glucosylglycerol and trehalose by salt-stressed cells of *Stenotrophomonas* strains. FEMS Microbiol. Lett., 243: 219-226.
- Schwan, W.R., L. Lehmann and J. McCormick, 2006. Transcriptional activation of the *Staphylococcus aureus* putP gene by low-proline-high osmotic conditions and during infection of murine and human tissues. Infect. Immunity. 74: 399-409.

- Singh, A.K., D. Chakravarty, 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 Environ.*, 19: 490-494.
- Sutherland, L., J. Cairney, M.J. Elmore, I.R. Booth and C.F. Higgins, 1986. Osmotic regulation of transcription: Induction of the proU betaine transport gene is determined by the accumulation of intracellular potassium. *J. Bacteriol.*, 168: 805-814.
- Warr, S.R.C., R.H. Reed and W.D.P. Stewart, 1988. The compatibility of osmotica in cyanobacteria. *Plant Cell Environ.*, 11: 137-142.
- Welsh, D.T., 2000. Ecological significance of compatible solute accumulation by micro-organisms: From single cells to global climate. *FEMS Microbiol. Rev.*, 24: 263-290.