Indian Journal of Experimental Biology
Vol.52, April 2014, pp. 352-358

Regulation of nitrogen metabolism in salt tolerant and salt sensitive *Frankia* strains

Amrita Srivastava & Arun Kumar Mishra*

Laboratory of Microbial Genetics, Department of Botany, Banaras Hindu University, Varanasi 221 005, India

Received 23 January 2013; revised 10 October 2013

Effect of salinity (0, 50, 100, 250, 500 and 750 mM NaCl) was observed on some important physiological parameters of nitrogen metabolism such as nitrate uptake, intracellular and extracellular ammonium status and activities of nitrogenase, nitrate reductase, nitrite reductase and glutamine synthetase among *Frankia* strains differing in their salt tolerance capacity. Nitrogenase activity closely followed the growth pattern with regular decline on NaCl supplementation. All the other enzymes showed optimum activity at 100 mM and declined further. Co-regulation of the nitrate uptake system and sequential enzyme activities plays a crucial role in governing the nitrogen status of strains during salt stress. HsIi10 experiencing minimum decline in enzyme activities and best possible nitrogen regulation under NaCl replete condition showed adequate nutritional management. Among all the strains, HsIi10 proved to be salt tolerant on account of above features while the salt sensitive strain HsIi8 lacked the ability to regulate various steps of nitrogen metabolism during salinity, and thus *Frankia* strain HsIi10 can potentially serve as a potential biofertilizer in the saline soil.

Keywords: *Frankia*, Nitrogen metabolism, Salinity, Salt sensitive, Salt tolerant

Nitrogen is an important bio-element and the assimilatory processes of microorganisms and plants carry out its incorporation into the biosphere. Nitrogen fixation is perhaps the most important biological phenomenon after carbon fixation. Biological nitrogen fixation (BNF) requires a complex set of enzymes and a huge expenditure of ATP[1]. Functionally, biological nitrogen fixation can be categorized as free-living or asymbiotic involving cyanobacteria, *Azotobacter* etc., associative as in lichens or leaf nodules and symbiotic such as legume-rhizobial and actinorhizal-frankial system[1,2]. Nitrogenase is the enzyme complex responsible for the above process and is of universal occurrence among nitrogen fixing microbes. The most easily available form of nitrogen i.e. nitrate is assimilated by the microbes through an active transport system and its sequential reduction to ammonium in a two-step reaction mediated by nitrate reductase and nitrite reductase enzymes[3]. Further, ammonium is incorporated into carbon skeleton by the sequential action of glutamine synthetase (GS) and glutamate synthase (GOGAT) pathway[4].

*Correspondent author
Telephone: 91-542-6701103(Office); +91-9335474142(Mobile)
Fax: 91-542-2366402
E-mail: akmishraau@rediffmail.com; akmishraau@hotmail.com

Frankia is a prokaryote belonging to the actinobacteria domain and is characterized by high G+C content (66-75%) along with its filamentous, gram positive nature. It generates experimental interest because of its capability to form nitrogen fixing root nodules in a variety of higher woody dicotyledenous plants known as actinorhizas[5-8]. Additionally, it survives through a variety of stresses such as extreme temperature variations, nitrogen deprivation and water deficiency during chilling seasons. Therefore, its application as a biofertilizer in soils suffering from some or the other form of abiotic stress is worth investigating. Salinity is the most widespread abiotic stress faced by biological systems. Apart from causing morphological, physiological and biochemical changes in a system, salt water present in rhizosphere induces water deficit condition[9] and interferes with nutrient uptake[10]. The effects of salt stress on nitrogen metabolism[11,12] and salt induced alterations in enzyme activities have been reported[13,14]. Along with other physiological parameters, the process of ammonium assimilation also gets inhibited due to salt stress[15] and there are corresponding changes in the amino acid pool[10].

The process of nitrogen fixation along with its further metabolism plays an important role in deciding the utility of microbe as a biofertilizer. The

efficacy of latter in salt affected areas depends additionally on the capacity of the microbe to withstand saline stress. Salt stress tolerance of *Frankia* strains isolated from *Hippöphae salicifolia* D. Don has been studied in detail along with screening of salt tolerant *Frankia* strain[16]. Although the morphology, histology and histochemistry of *Frankia* have been studied in detail[17], no reports are available on salt induced alterations of some physiological parameters related to nitrogen metabolism in *Frankia* strains. Therefore, study on regulation of nitrogen metabolism in *Frankia* strains under various levels of salinity stress would not be only helpful in understanding the mechanism(s) adapted by *Frankia* strains for their growth under saline conditions but also in developing *Frankia* strain as a potential biofertilizer. Further, an extrapolation of the present study can also be employed for utilizing currently prevalent biofertilizers in reclamation of salt affected areas. Therefore, the present communication reports regulation of nitrogen metabolism under different levels of salinity among frankial strains which are selected as salt tolerant and salt sensitive.

Materials and Methods

Organisms and culture conditions—*Frankia* strains—HsIi2 (NAIMCC-B-00726; accession no.JQ480013), HsIi8 (NAIMCC-B-00730; accession no.JQ480011), HsIi9 (NAIMCC-B-00731; accession no.JQ480009) and HsIi10 (NAIMCC-B-00732; accession no. JQ480012) were isolated from root nodules of *Hippöphae salicifolia* D. Don growing in North Sikkim, India. Reference strain CpI2 that was isolated from *Comptonia peregrine* was obtained from Dr. Johannes Pasi Haansuu, Department of Biological and Environmental Sciences, University of Helsinki, Finland. Liquid growth (BAP) medium, *p*H 7.4[18] was utilized for culturing *Frankia* strains in autoclaved 250 mL Erlenmeyer flasks containing 100 mL medium. Filter (0.45 µm) sterilized sodium pyruvate (10 mM) (carbon source) and antibiotics-cycloheximide (50 µg mL^{-1}) and nalidixic acid (10 µg mL^{-1}) were incorporated in the autoclaved media. Suspension (1000 µL) of *Frankia* cells growing at a concentration of 35 µg protein mL^{-1} was used as inoculum. B.O.D. incubator fitted with rotary shaker (120 rpm) was used for maintenance of homogenous cultures under dark conditions at 29 ± 0.5 °C.

Experimental design—*Frankia* strains were grown in nitrogen deficient BAP medium supplemented with different concentrations of NaCl i.e. 0, 50, 100, 250, 500 and 750 mM at standard growth conditions. Exponentially grown cultures were harvested and employed for following experiment. Experiments were performed in six replicates.

Nitrate uptake[19] and intracellular and extracellular ammonium[20] levels were estimated in exponentially grown *Frankia* cultures. Nitrogenase (EC 1.18.6.1)[21], nitrate reductase (EC 1.7.7.2)[4], nitrite reductase (EC 1.7.7.3)[22] and glutamine synthetase (EC 6.3.1.2)[23] were also assayed.

Statistical analysis—In all the graphs, bars indicate standard error of the six replicates (n = 6). Significance of quantitative changes in all the experimental parameters occurring on account of different NaCl treatments and strains were assessed by subjecting the results to two-way ANOVA. Duncan's multiple range test was performed as post hoc on parameters subjected to ANOVA (only if the ANOVA was significant). SPSS software (SPSS Inc., version 16.0) was used to perform all the statistical analyses.

Results and Discussion

In an earlier experiment[16] *Frankia* strains were subjected to varying concentrations of NaCl. On the basis of inhibition in growth as compared to their respective control (-NaCl grown cultures), salt sensitive and salt tolerant strains were screened out. Also, the regulation of sodium ions leading to varying salt sensitivity/tolerance and its impact on macronutrient concentrations was analytically studied. Similar results of salinity induced growth reduction were obtained by Pattanagul and Thitisaksakul[24] in plant system, *Oryza sativa* L. The four strains used in present study are those that have been screened for salt tolerance on the basis of growth in presence of NaCl[16] and reference strain CpI2. The capacity to tolerate saline medium increased from HsIi2, HsIi9 to HsIi10 while HsIi8 was the most sensitive strain.

The results exhibit two dimensions—the effect of NaCl on various physiological parameters and the differential salt sensitivity of the five experimental strains. NaCl showed an initial stimulatory effect on nitrate reductase, nitrite reductase and glutamine synthetase (up to 100 mM NaCl) followed by a decline at higher concentrations while still remaining higher than control at 250 mM NaCl.

Nitrogen fixation and ammonium assimilation—Nitrogenase activity showed remarkable variation among the strains (Fig. 1). On supplementing the medium with NaCl, there was a regular decline in nitrogenase activity in cultures treated with higher

NaCl concentration showing positive correlation with growth[16]. It is an established fact that nearly all phases of nodule development and eventually the process of nitrogen fixation are adversely affected by salinity[25,26]. Reduced *nif*H gene expression[26] and water stress are also known to cause loss of active nitrogenase and such condition has been reported in legumes[27] and actinorhizas[28]. At 50 mM NaCl, although the nitrogenase activity was maximum in HsIi8, the depreciation in activity was 25.77% as compared to its control. At the same concentration of NaCl (50 mM) the nitrogenase activity was lesser by 23.05, 20.43, 17.57 and 12.05% in HsIi2, CpI2, HsIi9 and HsIi10 respectively i.e. much lower than the decrease for HsIi8. At all the other NaCl concentrations, HsIi10 showed maximum nitrogenase activity among all the strains. Likewise, the decrease (with respect to control) in nitrogenase activity at 750 mM NaCl was maximum in HsIi8 (61%) and minimum in HsIi10 (46%).

Under salt deplete condition, maximum glutamine synthetase (GS) activity was found in HsIi2 followed by HsIi8, CpI2 and HsIi9. This order of GS activity was observed up to 250 mM NaCl (Fig. 2). Higher GS activity at initial concentrations of NaCl might be a way of providing more glutamine for various metabolic functions during salinity. At 500 mM NaCl, all the strains experienced sharp decrease in the GS activity except HsIi10 which experienced only a slight decrement of 18.87% as compared to the activity at 250 mM NaCl (Fig. 2 inset). On supplementation of NaCl beyond 500 mM, GS activity was found to be highly reduced in all the frankial strains. Decrease in GS activity could be because of several reasons such as alternative channelization of ATP and glutamate (for synthesis of organic osmotica such as proline, putrescine and betaine) as reported in other organisms[29] or decrease in the amount of GS protein due to NaCl toxicity[29,30].

Nitrate metabolism—The rate of nitrate uptake lied in a close range among all the strains (Fig. 3). The

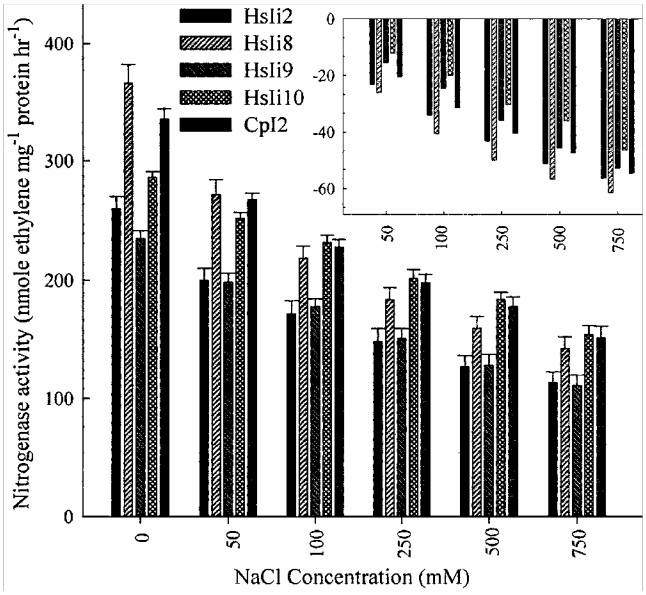

Fig. 1—Effect of different concentrations of NaCl on nitrogenase activity of *Frankia* strains HsIi2, HsIi8, HsIi9, HsIi10 and CpI2

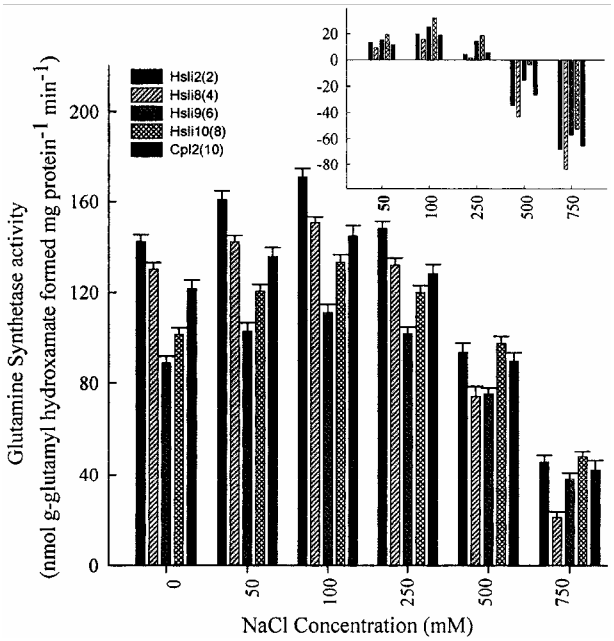

Fig. 2—Glutamine synthetase activity of different frankial strains exposed to a graded concentration of NaCl. Inset represents percentage change in enzyme activity at different NaCl concentrations

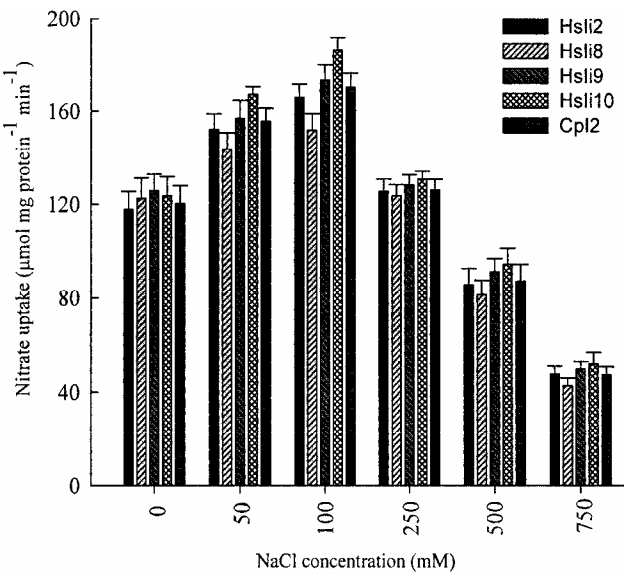

Fig. 3—Nitrate uptake by *Frankia* strains HsIi2, HsIi8, HsIi9, HsIi10 and CpI2 exposed to a gradient of NaCl concentrations

maximum and minimum rates of nitrate uptake were observed at 100 mM and 750 mM NaCl respectively. Among the experimental strains, maximum and minimum uptake rates at 100 and 750 mM NaCl were observed in HsIi10 and HsIi8 Under –NaCl conditions, HsIi10 showed maximum nitrate reductase (NR) activity followed by CpI2, HsIi2 and HsIi9 and minimum in HsIi8 (Fig. 4). The activity increased only up to 100 mM NaCl. At the later concentration, maximum and minimum increase in the enzyme activity as compared to control was observed in HsIi10 and HsIi8 respectively (Fig. 4 inset). Apart from its role in nitrate reduction NR also plays a role in nitrate uptake[3]. An initial rise in enzyme activity at low NaCl concentrations could be for better nitrate uptake; for fulfilment of additional amino acid requirement for the synthesis of stress combating proteins or for creation of low Na^+ environment through decreased influx and efflux of Na^+ [9]. At 500 mM NaCl, the NR activity in HsIi10 was the same as those under salt deplete condition demonstrating its high endurance capacity. Reduced NR activity of the other strains is in proper accordance with the earlier report[16,31]. In numerous cases, NO_3^- is known to play key role in regulating transcription, translation and activity of NR[32]. Beyond 100 mM NaCl, the decrease in NR activity could be due to lesser NO_3^- uptake because of energy limitation and competition from Cl^- for NO_3^- transporters[33] or by direct inactivation of NO_3^- transporter proteins[34]. Sensitivity of NR activity to water stress[35] induced due to salinity might be another probable reason.

Nitrite reductase (NiR) activity under the influence of NaCl followed a similar pattern as that has been observed for NR activity (Fig. 5). In NaCl deplete medium, maximum NiR activity was observed in HsIi10 followed by HsIi2 and CpI2, HsIi9 and HsIi8. The maximum activity as compared to control was found at 100 mM NaCl in HsIi10 while HsIi8 showed minimum activity (Fig. 5 inset). The initial rise might be for quenching increased metabolic demands along with elevated substrate level (nitrite ion) due to higher NR activity. Also, nitrate is known to act as an inducer for NiR through an increase in the amount of NiR mRNA[36]. Beyond 250 mM NaCl concentration, NiR activity declined steeply, as reported in other systems[37] and remained 5-10% lesser than the activity under standard growth conditions. Reduced activity can be attributed to reduction in nitrate (via uptake) and nitrite (via reduction by NR) ions. Throughout the experiment, NiR activity was always much higher

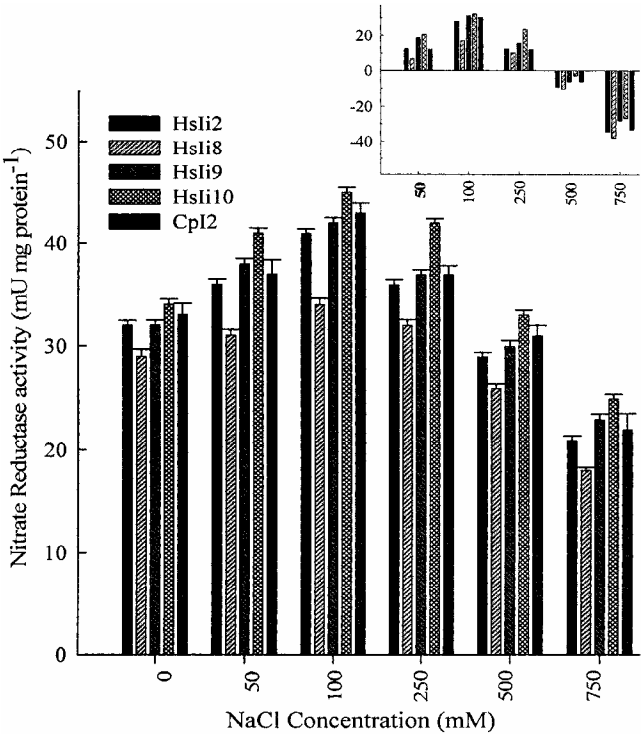

Fig. 4—Effect of NaCl on nitrate reductase activity in *Frankia* strains. Inset represents percentage change in enzyme activity at different NaCl concentrations

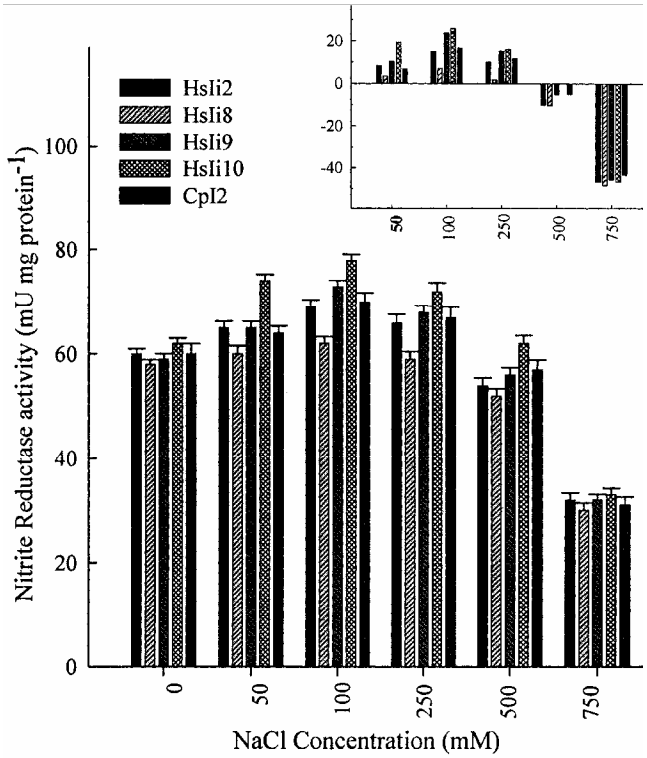

Fig. 5—Effect of NaCl on nitrite reductase activity in *Frankia* strains. Inset represents percentage change in enzyme activity at different NaCl concentrations

than NR activity i.e. approximately double at –NaCl condition. This must be for converting all the possible amount of nitrite ions, with potential cell toxicity, generated by NR[38].

Intracellular and extracellular ammonium status— Intracellular ammonium showed differential status among the five strains. Under NaCl deplete condition, intracellular ammonium content showed maximum and minimum values in HsIi9 and HsIi2 respectively with intermediate values in HsIi10, CpI2 and HsIi8 (Fig. 6). The intracellular status of ammonium changed abruptly during NaCl treatment whereby HsIi10 and HsIi8 showed maximum and minimum intracellular ammonium content throughout the experiment. Up to 100 mM NaCl, the ammonium content kept decreasing. Being one of the substrates for GS activity, the decrease in ammonium content could have been on account of rapid increase in the GS activity. A sudden higher value was observed at 250 mM NaCl while the ammonium content was considerably less at 500 mM and further diminished at 750 mM. At 250 mM NaCl, higher amount of intracellular ammonium corresponded accordingly with the fall in GS activity. At this concentration, since the activities of NR and NiR were lower as well, the amount of ammonium formed should have decreased. The percentage decline in the activities of NR and NiR from 100 to 250 mM NaCl was 6.67% and 7.69% respectively while the decrease in GS activity was found to be comparatively higher i.e. 10.03%. The amount of ammonium formed at 250 mM NaCl must have certainly been lesser but the rate and thus the amount of ammonium converted via GS must have declined more. At 750 mM NaCl, similar pattern of intracellular ammonium concentration was observed among the strains with maximum and minimum values in HsIi10 and HsIi8 respectively.

Ammonium excretion during –NaCl condition was quite close in strains HsIi2, HsIi8 and CpI2 and similarly in strains HsIi9 and HsIi10 (Fig. 7). The pattern became more obvious under the effect of NaCl where ammonium excretion decreased sequentially from HsIi10 to HsIi9, CpI2, HsIi2 and HsIi8 at all the tried NaCl concentrations. From control to 100 mM NaCl, ammonium excretion was found to be lesser as compared to control at each increasing NaCl concentration. Beyond 100 mM i.e. from 250 mM to 750 mM NaCl, ammonium excretion showed sequentially elevated values at each higher NaCl concentration. Extracellular release of ammonium was closely related with intracellular ammonium content as it is the unused ammonium that was excreted out of the cell. Sequentially lesser ammonium excretion up to 100 mM NaCl might have been because of elevated GS activity. Thus, the

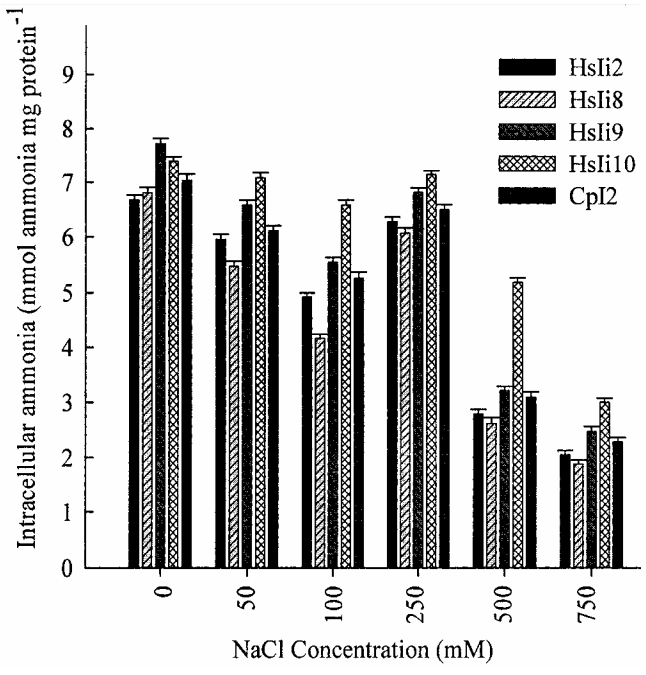

Fig. 6—Intracellular ammonium status in *Frankia* strains grown in different levels of NaCl

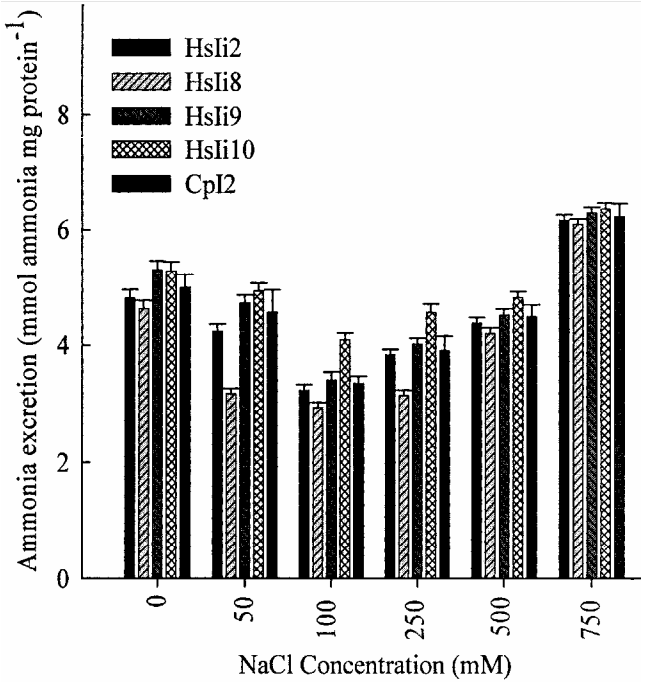

Fig. 7—Effect of NaCl on the extracellular release of ammonium in *Frankia* strains

Table 1—Results of analysis of variance (ANOVA) for repeated measures of NaCl concentrations (treatments), strains and their interactions for activities of nitrogenase, nitrate reductase, nitrite reductase and glutamine synthetase; nitrate uptake and intracellular and extracellular ammonium ion content.

Parameter	Treatment	Strain	Treatment×Strain
Nitrogenase	0.002092^{***}	637.092^{***}	36.253^{***}
Nitrate Uptake	0.001371^{***}	24.504^{***}	3.201^{***}
Nitrate Reductase	60.912^{***}	14.666^{***}	0.293^{ns}
Nitrite Reductase	331.656^{***}	25.615^{***}	1.654^{ns}
Glutamine Synthetase	0.001605^{***}	275.511^{***}	29.846^{***}
Intracellular NH_4^+	121.494^{***}	21.658^{***}	2.096^{*}
Extracellular NH_4^+	29.306^{***}	4.526^{**}	0.426^{ns}

P values * < 0.05; ** < 0.01; *** < 0.001; ns: not significant

minimum amount of ammonium excretion was observed at 100 mM NaCl. At 250 mM NaCl, decline in GS activity caused an increase in the amount of intracellular ammonium. Since free or unconverted ammonium is toxic to the cell, most of it must have been released out of the cell in order to ensure a congenial intracellular environment. Above 500 mM NaCl, direct effect of salinity has been visualized on the cellular membranes in the form of membrane disintegration and high electrolyte leakage[16].

Two-way ANOVA revealed that the response of all the experimental parameters varied significantly due to strain and treatment (NaCl incorporation) while significant variation due to treatment×strain was observed only for nitrogenase, nitrate uptake, glutamine synthetase and intracellular ammonium concentration (Table 1).

A better command over nitrogen metabolism machinery in HsIi10 under salt replete conditions, could have paved way for adequate nutritional supplement as an adaptation. Failure of such regulation could have led to higher sensitivity or least survival of HsIi8 during salt stress. Earlier results have shown that *Frankia* strain HsIi10 is well capable of sustaining its growth up to 500 mM NaCl, a considerably high salt concentration[16]. Better efficiency of nitrogen fixation and substantially adequate amounts of intracellular and extracellular ammonium indicate a probable use of this strain, subjected to further analyses, as a potent biofertilizer in salt stressed nitrogen deficient lands.

Acknowledgement

Thanks are due to the Department of Biotechnology, New Delhi for financial support and to the Head, Department of Botany, Banaras Hindu University, Varanasi, India for facilities. Amrita Srivastava is thankful to the CSIR, New Delhi for providing scholarship.

References

1 Postgate J R, *The fundamentals of nitrogen fixation* (Cambridge University Press, New York, USA) 1982.

2 Benson D R & Silvester W B, Biology of *Frankia* strains, actinomycete symbionts of actinorhizal plants, *Microbiol Rev*, 57 (1993) 293.

3 Herrero A, Muro-Pastor A M & Flores E, Nitrogen control in cyanobacteria, *J Bacteriol*, 183 (2001) 411.

4 Herrero A, Flores E & Guerrero M G, Regulation of nitrate reductase levels in the cyanobacteria *Anacystis nidulans*, *Anabaena* sp. strain 7119, and *Nostoc* sp. Strain 6719, *J Bacteriol*, 145 (1981) 175.

5 Sarma H K, Sharma B K, Singh S S, Tiwari S C & Mishra A K, Polymorphic distribution and phenotypic diversity of *Frankia* strains in nodulated lobes of *Hippöphae salicifolia* D. Don, *Curr Sci*, 90 (2006) 1561.

6 Singh S S, Singh A, Srivastava A, Singh P, Singh A & Mishra A K, Characterization of frankial strains isolated from *Hippophae salicifolia* D. Don, based on physiological, SDS-PAGE of whole cell proteins and RAPD PCR analyses, *W J Microbiol Biotechnol*, 26 (2010) 985.

7 Pawlowski K & Sirrenberg A, Symbiosis between *Frankia* and actinorhizal plants: root nodules of non-legumes, *Indian J Exp Biol*, 41 (2003) 1165.

8 Elumalai S & Raaman N, In vitro synthesis of *Frankia* and mycorrhiza with *Casuarina equisetifolia* and ultrastructure of root system, *Indian J Exp Biol*, 47 (2009) 289.

9 Singh S S, Upadhyay R S & Mishra A K, Physiological interactions in *Azolla-Anabaena* system adapting to the salt stress, *J Plant Interact*, 3 (2008) 145.

10 Debouba M, Maaroufi-Dghimi H, Suzuki A, Ghorbel M H & Gouia H, Changes in growth and activity of enzymes involved in nitrate reduction and ammonium assimilation in tomato seedlings in response to NaCl stress, *Annals Bot*, 99 (2007) 1143.

11 Carillo P, Mastrolonardo G, Nacca F & Fuggi A, Nitrate reductase in durum wheat seedlings as affected by nitrate nutrition and salinity, *Funct Plant Biol*, 32 (2005) 209.

12 Flores P, Botella M A, Martı´nez V & Cerda´ A, Ionic and osmotic effects of nitrate reductase activity in tomato seedlings, *J Plant Physiol*, 156 (2000) 552.

13 Masood A, Shah N A, Zeeshan M & Abraham G, Differential response of antioxidative enzymes to salinity stress in two varieties of *Azolla* (*Azolla pinnata* and *Azolla filiculoides*), *Environ Experiment Bot*, 58 (2006) 216.

14 Garratt L C, Janagoudar B C, Lowe K C, Anthony P, Power J B & Davey M R, Salinity tolerance and antioxidant status in cotton cultures, *Free Radical Biol Med*, 33 (2002) 502.

15 Kwinta J & Cal K, Effects of salinity stress on the activity of glutamine synthetase and glutamate dehydrogenase in triticale seedlings, *Pol J Environ Stud*, 14 (2005) 125.

16 Srivastava A, Singh S S & Mishra A K, Sodium transport and mechanism(s) of tolerance in *Frankia* strains, *J Basic Microbiol*, 52 (2013) 1.
17 Basu R K & Jothi M, Morphological variation, histology, histochemistry and nutrient contents of *Frankia* root nodules in *Casuarina equisetifolia*, *Indian J Exp Biol*, 44 (2006) 924.
18 Murry M A, Fontaine M S & Torrey J D, Oxygen protection of nitrogenase in *Frankia* sp. HFParI3, *Arch Microbiol*, 139 (1984) 162.
19 Cawse P, The determination of nitrate in soil solutions by ultraviolet spectrophotometry, *The Analyst*, 92 (1967) 311.
20 Soloranzo L, Determination of ammonia in natural waters by the phenol hypochlorite method, *Limnol Oceanogr*, 14 (1969) 799.
21 Postgate J R, The acetylene reduction test for nitrogen fixation, in *Meth Microbiol*, edited by J R Norris & D W Ribbons (Academic Press, London) 1972, 1.
22 Losada M & Paneque A, Nitrite Reductase, *Meth Enzymol*, 23 (1971) 487.
23 Shapiro B M, Stadtman E R, Glutamine synthetase (*Escherichia coli*), in *Methods in Enzymology*, edited by H Tabor & CW Tabor (Academic Press, New York) 1970, 911.
24 Pattanagul W & Thitisaksakul M, Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance, *Indian J Exp Biol*, 46 (2008) 736.
25 Swaraj K & Bishnoi N R, Effect of salt stress on nodulation and nitrogen fixation in legumes, *Indian J Exp Biol*, 37 (1999) 843.
26 Bolanos L, Martin M, El-Hamdaoui A, Rivilla R & Bonilla I, Nitrogenase inhibition in nodules from pea plants grown under salt stress occurs at the physiological level and can be alleviated by B and Ca, *Plant Soil* 280 (2006)135.
27 Sprent J I, Zahran H H, Infection, development and functioning of nodules under drought and salinity, in *Nitrogen fixation by legumes in Mediterranean agriculture*, edited by D P Beck & LA Materon, *Dev Plant Soil Sci*, 32 (1988) 145.
28 Sundstrom K R & Huss-Danell K, Effects of water stress on nitrogenase activity in *Alnus incana*, *Physiol Plant*, 70 (1987) 342.
29 Santa-Cruz A, Acosta M, Rus A & Bolarin M C, Short-term salt tolerance mechanisms in differentially salt tolerant tomato species, *Plant Physiol Biochem*, 37 (1999) 65.
30 Teixeira J & Fidalgo F, Salt stress affects glutamine synthetase activity and mRNA accumulation on potato plants in an organ dependent manner, *Plant Physiol Biochem*, 47 (2009) 807.
31 Lau P S, Tam N F Y & Wong Y S, Effect of carrageenan immobilization on the growth, physiology and nitrate reductase activity of *Chlorella vulgaris*, *Bioresource Technol*, 63 (1998) 115.
32 Wang R, Tischner R, Rodigo A G, Hoffman M, Xing X, Chen M, Coruzzi G & Crawford N M, Genomic analysis of the nitrate response using a nitrate reductase-null mutant of *Arabidopsis*, *Plant Physiol*, 136 (2004) 2512.
33 Deane-Drummond C E, A comparison of regulatory effects of chloride on nitrate uptake, and of nitrate on chloride uptake into *Pisum sativum* seedlings, *Physiol Plant*, 66 (1986) 115.
34 Lin H, Sandra S S & Schumaker K S, Salt sensitivity and the activities of the H-ATPase in cotton seedlings, *Crop Sci*, 37 (1997) 190.
35 Hanson H D & Hitz W D, Metabolic responses of mesophytes to plant water deficits, *Ann Rev Plant Physiol*, 33 (1982) 163.
36 Ogawa K, Soutome R, Hiroyama K, Hagio T, Ida S & Nakagawa H, Co-regulation of nitrate reductase and nitrite reductase in cultured spinach cells, *J Plant Physiol*, 157 (2000) 299.
37 Katiyar S & Dubey R S, Influence of NaCl salinity on behaviours of nitrate reductase and nitrite reductase in rice seedlings differing in salt tolerance. *J Agro Crop Sci*, 169 (1992) 289.