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Proteomic Responses of the Cyanobacterium *Nostoc Muscorum* under Salt and Osmotic Stresses

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Abstract. In this paper, we examined the effect of salt stress (NaCl) and osmotic stress (sucrose) on proteomic level in the diazotrophic cyanobacterium *Nostoc muscorum*. The aim of this study is to compare proteins appeared in control vs. salt treated, control vs. sucrose treated and salt treated vs. sucrose treated cultures. In the salt treated cultures about 37 proteins were expressed differentially out of these only 5 proteins have shown fold regulation of 1.5 or more. About 141 proteins were found to express independently in control and about 554 proteins were express independently in salt treated culture. When we compared proteins in control and sucrose treated cells, it was reported that about 37 protein spots were express differentially, out of these only 7 proteins have fold regulation 1.5 or more. The independently expressed proteins appeared on gel are 141 and 186 respectively. Similarly, when we compared proteins appeared in salt and sucrose treated cells, it was reported that about 54 proteins were express differentially, out of these 10 proteins have fold regulation 1.5 or more. About 537 protein spots were independently present in salt treated cells and about 186 proteins were independently present in sucrose treated cells. In addition, the differentially expressed proteins and their identification with their functional group have also been discussed.

Key words: *Nostoc muscorum*, osmotic stress, proteomic, salt stress

1 Introduction

Cyanobacteria are Gram negative eubacteria, their evolutionary history dated back to 2.7 billion years ago [1]. The origin of cyanobacteria and the evolution of oxygenic photosynthesis have been considered as the most important event in the evolution of aerobic atmosphere. Cyanobacteria are known to be found in almost all the ecological niches with diverse environmental conditions. The native cyanobacterial species present in such habitats confronted with cation toxicity and water loss. The microorganisms, including cyanobacteria that grow and multiply in such stressful habitats have ability to change their morphological and physiological parameters to cope up with such stressful conditions [2]. The ionic component of the stress factor is usually overcome by the efflux mechanism driven by Na^+/H^+ antiporter activity or by the Mrp system [3,4,2]. On the other hand the osmotic component of the stress factor is overcome by the synthesis/accumulation of low molecular weight organic compounds collectively known as compatible solutes [5,6].

The nature and the biosynthesis of compatible solutes depend upon the habitat in which cyanobacteria grow. The fresh water cyanobacterial strains are known to synthesized sucrose, trehalose and proline as an osmotic balancer [7,2,8]. Glucosyl-glycerol is a major compatible solute synthesized by moderately halotolerant strains [9,10]. On the other hand hyper saline strains produce glycine-betaine or glutamate-betaine as compatible solutes [11,12].

The modern molecular biology techniques such as genomics and proteomics have provided valuable databases for the better understanding of many physiological and biochemical processes including cyanobacterial adaptation to salt and osmotic stresses. It is known that during such stresses cellular proteins either denatured or inactivated followed by altering other metabolic activities. During such stresses molecular chaperones play a vital role in maintaining cellular homeostasis [13,14,15,16]. The initial signal of environmental changes perceived by cell surface and ultimately transferred this signal to the cells. In the cyanobacterium *Anabaena* sp PCC 7120 it has been reported that about 18 cell surface associated proteins were over-expressed under stress conditions. These over-expressed proteins have

involved in nucleic acid binding, protein synthesis, proteolytic activity, electron transfer and other proteins [17].

Salinity and osmotic stresses triggered distinct protein synthesis in the *Anabaena* species [18]. In this strain synthesis of several proteins was repressed by salinity stress. Similarly, some proteins were induced only under salinity stress. However, there are certain proteins which were induced by both salinity and osmotic stresses. In addition, salinity and osmotic stress have been known to induce some independently expressed proteins. In cyanobacteria, gene expression under salt and osmotic stresses, has been studied by Kanesaki, *et al.* [19]. Their findings indicate that about 28 genes were expressed only under salt stress condition, while those of 11 genes were expressed only in response to osmotic stress. In addition, 34 genes are expressed both under salinity and osmotic stresses. The products of some of these genes are hypothetical proteins whose functions have not been characterized so far.

In this study protein profile of the cyanobacterium *Nostoc muscorum* under salinity (NaCl) and osmotic (sucrose) stress was compared in terms of commonly and differentially expressed proteins (control vs. treated and salt vs. sucrose).

2 Materials and Methods

2.1 Organism and Growth Conditions

The cyanobacterium is *Nostoc muscorum,* used in the present study is fresh water, filamentous and diazotrophic cyanobacteria that is capable of oxygenic photosynthesis. This species was grown in modified Chu No. 10 medium [20] for routine as well as for experimental purposes. The cultures were routinely grown in 250 ml Erlenmeyer's flask containing 100 ml of liquid medium and incubated in a culture room set at a temperature of 24± 1°C and illuminated for 16 hrs per day with cool daylight fluorescent tubes (intensity approximately 10 - 50W/m^2). The culture medium was maintained at pH 7.5 with the help of 10mM HEPES-NaOH.

The survival studies revealed that NaCl, at the concentration of 100mM was found lethal to the cyanobacterium *N. muscorum*. The osmotic stress was generated by the sucrose. Sucrose at the concentration of 250mM was found lethal to the *N. muscorum*. The diazotrophically grown cultures were exposed to the lethal doses of NaCl and sucrose for 12 hrs and then inoculated into fresh diazotrophic growth medium for further use.

2.2 Total Protein Extraction

Exponentially grown cultures of the cyanobacterium were harvested by centrifugation (Remi C-24BL, India) and the cell suspension was washed thrice with culture medium. The cell pellets thus obtained were weighted and then mixed in five times their volume of extraction buffer (B1). Then the mixture was grind with mortar pestle in liquid nitrogen three times followed by Sonication (Sonic Vibra-cell, USA) 10 times (70% intensity) for 20s each with an ice bath, with 40s cooling breaks. The homogenate was centrifuged for 45 min at 16000 g at 4^{o}C [21]. The supernatant thus obtained designated as total soluble protein fractions. The precipitation of protein was done with the help of trichloroacetic acid (TCA). Protein quantification of the extracted protein was carried out with the help of standard curve (BSA).

2.3 TCA Precipitation

The TCA precipitated protein was free of various non-protein contaminants which can interfere with isoelectric focusing and electrophoresis, such as lipids and salts. Extracted impure protein was precipitated by a mixture of TCA and chilled acetone in the ratio of 1:1:8 (impure protein: TCA: Acetone) for more than 2 hours. Precipitated proteins were washed thrice, first wash with 70% chilled acetone containing 0.07% DTT and the rest of the two wash with 70% chilled acetone only [22].

2.4 2-Dimensional Gel Electrophoresis (2DE)

Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) (O'Farrell, 1975) is the method in which protein molecules are separated according to the charge (pI) by isoelectric focusing (IEF) in the first dimension and according to the size (Mw) by SDS-PAGE in the second dimension. 2-DE has a unique capacity for the resolution of complex mixtures of proteins, permitting the simultaneous analysis of hundreds or even thousands of gene products.

The protein sample was solubilized in appropriate amount of rehydration buffer and rehydration of immobilized pH gradient dry strip gel, IEF, equilibrium of IPG strip for proper protein transfer and SDS-PAGE were performed as described previously by Gupta et al [23].

2.5 Image Scanning and Image Acquisition

Gel imaging was performed on an Image Scanner III (GE Healthcare Bio-Sciences Ltd, India) and the image was saved in .tif (dot tif) and .mel (dot mel) format. Image acquisition was done using Image Master 2D Platinum 7 (IMP7, GE Healthcare, Freiburg, Germany) software. Protein spots of the gel were further analyzed using images of 2DE followed by calculation by Image Master 2D Platinum version 7.0 (GE Healthcare) software. The theoretical pI and molecular weight of overall functional annotation of the data were received by Expasy (http://web.expasy.org/compute_pi/Mw).

On the basis of their function these proteins are grouped into nine classes viz. (i) hypothetical, (ii) cellular processes, (iii) amino acid biosynthesis, (iv) photosynthesis and respiration, (v) energy metabolism, (vi) biosynthesis of cofactors, prosthetic groups, and carriers, (vii) cell envelope, (viii) central intermediary metabolism, (ix) fatty acid, phospholipid and sterol metabolism (http://www.kazusa.or.jp/cyano/ Anabaena /index.html).

3 Results and Discussion

In this study proteomics of the cyanobacterium *N. muscorum* under salt and osmotic stresses have been analyzed. This analysis has paved the way to compare protein spots in terms of differentially expressed and independently expresses proteins. The protein spots and multiple protein spots that showed fold regulation 1.5 or more [24] were further categorized into various functional groups and their role in salt and osmotic stresses. The 2-DE images showed that most of the protein spots were detected in a pH range of 4-7 and their molecular mass lies in the range of 10-90kDa.

3.1 2D Analysis of Proteins under Salt Stress

The protein spots appearing in control as well as in its salt treated cells were compared, as shown in table-1 about 37 proteins were expressed differentially. Out of these only 5 protein spots have showed fold regulation of 1.5 or more. The differentially expressed proteins and their identifications on the basis of their functional group are summarized in table-2. The spots which are marked by sign + in the Fig. 1 (G & H) are independently present in control (141 spots) and salt treated cells (554 spots). Out of these protein spots, some proteins were found to occur in two or more spots. These multiple spots have similar molecular masses, but different pI values. The variation in pI value reflects post translation modification in the concerned protein molecule. On the contrary, some multiple spots of the same protein showed difference in their molecular masses. The various functional categories of differentially expressed proteins are discussed below:

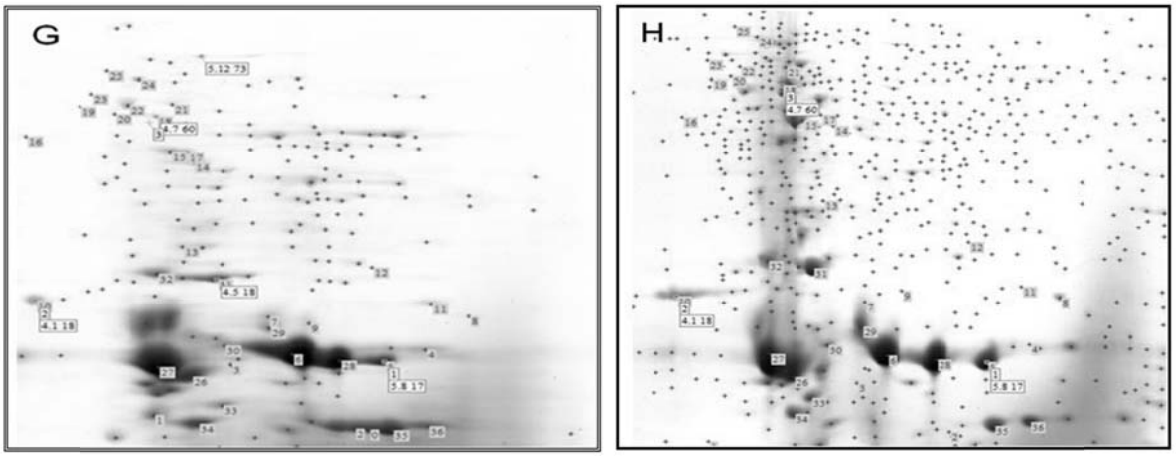

Figure 1. G and H Protein composition of total soluble protein fractionation from *N. muscorum* cells were grown under **control** (G,) and **salt condition** (H, 100mM NaCl); proteins were separated using 2D-PAGE and stained with Coomassie brilliant blue (CBB). Spot No: 0-36 (37 spots) are present in both control (G) and also in salt (H), but are differentially expressed. Other spots: marking by (+) are independently present in both.

3.1.1 Biosynthesis of Cofactors, Prosthetic Groups, and Carriers

Protein spot differentially expressed under this category was identified as 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase. This protein synthesized from 2-oxoglutrate and isochorismate in menaquinone biosynthesis (*menD*). In prokaryotes, menaquinone is an important component of the electron transport system [25]. As reported previously various genes involved in menaquinone biosynthesis help in maintaining balance between the two photosystems to work in a coordinate manner [26,27].

3.1.2 Cellular Processes

In cyanobacteria the function of the two component regulatory systems which consists of sensors and transducers of various abiotic stresses depends upon the degree of super-coiling of the genomic DNA [28]. This mechanism regulates transcription of stress induced genes for successful acclimatization of cells under stress conditions. In this study, differentially expressed protein Hsp70 identified as chaperones protein DnaK3. The role of molecular chaperones in maintaining protein conformational homeostasis is the key factor to the stress adaptability of cyanobacteria [29]. DNaK3 is a thylakoid membrane located protein and may be involved in protein folding in thylakoid [30]. Similar protein has also been induced under salt and osmotic stress in the unicellular cyanobacterium *Synechocystis* sp PCC 6803 [31], and also in the filamentous cyanobacteria *Anabaena* sp PCC 7120 [32].

3.1.3 Energy Metabolism

In *Synechocystis* sp PCC 6803, the operation of photorespiration has been reported by Bauwe, *et al,* [33]. They reported the existence of glycolate metabolism and glycerate pathway in the examined cyanobacterium. Like unicellular cyanobacteria glycolate metabolism has also been reported in filamentous cyanobacteria i. e. *Anabaena* sp. under salt stress [34,35]. In the present analysis similar to the S-layer RTX-protein found to express differentially, this involved in glycolate pathway. The study of Srivastava *et al.* [35] has pointed out the role of a glycolate oxydase gene (*all0170*) in salt acclimation. Therefore, it is suggested that genes involved in the glycolate pathway up regulated during salt shock. In addition, some cell surface-associated proteins (S-layer) also assembled into macromolecule structures that play an important role in cell physiology [17].

3.1.4 Unknown and Hypothetical

Phycobillisomes are the major light harvesting complexes of cyanobacteria. They are associated with photosytem II and constitute up to 50% of the total cellular proteins. Phycobillisomes are multiprotein assemblies and under diazotrophic growth, various genes involved in phycobillisome proteins are over expressed [36]. In consistence with the above findings, it was found that orf *viz. alr0021* which is annotated as allophycocyanin alpha subunit was over expressed under salt stress.

Another hypothetical protein identified as endodeoxyribonuclease RuvC over expressed under salt stress. This protein involved in DNA replication, DNA repair and endonuclease binding protein. Similar proteins were also reported to over express under heat shock stress in the cyanobacterium *Synechocystis* sp PCC 6803 [37]. In the filamentous cyanobacterium *Anabaena* sp. strain PCC7120, cell surface-associated proteins were also reported to involve in nucleic acid binding under stress conditions [17].

In addition to the above mentioned differentially expressed protein, there are a large number of proteins that were identified in the control as well as in salt treated cells, which were expressed independently. This observation suggested that salt stress caused over expression of certain genes and simultaneous repression of certain genes. This metabolic plasticity in terms of up regulation and down regulation of genes helps in surviving cells under the given stresses.

3.2 2D Analysis of Proteins under Sucrose Stress

The protein spots in control and its sucrose treated cells were compared, and it was reported that about 37 proteins were expressed differentially as shown in table-3. Out of these only 7 protein spots have fold regulation 1.5 or more. The differentially expressed proteins and their identifications with their functional group are summarized in table-4. The spots which are marked by sign + are independently present in control (141 spots) and sucrose treated cells (186 spots) Fig.2 (I and J). The various categories of differentially expressed proteins are given below:

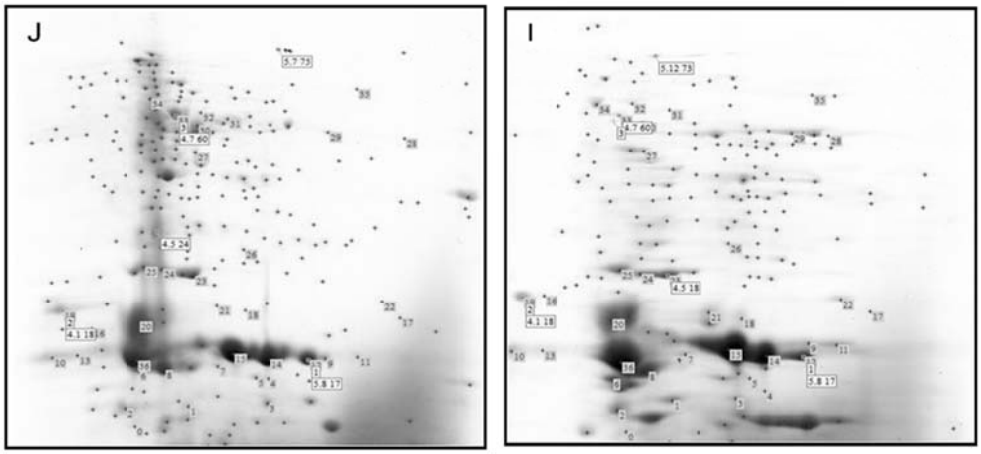

Figure 2. I and J. Protein composition of total soluble protein fractionation from *N. muscorum*. Cells were grown under **control** (I,) and **sucrose condition** (J, 250mM sucrose); proteins were separated using 2D-PAGE and stained with Coomassie brilliant blue (CBB). Spot No: 0-36 (37 spots) are present in both control (I) and also in sucrose (J), but are differentially expressed. Other spots: marking by (+) are independently present in both.

3.2.1 Cell Envelope

In this group penicillin binding protein, which is involved in the synthesis of the peptidoglycan layer of the cell wall has been differentially expressed. Since the sucrose stress was given to diazotropically grown culture, therefore it is suggested that over expression of penicillin binding proteins is essential for the formation of the peptidoglycan layer. Similar role of penicillin binding protein has also been elucidated by Lazaro *et al.* [38] in the cyanobacterium *Anabaena* sp PCC 7120 under normal condition. The role of penicillin binding protein in heterocyst development and in the remodeling of peptidoglycan layer has also been reported in the cyanobacterium *Anabaena* sp PCC 7120 [39].

3.2.2 Energy Metabolism

Phototrophic organisms like cyanobacteria use carbohydrates as carbon source to buildup cellular material and provide reductants. The carbohydrate molecules synthesized during the photosynthesis are broken down through various respiratory pathways. In our analysis the enzyme 2, 3-bisphosphoglycerate has been found to express differentially. This enzyme catalyses the inter conversion of 2-phosphoglycerate and 3- phosphoglycerate. It is a major regulator of glycolysis and regulates the flux of

carbon through the Kelvin Benson Cycle and its export in to glycolysis [40]. Another protein in this group identified as phosphoenolpyruvate synthase (*all3147*) catalyzes the phosphorylation of pyruvate and phosphoenolpyruvate in the presence of ATP molecules. The role of phosphoenolpyruvate synthase as an alternative phosphoenolpyruvate degradation has been reported in *Synechococcus* sp PCC 7002 under light stress condition [41]. The expression of genes involved in energy metabolism under stress condition is the key factors involved in cyanobacterial adaptation to stress factors [42].

3.2.3 Central Intermediary Metabolism

The expression level of *alr0692* was higher in the nitrogen depletion condition. This ORF identified as a NifU like protein, it harbors NifU like domain partially over lapping a thioredoxine like domain. Thioredoxine catalyzing the reduction of intermolecular disulphide bonds by this means it plays a major role in the formation of Fe-S clusters [43]. The differentially expression of this protein may be related to the assembly of a functional uptake hydrogenase. The gene involved in assembly of hydrogenase should be regulated differentially depending on strains, environment and type of hydrogenase [44]. The differential expressions of this protein in the present investigation are inconsistent with the above hypothesis.

Another enzyme of this group i,e. inorganic pyrophosphatase catalyses the conversions of diphosphate to phosphate, induced differentially. Its role in metabolism is thought to be the removal of inorganic pyrophosphate, which is a byproduct of many anabolic reactions. It is also believed that pyrophosphate also plays an important role in the bioenergetics under various biotic and abiotic stresses [45,46,47].

3.2.4 Unknown & Hypothetical

Phototrophs like cyanobacteria might use gas vesicle to expose them into appropriate light intensity. These gas vesicles are basically protein bodies and in prokaryotes they evolutionary most conserved bodies. In the cyanobacterium *Anabaena sp.* five additional proteins were identified (Gbp-F, Gbp-G, Gbp-j, Gbp-l and Gbp-M). These proteins are involved in the initiations of vesicle formation. In cyanobacteria buoyancy is regulated either by the formation of gas vesicle or synthesis/breakdown of carbohydrate molecules [48]. Our findings regarding the over expression of various proteins are inconsistent with the above finding.

The ATP binding protein i. e. alr2300 has identified as conserved hypothetical proteins in the present study. The over expression of this protein (HetY) suppresses the heterocyst formation [49]. In the sucrose treated cells heterocyst differentiations delayed as compared to the control. This delay in heterocyst differentiation correlated with the expression of alr2300 gene.

In addition, to the above mentioned differentially expressed protein, there are a number of proteins that were identified in the control as well as sucrose treated cells, which were expressed independently. This observation suggested that sucrose stress caused over expression of certain genes and simultaneous repression of certain genes. This up regulation and down regulation of certain genes helps in surviving cells under the given stresses.

4 2D Analysis of Protein under Salt and Sucrose Stress

In the next series of analysis we compared salt treated and osmotic treated samples in terms of commonly expressed proteins (Table 5). The protein spots with fold regulation 1.5 or more and their identification with functional group are given in table 6. The spots which are marked by sign + are independently present in salt (537 spots) and sucrose treated cells (186 spots), Fig. 3 (K and L).

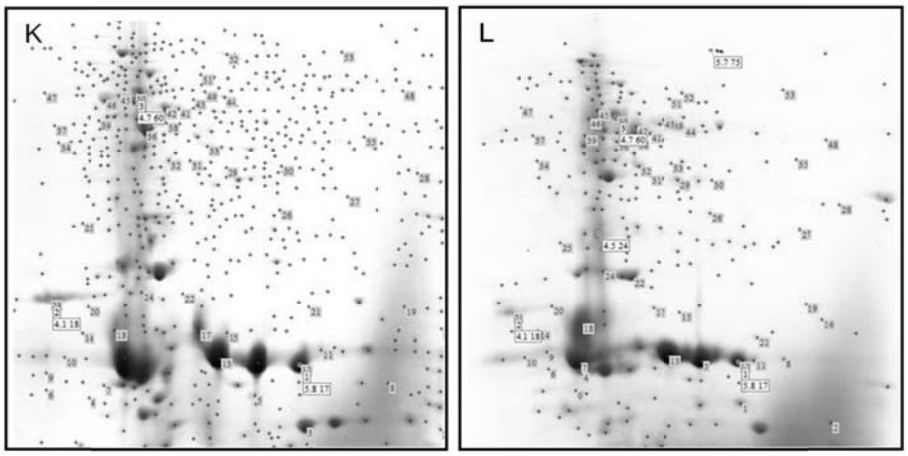

Figure 3. K and L Protein composition of total soluble protein fractionation from *N.muscorum*. Cells were grown under **salt condition** (K, 100mM NaCl) and **sucrose condition** (L, 250mM sucrose); proteins were separated using 2D-PAGE and stained with Coomassie brilliant blue (CBB). Spot No: 0-53 (54 spots) are present in both salt (K) and also in sucrose (L), but are differentially expressed. Other spots: marking by (+) are independently present in both.

4.1 Amino Acid Biosynthesis

In this category the only protein belongs to glutamate family i. e. arginine biosynthesis bifunctional protein ArgJ2 was found to express differentially. This protein involved in the cyclic version of arginine biosynthesis; the synthesis of N-acetylglutamate from glutamate and acetyl Co-A as the acetyl donor, and of ornithine by transacetylation between N(2)-acetyl ornithine and glutamate [50,51].

4.2 Biosynthesis of Cofactors, Prosthetic Groups, and Carriers

Biosynthesis of the PSI cofactor i. e. phylloquinone occurs in almost all photosynthetic organisms, including cyanobacteria. This cofactor is analogous to that of menaquinone a mobile electron carrier in many bacterial bioenergetic systems [25]. Any up shift or down shift in the environmental factor poses an additional energy burden in terms of cellular metabolism. Since the experimental organism exposed to salinity and osmotic stresses, therefore the over expression of MenD is justified. Similar role of *menD* operon in bacteria and in algae has also been reported [25,52].

4.3 Cellular Processes

The phenomenon of programmed cell death or apoptosis is very rare in prokaryotes. In cyanobacteria programmed cell death is associated with membrane integrity, leakage of proteases and DNA degradation. Studies on heamolysin produced by glucose tolerant strain of *Synechocystis* sp PCC 6803 suggested that heamolysin produced by this strain has no toxic activity [53]. In contrast, haemolysin obtained from wild type cells of *Synechocystis* sp PCC 6803 showed haemolytic activity against erythrocytes [54]. The haemolysin like protein was found to express differently in our study, however; we are unable to interpret the exact role of haemolysin production in this study.

The cyanobacterial heat shock response has already been studied both at the transcription level and expression level of specific genes and proteins [55]. The Hsp60/Hsp10 family also referred to as the GroE chaperone machinery in this study the experimental organism exhibit differential expression of two heat shock proteins encoded by Gro-EL1 and Gro-EL2 [56]. In addition, a 60kDa chaperonin 2 (Gro-EL2) was also found to express differentially in this study. It was also observed an increased in the expression level of protease (all2263). In photosynthetic organisms it has been reported that abiotic stresses not only over expressed proteins/enzyme involved in the main metabolic pathways, but also in the synthesis of Gro-EL1 and Gro-EL2 chaperonin and N-ATP dependent proteases [57,58]. The constitutive expression of these Hsps in the examined cyanobacterium suggests their role in stress tolerance.

4.4 Photosynthesis and Respiration

Cyanobacterial nitrogen fixation is an energy requiring process; it requires ATP and a reductant for efficient nitrogen fixation. The over expressions of NADH dehydrogenase under stress conditions produce more ATP and a reductant to support nitrogen fixation and other metabolic activities. The protein involved in energy metabolism (photosynthesis and respiration) e.g. NADPH quinone oxidoreductase and NADH-plastoquinone oxidoreductase was highly abundant in the present analysis. This suggested that more ATP and a reductant is available to the organism for nitrogen fixation. Similar finding has also been reported by many workers [35,36].

4.5 Unknown & Hypothetical

Arginyl-tRNA synthetase (ArgRS) is known to responsible for aminoacylating its cognate tRNA(s) with a unique amino acid in a two-step catalytic reaction. In the first step amino acid t-RNA ligases binds to the amino acid, ATP to activate the amino acid through the formation of N-aminoacyl-Adenylate. The second step involved the transfer of aminoacyl of the t-RNA.

Phycobillisomes are the major light harvesting complexes of cyanobacteria under nitrogen fixing condition and under salt stress conditions; major component of the phycobilisomes is strongly expressed [36,59]. The above findings are in agreement with our interpretations.

Phosphoglycerate kinase (PGK) is an enzyme that catalyzes the reversible transfer of a phosphate group from 1,3-bisphosphoglycerate (1,3-BPG) to ADP producing 3-phosphoglycerate (3-PG) and ATP during carbohydrate metabolism. The differentially expression of this protein suggested that the interaction of metabolic protein associated with the survival of the organism under stress condition. Similar role of carbohydrate metabolism in stress has also been reported in *Anabaena* sp. [60].

The enzyme 1,4-dihydroxy-2-naphthoyl-CoA hydrolase is known to be involved in the formation of a nephthaquonone ring of phylloquinone. In higher plants the cleavage of this enzyme leads to formation of phylloquinone; the cognate thioestrase of the same enzyme has been recently characterized in the cyanobacterium *Synechocystis* sp [61]. In photoautotrophic organisms, including certain species of cyanobacteria phylloquinone is a vital redox cofactor required for electron transfer in PSI and the formation of protein disulphide bond [62,63,64]. In consistence with the above findings, in cyanobacterium *Synechocystis* sp. PCC 6803, salt stress enhances the expression of genes of ribosomal proteins (*rpl2*, *rpl3*, *rpl4* and *rpl23*), on the other hand hyperosmotic stress, enhances the expression of genes for the synthesis of lipids and lipoproteins (*fabG* and *rlpA*) and for other functions. The over expression of these genes clearly indicates that *Synechocystis* sp. PCC 6803 recognizes salt stress and hyperosmotic stress as different signals. To the best of our knowledge this is the first report from the *Nostoc muscorum* investing proteomic responses under salt and osmotic stress.

5 Conclusion

The over expression of commonly induced proteins under salt and osmotic stress suggested that some factors might perceive and transducer such signals of the specific pathways that control the expression of a number of genes. Therefore; the role of various differently expressed proteins is to overcome given stress for the normal functioning of the cell. This metabolic adaptability of the cyanobacteria could be useful in the production of biofertilizer for stressful ecosystems and isolation of commercially important bioactive compounds.

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Appendix

Table 1. Spot details on commonly induced proteins under salt treated cells verses control cells of N. muscorum. NC=protein spots apparent on the gel of control cells of N. muscorum; NN=protein spots apparent on the gel of salt treated cells of N. muscorum

File Name	Spot ID	Match ID	Apparent pI	Apparent MW (kDa)	%Vol	Fold Regulation (T/C)	Protein Acc. No	Protein Identfication	Theoretical Mw (Da)	Theoretical pI
NN	8431	36	6.029	16	0.84788	1.19	Q8YQ24	Chorismate mutase	15706.02	6.91
NC	3279	36	6.133	16	0.71543					
NN	8432	35	5.836	16	1.54506	0.72	Q8YP58	Mannose-6-phosphate isomerase	15804.86	6.65
NC	3277	35	5.857	16	2.13274					
NN	8424	34	4.763	16	0.81515	0.56	P58703	Cyanate hydratase (Cyanase) (EC 4.2.1.104) (Cyanate hydrolase) (Cyanate lyase)	16398.99	4.97
NC	3276	34	5.091	16	1.46755					
NN	8408	33	4.856	16	0.66589	0.58	Q8YUT1	Gas vesicle protein GvpJ	16597.58	4.73
NC	3273	33	4.549	16	1.15037					
NN	8281	32	4.617	22	1.92456	1.28	P80562	Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase)	18960.61	4.69
NC	3228	32	4.710	19	1.50248					
NN	8289	31	4.867	21	1.83581	0.90	O52749	UPF0079 ATP-binding protein alr2300	17938.69	4.33
NC	3230	31	4.500	18	2.03955					
NN	8362	30	4.945	17	0.71796	0.66	O52751	Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)	17740.55	4.7
NC	3253	30	4.565	17	1.08462					
NN	8342	29	5.127	18	2.34606	**2.45**	P80555	Allophycocyanin subunit alpha 1	17214.47	4.92
NC	3249	29	4.914	18	0.95666					
NN	8359	28	5.529	17	5.84423	1.04	P80557	Allophycocyanin subunit beta	17173.56	5.46
NC	3256	28	5.451	17	5.63804					
NN	8358	27	4.628	17	7.99235	0.59	O52751	Crossover junction	17740.55	4.7

NC	3254	27	4.720	17	13.5579			endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)		
NN	8384	26	4.763	17	1.33462	0.52	P80556	Allophycocyanin subunit alpha-B	17680.3	5.06
NC	3263	26	5.022	17	2.56327					
NN	7850	25	4.436	84	0.06533	**1.59**	Q8YUA6	Chaperone protein dnaK3 (HSP70-3) (Heat shock 70 kDa protein 3) (Heat shock protein 70-3)	71181.38	4.6
NC	3116	25	4.460	70	0.041					
NN	7865	24	4.562	79	0.1323	0.78	Q8YW74	Chaperone protein dnaK2 (HSP70-2) (Heat shock 70 kDa protein 2) (Heat shock protein 70-2)	67907.54	4.84
NC	3120	24	4.617	68	0.16902					
NN	7903	23	4.282	69	0.02226	**2.43**	Q8YZZ2	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (SEPHCHC synthase) (EC 2.2.1.9) (Menaquinone biosynthesis protein MenD)	65729.18	5.83
NC	3125	23	4.382	65	0.00916					
NN	7918	22	4.463	67	0.02731	0.06	Q8YM86	NAD(P)H-quinone oxidoreductase chain 4-3 (EC 1.6.5.-) (NAD(P)H dehydrogenase I, subunit D-3) (NDH-1, chain 4-3)	61013.04	5.72
NC	3128	22	4.564	62	0.44259					
NN	7922	21	4.721	66	0.17323	1.15	Q8YP23	Peptide chain release factor 3 (RF-3)	61270.8	5.43
NC	3129	21	4.856	62	0.15004					
NN	7947	20	4.414	63	0.0671	0.53	Q8Z0E5	Penicillin-binding protein	60683.9	5.04
NC	3133	20	4.497	60	0.1261					
NN	7954	19	4.304	62	0.07786	**4.21**	Q8YR01	Alr3659 protein	61699.02	4.01
NC	3131	19	4.324	62	0.01849					
NN	7969	18	4.700	60	0.2005	1.16	Q8Z0E5	Penicillin-binding protein	60683.9	5.04
NC	3134	18	4.700	60	0.17293					
NN	8035	17	4.914	51	0.04325	0.19	Q8YPU6	NADH dehydrogenase	45675.2	4.95
NC	3163	17	4.983	46	0.23071					
NN	8042	16	4.139	50	0.05923	0.65	Q8Z064	Probable cytosol aminopeptidase (EC 3.4.11.1) (Leucine aminopeptidase) (LAP) (EC 3.4.11.10) (Leucyl aminopeptidase)	51918.33	4.87
NC	3146	16	4.058	52	0.09061					
NN	8046	15	4.815	49	0.30447	0.65	Q8YRB0	Enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (2-phosphoglycerate dehydratase)	45965.05	5.03
NC	3162	15	4.837	46	0.47115					
NN	8066	14	4.976	48	0.07466	0.12	Q8YP49	1-deoxy-D-xylulose 5-	43200.83	5.05

NC	3168	14	5.052	43	0.63857			phosphate reductoisomerase (DXP reductoisomerase) (EC 1.1.1.267) (1-deoxyxylulose-5-phosphate reductoisomerase) (2-C-methyl-D-erythritol 4-phosphate synthase)		
NN	8205	13	4.924	31	0.03652	0.10	Q8YNC5	Peroxiredoxin	22630.61	4.87
NC	3217	13	4.944	23	0.37055					
NN	8267	12	5.701	25	0.03946	0.94	Q8YLJ6	50S ribosomal protein L10	19438.44	5.71
NC	3227	12	5.711	20	0.04217					
NN	8309	11	5.982	19	0.01492	0.03	Q8YNU3	Alr4468 protein	18080.98	6.9
NC	3243	11	6.158	18	0.48412					
NN	8313	10	4.100	18	0.63671	1.08	O52749	UPF0079 ATP-binding protein alr2300	17938.69	4.33
NC	3241	10	4.100	18	0.58712					
NN	8315	9	5.341	19	0.04354	0.07	Q8YYZ9	Alr0692 protein	17425.21	5.37
NC	3248	9	5.215	18	0.64891					
NN	8325	8	6.191	18	0.28307	1.16	Q8YWH5	Molybdopterin synthase catalytic subunit (EC 2.8.1.12) (MPT synthase subunit 2) (Molybdenum cofactor biosynthesis protein E) (Molybdopterin-converting factor large subunit) (Molybdopterin-converting factor subunit 2)	18097.81	7
NC	3247	8	6.442	18	0.24498					
NN	8328	7	5.143	18	0.39368	0.84	Q8YSE1	Phosphoenolpyruvate synthase	18033.87	4.85
NC	3246	7	4.898	18	0.46624					
NN	8356	6	5.263	17	5.98757	0.49	P80555	Allophycocyanin subunit alpha 1	17214.47	4.92
NC	3252	6	5.085	17	12.2443					
NN	8360	5	5.800	17	3.38518	**1.53**	O52751	Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)	17740.55	4.7
NC	3260	5	5.800	17	2.21081					
NN	8365	4	6.029	17	0.11362	0.05	Q93SX1	Cytochrome b6-f complex subunit 4 (17 kDa polypeptide)	17535.91	7.85
NC	3257	4	6.117	17	2.14779					
NN	8395	3	5.101	17	0.08298	1.24	O52753	Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)	17740.55	4.7
NC	3262	3	4.614	17	0.06696					
NN	8441	2	5.597	16	0.01524	0.02	Q8Z0K8	Alr0083 protein	15901.34	5.64

NC	3280	2	5.564	16	0.72198					
NN	8450	1	4.287	16	0.06125	0.03	Q8YUT1	Gas vesicle protein GvpJ	16597.58	4.73
NC	3274	1	4.690	16	2.06072					
NN	8452	0	5.737	16	0.14889	0.22	Q8Z0K8	Alr0083 protein	15901.34	5.64
NC	3281	0	5.678	16	0.67488					

Table 2. Showing identical protein with differential expression (>1.5 Fold Regulation) in the control and salt treated cells. The putative gene products are also given in the table.

S.N.	Functional Group	Protein Identfication	Sub function	Gene Name	Match ID
1	Biosynthesis of cofactors, prosthetic groups, and carriers	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (SEPHCHC synthase) (EC 2.2.1.9) (Menaquinone biosynthesis protein MenD)	Menaquinone and ubiquinone	alr0312	23
2	Cellular processes	Chaperone protein dnaK3 (HSP70-3) (Heat shock 70 kDa protein 3) (Heat shock protein 70-3)	Chaperones	alr2446	25
3	Energy metabolism	similar to S-layer-RTX protein	Glycolate pathway	alr3659	19
4	Unknown & Hypothetical	Allophycocyanin subunit alpha 1		alr0021	29
		Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)		all2297	5

Table 3. Spot details on commonly induced proteins under sucrose treated cells verses control cells of *N. muscorum.* NC=protein spots apparent on the gel of control cells of *N. muscorum*; NS=protein spots apparent on the gel of sucrose treated cells of *N. muscorum.*

File Name	Spot ID	Match ID	Apparent pI	Apparent MW (kDa)	%Vol	Fold Regulation Value (t/c)	Protein Acc. No	Protein Identfication	Theoretical Mw (Da)	Theoretical pI
NS	3770	36	4.414	17	9.6733	0.71	O52751	Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)	17740.6	4.7
NC	3254	36	4.720	17	13.5579					
NS	3596	35	5.964	66	0.0245	0.27	Q8YZZ2	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (SEPHCHC synthase) (EC 2.2.1.9) (Menaquinone biosynthesis protein MenD)	65729.2	5.83
NC	3126	35	5.889	64	0.0898					
NS	3603	34	4.470	64	0.4829	1.09	Q8YM86	NAD(P)H-quinone oxidoreductase chain 4-3 (EC 1.6.5.-) (NAD(P)H dehydrogenase I, subunit D-3) (NDH-1, chain 4-3)	61013	5.72
NC	3128	34	4.564	62	0.4426					
NS	3612	33	4.700	60	0.8317	**4.81**	Q8Z0E5	Penicillin-binding protein	60683.9	5.04
NC	3134	33	4.700	60	0.1729					
NS	3614	32	4.941	61	0.1040	0.69	Q8YP23	Peptide chain release	61270.8	5.43

NC	3129	32	4.856	62	0.1500			factor 3 (RF-3)		
NS	3620	31	5.200	59	0.1628	1.46	Q8YQZ0	Urease subunit alpha (EC 3.5.1.5) (Urea amidohydrolase subunit alpha)	61155.6	5.23
NC	3132	31	4.524	61	0.1116					
NS	3623	30	4.907	56	0.7416	**7.08**	Q8YPL2	2,3-bisphosphoglycerate-independent phosphoglycerate mutase (BPG-independent PGAM) (Phosphoglyceromutase) (iPGM) (EC 5.4.2.1)	57677.3	4.99
NC	3135	30	4.973	57	0.1047					
NS	3636	29	5.867	53	0.0964	0.16	Q8YM64	Light-independent protochlorophyllide reductase subunit N (DPOR subunit N) (LI-POR subunit N) (EC 1.18.-.-)	52534.9	5.69
NC	3141	29	5.694	52	0.5954					
NS	3643	28	6.124	50	0.0326	0.44	Q8YLT5	Alr5211 protein	52011.7	8.78
NC	3149	28	6.036	52	0.0737					
NS	3655	27	4.890	45	0.1636	0.71	Q8YPU6	NADH dehydrogenase	45675.2	4.95
NC	3163	27	4.983	46	0.2307					
NS	3728	26	5.355	23	0.1012	0.63	Q8YVB5	Uracil phosphoribosyltransferase (EC 2.4.2.9) (UMP pyrophosphorylase) (UPRTase)	23364.1	5.08
NC	3220	26	5.085	23	0.1604					
NS	3735	25	4.444	21	1.5199	1.01	P80562	Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase)	18960.6	4.69
NC	3228	25	4.710	19	1.5025					
NS	3736	24	4.548	21	2.4107	**3.94**	Q8YSE1	Phosphoenolpyruvate synthase	18033.9	4.85
NC	3231	24	4.944	18	0.6115					
NS	3738	23	4.872	20	1.1271	0.55	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NC	3230	23	4.500	18	2.0396					
NS	3749	22	6.048	19	0.1077	0.22	Q8YNU3	Alr4468 protein	18081	6.9
NC	3243	22	6.158	18	0.4841					
NS	3752	21	5.097	18	0.2333	0.50	Q8YSE1	Phosphoenolpyruvate synthase	18033.9	4.85
NC	3246	21	4.898	18	0.4662					
NS	3753	20	4.422	18	10.2086	**1.96**	P80562	Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase)	18960.6	4.69
NC	3244	20	4.653	18	5.2084					
NS	3755	19	4.100	18	0.6886	1.17	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NC	3241	19	4.100	18	0.5871					
NS	3756	18	5.372	18	0.1126	0.17	Q8YYZ9	Alr0692 protein	17425.2	5.37
NC	3248	18	5.215	18	0.6489					
NS	3757	17	6.109	18	0.3474	1.42	Q8YWH5	Molybdopterin synthase catalytic subunit (EC 2.8.1.12) (MPT synthase subunit 2) (Molybdenum	18097.8	7
NC	3247	17	6.442	18	0.2450					

								cofactor biosynthesis protein E) (Molybdopterin-converting factor large subunit) (Molybdopterin-converting factor subunit 2)		
NS	3759	16	4.223	18	0.1822	**1.63**	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NC	3242	16	4.236	18	0.1118					
NS	3765	15	5.252	17	7.0583	0.58	P80555	Allophycocyanin subunit alpha 1	17214.5	4.92
NC	3252	15	5.085	17	12.2443					
NS	3767	14	5.605	17	4.2938	0.76	P80557	Allophycocyanin subunit beta	17173.6	5.46
NC	3256	14	5.451	17	5.6380					
NS	3771	13	4.164	17	0.3574	0.55	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NC	3259	13	4.225	17	0.6500					
NS	3772	12	5.800	17	2.6184	1.18	O52751	Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)	17740.6	4.7
NC	3260	12	5.800	17	2.2108					
NS	3773	11	5.967	17	0.6276	0.29	Q93SX1	Cytochrome b6-f complex subunit 4 (17 kDa polypeptide)	17535.9	7.85
NC	3257	11	6.117	17	2.1478					
NS	3774	10	4.059	17	1.4799	**2.06**	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NC	3258	10	4.037	17	0.7195					
NS	3775	9	5.858	17	0.3655	0.60	Q8YU89	1,4-dihydroxy-2-naphthoyl-CoA hydrolase (DHNA-CoA hydrolase) (EC 3.1.2.28) (DHNA-CoA thioesterase)	16864.4	5.8
NC	3255	9	5.849	17	0.6103					
NS	3777	8	4.576	17	2.6173	1.02	P80556	Allophycocyanin subunit alpha-B	17680.3	5.06
NC	3263	8	5.022	17	2.5633					
NS	3779	7	5.105	17	0.2424	0.80	O52752	Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC)	17740.6	4.7
NC	3261	7	4.671	17	0.3047					
NS	3781	6	4.422	17	0.6460	0.49	Q8YUT2	Protein GvpK	16947.7	4.65
NC	3266	6	4.658	17	1.3085					
NS	3783	5	5.476	17	0.1593	**1.78**	Q8YYZ9	Alr0692 protein	17425.2	5.37
NC	3268	5	5.296	17	0.0893					
NS	3784	4	5.597	17	0.1166	0.62	Q8Z017	Small heat shock protein	17122.6	5.5
NC	3271	4	5.434	16	0.1874					
NS	3793	3	5.588	16	0.3697	0.44	Q8YRG9	Alr3479 protein	16687	5.26
NC	3272	3	5.158	16	0.8422					
NS	3794	2	4.365	16	1.1991	0.58	Q8YUT1	Gas vesicle protein GvpJ	16597.6	4.73
NC	3274	2	4.690	16	2.0607					
NS	3795	1	4.821	16	0.1921	0.17	Q8YUT1	Gas vesicle protein GvpJ	16597.6	4.73
NC	3273	1	4.549	16	1.1504					
NS	3805	0	4.407	16	0.3505	**3.33**	Q8YUT1	Gas vesicle protein GvpJ	16597.6	4.73

NC	3285	0	4.778	16	0.1053					

Table 4. Showing identical protein with differential expression (>1.5 Fold Regulation) in the control and sucrose treated cells. The putative gene products are also given in the table.

S.N.	Functional Group	Protein Identification	Sub function	Gene Name	Match ID
1	Cell envelope	Penicillin-binding protein	Murein sacculus and peptidoglycan	alr0153	33
2	Energy metabolism	2,3-bisphosphoglycerate-independent phosphoglycerate mutase (BPG-independent PGAM) (Phosphoglyceromutase) (iPGM) (EC 5.4.2.1)	Glycolysis	all4182	30
		Phosphoenolpyruvate synthase	Pyruvate and acetyl-CoA metabolism	alr3147	24
3	Central intermediary metabolism	similar to NifU protein	Nitrogen fixation	alr0692	5
		Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase)	Phosphorus compounds	all3570	20
4	Unknown & Hypothetical	Gas vesicle protein GvpJ		all2250	0
		UPF0079 ATP-binding protein alr2300		alr2300	16, 10

Table 5. Spot details on commonly induced proteins under salt and sucrose treated cells of *N. muscorum.* NS=protein spots apparent on the gel of sucrose treated cells of *N. muscorum*; NN=protein spots apparent on the gel of salt treated cells of *N. muscorum*

File Name	Spot ID	Match ID	Apparent pI	Apparent MW (kDa)	%Vol	Fold Regulation (T/C)	Protein Acc. No	Protein Identfication	Theoretical Mw (Da)	Theoretical pI
NN	7877	53	6.08	77	0.045	**1.83**	Q8YZZ2	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (SEPHCHC synthase) (EC 2.2.1.9) (Menaquinone biosynthesis protein MenD)	65729.2	5.83
NS	3596	53	5.96	66	0.024					
NN	7881	52	5.32	76	0.133	**2.24**	Q8YQU9	Arginine--tRNA ligase (EC 6.1.1.19) (Arginyl-tRNA synthetase) (ArgRS)	65814.9	5.3
NS	3597	52	5.40	65	0.059					
NN	7916	51	5.14	67	0.058	0.81	Q8YXJ6	L-aspartate oxidase (LASPO) (EC 1.4.3.16) (Quinolinate synthase B)	63173	6.17
NS	3604	51	5.27	64	0.072					
NN	7969	50	4.70	60	0.200	0.24	Q8YSY8	All2941 protein	62501	9.2
NS	3612	50	4.70	60	0.832					
NN	7972	49	5.17	61	0.041	0.19	P48575	2-isopropylmalate synthase (EC 2.3.3.13) (Alpha-IPM synthase) (Alpha-isopropylmalate synthase)	57761.5	5.38
NS	3621	49	5.29	58	0.216					
NN	7973	48	6.48	61	0.025	0.77	Q8YXT4	NADH dehydrogenase	49725.4	6.13
NS	3643	48	6.12	50	0.033					
NN	7977	47	4.07	60	0.036	0.40				

NS	3610	47	4.14	62	0.089					
NN	7978	46	4.49	58	0.591	**5.02**	Q8YVS8	60 kDa chaperonin 2 (GroEL protein 2) (Protein Cpn60 2)	58969.6	4.93
NS	3618	46	4.46	59	0.118					
NN	7979	45	4.59	59	0.144	0.28	Q8Z0C1	Putative diflavin flavoprotein A 5	62572.6	6.1
NS	3611	45	4.49	61	0.508					
NN	7984	44	5.30	59	0.157	**4.55**	Q8YMQ0	NAD(P)H-quinone oxidoreductase subunit 2 (EC 1.6.5.-) (NAD(P)H dehydrogenase subunit 2) (NADH-plastoquinone oxidoreductase subunit 2) (NDH-1, subunit 2)	55464.6	5.6
NS	3627	44	5.42	55	0.034					
NN	7988	43	5.09	58	0.012	0.07	Q8YZR2	L-2,4-diaminobutyrate decarboxylase	58500.6	5.01
NS	3620	43	5.20	59	0.163					
NN	7994	42	4.90	55	0.435	0.59	Q8YZX6	Anthranilate synthetase alpha-subunit	56214.2	5.16
NS	3623	42	4.91	56	0.742					
NN	8000	41	4.99	55	0.141	0.52	Q8YWF0	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase	53473.8	5.17
NS	3634	41	5.05	53	0.270					
NN	8016	40	4.81	53	0.119	0.25	Q8Z064	Probable cytosol aminopeptidase (EC 3.4.11.1) (Leucine aminopeptidase) (LAP) (EC 3.4.11.10) (Leucyl aminopeptidase)	51918.3	4.87
NS	3639	40	4.79	51	0.468					
NN	8026	39	4.45	51	0.056	0.17	Q8YQX9	Trigger factor (TF) (EC 5.2.1.8) (PPIase)	52381.9	4.43
NS	3640	39	4.44	52	0.342					
NN	8035	38	4.91	51	0.043	0.03	Q8YN91	tRNA modification GTPase MnmE (EC 3.6.-.-)	49783	4.9
NS	3637	38	4.92	50	1.662					
NN	8042	37	4.14	50	0.059	0.39	Q8YQX9	Trigger factor (TF) (EC 5.2.1.8) (PPIase)	52381.9	4.43
NS	3638	37	4.20	52	0.150					
NN	8045	36	4.77	48	1.180	**10.77**	Q8YLT0	Alr5216 protein	48421.9	4.58
NS	3647	36	4.71	49	0.110					
NN	8077	35	6.22	47	0.015	0.80	Q8YQU4	Precorrin-6y-dependent methyltransferase	43288.1	6.33
NS	3658	35	6.01	43	0.019					
NN	8084	34	4.17	45	0.073	0.87		UNKNOWN		
NS	3659	34	4.22	42	0.084					
NN	8094	33	5.18	44	0.135	**2.27**	Q8YPR1	Phosphoglycerate kinase (EC 2.7.2.3)	42441.5	5.15
NS	3663	33	5.29	41	0.059					
NN	8126	32	4.93	41	0.109	0.24	P58571	Magnesium-chelatase subunit ChlI (EC 6.6.1.1) (Mg-protoporphyrin IX chelatase)	41245.2	5.03
NS	3666	32	4.94	40	0.454					
NN	8127	31	5.07	41	0.023	0.39	P70801	Glucanase	37896.1	5.13
NS	3672	31	5.05	38	0.060					
NN	8135	30	5.68	39	0.021	0.21	Q8YQG6	Cyclic pyranopterin monophosphate synthase (EC 4.1.99.18)	36878.3	6.25
NS	3679	30	5.70	36	0.098					

								(Molybdenum cofactor biosynthesis protein A)		
NN	8139	29	5.31	39	0.048	0.18	Q8YUM5	Ketol-acid reductoisomerase (EC 1.1.1.86) (Acetohydroxy-acid isomeroreductase) (Alpha-keto-beta-hydroxylacyl reductoisomerase)	36010.9	5.4
NS	3680	29	5.36	36	0.273					
NN	8149	28	6.58	38	0.086	**1.64**	Q8YUS1	Protease HtpX homolog (EC 3.4.24.-)	30638.5	9
NS	3699	28	6.17	30	0.052					
NN	8189	27	6.11	33	0.020	0.44	Q8YS90	Mg-protoporphyrin IX methyl transferase	25344.8	6.23
NS	3712	27	6.02	25	0.045					
NN	8208	26	5.67	30	0.071	0.70	Q8YT99	Glucose-1-P cytidylyltransferase	29391.6	5.69
NS	3704	26	5.69	28	0.101					
NN	8232	25	4.33	28	0.061	0.40	Q8YLN8	Riboflavin synthase alpha chain	23518.8	4.75
NS	3719	25	4.32	23	0.153					
NN	8310	24	4.75	19	0.244	0.10	Q8YUQ7	Alr2278 protein	21191.7	4.63
NS	3736	24	4.55	21	2.411					
NN	8313	23	4.10	18	0.637	0.92	P07120	C-phycocyanin subunit beta	18255.6	5
NS	3755	23	4.10	18	0.689					
NN	8314	22	5.02	19	0.040	0.04	Q8YNA6	Glutathione S-transferase	20774.1	4.89
NS	3738	22	4.87	20	1.127					
NN	8329	21	5.86	18	0.060	0.40	Q8YNU3	Alr4468 protein	18081	6.9
NS	3763	21	5.87	17	0.149					
NN	8330	20	4.37	18	0.052	0.24	Q06881	Biotin carboxyl carrier protein of acetyl-CoA carboxylase (BCCP)	19048.7	4.63
NS	3750	20	4.29	18	0.212					
NN	8332	19	6.49	18	0.036	0.34	Q8YME4	Methylated-DNA--protein-cysteine methyltransferase (EC 2.1.1.63) (6-O-methylguanine-DNA methyltransferase) (O-6-methylguanine-DNA-alkyltransferase)	19730.8	7.72
NS	3749	19	6.05	19	0.108					
NN	8341	18	4.56	18	1.556	0.15	P80562	Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase)	18960.6	4.69
NS	3753	18	4.42	18	10.209					
NN	8342	17	5.13	18	2.346	**10.06**	P07120	C-phycocyanin subunit beta	18255.6	5
NS	3752	17	5.10	18	0.233					
NN	8345	16	6.74	18	0.028	0.08	Q8YNU3	Alr4468 protein	18081	6.9
NS	3757	16	6.11	18	0.347					
NN	8350	15	5.32	17	0.325	**2.89**	Q8YPF9	Arginine biosynthesis bifunctional protein ArgJ	18553.3	5.39
NS	3756	15	5.37	18	0.113					
NN	8352	14	4.33	17	0.063	0.35	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NS	3759	14	4.22	18	0.182					
NN	8356	13	5.26	17	5.988	0.85	Q8YYZ9	Alr0692 protein	17425.2	5.37
NS	3765	13	5.25	17	7.058					

NN	8360	12	5.80	17	3.385	1.29	P35796	Phycoerythrocyanin alpha chain	17454.5	6.27
NS	3772	12	5.80	17	2.618					
NN	8363	11	5.94	17	0.422	1.16	Q93SX1	Cytochrome b6-f complex subunit 4 (17 kDa polypeptide)	17535.9	7.85
NS	3775	11	5.86	17	0.365					
NN	8373	10	4.20	17	0.328	0.92				
NS	3771	10	4.16	17	0.357					
NN	8380	9	4.08	17	0.094	0.11	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NS	3768	9	4.26	17	0.882					
NN	8393	8	6.39	17	0.010	0.02	Q93SX1	Cytochrome b6-f complex subunit 4 (17 kDa polypeptide)	17535.9	7.85
NS	3773	8	5.97	17	0.628					
NN	8396	7	4.49	17	0.118	0.01	Q8YUT2	Protein GvpK	16947.7	4.65
NS	3770	7	4.41	17	9.673					
NN	8399	6	4.08	17	0.014	0.07	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NS	3780	6	4.27	17	0.206					
NN	8403	5	5.50	17	0.185	0.04	Q8YQF0	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MECDP-synthase) (MECPP-synthase) (MECPS) (EC 4.6.1.12)	17873.6	5.56
NS	3767	5	5.61	17	4.294					
NN	8410	4	4.38	17	0.078	0.12	O52749	UPF0079 ATP-binding protein alr2300	17938.7	4.33
NS	3781	4	4.42	17	0.646					
NN	8432	3	5.84	16	1.545	**9.55**	Q8YU89	1,4-dihydroxy-2-naphthoyl-CoA hydrolase (DHNA-CoA hydrolase) (EC 3.1.2.28) (DHNA-CoA thioesterase)	16864.4	5.8
NS	3785	3	5.81	17	0.162					
NN	8438	2	6.73	16	0.151	1.25	Q8Z033	Diacylglycerol kinase	16338.5	6.81
NS	3801	2	6.14	16	0.120					
NN	8449	1	5.85	16	0.150	0.30	Q8YYW0	Urease accessory protein UreE	16559.1	5.86
NS	3791	1	5.82	16	0.501					
NN	8450	0	4.29	16	0.061	0.22	Q8YUT2	Gas vesicle protein GvpJ	16597.6	4.73
NS	3788	0	4.40	16	0.276					

Table 6. Showing identical protein with differential expression (>1.5 Fold Regulation) in the salt treated and sucrose treated cells. The putative gene products are also given in the table.

S.N.	Functional Group	Protein Identification	Sub function	ORF'S	Match ID
1	Amino acid biosynthesis	Arginine biosynthesis bifunctional protein ArgJ 2 [Cleaved into: Arginine biosynthesis bifunctional protein ArgJ alpha chain; Arginine biosynthesis bifunctional protein ArgJ beta chain] [Includes: Glutamate N-acetyltransferase (EC 2.3.1.35) (Ornithine acetyltransferase) (OATase) (Ornithine transacetylase); Amino-acid acetyltransferase (EC 2.3.1.1) (N-acetylglutamate synthase) (AGSase)]	Glutamate family / Nitrogen assimilation	alr4235	15
2	Biosynthesis of cofactors, prosthetic groups, and carriers	2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (SEPHCHC synthase) (EC 2.2.1.9) (Menaquinone biosynthesis protein MenD)	Menaquinone and ubiquinone	alr0312	53

3	Cellular processes	probable hemolysin	Cell killing	alr5216	36
		Protease HtpX homolog (EC 3.4.24.-)heat shock protein X	Chaperones	all2263	28
		60 kDa chaperonin 2 (GroEL protein 2) (Protein Cpn60 2)	Chaperones	alr1896	46
4	Photosynthesis and respiration	NAD(P)H-quinone oxidoreductase subunit 2 (EC 1.6.5.-) (NAD(P)H dehydrogenase subunit 2) (NADH-plastoquinone oxidoreductase subunit 2) (NDH-1, subunit 2)	NADH dehydrogenase	all4883	44
5	Unknown & Hypothetical	Arginine--tRNA ligase (EC 6.1.1.19) (Arginyl-tRNA synthetase) (ArgRS)		all3717	52
		C-phycocyanin subunit beta		alr0528	17
		Phosphoglycerate kinase (EC 2.7.2.3)		all4131	33
		1,4-dihydroxy-2-naphthoyl-CoA hydrolase (DHNA-CoA hydrolase) (EC 3.1.2.28) (DHNA-CoA thioesterase)		alr2465	3

