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/m²). 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°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:
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-oxoglutarate 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 oxidase 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

Phycobilisomes are the major light harvesting complexes of cyanobacteria. They are associated with photosystem II and constitute up to 50% of the total cellular proteins. Phycobilisomes are multiprotein assemblies and under diazotrophic growth, various genes involved in phycobilisome proteins are over expressed [36]. In consistence with the above findings, it was found that orf viz. abr0021 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:

![Image](image.jpg)

**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 protein 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 (alr3147) 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 thioredoxin like domain. Thioredoxin 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 initia tions 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).
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 haemolysin produced by glucose tolerant strain of *Synechocystis* sp PCC 6803 suggested that haemolysin 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.

Phycobilisomes 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 Identification | Theoretical Mw (Da) | Theoretical pI |
|-----------|---------|----------|-------------|-------------------|------|----------------------|-----------------|-----------------------|-------------------|-----------------|
| NN 8431   | 36      | 6.029    | 16          | 0.84788           |      | 1.19 Q8YQ24          | Chorismate mutase | 15760.62              | 6.91              |
| NN 8432   | 35      | 5.836    | 16          | 1.54506           | 0.72 | Q8YP58               | Mannose-6-phosphate isomerase | 15804.86              | 6.65              |
| NN 8424   | 34      | 4.763    | 16          | 0.81515           | 0.56 | P58703               | Cyanate hydratase (Cyanase) (EC 4.2.1.104) (Cyanate hydrolyase) (Cyanate lyase) | 16398.99              | 4.97              |
| NN 8408   | 33      | 4.856    | 16          | 0.66589           | 0.58 | Q8YUT1               | Gas vesicle protein GvpJ | 16597.58              | 4.73              |
| 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              |
| NN 8289   | 31      | 4.867    | 21          | 1.83581           | 0.90 | O52749               | UFP0079 ATP-binding protein alr2300 | 17938.69              | 4.33              |
| 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               |
| NN 8342   | 29      | 5.127    | 18          | 2.34606           | **2.45** | P80555               | Allophycocyanin subunit alpha 1 | 17214.47              | 4.92              |
| NN 8359   | 28      | 5.529    | 17          | 5.84223           | 1.04 | P80557               | Allophycocyanin subunit beta | 17173.56              | 5.46              |
| NN 8358   | 27      | 4.628    | 17          | 7.99235           | 0.59 | O52751               | Crossover junction | 17740.55              | 4.7               |
| Accession | Start | End | Score | E-value | Description |
|-----------|-------|-----|-------|---------|-------------|
| 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 | P80556 Allophycocyanin subunit alpha-B |
| NC 3263   | 26    | 5.022 | 17    | 2.56327 |
| NN 7850   | 25    | 4.436 | 84    | 0.06533 | |
| NC 3116   | 25    | 4.460 | 70    | 0.041 |
| NN 7865   | 24    | 4.562 | 79    | 0.1323 |
| NC 3120   | 24    | 4.617 | 68    | 0.16902 |
| NN 7903   | 23    | 4.282 | 69    | 0.02226 | 2.43 Q8YZ2 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase (SEPCHC synthase) (EC 2.2.1.9) (MenAquinone biosynthesis protein MenD) |
| NC 3125   | 23    | 4.382 | 65    | 0.00916 |
| NN 7918   | 22    | 4.463 | 67    | 0.02731 | |
| 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) |
| NC 3129   | 21    | 4.856 | 62    | 0.15004 |
| NN 7947   | 20    | 4.414 | 63    | 0.0671 |
| NC 3133   | 20    | 4.497 | 60    | 0.1261 |
| NN 7954   | 19    | 4.304 | 62    | 0.07786 | 4.21 Q8YR01 Alk3659 protein |
| NC 3131   | 19    | 4.324 | 62    | 0.01849 |
| NN 7969   | 18    | 4.700 | 60    | 0.2005 |
| NC 3134   | 18    | 4.780 | 60    | 0.17290 |
| NN 8035   | 17    | 4.914 | 51    | 0.04325 | |
| NC 3163   | 17    | 4.983 | 46    | 0.23071 |
| NN 8042   | 16    | 4.139 | 50    | 0.05923 | |
| NC 3146   | 16    | 4.058 | 52    | 0.09061 | 0.65 Q8Z064 Probable cytosol aminopeptidase (EC 3.4.11.1) (Leucine aminopeptidase) (LAP) (EC 3.4.11.10) (Leucyl aminopeptidase) |
| NN 8046   | 15    | 4.815 | 49    | 0.30447 | |
| NC 3162   | 15    | 4.837 | 46    | 0.47115 | 0.65 Q8YR80 Endoase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase) (2-phosphoglycerate dehydratase) |
| NN 8066   | 14    | 4.976 | 48    | 0.07466 | 0.12 Q8YP49 1-deoxy-D-xylulose 5- |
| NC  | 3168 | 14  | 5.052 | 43  | 0.63857 | phosphate reductoisomerase (DXP reductoisomerase) (EC 1.1.1.267) (1-deoxyxylose-5-phosphate reductoisomerase) (2-C-methyl-D-erythritol 4-phosphate synthase) |
| NN  | 8205 | 13  | 4.921 | 31  | 0.03652 | Q8YNC5 Peroxiredoxin |
| NC  | 3217 | 13  | 4.944 | 23  | 0.37055 | |
| NN  | 8267 | 12  | 5.701 | 25  | 0.03946 | Q8YLJ6 3OS ribosomal protein L10 |
| NC  | 3227 | 12  | 5.711 | 20  | 0.04217 | |
| NN  | 8309 | 11  | 5.982 | 19  | 0.01492 | Q8YNU3 Alr4468 protein |
| NC  | 3243 | 11  | 6.158 | 18  | 0.48412 | |
| NN  | 8312 | 10  | 4.100 | 18  | 0.63671 | O52749 UPF0079 ATP-binding protein alr2300 |
| NC  | 3241 | 10  | 4.100 | 18  | 0.58712 | |
| NN  | 8315 | 9   | 5.341 | 19  | 0.04354 | Q8YYZ9 Alr0692 protein |
| NC  | 3248 | 9   | 5.215 | 18  | 0.64891 | |
| NN  | 8325 | 8   | 6.191 | 18  | 0.28307 | 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) |
| NC  | 3247 | 8   | 6.442 | 18  | 0.24498 | |
| NN  | 8328 | 7   | 5.143 | 18  | 0.39368 | Q8YSE1 Phosphoenolpyruvate synthase |
| NC  | 3246 | 7   | 4.908 | 18  | 0.46624 | |
| NN  | 8356 | 6   | 5.263 | 17  | 0.98577 | P80555 Allophycocyanin subunit alpha 1 |
| NC  | 3252 | 6   | 5.085 | 17  | 1.23443 | |
| NN  | 8360 | 5   | 5.800 | 17  | 1.38518 | O52751 Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC) |
| NC  | 3260 | 5   | 5.800 | 17  | 2.21081 | |
| NN  | 8365 | 4   | 6.029 | 17  | 1.11362 | Q93SX1 Cytochrome b6-f complex subunit 4 (17 kDa polypeptide) |
| NC  | 3257 | 4   | 6.117 | 17  | 2.14779 | |
| NN  | 8395 | 3   | 5.101 | 17  | 0.08298 | O52753 Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC) |
| NC  | 3262 | 3   | 4.614 | 17  | 0.06696 | |
| NN  | 8441 | 2   | 5.597 | 16  | 0.01524 | Q8Z0K8 Alr0083 protein |

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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 Identification                                                                 | 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                                                       | Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4) (Holliday junction nuclease RuvC) (Holliday junction resolvase RuvC) | alr0021            | 29       |

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 Identification                                                                 | Theoretical Mw (Da) | Theoretical pI |
|-----------|---------|----------|--------------|-------------------|------|--------------------------|-----------------|---------------------------------------------------------------------------------------|-------------------|--------------|
| NS        | 3770    | 36       | 4.141        | 17                | 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        | 3254    | 36       | 4.720        | 17                | 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         |
| NS        | 3596    | 35       | 3.964        | 66                | 0.8317 | 4.81                     | Q8Z0E5          | Penicillin-binding protein                                                             | 60683.9           | 5.04         |
| NC        | 3128    | 34       | 4.564        | 62                | 0.1729 | 0.69                     | Q8YP23          | Peptide chain release                                                                    | 61270.8           | 5.43         |
| NS        | 3612    | 33       | 4.700        | 60                | 0.1040 |                          |                 |                                                                                        |                   |              |
| NC        | 3134    | 33       | 4.700        | 60                | 0.8317 |                          |                 |                                                                                        |                   |              |
| NS        | 3614    | 32       | 4.941        | 61                | 0.06125 |                          |                 |                                                                                        |                   |              |
| Accession | Description                                                                 | F织 | Factor (RT-3) | M织 | Description                                                                 | Value  |
|-----------|------------------------------------------------------------------------------|-----|--------------|-----|------------------------------------------------------------------------------|--------|
| NC 3129   | 32 4.856 62 0.1500 factor 3 (RF-3)                                           |     |              |     | Urease subunit alpha (EC 3.5.1.5) (Urea amidohydrolase subunit alpha)       | 61155.6 | 5.23 |
| NS 3620   | 31 5.200 59 0.1628                                                          |     |              |     | 2.3-bisphosphoglycerate-independent phosphoglycerate mutase (BPG-independent PGAM) (Phosphoglyceromutase) (iPGM) (EC 5.4.2.1) | 57677.3 | 4.99 |
| NC 3132   | 31 4.524 61 0.1116                                                          |     |              |     | Light-independent protochlorophyllide reductase subunit N (DPOR subunit N) (LI-POR subunit N) (EC 1.18.-.-) | 52534.9 | 5.69 |
| NS 3636   | 29 5.867 53 0.0964                                                          |     |              |     | Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase) | 18960.6 | 4.69 |
| NC 3141   | 29 5.604 52 0.5954                                                          |     |              |     | Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase) | 18960.6 | 4.69 |
| NS 3643   | 28 6.124 59 0.0326                                                          |     |              |     | Light-independent phosphoribosyltransferase (EC 2.4.2.9) (UMP pyrophosphorylase) (UPRTase) | 23364.1 | 5.08 |
| NC 3149   | 28 6.036 52 0.0737                                                          |     |              |     | Light-independent phosphoribosyltransferase (EC 2.4.2.9) (UMP pyrophosphorylase) (UPRTase) | 23364.1 | 5.08 |
| NS 3655   | 27 4.800 45 0.1636                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NC 3163   | 27 4.983 46 0.2307                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NS 3728   | 26 3.355 23 0.1012                                                          |     |              |     | Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase) | 18960.6 | 4.69 |
| NC 3220   | 26 3.085 23 0.1604                                                          |     |              |     | Inorganic pyrophosphatase (EC 3.6.1.1) (Pyrophosphate phospho-hydrolase) (PPase) | 18960.6 | 4.69 |
| NS 3735   | 25 4.444 21 1.5199                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NC 3228   | 25 4.710 19 1.5025                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NS 3736   | 24 4.548 21 2.4107                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NC 3231   | 24 4.944 18 0.6115                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NS 3738   | 23 4.872 20 1.1271                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NC 3230   | 23 4.500 18 2.0396                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NS 3749   | 22 6.048 19 0.1077                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NC 3243   | 22 6.158 18 0.4841                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NS 3752   | 21 5.097 18 0.2333                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NC 3246   | 21 4.808 18 0.4662                                                          |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NS 3753   | 20 4.422 18 10.2086                                                         |     |              |     | Phosphoenolpyruvate synthase                                                | 18033.9 | 4.85 |
| NC 3244   | 20 4.653 18 5.2084                                                          |     |              |     | Phosphoenolpyr...
|    | Accession Number | Description                                                                 | E-value | Cofactor Biosynthesis Protein E | Protein Name/Description                                                                 |
|----|------------------|------------------------------------------------------------------------------|---------|--------------------------------|------------------------------------------------------------------------------------------|
| NS | 3759             | 4.233                                                                       | 0.0182  | 1.63                           | O52749 UPF0079 ATP-binding protein alr2300                                               |
| NC | 3242             | 4.236                                                                       | 0.1118  |                                |                                                                                           |
| NS | 3765             | 5.252                                                                       | 7.0583  | 0.58                           | P80555 Allophycocyanin subunit alpha 1                                                   |
| NC | 3252             | 5.085                                                                       | 12.2443 |                                |                                                                                           |
| NS | 3767             | 5.605                                                                       | 4.2938  | 0.76                           | P80557 Allophycocyanin subunit beta                                                      |
| NC | 3256             | 5.451                                                                       | 5.6380  |                                |                                                                                           |
| NS | 3771             | 4.164                                                                       | 0.3574  | 0.55                           | O52749 UPF0079 ATP-binding protein alr2300                                               |
| NC | 3259             | 4.225                                                                       | 0.6500  |                                |                                                                                           |
| NC | 3260             | 5.800                                                                       | 2.6184  | 1.18                           | O52751 Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4)                        |
| NC | 3263             | 5.117                                                                       | 2.1478  |                                |                                                                                           |
| NC | 3257             | 6.059                                                                       | 1.4799  | 2.06                           | O52749 UPF0079 ATP-binding protein alr2300                                               |
| NC | 3258             | 4.017                                                                       | 0.7195  |                                |                                                                                           |
| NS | 3775             | 5.858                                                                       | 0.3655  | 0.60                           | Q8YU89 1,4-dihydroxy-2-naphthoyl-CoA hydrolase (DHNA-CoA hydrolase) (EC 3.1.2.28)        |
| NC | 3255             | 5.849                                                                       | 0.6103  |                                |                                                                                           |
| NS | 3777             | 4.576                                                                       | 0.6276  | 0.29                           | Q93SX1 Cytochrome b6-f complex subunit 4 (17 kDa polypeptide)                            |
| NC | 3257             | 6.117                                                                       | 2.1478  |                                |                                                                                           |
| NS | 3774             | 4.059                                                                       | 1.4799  | 2.06                           | O52749 UPF0079 ATP-binding protein alr2300                                               |
| NC | 3258             | 4.017                                                                       | 0.7195  |                                |                                                                                           |
| NC | 3261             | 5.800                                                                       | 2.6184  | 1.18                           | O52751 Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4)                        |
| NC | 3263             | 5.117                                                                       | 2.1478  |                                |                                                                                           |
| NS | 3263             | 5.022                                                                       | 2.5633  |                                |                                                                                           |
| NS | 3779             | 5.105                                                                       | 0.2424  | 0.80                           | O52752 Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4)                        |
| NC | 3261             | 4.671                                                                       | 0.3047  |                                |                                                                                           |
| NS | 3777             | 4.576                                                                       | 2.6173  | 1.02                           | P80556 Allophycocyanin subunit alpha-B                                                    |
| NC | 3263             | 5.022                                                                       | 2.5633  |                                |                                                                                           |
| NS | 3779             | 5.105                                                                       | 0.2424  | 0.80                           | O52752 Crossover junction endodeoxyribonuclease RuvC (EC 3.1.22.4)                        |
| NC | 3261             | 4.671                                                                       | 0.3047  |                                |                                                                                           |
| NS | 3781             | 4.422                                                                       | 0.6460  | 0.49                           | Q8YUT2 Protein GvpK                                                                       |
| NC | 3266             | 4.658                                                                       | 1.3085  |                                |                                                                                           |
| NS | 3783             | 5.476                                                                       | 0.1593  | 1.78                           | Q8YVZ9 Ahr0692 protein                                                                    |
| NC | 3268             | 5.296                                                                       | 0.0893  |                                |                                                                                           |
| NS | 3784             | 5.597                                                                       | 0.1166  | 0.62                           | Q8Z017 Small heat shock protein                                                           |
| NC | 3271             | 5.434                                                                       | 0.1874  |                                |                                                                                           |
| NS | 3793             | 5.588                                                                       | 0.3697  | 0.44                           | Q8YRG9 Ahr3479 protein                                                                    |
| NC | 3272             | 5.518                                                                       | 0.8422  |                                |                                                                                           |
| NS | 3794             | 4.365                                                                       | 1.1991  | 0.58                           | Q8YUT1 Gas vesicle protein GvpD                                                            |
| NC | 3274             | 4.690                                                                       | 2.0607  |                                |                                                                                           |
| NS | 3795             | 4.821                                                                       | 0.1921  | 0.17                           | Q8YUT1 Gas vesicle protein GvpD                                                            |
| NC | 3273             | 4.549                                                                       | 1.1504  |                                |                                                                                           |
| NS | 3805             | 4.407                                                                       | 0.3505  | 3.33                           | Q8YUT1 Gas vesicle protein GvpD                                                            |
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 | all182 | 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        |

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 Identification | 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 |
| 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 | 3.3 |
| 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 |
| NN 7969 | 50 | 4.70 | 60 | 0.200 | 0.24 | | Q8YSYS8 | All2941 protein | 62501 | 9.2 |
| NN 7972 | 49 | 4.70 | 60 | 0.832 | | | | | |
| NN 7973 | 48 | 6.48 | 61 | 0.025 | | | Q8YXT4 | NADH dehydrogenase | 49725.4 | 6.13 |
| NN 3643 | 48 | 6.12 | 50 | 0.033 | | | | | |
| NN 3621 | 47 | 4.07 | 60 | 0.036 | | | | | |
| Accession | E Value | Identity | Query Length | Target Length | Description | E Value |
|-----------|---------|----------|--------------|---------------|-------------|---------|
| NS 3610   | 47      | 1.14     | 62           | 0.089         |             |         |
| NS 3618   | 46      | 1.46     | 59           | 0.118         |             |         |
| NS 3611   | 45      | 1.49     | 61           | 0.508         |             |         |
| NS 3627   | 44      | 5.42     | 55           | 0.034         |             |         |
| NS 3620   | 43      | 5.20     | 59           | 0.163         |             |         |
| NS 3623   | 42      | 1.91     | 56           | 0.742         |             |         |
| NS 3634   | 41      | 5.05     | 53           | 0.270         |             |         |
| NS 3639   | 40      | 1.81     | 53           | 0.468         |             |         |
| NS 3633   | 41      | 4.79     | 51           | 0.468         |             |         |
| NS 3640   | 39      | 1.45     | 51           | 0.056         |             |         |
| NS 3649   | 39      | 1.44     | 52           | 0.342         |             |         |
| NS 3637   | 38      | 1.91     | 51           | 0.043         |             |         |
| NS 3638   | 38      | 1.92     | 50           | 1.662         |             |         |
| NS 3642   | 37      | 1.14     | 50           | 0.059         |             |         |
| NS 3637   | 37      | 1.20     | 52           | 0.150         |             |         |
| NS 3645   | 36      | 1.77     | 48           | 1.180         |             |         |
| NS 3647   | 36      | 1.71     | 49           | 0.110         |             |         |
| NS 3677   | 35      | 6.22     | 47           | 0.015         |             |         |
| NS 3658   | 35      | 6.01     | 43           | 0.019         |             |         |
| NS 3684   | 34      | 1.17     | 45           | 0.073         |             |         |
| NS 3659   | 34      | 1.22     | 42           | 0.084         |             |         |
| NS 3694   | 33      | 5.18     | 44           | 0.135         |             |         |
| NS 3663   | 33      | 5.29     | 41           | 0.059         |             |         |
| NS 3666   | 32      | 4.91     | 40           | 0.454         |             |         |
| NS 3672   | 31      | 5.07     | 41           | 0.023         |             |         |
| NS 3672   | 31      | 5.05     | 38           | 0.060         |             |         |
| NS 3679   | 30      | 5.70     | 36           | 0.098         |             |         |

**Note:** The table lists protein accession numbers, E-values, identity percentages, query and target lengths, and descriptions. The descriptions include various functions and annotations such as chaperonin, flavoprotein, dehydrogenase, oxidoreductase, decarboxylase, synthetase, ligase, and others, indicating diverse molecular functions and roles. The E-values and identity percentages are crucial for assessing the similarity and reliability of the protein alignments.
| Entry | Accession | Description | Score | Protein Name | Function | Score |
|-------|------------|-------------|-------|--------------|----------|-------|
| NN 8139 29 | 3.31 39 | 0.048 | 0.18 | Q8YUS5 | Ketol-acid reductoisomerase (EC 1.1.1.86) (Acetohydroxyacid isomeroreductase) (Alpha-keto-beta-hydroxylacyl reductoisomerase) | 36010.9 | 3.4 |
| 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 28 | 0.071 | 0.70 | Q8YT99 | Glucose-1-P cytidylyltransferase | 29391.6 | 6.69 |
| NS 3704 26 | 5.69 28 | 0.101 | | | | |
| NN 8232 25 | 4.33 28 | 0.061 | 0.40 | Q8YNL8 | Riboflavin synthase alpha chain | 23518.8 | 4.75 |
| NS 3719 25 | 4.32 23 | 0.153 | | | | |
| NN 8310 24 | 1.75 19 | 0.244 | 0.10 | Q8YUQ7 | Alr2278 protein | 21191.7 | 4.63 |
| NS 3736 24 | 1.55 21 | 2.411 | | | | |
| NN 8333 23 | 1.10 18 | 0.637 | 0.92 | P07120 | C-phycocyanin subunit beta | 18255.6 | 5 |
| NS 3755 23 | 1.10 18 | 0.689 | | | | |
| NN 8314 22 | 5.02 19 | 0.040 | 0.04 | Q8YNAA6 | Glutathione S-transferase | 20774.1 | 4.89 |
| NS 3738 22 | 1.87 20 | 1.127 | | | | |
| NN 8329 21 | 5.86 18 | 0.060 | 0.40 | Q8YNAA3 | Alr4468 protein | 18081 | 6.9 |
| NS 3763 21 | 5.87 17 | 0.149 | | | | |
| NN 8330 20 | 1.37 18 | 0.052 | 0.24 | Q06881 | Biotin carboxyl carrier protein of acetyl-CoA carboxylase (BCCP) | 19048.7 | 4.63 |
| NS 3750 20 | 1.29 18 | 0.212 | | | | |
| NN 8332 19 | 6.49 18 | 0.036 | 0.34 | Q8YMIE1 | 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 | 1.56 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 | Q8YNAA3 | 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 | 1.33 17 | 0.063 | 0.35 | O52749 | ATP-binding protein alr2300 | 17938.7 | 4.33 |
| NS 3759 14 | 1.22 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 | | | | |
## 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 ID | 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 transacylase); 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 (SEPHIC synthase) (EC 2.2.1.9) (Menaquinone biosynthesis protein MenD) | Menaquinone and ubiquinone | alr0312 | 53 |
|   | Cellular processes                                      | Cell killing | alr5216 | 36 |
|---|--------------------------------------------------------|--------------|---------|----|
| 3 | probable hemolysin                                     |              |         |    |
|   | Protease HtpX homolog (EC 3.4.24.-) heat shock protein X|              | alr2263 | 28 |
|   | 60 kDa chaperonin 2 (GroEL protein 2) (Protein Cpn60 2)|              |         |    |
|   | Chaperones                                             |              |         |    |

|   | Photosynthesis and respiration                         | NADH dehydrogenase | alr1896 | 46 |
|---|--------------------------------------------------------|-------------------|---------|----|
| 4 | 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)                                   |                   |         |    |

|   | Unknown & Hypothetical                                  |                   | alr3717 | 52 |
|---|--------------------------------------------------------|-------------------|---------|----|
| 5 | Arginine-tRNA ligase (EC 6.1.1.19) (Arginyl-tRNA synthetase) (ArgRS) |                   |         |    |
|   | C-phycocyanin subunit beta                             |                   |         |    |
|   | Phosphoglycerate kinase (EC 2.7.2.3)                   |                   |         |    |
|   | 1,4-dihydroxy-2-naphthoyl-CoA hydrolase (DHNA-CoA hydrolase) (EC 3.1.2.28) (DHNA-CoA thioesterase) |                   |         |    |
|   |                                                        |                   |         |    |