Research Article

Molecular mechanisms adopted by abiotic stress tolerant *Pseudomonas fluorescens* (NBAII-PFDWD) in response to in vitro osmotic stress

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ABSTRACT: Water stress in one of the limiting factors which influences the plant growth. Microbes being as a partner are an integral part of the ecosystem which influences the plant growth under stress. In the present study, *Pseudomonas fluorescens* (NBAII- PFDWD) subjected to osmotic stress by altering osmotic potential (-10.28 MPa and -26.82 MPa) using Polyethylene Glycol (PEG) 6000 in its growth media revealed expression of proteins which modulates its cell processes. MALDI TOF studies of selected spots from 2D gel analysis of *P. fluorescens* (NBAII- PFDWD) grown under different osmotic stresses revealed that the stress kindled genes which were involved in production of osmoprotectants, genes encoding DNA damage repair and increased the translational accuracy. The studies also showed that *P. fluorescens* possesses unique mechanisms for survival under osmotic stress. The studies indicate the diverse expression of proteins in *P. fluorescens* (NBAII- PFDWD) under different osmotic potentials which helped them to mitigate impact of osmotic stress. The present method unravelled the mechanisms adopted by *P. fluorescens* (NBAII- PFDWD) to thrive under osmotic stress. The bacterium is potential stress tolerant isolate which can be exploited as a plant growth promoting rhizobacteria for agricultural crops grown in stressed soils.

KEY WORDS: MALDI- TOF, osmotic potential, osmotic stress, *Pseudomonas fluorescens*, PEG 6000

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INTRODUCTION

Bacteria surviving in soil are continuously exposed to number of stresses *viz*., flooding, high and low temperatures, drought and chemicals. The scientific studies to understand the mechanisms followed by bacteria are interesting and bring forward the survival strategy ratified by them. Bacteria alter their gene expression patterns producing proteins which help them to cope these stresses (Boor, 2006). Stress induced alteration in metabolic pathways including translation is one of the multitude ways to survive under stressful conditions. Protein synthesis is a vital part in cell function as it maintains the fidelity of genetic information (Karas, 2015).

The lipid membrane of Gram negative bacteria maintains stability by balancing its solute content and the osmotic pressure of environment. *Pseudomonas* spp. managed the variation in homeostasis by accumulating compatible solutes by *de novo* synthesis, trehalose, glutamate, mannitol, glucosylglycerol, ectoine, and hydroxyectoine; other osmotolerance mechanisms include accumualtion of K+ ions, proline, gamma aminobutyrate, -aminobutyrate, alanine, the quaternary amines glycinebetaine, fully N-methylated amino acid derivatives, sucrose, trehalose (-D-glucopyrano-syl- -D-glucopyranoside) and glucosylglycerol (Kets *et al*., 1996; Freeman *et al*., 2010, 2013). Transcriptional regulation of certain genes like ProU, OmpF, OmpC also occur under osmotic changes (Csonka, 1989). Bacterial sigma factors support bacterial growth and regulate physiological development under stress (Boor, 2006). In addition, membrane transporter and porins facilitate translational stress response under osmotic stress (Wood, 2015). Osmotic potential is used to measure the osmotic property of the bacterial environment.

Pseudomonas spp. is ubiquitous and flourishes in diverse habitats. They produce wide variety of stress response system to adapt under environmental changes (Udaonda *et al*., 2012). *Pseudomonas* spp. confers drought resistance by

accumulation of exopolysaccharides and osmolytes which make it notable to use as efficient PGPR in soils (Sandhya *et al.*, 2009).

Pseudomonas fluorescens is widely used as a biological control agent but the mechanism it opts to survive under low moisture conditions is less understood. In the present investigation, our objective was to understand mechanisms executed by *Pseudomonas fluorescens* (NBAII- PFDWD) to survive under osmotic imbalance. *Pseudomonas fluorescens* (NBAII- PFDWD) isolated from rhizosphere soil is tolerant to high temperature, salinity, pH and osmotic stress (Ashwitha *et al.*, 2013). *In vitro* studies were designed to grow the culture in PEG 6000 mediated osmotic stress in media. PEG, is a polymeric co solvent results in osmotic shifts which lead to change in membrane structure by regulating efflux or influx of solvents to maintain balance (Wood, 1999). *Pseudomonas fluorescens* (NBAII-PFDWD, GenBank HM439956) was selected as an abiotic stress tolerant organism during screening of several isolates. It was found tolerant to 45ºC, salinity (1.5M NaCl), pH 9.0 and also moisture stress. The protein profiling of the *P. fluorescens* (NBAII- PFDWD) grown under stress condition was done using 2D gel electrophoresis and major spots corresponding to stress genes were identified using MALDI-TOF analysis.

MATERIAL AND METHODS

Osmotic stress mediated growth of *Pseudomonas fluorescens* (NBAII- PFDWD)

Pseudomonas fluorescens (NBAII- PFDWD GenBank HM439956) was used in the study for investigating the growth under osmotic stress. 50 μl of mid-log-phase culture (12 h) of *P. fluorescens* (NBAII- PFDWD) was inoculated to 250 ml Erlenmeyer flask containing 100 ml of nutrient broth (g/L^{-1}) Peptone, 5g; Beef extract, 3g; NaCl, 5g)

Table 1. List of selected MALDI- TOF identified proteins expressed by *Pseudomonas fluorescens* (NBAII-PFD WD) under different osmotic potentials

Stress condition	Spot no.	Protein name (Swiss Prot Database)	Log Normalized Values		
			0 MPa	-10.28 MPa	-26.82 MPa
Normal growth condition	241	2-isopropylmalate synthase OS	5.34	5.11	4.82
	240	Probable dual-specificity RNA methyltransferase RlmN OS	4.95	4.64	4.46
	477	30s ribosomal protein S8 OS	5.54	5.12	5.11
	482	Exodeoxyribonuclease 7 small subunit OS	5.62	5.19	5.05
	336	Bifunctional pantoate ligase/cytidylate kinase OS	4.18	3.94	3.78
	391	Cellular nucleic acid-binding protein homolog OS	4.84	4.34	4.36
	392	Threonine--tRNA ligase OS	4.72	4.48	4.40
30% PEG stress (-10.28 OP)	496	Dihydrorotate dehydrogenase (quinone) OS	5.43	5.54	5.56
	90	Ribosome-recycling factor OS	2.95	2.61	3.18
	147	L-ectoine synthase	4.01	4.44	4.00
	156	Arginine deiminase OS	5.49	5.50	5.48
	222	N-acetylmannosamine kinase OS	4.88	4.91	4.96
	347	DNA replication and repair protein RecF OS	4.56	4.53	4.46
	354	Phosphate acyltransferase OS	5.08	4.99	4.98
	367	DNA repair protein RecO OS	5.14	5.01	4.95
50% PEG stress (-26.82 OP)	429	Transcription elongation factor GreA OS	4.36	4.72	5.01
	426	Elongation factor Ts OS	5.16	5.17	5.23
	486	Crossover junction endodeoxyribonuclease RuvC OS	4.87	4.92	4.93
	184	Pantothenate synthetase OS	4.08	4.31	4.43
	218	60 kDa chaperonin OS	4.20	4.40	4.43
	206	DNA mismatch repair protein MutL OS	4.97	4.73	4.98
	248	V-type proton ATPase subunit E OS ,	4.50	4.13	4.32
	263	Gamma-glutamyl phosphate reductase OS	4.77	4.81	4.89
	439	Ribonuclease HII OS	4.87	4.85	4.83
	326	Transcriptional repressor NrdR OS	4.75	4.84	4.94
	355	Leu operon leader peptide OS	4.28	4.44	4.53
	373	Fructose-1,6-bisphosphatase class 2 OS	3.96	4.23	4.37
	396	Putative ATP-dependent Clp protease proteolytic subunit-like OS	4.60	4.47	4.56
	390	Bifunctional protein GlmU OS	4.08	4.34	4.44
	368	NAD(P)H-quinone oxidoreductase subunit O OS	4.51	4.67	4.68
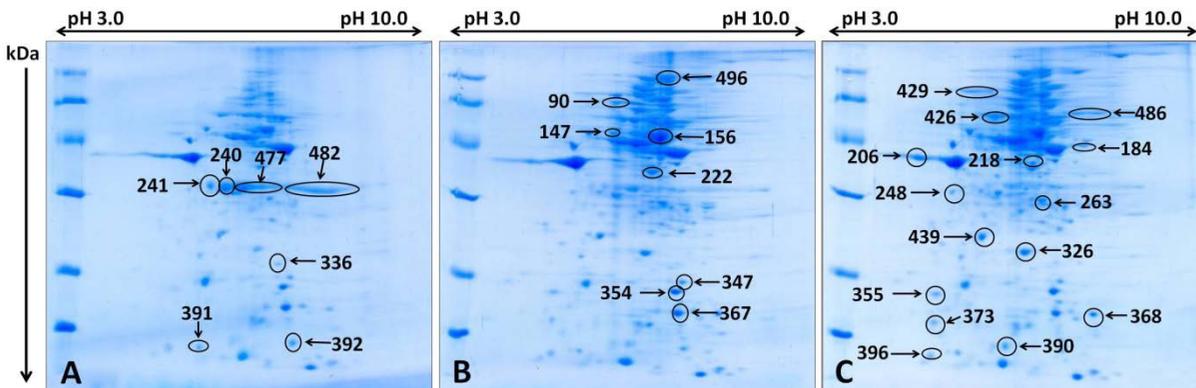
Fig. 1. Ezee blue stained SDS PAGE gel after IEF showing different protein spots expressed by *Pseudomonas fluorescens* (NBAII-PFDWD) at different osmotic potentials, A; 0 MPa, B; -10.26 MPa and C; -26.82 MPa. The spots selected for MALDI-TOF are circled in black.

amended with PEG 6000 to attain different osmotic potentials ( ) of 0 MPa, -10.28 MPa and -26.82 MPa concentrations. The pH of the media was adjusted to 7.2 ± 2. The cultures were incubated at 28 ± 2 °C under constant shaking.

Extraction of bacterial proteins

The bacterial cells were collected by centrifugation (10,000 g for 10 min) and washed three times with isotonic solution. The proteins were precipitated according to method described by Paul *et al*. (2006). The protein extracts were frozen until further use.

2D Gel analysis for stress-related proteins

The first dimension was carried out at Genei TM Merck Biosciences, Bangalore, India as per standardized protocol. Gel mixture was prepared containing 10.8 M urea, 30% acrylamide, 20 μl of 100 % Triton-X, 130 μl ampholyte pH (3–10), 3 μl TEMED and APS (10%). The 1D was carried out in EttanIPGphor3 (GE Health care). The gel mixture was poured in tube by using (18-gauge needle) syringe up to 15 cm without any air bubble (remaining 1 cm for sample loading space). Once the gel was set the casted gel tube was fixed to the tank with rubber grommet very tightly (sample loading space of tube facing toward the top reservoir). The reservoir top was filled with 1X cathode buffer (400 mM NaOH) and bottom reservoir with 1X anode buffer (100 mM H_3PO_4). The gel was pre-focused by adding 1–3 μl of 2D SLB [9.8 M urea, 2% NP-40 (10% in distilled water), 2% carrier ampholytes 8/10, 25 mM DTT] into sample loading space and power cords were connected to the power supply. Electrophoretic conditions of the rod gels during the IEF (pre-run) were 200 V for 15 min, 300 V for 30 min, and 400 V for 1 h. After IEF (pre-run), 350 μg of sample was loaded and gel was run at 400 V for 16 h. After 16 h, the gels were transferred to a small tray containing 2D equilibration buffer [6 M urea,75 mM Tris–HCl (pH 8.8), 29.3% glycerol, 2% SDS, 0.002% bromophenol blue] and left for 30 min.

The second dimension was carried out on 12.5% SDS PAGE. The equilibrated gel was placed on 1% agarose added on the top of the polymerized SDS-gel. Electrophoresis was carried out at 150 V in Genei™ Vertical Maxi Gel system. Protein spots were visualized using Ezee blue gel stainer (Genei™) and the well-resolved spots were numbered. The Mr of individual protein spots was determined by comparison with molecular weight markers. Spot detection was carried out using the software, Progenesis (Nonlinear Dynamics available at www.nonlinear.com). The two-dimensional gels of *P. fluorescens* (NBAII- PFDWD) grown in all three osmotic conditions were compared using this software.

Protein identification

Selected proteins were subjected to in-gel proteolytic digestion by excising 1-mm-thick gel and washing for 30 min at room temperature under vigorous shaking with 400 μl of 10 mM ammonium bicarbonate solution containing 50% (v/v) acetonitrile. The gel pieces were dried for 15 min in a vacuum concentrator. The rehydrated gel pieces were incubated in 150 μl reduction solution (10 mM DTT, 100 mM ammonium bicarbonate) for 30 min at 56°C. The reduction solution was then discarded and 100 μl alkylation solution (50 mM iodoacetamide, 100 mM ammonium bicarbonate) was added for 30 min in dark at room temperature. The gel pieces were digested in 5 μl trypsin solution (sequencing grade modified trypsin, Promega, Madison and 10 ng/μl in 5 mM ammonium bicarbonate/5% acetonitrile). The samples were incubated for 5 h at 37°C. The reaction was stopped by adding 1 μl of 1 % trifluoroacetic acid. For better extraction of peptides, the samples were stored overnight at 5 °C.

MALDI-TOF MS Identification of Protein

The analysis was carried out in MALDI-TOF mass spectrometer (Bruker-Daltonics, Germany) at Merck Biosciences, Bangalore, India. The 1 μl of concentrated peptides from the supernatant was mixed with 2 μl of matrix solution [5 mg a-cyano-4-hydroxycinnamic acid in 40 % (v/v) acetone, 50 % (v/v) acetonitrile, 9.9 % (v/v) water, and 0.1 % (w/v) TFA in water]. From this mix, 1 μl was deposited onto the MALDI target. Tryptic peptides were analyzed in positive mode. Background ions from trypsin autolysis and contamination by keratins were removed from mass lists. The proteins were analysed using MASCOT search engine in latest version of the NCBInr database with following relevant search parameters viz., monoisotopic mass accuracy, peptide mass tolerance (0.1 Da), peptide charge state, allowed variable modifications, oxidation of methionine and fixed modification, carbamidomethylation of cysteine. Fragmentation of selected peptides was measured using the PSD mode. The percentage similarity of amino acids, Mr and pI were taken into consideration for identification of the proteins from bacteria of related species/genera.

RESULTS AND DISCUSSION

Capability of *P. fluorescens* (NBAII- PFDWD) to survive osmotic stress is well addressed in the protein profiling technique. The stress response of *P. fluorescens* (NBAII-PFDWD) subjected to osmotic stress at different osmotic potential using PEG 6000 revealed wide array of proteins produced by translational modification. Overall more than 300 proteins were expressed in all the three growth conditions. Thirty distinctive spots were selected based on Prolinear software from protein profile gels of all the three growth conditions subjected *viz.*, 0 MPa, -10.28 MPa and -26.82 MPa (Fig. 1) and identified using MALDI-TOF MS. The proteins were abundant under osmotic stress conditions, -10.28 MPa and -26.82 MPa when compared to the normal conditions. Seven spots were detected from protein gel of 0 MPa which were down regulated under osmotic stress (Fig. 1). Eight proteins and 15 proteins which were up regulated under -10.28 MPa and -26.82 MPa were selected for identification respectively. The fold increase of all these proteins are tabulated (Table 1). Comparison of proteins revealed that the expression levels of bifunctional pantoate ligase/cytidylate kinase OS decreased under -10.28 MPa and -26.82 MPa induced osmotic stress. L-ectoine synthase expression was higher under -10.28 MPa induced osmotic stress, however, its expression was almost on par under normal conditions and -26.82 MPa induced stress.

The spot details obtained from Progenesis software clearly distinguishes spots corresponding to transcription elongation factor GreA OS and fructose-1,6-bisphosphatase class 2 OS were up regulated under both cases of osmotic stress. Their expression under normal condition was barely visible in gel. In both osmotic stress conditions, exodeoxyribonuclease 7 small subunit OS was down regulated. Comparison among all the three gels showed that, arginine deaminase OS, DNA replication and repair protein RecF OS, phosphate acyltransferase OS and DNA repair protein RecO OS showed no significant difference in their expression under normal and osmotic stress conditions (Fig. 2, 3). It was noticed that the 11 proteins selected from both osmotic stress conditions were on par except four identified as transcription elongation factor GreA OS, 60 kDa chaperonin OS, DNA mismatch repair protein MutL OS and Gamma-glutamyl phosphate reductase OS which showed slight increase under -26.82 MPa induced osmotic stress.

In order to survive under moisture stress, *Pseudomonas fluorescens* (NBAII-PFDWD) produced an array of proteins which helped them mitigate the stress conditions. Most of the proteins shown to over express under moisture condition by *P. fluorescens* (NBAII- PFDWD) are not reported so far. The current study is novel and has provided an initial platform to explore microbial interaction under stressed ecosystem. Literature studies reveal that all these genes are expressed as stress response in different microorganisms. 2-isopropylamalate synthase was expressed in osmotic stress subjected in *Campylobacter jejuni* and it belongs to transferases involved in L-leucine and pyruvate metabolism (Cameron *et al.*, 2012). RlmN is associated with catalysis of m2A sythesis with both rRNA nd tRNA. The translation accuracy decreased with inactivation of RlmN in *E.coli* (Benitez- Paez and Villarrayo, 2012). This function reduces the chances of misreading of translational reading frame and further balance bacterial phenotype which could impair under stress conditions. Many genes are expressed for bacterial growth under a stable environment however, its expression varies when the bacteria challenges a distressed milieu. Ribosome profiling provides an insight to its role in expression of bacterial proteins under environmental fluctuations (Starosta *et al.*, 2014). Expression of 30s ribosomal proteins were found to be down regulated in carnocyclin treated *Listeria monocytogenes* (Liu *et al.,* 2014). DNA repair mechanisms play a pivotal role in altering stressed genes for a normal functioning of a cell. Exodeoxyribonuclease 7 small subunit plays important role in DNA repair by avoiding mutation occurring under environment instability (Lau *et al*., 2011; Lovett, 2011). DNA binding protein modulate biochemical functions, DNA binding and ferroxidase activity to protect cells from damage due to various stresses when in exponential growth and majorly when they enter stationary phase (Karas *et al.*, 2015). No

functional activity was reported for bifunctional pantoate ligase/cytidylate kinase OS under stress conditions. Hence precise regulation of these proteins is important under adverse conditions.
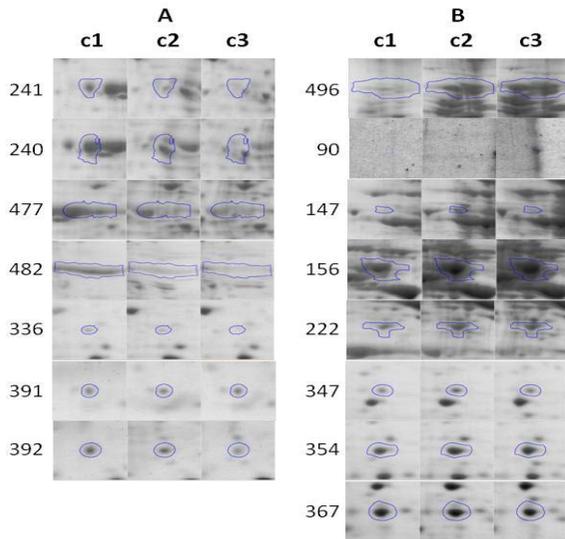
Fig. 2. A, Spots which are expressed under normal growth conditions (c1) analysed using Progenesis Linear Dynamics Software. Spots 241-392 are shown to be down regulated under conditions c2 (-10.28MPa) and c3 (-26.82 MPa). B, differentially expressed genes under c2.

Stress involved proteins expression levels switch either by down regulating or up regulating. Eight spots were selected from gel profile of -10.28 MPa induced osmotic stress and fifteen from -26.82 MPa induced osmotic stress, all of which were differentially regulated under stress (Fig. 2, 3). Dihydrorotate dehydrogenase (quinone) OS and arginine deiminase OS were expressed in higher folds under stress and dihydroorotate dehydrogenase (quinone) is involved in pyrimidine biosynthesis however, no function in stress response was observed (Jensen and Larsen, 2003; Kawasaki *et al*., 2009). Arginine deiminase stimulates the growth of *Pseudomonas* spp. and reverses the adverse effects in acidic environment by serving as energy source (Marquis *et al*., 1987). Ammonia derived from arginine deiminase breakdown reduced the effect of pH stress in *Staphylococcus epidermidis* (Lindgren *et al*., 2014). Ribosome-recycling factor OS, L-ectoine synthase, N-acetylmannosamine kinase OS and phosphate acyltransferase OS were over expressed under this stress. Ribosome recycling is closely associated with protein synthesis which is required for bacterial growth. In *E. coli* it was shown that this gene is involve in dissociation of ribosome from mRNA after translation and in turn facilitates next cycle of protein synthesis (Borovinskaya *et al*., 2007; Li *et al*., 2012). Increase or decrease in cytoplasmic solutes is primary response to osmotic swing on bacteria. Ectoines belong to an array of compatible solutes produced majorly by halophilic bacteria and are in-

volved in protein stabilization to prevent cell damage under osmotic or temperature stresses (Louis and Galinski, 1997; Kuhlmann *et al*., 2011). In addition ectoines serve as carbon and nitrogen source or as intracellular reserves (Pastor *et al*., 2010). Enzymes catalyse metabolic reaction in cell biosynthesis, N-acetylmannosamine kinases catalyse cell biosynthesis and cellular response to DNA damages stimulus. No reports are found with respect to expression of these stress proteins in *P. fluorescens* (NBAII-PFDWD). Acyltransferase utilizes fatty acid to form membrane phospholipids and further helps in membrane expansion. They regulate membrane biogenesis and cell growth. Notable function under stress is not known (Zhang and Rock, 2008). DNA replication is realm of bacterial survival. Osmotic stress induces perturbations in replication cycle. RecF influence the repair mechanism in responsible damage and replication fork stress in *E. coli* and *B. subtilis* (Lenhart *et al*., 2014; Villarrayo *et al*., 1998). Bacteria regulate membrane biogenesis of coordinate cell growth by synthesis of fatty acid. RecO are recombinant mediator protein involved in restarting of stalled replication in ssDNA gaps repair and in repairing dsDNA breaks. It is assisted in rapid repair of chromosome breaks in *Deinococcus radiodurans* under radiation and desiccation stress (Lusetti and Cox, 2002; Ryzhikov *et al*., 2011). Surprisingly, in the present study DNA replication and repair protein RecF OS and DNA repair protein RecO OS were found to be repressed under osmotic stress.
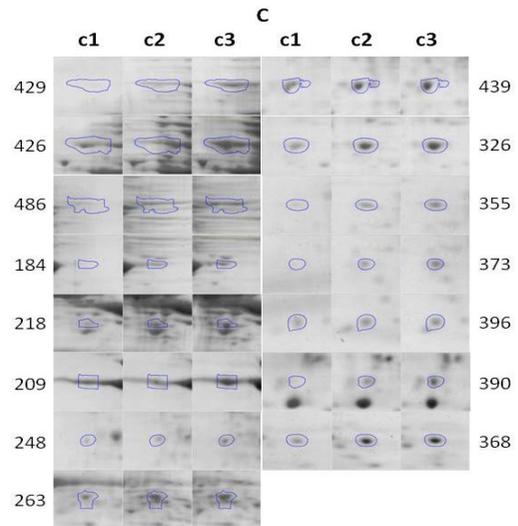
Fig. 3. Differentially expressed genes as analysed using Progenesis Linear software under c3 (-26.82 MPa).

A slight variation in osmotic potential of media prompted expression of proteins to express at higher folds. Nine proteins were up regulated when only 50% water availability was provided in media. GreA cleaves complexes formed at site of transcriptional arrest and resumes the transcriptional process additionally and they are up regualted in environmen-

tal perturbations (Kusuya *et al.,* 2011; Li *et al.*, 2012). The genes which were up regulated were transcription elongation factor GreA OS, elongation factor Ts OS, crossover junction endodeoxyribonuclease RuvC OS, crossover junction endodeoxyribonuclease RuvC OS, pantothenate synthetase OS, 60 kDa chaperonin OS, Gamma-glutamyl phosphate reductase OS, transcriptional repressor NrdR OS, Leu operon leader peptide OS, fructose-1,6-bisphosphatase class 2 OS, bifunctional protein GlmU OS and NAD(P)H-quinone oxidoreductase subunit O OS. RuV is involved in repair of DNA facing damage due to mutations. They encode endonucleases activities which resolve Holliday structure during recombination (Takahagi *et al.*, 1991). No significant function was reported in stress mechanism however its major function lies in protein biosynthesis. 60 KDa chaperonin or commonly referred *E. coli* GroEL is generally exhibited under temperature stress and is involved in unfolding the misfolded protein to its natural state (Maguire *et al.*, 2002; Mande and Kumar, 2013). Gamma glutamyl phosphate reductase catalyses metabolic reaction of a major osmoprotectant proline encoded by ProA. Artificial bifunctional enzyme gamma-GK/gamma GPR expression in *E.coli* increased production of proline and osmotolerance of host (Chen *et al.*, 2006; Liang *et al*. 2013). It is also associated with production of small amount of glutathione which is linked to proline biosynthesis under oxidative stress (Perez-Arellano *et al.*, 2010). NrdR controls the transcription of genes by binding to promoter region. This regulatory protein controls expression of ribonucleoside reductases which are prescursors of DNA synthesis and repair during vegetative growth. These are important for aerobic growth of *E. coli* (Torrents *et al.,* 2007). Fructose 1, 6-biphosphate class 2 OS play central function in glycolysis. Macomber *et al*. (2011) reported that the cellular over expression of fructose 1,6-biphosphate in *E .coli* reduced inhibitory effects of nickel. GlmU is a precursor for bacterial cell wall synthesis components like lipopolysaccharides and peptidoglycan. Inhibition of GlmU adversly affects cell viability (Larsen *et al*., 2012). Hence, this has been utilized as target for antibiotic drug discovery (Mochalkin *et al.*, 2007). EF-Ts serves as the guanine nucleotide exchange factor for EF-Tu which is associated in response to nutrient deprivation and protect from stress (Caldas *et al.*, 1998). NADPH- quinone oxidoreductase subunit is induced to protect cell from oxidative stress along with many other detoxifying enzymes (Atia *et al*., 2014). In *E. coli* they adapt and overcome a strong imbalance in NADPH redox state (Auriol *et al*., 2011). Down regulation of three proteins (V-type proton ATPase, Ribosome nuclease II, and Putative ATP-dependent Clp protease proteolytic subunit-like OS) at -26.82 MPa osmotic potential was observed in our study. V-type proton ATPase subunit is a membrane subunit whose major role is not much elucidated. Their function ranges from protein sorting to membrane trafficking. They drive the active transport systems in membrane. These V-type ATPases restrict the influx of external solutes so as to reduce risk of membrane integrity loss (Beyenback and Wiezoerek, 2006; Cohen, 2014). Ribosome nuclease II functions as exoribonucleolytic which is important for cell response to stress conditions. In *E. coli* activity of ribonuclease II was reduced under conditions of slow growth (Song *et al.,* 2016). Similar case was observed in *P. fluorescens* (NBAII- PFDWD). RNA polymerases transcribe few genes to up regulate when bacteria reach its stationary phase and when its regular process shut downs (Lau *et al.*, 2011). Clp mediates proteolysis of misfolded proteins subsequent to translation (Schelin *et al.,* 2002). They exhibit adaptive response in *B. subtilis* against stationary phase phenomena like sporulation (Msadek *et al.*, 1998). DNA repair was active through other set of genes like MutL. MutL belonging to mismatch repair system suppress chromosomal aberration by correcting mismatch bases during replication error occurring under various stress conditions (Harris *et al*., 1997) and helps in maintaining genetic stability (Oliver *et al.*, 2002). Unlike other methyl directed mismatch proteins, MutL was functional even in stationary phase of *E. Coli* (Feng *et al*., 1996). In studies using *P. syringae* it was shown that osmotolerance was influenced by synthesis of N-acetylglutaminylglutamine amide (NAGGN) that causes reduction of cellular glutamine indicating lower glutamine synthetase activity thereby causing glutamate accumulation (Freeman *et al.*, 2013). In our studies up regulation of Gamma-glutamyl phosphate reductase OS was seen and this protein is involved in synthesizes of L-glutamate 5-semialdehyde from L-glutamate. Literature on stress response proteins expressed by a PGPR like *P. fluorescens* (NBAII-PFDWD) is very limited. The study has shown that *P. fluorescens* expressed a wide array of stress response genes which ameliorate its survival in environmental perturbations. The stress response genes identified are complex and *P. fluorescens* does possess unique pathways to survive under osmotic stress. The culture is being promoted as a useful biological control agent in stressed soils. Application of stress tolerant strains of microorganisms could be considered as a feasible choice in stress prone areas (Meena *et al.*, 2017).

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