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An ABC Transporter Protein from Pseudomonas pseudoalcaligenes Involved in Improved Plant Growth under Salt Stress

Dhara Patel* and Meenu Saraf

Department of Microbiology and Biotechnology, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India

ABSTRACT

Bacterial isolate Pseudomonas pseudoalcaligenes was isolated from the saline areas of Gujarat. This isolate showed efficient production of PGPR attributes like phosphate solubilization, Siderophore production and IAA production under salinity stress both in vivo and in vitro. Field studies revealed that they have the potential to increase vegetative growth of Jatropha curcas plant under salinity stress, and increase the soil fertility. To understand the mechanism of salt tolerance, proteome analysis of Pseudomonas was carried out using SDS PAGE. Salt stress proteins associated with cell membrane were analyzed by MALDI-TOF. This technique was used to investigate the regulation of gene product expression of P. pseudoalcaligenes grown under high osmolarity. Peptide mass fingerprinting and in silico investigation was used to identify proteins with altered expression. Among them one newly induced protein was assigned to protein with known function. Bioinformatics tools were then employed to identify the protein to understand the proteomics of salt stress in the bacteria. The protein showed high homology to the enzyme, ABC transporter, and iron.B12.siderophore. hemin, ATP-binding component.

Keywords: Proteome analysis, *Pseudomonas pseudoalcaligenes*, Salt stress proteins

INTRODUCTION

Microbial population and their activities are greatly affected by the change in the osmotic concentration of the medium. Osmotic pressure retards bacterial activity by changing the nature of cell cytoplasm. Microbes have developed diverse resistance strategies towards toxic environments. Under high salt concentration conditions many gram negative bacteria from hypersaline soils have been reported to have increased synthesis and accumulation of glutamine synthetase and glutamate synthetase [1]. Microbes also synthesize non enzymatic antioxidants such as glutathione, tocopherol, ascorbate etc. Bacterial cells may also show high accumulation of osmoprotectants like betaine, glycine betaine and proline in presence of osmotic shock as a result of high salt concentration in the environment [2].

Accumulation of excess Na^+ may cause metabolic disturbances in processes where low Na^+ ansd high K^+ or Ca^{+2} are required for optimum function [3]. A decrease in nitrate reductase activity, inhibition of photosystem II and chlorophyll breakdown are all associated with increased Na^+ concentration. Cell membrane function may be compromised as a result of Na^+ replacing Ca^+ resulting in cell leakiness. Uptake and accumulation of Cl^- may disrupt photosynthetic function through the inhibition of nitrate reductase activity [4]. Once the capacity of cells to store salts is exhausted, salts build up in the intercellular space leading to cell dehydration and death [5].

It has been observed that plants inoculated with bacteria having different PGPR traits are more resistant to deleterious effect of stress ethylene synthesized as a consequence of stress conditions [6]. In addition to facilitating plant growth, PGPR can protect plants from deleterious effects of environmental stresses including flooding, drought, salinity and phytopathogens [7].Bacteria are able to adapt to a certain range of changes in external osmolarity. One of its adaptations to counterbalance this osmotic difference is accumulation of low molecular weight hydrophilic molecules, which do not interfere with cell metabolism. Bacteria also initiate a program of gene expression in response to osmotic stress by high NaCl concentrations, which are manifested as a set of proteins produced in increased amounts in response to the stress [8]. There are large numbers of specific proteins reported in various genera of bacteria that showed increase in their level of expression, upon adverse conditions such as heat, salt and nutrient limitations.

In a post-genomic era, proteomics is one of the best strategies used to reveal the dynamic expressions of whole

proteins in cells and their interactions. The term "proteome" is used here to describe the complex state of an organism under defined conditions rather than its complete protein repertoire. Due to its high resolution, PAGE, combined with high throughput mass spectrometry and bioinformatics, is widely used for protein separation and identification, which is considered sufficiently discriminating to allow the unique identification of unknown proteins [9]. Identification of differently displayed proteins could be used to ascertain the genes responding to relative physiological actions, and clarify the functions of genes.

Here, selected PGPR isolated from the saline soil of Gujarat was adapted to 500 mM NaCl concentration and was able to solubilize phosphate, produce siderophore and indole acetic acid under saline stress. These isolate also proved to be the best PGPR for *Jatropha* plants by reducing salt uptake and accumulation in plants and thereby enhancing plant growth. The present study describes the proteins that are induced or repressed in *P. pseudoalcaligens* upon salt stress and by using SDS PAGE separation followed by matrix-assisted laser desorption ionization time-of-flight/mass spectrometry (MALDI-TOF/MS) to generate a distinctive peptide mass fingerprint. The whole experiment was done in duplicate and the results verified. Bioinformatics tools were employed to identify the proteins to understand the proteomics of salt stress in the bacterium.

MATERIALS AND METHODS

Culture collection and influence of different NaCl concentration on growth of PGPR

Isolate *Pseudomonas pseudoalcaligens* (accession number GU564407) was procured from the departmental laboratory. The tolerance of isolate to salinity was tested by growing the strains on Nutrient broth supplemented with increasing concentration of NaCl from 300-1000 mM. Salt tolerant strain was maintained on nutrient agar supplemented with 500 mM and then were further studied for their PGPR potential [10].

Influence of 500 mm NaCl concentration on growth profile of PGPR

All the bacterial cultures were maintained and activated in nutrient broth. NaCl was added to 100 ml growth medium so as to give a final concentration of 500 mm NaCl in the medium. 1% inoculum of actively growing culture suspension was added and incubated at 37 ºC on shaker. Bacterial growth was measured after every 2 hour at 540 nm. Mean growth rate constant (K) was calculated using the formula: K=3.322 (logZt – logZ0)/Dt; where Z0 and Zt are the initial and final cell populations, while Dt is difference in culture time [11].

PGP attributes under salinity stress

Phosphate solubilization by PGPR under salinity stress: Phosphate solubilization was quantified in liquid Pikovskyaya's medium containing 500 mM NaCl in flasks for 21 days. Inoculate 1 ml of culture suspension of *Pseudomonas pseudoalcaligens* in respective flasks. The concentration of the soluble phosphate in the supernatant was estimated at every third day by the Stannous Chloride ($SnCl_2$. $2H_2O$) method [12]. A simultaneous change in the pH was also recorded in the supernatant by a Systronics digital pH meter.

Siderophore production by PGPR under salinity stress: The siderophore production was determined by performing the chrome azurol S (CAS) assay [13]. Quantitative estimation was carried out by inoculating 1 ml of actively growing isolate in 50 ml of deferrated succinic acid medium containing 500 mM NaCl in 250-ml Erlenmeyer flasks. After 24 h of incubation, supernatant was collected and tested for pH, fluorescence, and hydroxymate type of siderophore production [14].

Indole acetic acid production by PGPR under salinity stress: Indole acetic acid production was studied by growing isolate in glycerol-peptone broth containing 500 mM NaCl and tryptophan (500 mg ml^{-1}) and incubated at 28°C. Fully grown cultures were centrifuged at 3,000 rpm for 30 min. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml Salkowsky's reagent (50 ml, 35% perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink color was measured at 536 nm by a spectrophotometer [15].

Seed bacterization and growth promotion of Jatropha under salinity stress: Pot study was carried out in triplicates on *Jatropha* under saline condition. Seeds were surface sterilized by soaking in 0.02% sodium hypochlorite for 2 min. and washed five times with sterilized distilled water. Seeds were coated with 1% carboxymethylcellulose as adhesive. Then seeds were treated with active culture of isolate for 30 min [16]. After germination 5 seeds were planted in the pots supplemented with saline solution for three consecutive days as according to Tank & Saraf, [17] so as to maintain the salinity level at 300 mM. Two sets of control were kept, one was negative control which was uninoculated without any stress and bacteria and one was positive control which was uninoculated and contains salt stress. After 60 days all

plants were carefully uprooted and its various vegetative parameters like root length, shoot length, number of leaves, chlorophyll content, fresh weight, dry weight were studied [18].

Statistical analyses

To evaluate the efficiency of rhizobacteria in pot experiments under saline conditions, a completely randomized block design was used. To identify significant treatment, analysis of variance (ANOVA) was carried out. Mean values were compared at significance levels of 1% and 5%. The ANOVA indicated significances of treatment and effects.

Estimation of protein under salinity stress

Protein estimation was carried out by inoculating 1 ml of actively growing culture of isolate in respective 100 ml nutrient broth containing 0 mM, 200 mM, 400 mM and 500 mM NaCl and incubated at 37ºC on rotary shaker. After every 24 h samples were withdrawn from each flask and were estimated by the method of Folin's Lowry [19].

SDS PAGE for salt stress proteins (SSPs)

Mid-log- phase culture (50 µl) of *Pseudomonas pseudoalcaligens* was inoculated on to two flasks containing 100 ml of minimal medium (MM). Upon mid-log-phase growth at 28ºC, to one of the flasks, solid NaCl was added to a final concentration of 500 mM in the medium. One hour after addition of NaCl the cells were pelleted at 5,000 × g for 10 min, washed thrice with isotonic solution and pelleted again. The pellets were suspended in 50 mM Tris-HCl buffer (pH 7.0). The cells were sonicated in ice bath using an ultrasonic probe with amplitude of 35% at 50 W with 9 sec pulses and 9 sec off mode for 10 min. The suspension was vortexed well and incubated at -20ºC for 1 h. The crude cell lysate was centrifuge at 11,500 x g for 10 min. The supernatant was transferred to a fresh tube and the soluble proteins were precipitated with two volumes acetone. The precipitation was done at -20ºC for overnight. The precipitated protein was pelleted at 11,500 x g for 10 min. The pellet was resuspended in in 0.3 ml of 0.1 M phosphate buffer, pH 7.0 and 20 µl of the suspension was resolved in a 12% polyacrylamide gel. After silver staining, the gel was observed under a transilluminator [20].

Studies on SSPS associated extracellular of Pseudomonas pseudoalcaligens

Mid-log- phase culture (50 µl) of *Pseudomonas pseudoalcaligens* was inoculated on to two flasks containing 100 ml of minimal medium (MM). The salt shock induction was performed as described earlier. The cells were pelleted at 5,000 × g for 10 min, the supernatant was transferred to a fresh tube and the soluble proteins were precipitated with two volumes acetone. The precipitation was done at -20ºC for overnight. The precipitated protein was pelleted at 11,500 × g for 10 min. The pellet was resuspended in 0.3 ml of 0.1 M Phosphate buffer, pH 7.0 and 20 µl of the suspension was resolved in a 12% polyacrylamide gel. After silver staining, the gel was observed under a transilluminator [20].

MALDI-TOF MS

Individual proteins were excized from the gel. Gel pieces were stored in 1% glacial acetic acid. Trypsin digestion of the respective gel piece were carried out and analyzed in MS mode MALDI TOF instrument (Molecular Biophysics Unit, Indian Institute of Science, Bangalore).

Protein identification by peptide mass fingerprinting (PMF)

The peptides mass spectrum of each protein was analyzed and identified with database matching through http://www.matrixsciences.com. All searches were performed using a mass window between 1 and 100 kDa. The search parameters allowed for oxidation of methionine and carbamidomethylation of cysteine. The percentage similarity of aminoacids, Mr and pI were taken into consideration for identification of the proteins from bacteria of related species/genera [21].

RESULTS

Influence of different NaCl concentration and growth profile study under salinity stress

Maximum growth of isolate was found to be at 300 mM which was higher than that observed in absence of NaCl but after that as the concentration of NaCl increases there was continuous decline in the growth. The minimum inhibitory concentration of NaCl was 300 mM whereas 1000 mM could be considered as lethal concentration (LD) of NaCl for isolate beyond which no growth was observed (Figure 1).This reduction in growth is due to the NaCl toxicity above 300 mM concentration to many bacteria. As the concentration increased the growth of bacteria was found to decrease. Increase in salt concentration outside the cell membrane increases osmotic potential by creating hyper osmotic pressure.

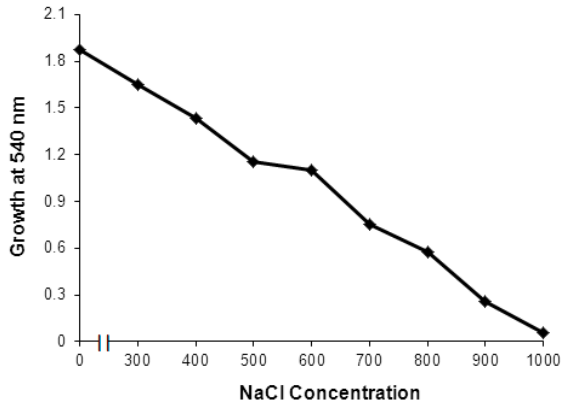

Figure 1: Growth of *Pseudomonas pseudoalcaligenes* at different NaCl concentration.

Pseudomonas pseudoalcaligens was grown at 500 mM NaCl concentration. *K* value of *Pseudomonas pseudoalcaligens* was 0.87 ± 0.04 in single-species cultures. When grown under salinity stress, *K* value of *Pseudomonas pseudoalcaligens* was 0.69 ± 0.06 (Figure 2). This shows that growth profile was decreased under salinity stress as compared to growth profile in absence of NaCl. Maximum growth was observed after 24 h of growth then after growth was decreased.

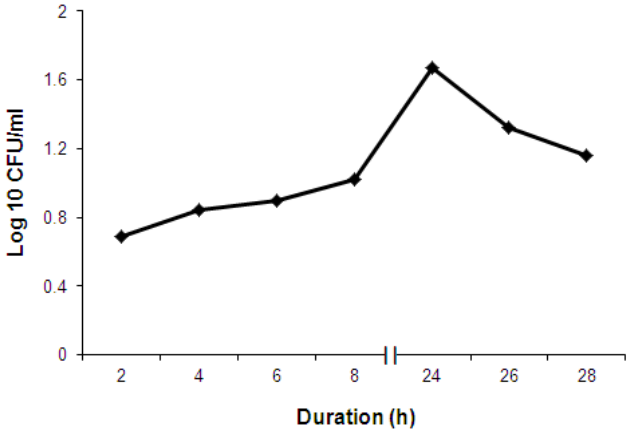

Figure 2: Growth profile study of *Pseudomonas pseudoalcaligenes* under 500 mM NaCl concentration

PGP attributes under salinity stress

Phosphate solubilization under salinity stress: Isolate showed phosphate solubilization on Pikovskaya's medium containing 500 mM NaCl. Maximum solubilization was observed after 7 days of incubation. Isolate showed phosphate solubilization in liquid medium 16.9 µg/ml after 7 days of incubation (Figure 3) pH was also observed to be decreased after 7 days of incubation from 7.2 to 4.16.

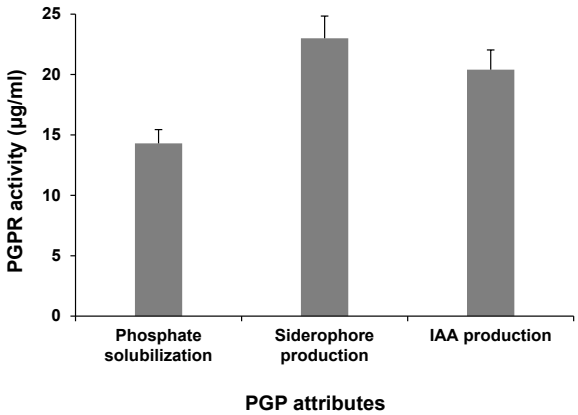

Figure 3: PGPR potential of *Pseudomonas pseudoalcaligenes* under salinity stress

Siderophore production under salinity stress: Quantitative analysis observed to produce hydroxymate type of siderophore. Isolate showed siderophore production 23 µg/ml after 26 hours of incubation under salinity stress (Figure 3). This isolate showed no significant difference in siderophore production in absence and presence of NaCl.

IAA production under salinity stress: Indole acetic acid production was estimated in isolate in tryptone yeast medium under salinity stress. IAA production was observed in isolate (18 µg/ml) after 96 hours of incubation under salinity stress (Figure 3).

Growth promotion of Jatropha under salinity conditions: Comparisons between all the vegetative parameters were made between normal soil (control -ve), saline soil (control +ve), and saline soil inoculated with *Pseudomonas pseudoalcaligens*. Plants grown in saline soil showed a reduction in root length to about 23.80% of that of *Jatropha* plants grown in normal soil. A decrease of 28.57% was observed in the shoot length of plants in soil treated with NaCl compared to plants in untreated soil. Plants grown in the presence of NaCl without treatment with PGPR showed an almost 76.19% reduction in root length and about a 47.62% reduction in shoot length in comparison to *Jatropha* plants grown in the presence of isolate. A reduction was also observed in the number of leaves and lateral roots. Increase in the number of leaves (36.36%) was observed in plants treated with *Pseudomonas pseudoalcaligens*. Treated plants also showed an increase in the number of branches (33.3%) compared to untreated control. In treated plants, fresh weight and dry weight were higher than uninoculated NaCl added plants. Plants treated with *Pseudomonas pseudoalcaligens* also showed increase in fresh weight and dry weight of shoot compared to untreated controls with and without salinity stress. The increase in chlorophyll content (Chla and Chlb)in treated plants (55.1% and 147.1%) at 60 DAS as compared to control with added stress. While control without salinity stress and PGPR also showed increase in chlorophyll content (Chla and Chl b) was found (7.18% and 26.42%) at 60 DAS, which was much less than all the three treatments (Table 1).

Table 1: Influence of PGPR treatments on vegetative parameters of *Jatropha* plant in presence of 300 mM NaCl. C -ve control plants without added stress and PGPR; C +ve control plants with added salinity but without PGPR Values are the mean of triplicates. ns nonsignificant * Significant at 5% LSD; ** significant at 1% level of LSD as compared to control

Vegetative parameter	Isolate	Control +ve	Control –ve
No. of leaves	15 ± 0.24 ^{ns}	11 ± 0.12 *	11 ± 0.14 *
No. of branches	12 ± 0.18 ^{ns}	9 ± 0.07 ^{ns}	10 ± 0.12 *
No. of lateral roots	50 ± 1.35 *	35 ± 1.15 ^{ns}	41 ± 1.39 ^{ns}
Root length (cm)	18.5 ± 1.04 ^{ns}	10.5 ± 1.14 ^{ns}	13 ± 1.06 ^{ns}
Shoot length (cm)	31 ± 1.24 ^{ns}	21 ± 1.26 *	27 ± 1.22 *
Fresh weight of root (g)	1.50 ± 0.02 ^{ns}	1.04 ± 0.04 ^{ns}	1.45 ± 0.03 ^{ns}
Dry weight of root (g)	0.60 ± 0.01 **	0.42 ± 0.01 ^{ns}	0.59 ± 0.02 ^{ns}
Fresh weight of shoot (g)	19.21 ± 1.29 ^{ns}	10.92 ± 1.41 ^{ns}	12.21 ± 1.38 ^{ns}
Dry weight of shoot (g)	7.61 ± 0.45 ^{ns}	4.47 ± 0.42 ^{ns}	5.11 ± 0.29 *
Chlorophyll a (mg/g wt)	0.52 ± 0.03 *	0.33 ± 0.01 ^{ns}	0.36 ± 0.01 ^{ns}
Chlorophyll b (mg/g wt)	0.35 ± 0.02 ^{ns}	0.14 ± 0.008 ^{ns}	0.17 ± 0.01 ^{ns}

Estimation of protein content in PGPR at different NaCl concentration

Pseudomonas pseudoalcaligens showed that protein content was maximum after 48 h of growth after that decrease in protein content was observed. *Pseudomonas pseudoalcaligens* showed maximum protein content 80 µg/ml at 200 mM concentration after 48 h of growth. However, isolate showed protein content 62 µg/ml at 0 mM NaCl concentration (Figure 4). This showed that protein content in adapted culture was increased in the presence of salinity stress compared to the original isolate.

SDS PAGE for salt stress proteins

Protein profile of the normal and induced *Pseudomonas pseudoalcaligens* isolate showed that there was one newly induced protein, eight over expressed and two repressed proteins (Figure 5). These induced and repressed proteins need to be further characterized for understanding their role in salt adaptation.

Salt stress proteins associated with cell membrane of bacteria

Protein profile of salt stress proteins (SSPs) associated with cell membrane of normal and induced bacterial cells showed three repressed proteins in *Pseudomonas pseudoalcaligens* isolate (Figure 6).

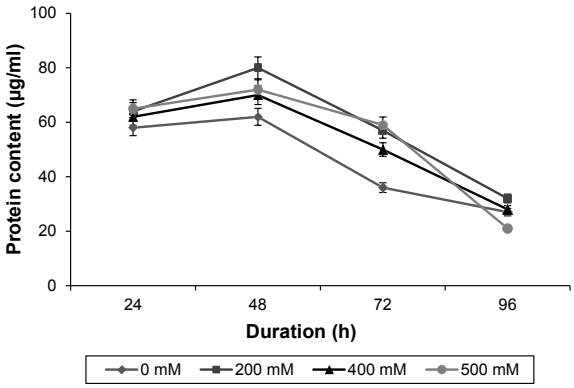

Figure 4: Estimation of protein content in MSC isolate at different NaCl concentration.

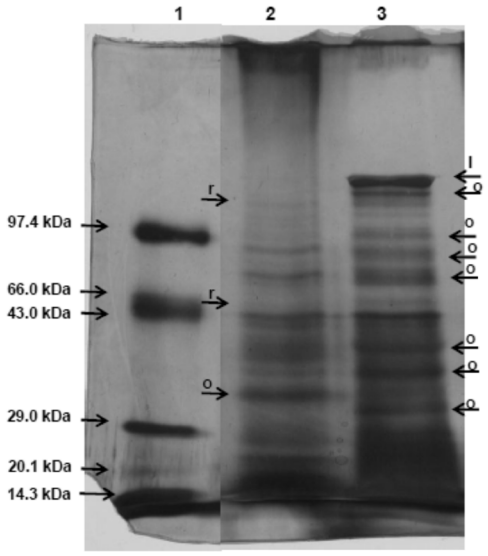

Figure 5: Profile of salt stress proteins in normal and induced *Pseudomonas pseudoalcaligenes* (MSC). Lane 1: marker, lane 2: normal cells, lane 3: cells stressed with 500 mM of NaCl. 'I' represents newly induced protein 'r' represents repressed protein and 'o' represents over-expressed protein.

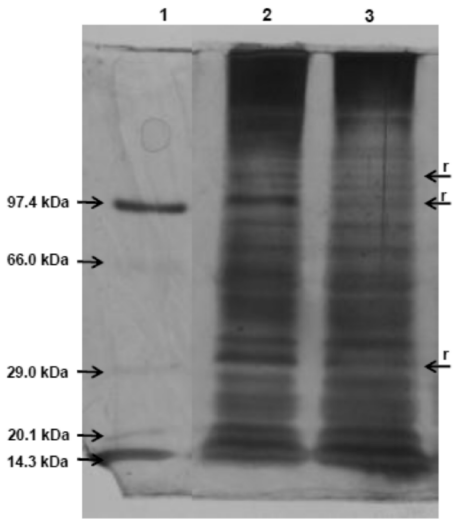

Figure 6: Profile of salt stress proteins in the cell membrane of normal and induced *Pseudomonas pseudoalcaligenes*. Lane 1: marker, lane 2: normal cells, lane 3: cells stressed with 500 mM of NaCl. 'I' represents newly induced protein 'r' represents repressed protein and 'o' represents over-expressed protein.

Studies on proteome analysis

The present study showed significant changes in the protein patterns in the SDS PAGE profiles of salt-stressed and normal cells. The newly induced protein band of *Pseudomonas pseudoalcaligens* was analyzed by MALDI-TOF analyzer (Figure 7). With peptide mass fingerprinting and using bioinformatics tools, function was assigned to one newly induced protein. The protein showed high homology to the enzyme, ABC transporter, iron. B12. siderophore. hemin, ATP-binding component (Table 2).

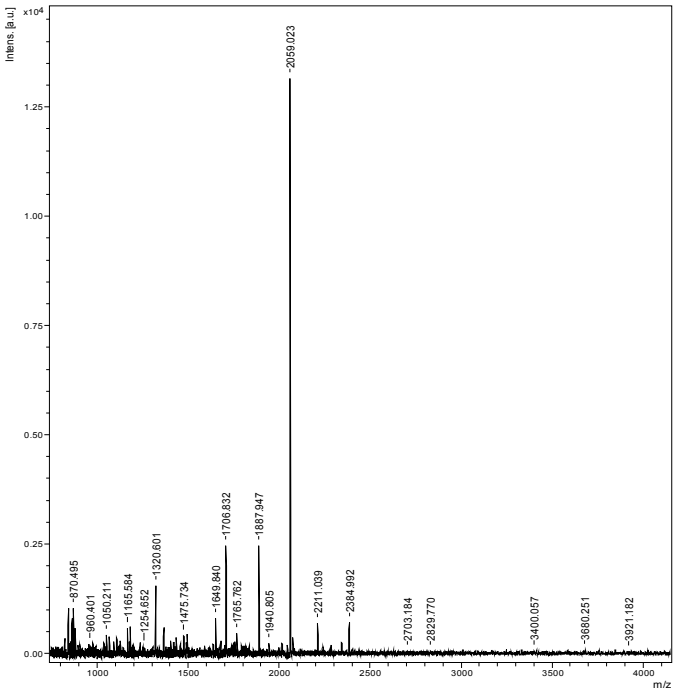

Figure 7: Intensity and m/z value of newly induced protein of *Pseudomonas pseudoalcaligenes* upon osmotic shock at late logarithmic phase of growth.

Table 2: Peptide mass fingerprinting of *Pseudomonas pseudoalcaligenes* newly induced protein upon 500 mM osmotic shock at late logarithmic phase of growth**.**

SI No.	NCBI ACC No.	Functional category	A Coverage (%)	pI	Mr	B Peptides matched	Start-end positions	Peptide sequence of matched fragment
1	gi|358070980	ABC transporter, iron.B12.siderophore. hemin, ATP-binding component-Burkholderia cenocepacia (strain H111).	34	6.36	28.4	6	01-Nov	MTHMLDIERVR
							54-75	YARPDAGRVALD TQDVWRMRPR
							109-149	RPFDAESADDR RFASLSGGEKQR
							176-185	HQLELLARVR
							208-215	LHVLAHGR
							244-258	HPVTGRPRITPLHPE

DISCUSSION

Present study revealed that the reduction in growth is due to the NaCl toxicity above 300 mM concentration to the selected isolate. As the salt concentration increased the growth of bacteria was found to decrease. Increase in salt concentration outside the cell membrane increases osmotic potential by creating hyper osmotic pressure. In this condition cells must maintain an outward oriented cytoplasmic pressure which is driving force for cell growth [1]. High level of salt tolerance might be due to the high salt concentration in soil from where the PGPR were isolated. Growth profile of the isolate was also found to be decreased under salinity stress as compared to growth profile in absence of NaCl. Maximum growth was observed after 24 h of growth then after growth was decreased. Phosphate solubilization and IAA production efficiency of the isolate decreases in the presence of NaCl. However, isolate showed no significant difference in siderophore production in absence and presence of NaCl.

Similarly, results were obtained with *Streptomyces i*solate C showing good solubilization of TCP in culture medium

without NaCl with 92 µg/ml. Solubilization decreased in presence of NaCl [22]. On the other hand, results showed that bacteria like *Bacillus subtilis* and *Bacillus megaterium* isolated from the saline belt of the Purna River Basin were able to solubilize phosphate and release a minute quantity of acid that reduced the salinity of the soil by neutralization [23]. However, a varied phosphate solubilization potential among the *Streptomyces* isolates. They reported that the requirement of NaCl for better solubilization of TCP was observed in all *Streptomyces*. Also salt concentration more than 0.2% decreased soluble phosphate content [24]. Similarly, *Pseudomonas putida* produced siderophore and induced systemic resistance in watermelon against gummy stem rot, whereas the siderophore-negative mutants failed to induce resistance [25]. Tank & Saraf, [17] show that PGPRs which are able to produce siderophore and IAA in salt condition promote growth of tomato plants under 2% NaCl stress. Similarly, it was reported that isolates PGB4, PGT1, PGT2, PGT3, PGG1 and PGG2 induce the production of indole acetic acid under salinity stress [26].

In the present study isolate *P. pseudoalcaligens* showed substantial increase in all the vegetative growth parameter in comparison to control with presence and absence of salinity stress. Substantial increase in root length may be responsible for increase in nutrient uptake. The seeds treated with *Pseudomonas pseudoalcaligens* showed maximum growth of roots and shoots, resulting in growth promotion and higher yields. Similarly, enhanced root and shoot growth of lettuce were obtained when inoculated with *Bacillus* spp. under dry salt stress conditions [27]. Munns, [28] mentioned that suppression of plant growth under saline stress may be due to the decreasing availability of water or toxicity of high salt concentrations. Increase in the shoot length, fresh weight, and number of leaves per pea plant were obtained [6]. This result is in accordance with our results where we observed an increase in root length, shoot length, number of leaves, and lateral root count of *Jatropha curcas* when inoculated with *Pseudomonas pseudoalcaligens* under salt stress. An increase in chlorophyll content in all plants treated with PGPR under saline stress has also been reported [29]. PGPR under salinity stress are able to enhance the production of IAA and solubilization of phosphorus, thereby improving root length, shoot length, and dry weight of roots and shoots of *C. arietinum* L. plants [26]. Similarly, application to chickpea of bioinoculants with phosphate solubilization and siderophore production potential yielded increases in all vegetative growth parameters under saline conditions [30].

There are various reports on the induced proteins in gram negative and gram positive bacteria upon salt stress [31]. In accordance with in this present study *P. pseudoalcaligens* showed newly induced, over expressed and repressed proteins under salinity stress. There has also been reported a down regulation of proteins in response to salt stress [32]. Proteins induced by stress have been shown to play an essential role in bacterial physiology. For example, GroEL, GroES, DnaK and DnaJ of *E. coli* are well known for their roles in protein folding as molecular chaperones [33]. Protein profile of the normal and induced *Pseudomonas pseudoalcaligens* MSP-538 isolate showed that there is one newly induced protein, six over repressed and five repressed proteins. These induced and repressed proteins need to be further characterized for understanding its role in salt adaptation. Similarly, reported one newly induced protein, one repressed and six over expressed protein in *Pseudomonas pseudoalcaligens* MSP-538 [20]. Because of its location and components, the cytoplasmic membrane has been traditionally suggested to sense environmental changes through certain proteins that expand into periplasm to interact with stress [34]. The primary response of bacteria to osmotic upshifts involves the activation of transporters in the cell membrane, which are mechanosensitive of osmoprotectants, and sensor kinases, to increase the transport and/or biosynthetic capacity for these solutes [35]. There are specific reports on modification in the membrane proteins due to salt shock. The omp F and omp C proteins are membrane proteins that determine permeability of the outer membranes. Omp F expression has been reported to be repressed by high osmolarity and omp C, repressed [36].

With peptide mass fingerprinting and using bioinformatics tools, function was assigned to one newly induced protein. The protein showed high homology to the enzyme, ABC transporter, iron. B12. siderophore.hemin, ATP-binding component. Our previous studies showed that this strain produced siderophore upon exposure to 500 mm of NaCl. The results clearly demonstrated that the expression of protein from a functional category is modulated in *Pseudomonas pseudoalcaligens* at high osmolarity. This stress-alleviation protein must be playing a major role in helping the bacterium to maintain its metabolism unaltered considerably, thus delivering the plant growth promoting properties in saline soils. Similarly, changes in protein pattern in two-dimensional PAGE profile of salt stressed and normal cells of *P. fluorescens* MSP-393 was also reported [21]. Peptide mass fingerprinting and bioinformatics tools function was assigned to 13 induced proteins and two repressed proteins. Significant modulation (approximately 32 proteins) in protein expression under hyper osmotic conditions (3.5% to 6.5% NaCl concentration) was first visualized in preparative 2DE gels in *Listeria monocytogenes* [37].

CONCLUSION

Salt tolerance is an inevitable property for a PGPR aimed for use in saline agricultural soils. Understanding the mechanism of osmoadaptation in rhizobacteria will contribute to the long-term goal of enhancing plant-microbe interaction for the improvement of crops grown in coastal agricultural niche. The selected PGPR in this study was able to tolerate NaCl concentration upto 1000 mM. This PGPR were adapted to 500 mM NaCl concentration and were able to solubilize phosphate, produce siderophore and indole acetic acid under saline stress. Isolate *Pseudomonas pseudoalcaligens* proved to be the best PGPR once again for *Jatropha* plants by reducing salt uptake and accumulation in plants and thereby enhancing plant growth. Protein concentration in *Pseudomonas pseudoalcaligens* increases as NaCl concentration increases. The present study revealed the accumulation of various osmolytes in the bacterium upon increasing salinity. Extracellular protein profile of normal and induced bacterial cells showed one newly induced protein, eight over expressed protein and two repressed protein. Intracellular protein profile showed three repressed protein. The experimental evidence also demonstrated the expression of protein from a number of functional category in *Pseudomonas pseudoalcaligens* at high osmolarity. These stress alleviation proteins must be playing a major role in helping the bacteria to maintain its metabolism unaltered considerably, thus delivering the plant growth promoting properties in saline soils. Plant growth promotion by rhizobacteria has been reported to be by the production of plant growth regulators, suppression of deleterious organisms and promotion of the availability and uptake of mineral nutrients. It could be ascertained that the osmotolerance mechanisms of salt stress proteins effectively nullified the detrimental effects of high osmolarity.

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