BVG Trust

J. Sci. Trans. Environ. Technov. 2017, 11(2) : 59-68

Scientific Transactions in Environment and Technovation

Screening of plant growth promoting potential of salt tolerant rhizobacteria isolated from halophytes of Thiruvanmiyur beach, Tamil Nadu.

P. Deepthy and P.Nirmala*

https://doi.org/10.56343/STET.116.011.002.001

http://stetjournals.com

PG and Research Department of Microbiology, Mohamed Sathak College of Arts and Science, Sholinganallur, Chennai.

Abstract

The use of soil rhizosphere bacteria possessing the traits of plant growth promotion under saline stress is becoming prevalent worldwide to achieve sustainable agriculture along with soil reclamation through phytoremediation as well as bioremediation. A detailed study of the natural population in the rhizosphere of three halophytes grown in saline soils was carried out to isolate and identify salt tolerant bacteria that could express plant growth promotion (PGP) traits at high salt concentrations. In the present study, 15 bacteria were isolated from the rhizosphere of halophytes grown in beach soils. Majority of the bacterial isolates were identified as *Vibrio* sp, *Bacillus* sp and *Pseudomonas* sp based on biochemical and morphological observations. The pH and electrical conductivity of the soil were found to be 8 and 6.7 dS m^{-1} respectively. Out of the 15 bacteria isolated from saline rhizospheres, six isolates showed tolerance to high salt concentration (12.5% NaCl). The potential salt tolerant bacterial isolates were further analyzed for the sodium uptake pattern which showed successive increase in the amount of sodium uptake pattern from 0.1M concentration up to 3M concentration of NaCl. The selected PGPR in this study were adapted to 12.5% NaCl stress and were able to produce IAA, ammonia, HCN, amylase and protease. They also exhibited the ability of phosphate solubilization and nitrogen fixation which would help plants to sequester inorganic phosphate available in the soil and to fix atmospheric nitrogen. The data represented here is quite encouraging to use these PGPR for enhancing plant growth under saline stress conditions..

Key words: Plant Growth Promoting Rhizobacteria, Halophytes, NaCl, Rhizosphere

Received : January 2017 Revised and Accepted : October 2017

INTRODUCTION

World population is growing at an alarming rate and is anticipated to reach about six billion by the end of year 2050. On the other hand, agriculture productivity is not increasing at a required rate to keep up with the food demand. The reasons for this are water shortage, depleting soil fertility and mainly various abiotic stresses.

Soil salinity is one of the most significant abiotic stresses among adverse environmental factors that affect the agricultural sector (Yadav *et al*., 2011). It can be described as high ion concentration in the soil solution that restricts plant growth, due to high osmotic potential of the solution that inhibits plant water uptake. The saline soil is considered when the electrical conductivity (EC) of the soil solution is over 4 dS m^{-1} (equivalent to 40 mM NaCl) (El-Swaify, 2000). The detrimental effects of salinity on plant growth can be divided into three broad categories: a) reduction in the soil osmotic potential thus reducing the amount of water available to plants, b) specific sodium ion toxicity, and c) inhibition of nutrient uptake resulting in nutrient imbalance (Ahl and Omer, 2011), together influencing plant growth and yield, which makes it necessity to look for alternate strategies that help plant to mitigate saline stress. Special effort is necessary for the maintenance of crop production under salinity stress and for the expansion of area under irrigation (Batlle- Sales, 2010).

Most of the rhizosphere microorganisms have been reported to have imparted some degree of tolerance to plants that are growing under abiotic stresses like temperature, water and osmotic stress (Grover *et al*., 2011). One of the recently gaining practices of counteracting the adverse effects of salinity on plant growth includes the implementation of salt tolerant bacteria which exhibit natural plant growth promoting ability in such conditions. Selection of microbial isolates from naturally stressed environments or rhizosphere is considered as possible measures for improving crop health which can control diseases and also promote plant growth.

Coastal ecosystem is one of the least studied marine ecosystems, which has fluctuating pH, salinity,

*Corresponding Author :
email: *nirmala_dineshkumar@yahoo.com*

P - ISSN 0973 - 9157
E - ISSN 2393 - 9249

temperature and physical structures. Plants of the coastal ecosystems are categorized into halophytes. This small group of plants consists only of few plant species which can grow in extremely unfavourable conditions, e.g.: salt marshes, estuaries, cliffs, dunes or deserts (Gago *et al*., 2011). Microorganisms associated with halophytes may contribute to the promotion of plant growth under stress conditions. However, reports on the exploration of bacteria associated with halophyes and their beneficial activities are very scarce despite of their potential.

Plant growth promoting rhizobacteria (PGPR) are the potent agents which can imply 'immunity' to the host plant species through active colonization over the plant roots and increase plant growth and yield (Heidari *et al*., 2011) through direct or indirect mechanisms (Verma *et al*., 2010). The mechanisms of plant growth promotion by PGPR include: the ability to produce phytohormones (Egamberdiyeva, 2007), nitrogen fixation (Salantur *et al*., 2006), antagonism against phytopathogens (Ahmed *et al*., 2008) and solubilization of insoluble phosphates (Bulgarelli *et al*., 2013). It is also suggested that PGPR can also prevent deleterious effects of stresses from the environment (Paul and Nair, 2008). Many plant growth promoting rhizobacteria (PGPR) isolated from saline environment are known for providing protection to the plants under saline condition (Upadhyay *et al.,* 2011, 2012).

The bacteria obtained from saline environment include *Flavobacterium, Azospirillum, Alcaligenes, Acinetobacterium, Pseudomonas* (Reinhold *et al.,* 1987; Ilyas *et al.,* 2012) *Sporosarcina, Planococcus* (Ventosa *et al.,* 1983), *Bacillus* (Upadhyay *et al.,* 2009) *Thalasobacillus, Halomonas*, *Brevibacterium, Oceanobacillus, Terribacillus, Enterobacter, Halobacillus, Staphylococcus* and *Virgibacillus* (Roohi *et al.,* 2012). Thus it becomes obvious that the saline environments too have bacterial diversity which could potentially colonize the diverse microhabitats and play vital role in the plant and soil health. Keeping these points in view investigation was made to isolate salt tolerant bacteria from the rhizosphere of halophytes, characterize them both morphologically and biochemically and to screen the isolates to determine the plant growth promoting and salt tolerant traits, and the results are discussed in this article.

MATERIALS AND METHODS

Collection of Rhizosphere Soil Samples

The roots of the halophytes which were grown wild in Thiruvanmiyur beach, Chennai, India were collected in sterile polythene bags in replicates from 0-15cm depth in three different locations. Rhizosphere soil samples were obtained by separating soil from plants roots (similar soil depth), focusing on a soil layer not thicker than 2 mm from the roots surface. The rhizospheric soil samples of each plant were brought to the laboratory and stored at 4°C until further investigation. The roots of the three halophytes collected were identified and labeled.

Isolation of Rhizosphere Bacteria from Soil Sample

Rhizospheric bacteria were isolated from 1 g soil tightly adhering to the root by serial dilution plating on Luria–Bertani (LB) agar plates as described by Somasegaran and Hoben (1994). Individual, distinct colonies were isolated from the incubated plates and repeatedly plated onto a fresh nutrient agar to obtain pure colonies and maintained in slant at 4°C for further use.

Iidentification of Bacterial isolates based on Morphological and Biochemical Characteristics

The pure isolates obtained from the serial dilution technique were identified based on the standard morphological and biochemical properties according to Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). Pure cultures of the isolates were streaked on to Nutrient, Mac conkey and Blood agar plates respectively. The individual colonies were then examined for shape, colour, texture, opacity and mucosity. Gram reactions and motility of all the isolates were recorded.

Further identification of the isolates was carried out based on the biochemical properties such as, Indole, Methyl Red, Voges-Proskauer, Citrate utilization, Catalase, Oxidase, Urease, Triple Sugar Iron agar, Mannitol and fermentation of Sugars (Glucose, Lactose, Dextrose, Maltose).

Screening of Bacterial isolates for the highest degree of salt tolerance using NaCl. (Damodaran *et al*., 2013)

The salinity tolerance of the isolated bacteria was determined by amending the basal medium with NaCl in different concentrations. Nutrient agar was supplemented with 0.5%, 5%, 7.5%, 10%, 12.5% and 15% of NaCl which served as the selective medium. A loopful of overnight broth cultures was streaked on to the nutrient agar plates amended with different concentrations of sodium chloride. The plates were incubated at 28 ± 2°C for 24 - 48 hours. After incubation, the plates were observed for the growth of bacteria. The appearance of colonies up to the highest concentration level of sodium chloride incorporated in the medium indicates the level of salt tolerance of the isolates.

Sodium uptake pattern of potential salt tolerant bacterial isolates (Damodaran *et al*., 2013)

Isolates growing luxuriantly in 12.5% NaCl were screened for sodium uptake pattern in Luria Bertani

broth containing different NaCl concentration (0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 M). The broth was inoculated with overnight cultures and incubated at 37°C for 24 hours. After 24 hours, the broth was centrifuged and the supernatant was discarded. The cell pellet was harvested and washed with sterile distilled water. The pellet was digested with 0.1 N HCl overnight at room temperature. The samples were centrifuged and the supernatant was collected for further analysis of sodium uptake pattern. The sodium uptake by bacterial cells was estimated using Flame photometer.

In vitro analysis for the plant growth promoting traits of potential salt tolerant bacterial isolates

***Production of Indole Acetic Acid* (IAA) (Brick *et al.*, 1991)**

Indole acetic acid production was detected in nutrient broth. The nutrient broth was inoculated with overnight broth cultures and incubated at 36 ± 2°C for 72 hours. Fully grown cultures were centrifuged at 3000 rpm for 30 min. 2ml of the supernatant was mixed with 2 drops of ortho phosphoric acid and 4ml of Salkowski reagent (50ml of 35% of perchloric acid plus 1ml of 0.5M $FeCl_3$ solution). The reaction was allowed to proceed until adequate color was developed. Development of pink color indicates the production of Indole acetic acid.

Phosphate Solubilization (Priyanthi *et al.*, 2007)

Phosphate solubilization of isolates was evaluated from the ability of the organism to solubilize inorganic phosphate. In this assay, Sperber's medium containing calcium phosphate as the inorganic form of phosphate was used. A loopful of overnight broth culture was streaked on to the plates. The plates were incubated at 28 ± 2°C for 24-48 hrs. After incubation, the plates were observed for the zone of clearance around the colonies indicating the phosphate solubilizing ability of the isolates.

Production of Ammonia (Cappucino and Sherman, 1992)

The strains were tested for ammonia production. The production of ammonia was tested in peptone water. 100µl inoculum of overnight broth culture was inoculated in 10ml peptone water and incubated at 30°C for 48–72 h. After incubation, 0.5 ml of Nessler's reagent was added to each tube. The tubes were observed for any prominent colour change. Development of brown to yellow color was recorded as a positive result for ammonia production.

Production of Hydrocyanic acid (HCN) (Lorck, 1948)

Nutrient agar plates were amended with 4.4 g glycine per liter. Point inoculation with overnight broth cultures was done on modified agar plate. A sterile Whatman No. 1 filter paper was soaked in 2% sodium carbonate and 0.5% picric acid solution was placed on to the surface of the agar. The plates were sealed with parafilm in order to maintain the gaseous metabolites and the reaction was allowed to take place. The plates were incubated at 28 ± 2°C for 4 to 7 days. The plates were observed for visible colour change of the filter paper, and the results were interpreted based on the colour change which was as tabulated below:

S. No.	Color change	Interpretation
1	No change	No HCN production
2	Yellow to brown	Weak HCN production
3	Yellow to brownish orange	Moderate HCN production
4	Yellow to complete orange	Strong HCN production

Nitrogen Fixation by the bacterial isolates (Priyanthi *et al.*, 2007)

The ability of the bacteria to fix nitrogen under free living conditions was tested on Norris-N free medium. A loopful of bacterial culture was streaked on to the plates and incubated at 28°C for 24-48 hrs. After incubation, the plates were observed for growth of the colonies which indicates the ability of the organism to fix nitrogen under free living conditions.

Enzyme production

Production of Amylase

The isolates were streaked on the starch agar plates and incubated at 28 ± 2°C for 24-48 hrs. After incubation, the plates were flooded with iodine solution. The plates were observed for the presence of clear zone around the colonies which indicates the ability of the organism to hydrolyze starch present in the medium.

Production of Protease

The isolates were streaked on the skim milk agar plates and incubated at 28 ± 2°C for 24-48 hrs. After incubation, the plates were observed for the zone of clearance around the colonies. Production of halo zone indicated the ability of the organism to hydrolyze casein present in the medium.

RESULTS

The rhizosphere soil samples of three halophytes were collected. The three halophytes were identified as *Wedilia biflora*, *Crinum asiaticum,* and *Ipomoea biloba* (Fig. 1). The pH and electrical conductivity of the soil sample was known to be 8 and 6.7 dS m^{-1} respectively.

a) Crinum asiaticum

b) Wedilia biflora

c) Ipomoea biloba

Fig. 4. Identification of three Halophytes

Isolation and identification of Bacterial isolates

A total of fifteen isolates were obtained in this study. Among the fifteen isolates, two were isolated from the sample collected from *Wedilia biflora,* and designated as WB. Eight were obtained from *Crinum asiaticum* and were designated as CA. Five isolates were obtained from *Ipomoea biloba* and designated as IB.

Fifteen bacterial isolates were identified based on staining reaction, morphological and biochemical characteristics according to the Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1993).

The fifteen bacterial isolates were identified as *Achromobacter sp, Pseudomonas sp* 1 and 2, *Vibrio sp* 1,2,3,4 and 5, *Bacillus sp* 1,2,3,4 and 5, *Staphylococcus sp* and *Photobacterium sp.* (Table 1 and 2).

Screening of bacterial isolates for the highest degree of salt tolerance using NaCl

The salt tolerance ability of each bacterial isolate was tested at different NaCl concentration. All the 15 isolates grew luxuriously at 0.5% NaCl concentration. 80% of the isolates could tolerate 5% NaCl and 73% of the isolates tolerated 7.5% NaCl concentration. 66% of the isolates were tolerant to 10% NaCl concentration. 40% of the isolates were found tolerant up to 12.5% NaCl concentration. None of the isolates was tolerant to 15% NaCl concentration, (Table 3).

Hence in the current study, 6 isolates which showed maximum NaCl tolerance (up to 12.5%) were subjected to further investigation. The isolates were found to be

Table.1. Identification of Bacterial isolates based on Morphological characteristics on different agar plates

Bacterial isolates	Nutrient agar	Macconkey agar	Blood agar
IB1	Off white, rough, raised, no pigmentation	Non lactose fermenting	Non haemolytic
IB2	Off white, round colonies, low convex.	Non lactose fermenting	Heamolytic
CA1	Orange, translucent, smooth,	lactose fermenting	Non haemolytic
IB3	Off white, translucent, smooth,	lactose fermenting	Non haemolytic
CA2	Off white, rough, raised, no pigmentation	lactose fermenting	Heamolytic
IB4	Orange, translucent, smooth,	lactose fermenting	Non haemolytic
CA3	Yellow, spherical, opaque, raised colonies	lactose fermenting	Non haemolytic
CA4	Off white, translucent, smooth	lactose fermenting	Non haemolytic
WB2	Off white, translucent, smooth	lactose fermenting	Non haemolytic
CA5	Off white	lactose fermenting	Non haemolytic
CA6	Off white, round colonies, low convex.	Non lactose fermenting	Non haemolytic
IB5	Off white, rough, raised, no pigmentation	lactose fermenting	No growth
WB1	Off white, rough, raised, no pigmentation	Non lactose fermenting	Non haemolytic
CA7	Off white, rough, raised, no pigmentation	lactose fermenting	Heamolytic
CA8	Off white, rough, raised, no pigmentation	Non lactose fermenting	Partially Heamolytic

Table.2. Identification of Bacterial isolates based on Biochemical Test

BACTERIAL ISOLATES	IB1	IB2	IB3	IB4	IB5	CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	WB1
GRAM STAINING	-	-	-	-	-	-	-	+	-	-	-	+	-	-
SHAPE	Short rod	Thin slender	Short rod	Rod	Rods in short chains	Minute Rods	Rods in chains	Cocci	Short rod	Thin slender long rods	Rods	Rod	Rod	Slender Rod
INDOLE	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	+	+	+	-	-	+	+	-	+	+	-	-	-	-
CITRATE	+	-	-	-	-	-	-	-	-	-	+	-	-	-
UREASE	-	+	-	-	-	-	-	-	-	-	-	+	-	-
TSI	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	A/K
MANITOL MOTILITY	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	+/+	-/-	-/+	+/+	-/+	-/-
GLUCOSE	+	+	+	+	Partially Positive	+	+	Partially Positive	+	Partially Positive	+	+	+	Partially Positive
LACTOSE	-	Partially positive	Partially positive	Partially positive	+	Partially positive	+	Partially Positive	+	Partially Positive	+	Partially Positive	+	Partially Positive
DEXTROSE	-	+	+	+	Partially Positive	+	Partially positive	+	+	Partially Positive	+	+	+	Partially Positive
MALTOSE	+	+	+	+	Partially Positive	+	+	Partially Positive	+	Partially Positive	+	+	+	Partially Positive
CATALASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OXIDASE	-	+	+	+	+	-	+	+	+	+	-	+	+	+
IDENTIFICATION	*Achromobacter sp*	*Pseudomonas sp 1*	*Vibrio sp 1*	*Vibrio sp 2*	*Bacillus sp 1*	*Vibrio sp 3*	*Bacillus sp 2*	*Staphylococcus sp*	*Vibrio sp 4*	*Photobacterium sp*	*Pseudomonas sp 2*	*Bacillus sp 3*	*Bacillus sp 4*	*Bacillus sp 5*

+' Indicates Positive
-' Indicates Negative
A/A Indicates Acid by Acid
A/K Indicates Acid by Alkaline

P - ISSN 0973 - 9157
E - ISSN 2393 - 9249
October to December 2017
Scientific Transactions in Environment and Technovation
www.stetjournals.com

Table.3. Screening of bacterial isolates for the highest degree of salt tolerance using NaCl

BACTERIAL ISOLATES	NaCl concentration					
	0.50%	5%	7.50%	10%	12.50%	15%
Achromobacter sp	++++	-	-	-	-	-
Pseudomonas sp 1	++++	+++	+++	+	-	-
Vibrio sp 3	++++	+++	+++	+	-	-
Vibrio sp 1	++++	+++	+++	+	-	-
Bacillus sp 2	++++	-	-	-	-	-
Vibrio sp 2	++++	+++	+++	+	+	-
Staphylococcus sp	++++	+++	+++	+	+	-
Vibrio sp 4	++++	+++	+++	+	+	-
Vibrio sp 5	++++	-	-	-	-	-
Photobacterium sp	++++	+++	+++	-	-	-
Pseudomonas sp 2	++++	+++	+++	+	+	-
Bacillus sp 3	++++	+++	-	-	-	-
Bacillus sp 5	++++	+++	+++	+	-	-
Bacillus sp 1	++++	+++	+++	+	+	-
Bacillus sp 4	++++	++++	++++	+++	+++	-

++++ Luxuriant growth **+++ Moderate growth**
+ Less Growth **- No Growth**

Bacillus sp 1 and 4, *Vibrio* sp 2, and 4, *Staphylococcus* sp and *Pseudomonas* sp 2.

Sodium uptake pattern of potential salt tolerant bacterial isolates

All the six bacterial isolates showed the increased amount of sodium uptake pattern from 0.1M to 3M concentration of NaCl. Among the six bacterial isolates, two isolates namely *Bacillus* sp 1 and *Vibrio* sp 2 showed maximum sodium uptake of 3M concentration of NaCl (96.3 ppm and 105 ppm) respectively. The bacterial isolate *Bacillus* sp 4 showed least amount of sodium uptake (46.4 ppm) at 3M NaCl concentration (Table 4).

Table.4. Sodium uptake pattern of potential saly tolerant bacterial isolates

Bacterial Isolates	NaCl concentration (ppm)						
	0.1M	0.5M	1M	1.5M	2M	2.5M	3M
Bacillus sp 1	32.1	42.2	56.4	72.7	76	84.2	96
Bacillus sp 4	10.1	18.2	21.6	29.8	35	37.2	46
Pseudomonas sp 2	31.2	36.1	37.1	40	43	49.7	51
Vibrio sp 4	6.2	8.3	20.1	21	21	40.4	55
Staphylococcus sp	11.5	15.8	16.5	45.6	53	60.4	85
Vibrio sp 2	31.7	33	54.5	56.5	67	70.6	105

M Molar concentration
PPM Parts Per Million

In vitro analysis for the plant growth promoting traits of potential salt tolerant bacterial isolates

The plant growth promoting traits such as IAA production, HCN production, Ammonia production, Phosphate solubilization, Nitrogen fixtion and enzyme production were analysed (Table 5).

All the six isolates, *Bacillus* sp 1 and 4, *Vibrio* sp 2, *Vibrio 2* and 4, *Staphylococcus* sp *and Pseudomonas* sp 2 were considered as positive for IAA production. 100% of isolates were positive for IAA production.

Only three isolates, *Bacillus* sp 1, *Vibrio* sp 2 and 4 solubilized insoluble inorganic phosphate present in the medium after 48 hours of incubation. Hence, 50% of isolates were considered as positive for phosphate solubilization.

Among the six salt tolerant strains, only four isolates (67%) namely *Bacillus* sp 1 and 4, *Pseudomonas* sp 2, and *Vibrio* sp 2 showed the development of brown colour on addition of Nessler's reagent. 67% of isolates were positive for ammonia production.

Three out of six isolates namely, *Bacillus* sp 1, *Staphylococcus* sp and *Vibrio* sp 2 showed prominent

color change from yellow to complete orange. Hence, 50% isolates were considered as positive for HCN production.

Three out of six isolates were grown on Norris N free agar medium. The isolates were namely, *Bacillus* sp 4, *Pseudomonas* sp 2 and *Vibrio* sp 2. Hence, 50% of the salt tolerant isolates were able to fix atmospheric nitrogen.

Four out of six isolates were found to hydrolyze starch present in the medium by producing the enzyme amylase. The following isolates such as *Bacillus* sp 1 and 4, *Pseudomonas* sp 2, and *Staphylococcus* sp were positive for amylase production. Approximately 67% of the isolates were considered positive for amylase production.

Table.5. *In vitro* analysis for the plant growth promoting traits of potential salt tolerant bacterial isolates

CULTURE NUMBER	IAA PRODUCTION	HCN PRODUCTION	AMMONIA PRODUCTION	PHOSPHATE SOLUBILIZATION	NITROGEN FIXATION	AMYLASE PRODUCTION	PROTEASE PRODUCTION
Bacillus sp1	+	+	+	+	-	Hydrolysis	+
Bacillus sp4	+	-	+	-	+	Partial hydrolysis	-
Pseudomonas sp2	+	-	+	-	+	Hydrolysis	+
Vibrio sp 4	+	-	-	+	-	No hydrolysis	+
Staphylococcus sp	+	+	-	-	-	Partial hydrolysis	+
Vibrio sp 2	+	+	+	+	+	No hydrolysis	-

+ Production of the growth promoting substance
- No production of the growth promoting substance

DISCUSSION

A detailed study of the natural population in the rhizosphere of three halophytes grown in saline soils was carried out. In the present study, salt tolerant bacterial species were isolated, identified and screened for the expression of plant growth promoting (PGP) traits at high salt concentrations. Our study established that the bacterial diversity was reduced with increase in soil pH under natural selection sites. It has been reported earlier that soil salinity plays a prominent role in the microbial selection process as environmental stress leads to reduce bacterial diversity (Borneman *et al.,* 1996). In our study, three halophytic plants which were grown wild were selected for the collection of rhizospheric soil samples in order to isolate salt tolerant organisms. Earlier studies also reported that grasses (Damodharan *et al.,* 2013) and weeds (Naz *et al.,* 2009) were selected for the isolation of salt tolerant organisms.

In our study, 15 isolates were obtained from natural selection in the rhizosphere of halophytes grown in beach soils. The bacterial isolates identified were *Vibrio* sp, *Bacillus* sp and *Pseudomonas* sp based on the morphological and biochemical observations. Earlier studies showed that the genera such as *Bacillus* and *Pseudomonas* tend to be predominant in saline soils (Tank and Saraf, 2010; Damodharan *et al.,* 2013). Earlier studies reported that bacteria obtained from saline environment include *Flavobacterium*, *Azospirillum*, *Alcaligenes*, *Acinetobacter*, *Pseudomonas* (Ilyas *et al.,* 2012); *Sporosarcina*, *Planococcus*, *Bacillus* (Upadhyay *et al.,* 2009), *Thalasobacillus*, *Halomonas*, *Brevidobacterium*, *Enterobacter*, *Halobacillus*, *Staphylococcus* and *Virgibacillus* (Roohi *et al.,* 2012).

In our study, the pH and electrical conductivity of the soil were 8 and 6.7 dS m^{-1}. Hence, the soil was saline in nature. The soil in general is said to be saline, if the conductivity exceeds 4 dS m^{-1} or the concentration of salts is higher than 0.1% (Juniper and Abbot, 1993).

Out of the 15 bacteria isolated from saline rhizospheres, six isolates showed tolerance to high salt concentration (12.5% NaCl). The isolates were *Bacillus* sp 1, *Bacillus* sp 4, *Vibrio* sp 2, *Vibrio* sp 4, *Staphylococcus* sp *and Pseudomonas* sp 2. As the concentration of NaCl increases, there was a constant decrease in the number of organisms. Our results are in line with the results of other researchers (Tank and Saraf, 2010) who reported that the bacterial strains isolated from tomato rhizosphere could tolerate 8% of NaCl stress. Hence, LD_{95} of NaCl for all PGPR was 8%. Kataoka *et al., 2017* also suggested that there was down regulation of growth under successive increase of NaCl concentration. The isolates, *Exiguobacterium* sp and *Serratia* sp had maximum tolerance up to 10% NaCl concentration. Deshwal and Kumar (2013) also suggested that all *Pseudomonas* strains tolerated 1.25% NaCl in medium. It is also suggested that 23 *Bacillus* isolates were grown at 10% NaCl.

The potential salt tolerant bacterial isolates were further analyzed for the sodium uptake pattern. It was found that all the six bacterial isolates showed the successive increase in the amount of sodium uptake pattern from 0.1M to 3M concentration of NaCl. Among them two bacterial isolates namely *Bacillus* sp 1 and *Vibrio* sp 2 showed maximum uptake of sodium when cultured under invitro conditions. The results were

contradictory to the study conducted by Damodaran *et al.,* (2013) which stated that at different molar (M) concentration of NaCl the bacterial isolates showed increasing sodium (Na^+) uptake up to 1M NaCl beyond which there was a significant decline. Kataoka *et al.,* (2017) also reported 8 strains (Species of *Arthobacter* and *Bacillus*) of sodium resistant plant growth promoting rhizobacteria from halophyte *Salsola grandis* in saline alkaline soils in Turkey

PGP activity of the bacteria present in the rhizosphere was found to exert beneficial effects on plant growth mechanism. Several mechanisms such as production of phytohormones, suppression of deleterious organisms, production of IAA, activation of phosphate solubilization and promotion of the mineral nutrient uptake were believed to involve in plant growth promotion by PGPR.

IAA, the most common auxin, functions as important signal molecule in the regulation of plant development (Usha *et al.,* 2012). The synthesis of phytohormone IAA is a frequently used mechanism of PGPR to enhance plant growth (Glick, 2012). IAA regulates several aspects of growth and development by controlling critical biological process, such as lateral root initiation, cell enlargement, cell division and increase in the root surface area that helps in an uptake of soil nutrients (Zhao, 2010). All bacterial isolates, studied here, produced significant quantity of IAA and their levels were similar or, even, higher than other PGPR that promote growth in various crop species (Majeed *et al.,* 2015; Zahid *et al.*, 2015).

Phosphorus is an essential nutrient for plant growth and development and is typically insoluble or poorly soluble in soils under salt stressed conditions (Harrison *et al.,* 2002). Some of the bacteria are known to improve the solubilization of the fixed soil phosphorus and applied phosphates, resulting in higher yields even under stress conditions (Banerjee *et al.,* 2010). In our experiment, rhizobacterial isolates *Bacillus* sp 1, *Vibrio* sp 4 and 2 showed *invitro* phosphate solubilizing efficiency. Ability to solubilize various insoluble phosphates is always a desirable attribute for a competent PGPR. Phosphate solubilization by *Bacillus* sp, isolated from salt stressed environment had been observed by earlier researchers (Son *et al.,* 2006). Ullah and Bano (2015) also reported that *Bacillus* sp., isolated from rhizospheric soil of *Atriplex leucoclada,* and *Arthrobacter pascens*, isolated from rhizospheric soil of *Suaeda fruticosa*, were active phosphate solubilizers, bacteriocin and siderophore producers.

Production of ammonia (Wani *et al.,* 2007) and HCN (Schippers *et al.,* 1990) were the important attributes of PGPR that influences plant growth indirectly and strengthen the host disease resistance mechanism respectively. In our present study, *Bacillus* sp 1 and 4, *Vibrio* sp 2, *and Pseudomonas* sp 2 produced ammonia. Majority of the ammonia producing bacteria identified were belonged to the genus *Bacillus* sp. Earlier studies reported that production of ammonia was commonly detected in the isolates of *Bacillus* sp and *Pseudomonas* (Joseph *et al.,* 2007; Damodaran *et al.,* 2013).

In our present study, *Bacillus* sp 1, *Vibrio* sp 2, and *Staphylococcus* sp produced HCN. It has been reported that hydrocyanic acid indirectly influenced the growth promotion of plant. Studies have shown that isolates from rhizosphere of rice (Suresh *et al.,* 2010), mangrove and effluent contaminated soil, chick pea showed the production of HCN (Samuel and Muthukkarppan, 2011). For many Pseudomonads, production of metabolites, such as antibiotics, siderophores and HCN is the primary mechanism for biocontrol (Kremmydas *et al.,* 2013).

50% of the salt tolerant isolates namely, *Bacillus* sp 4, *Pseudomonas* sp 2 and *Vibrio* sp 2 were not able to fix atmospheric nitrogen in contrary to the results of the study conducted by Tennakoon (2007) which stated that none of the isolates grown on Norris N-free medium were considered as non- nitrogen fixers.

It has also been reported previously that bacteria isolated from saline soil are more likely to withstand saline conditions. On the other hand, if such bacteria also possess plant growth promoting traits, they would be ideal for use in sustainable agriculture (Egamberdiyeva and Islam, 2008).

CONCLUSION

The use of soil rhizosphere bacteria possessing the traits of plant growth promotion under saline stress is becoming prevalent worldwide to achieve sustainable agriculture along with soil reclamation through phytoremediation as well as bioremediation. The selected PGPR in this study adapted to 12.5% NaCl stress and were able to produce IAA, ammonia, HCN, Amylase and protease. They also exhibited phosphate solubilizing and nitrogen fixing abilities which help the plants to sequester inorganic phosphate available in the soil and to fix atmospheric nitrogen. The data represented here are quite encouraging to use these PGPR for enhancing plant growth under saline stress conditions.

REFERENCES

Ahl, H.A., and Omer, E.A. 2011. "Medicinal and aromatic plants production under salt stress". *A review. Herba Polonica*. 57(1): 72-87.

Ahmad, F., Ahmad, I. and Khan, M.S. 2008. "Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities". *Microbiol. Res*. 168: 173-181. PMid:16735107 https://doi.org/10.1016/j.micres.2006.04.001

Banerjee, G., Scott-Craig, J.S. and Walton, J.D. 2010. "Improving enzymes for biomass conversion: a basic research perspective". *Bioenerg. Res*. 3:82-92. https://doi.org/10.1007/s12155-009-9067-5

Batlle-Sales, J. 2010. "Salinization: An environmental concern under climate change scenarios". Abstract - 10. Thomas, R.P. (Ed). *Proceedings of the global forum on salinization and climate change* (GFSCC2010) Valencia, 25–29 October

Borneman, J., Skroch, P.W., O'Sullivan, K.M., Paulus, J.A., Rumjanek, N.G., Jansen, J.L., Nienhuis, J. and Triplett, E.W. 1996. "Molecular microbial diversity of an agricultural soil in Wisconsin". *Appl. Environ. Microbiol*. 57:535-538.

Brick, J.M., Bostock, R.M. and Silverstone, S.E. 1991. "Rapid insitu assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane". *Appl. Environ. Microbiol*. 57:535-538 PMid:16348419 PMCid:PMC182744 https://doi.org/10.1128/aem.57.2.535-538.1991

Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E. and Schulze- Lefert P. 2013. "Structure and functions of the bacterial microbiota of plants". *Annu Rev Plant Biol* 64:807-38. PMid:23373698 https://doi.org/10.1128/aem.57.2.535-538.1991

Cappuccino, J.C. and Sherman, N. 1992. In: Microbiology: A Laboratory Manual 3rd ed New York: Benjamin/ Cumming Pub. Co.

Damodaran, T., Mishra, V.K., Sharma, D.K., Jha, S.K., Verma, C.L., Rai, R.B., Kannan, R., Nayak, A.K. and Dhama, K. 2013. "Management of subsoil sodicity for sustainable banana production in sodic soil – an approach". *Int. J. Curr. Res*. 5(7):1930-1934

Damodaran, T., Sah, V., Rai, R.B., Sharma, D.K., Mishra, V.K., Jha, S.K. and Kannan, R. 2013. "Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for plant growth-promoting rhizobacteria (PGPR) and growth vigour in tomato under sodic environment". *African Journal of Microbiology Research*. 7 (44): 5082-5089

Deshwal, V.K. and Kumar, P. 2013. "Effect of salinity on growth and PGPR activity of Pseudomonads". *Journal of Academia and Industrial Research (JAIR).* 2(6): 353-356.

Egamberdieva, D. and Islam, K.R. 2008. "Salt tolerant rhizhobacteria: plant growth promoting traits and physiological characterization within ecologically stressed environments". In: Ahmad, I., Pichtel, J. and Hayat, S. (eds) *"Plant bacteria interactions- strategies and techniques to promote plant growth"*. *Wiley* 257-281. https://doi.org/10.1002/9783527621989.ch14

Egamberdiyeva, D. 2007. "The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils". *Applied Soil Ecol*. 36: 184-189 https://doi.org/10.1016/j.apsoil.2007.02.005

El-Swaify, S. A. 2000. Soil and Water Salinity, [in:] J. A. Silva, R. Uchida (eds), Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa: 151-158.

Gago, C., Sousa, A.R., Juliao, M., Miguel, G., Antunes, D.C. and Panagopoulos, T. 2011. "Sustainable use of energy in the storage of halophytes used for food". *International Journal of Energy and Environment* 5: 592-599

Glick, B.R. 2012. "Plant growth-promoting bacteria: mechanisms and applications". *Scientifica (Cairo).* 963401–963401. PMid:24278762 PMCid:PMC3820493 https://doi.org/10.6064/2012/963401

Grover, M., Ali, S.Z., Sandhya, V., Rasul, A. and Venkateswarlu, B. 2011. "Role of microorganisms in adaptation of agriculture crops to abiotic stresses". *World Journal of Microbiology and Biotechnology*. 27: 1231-1240. https://doi.org/10.1007/s11274-010-0572-7

Harrison, M.J., Dewbre, G.R. and Liu, J. 2002. "A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi". *Plant cell.* 14: 2413-2429. https://doi.org/10.1105/tpc.004861 PMid:12368495 PMCid:PMC151226

Heidari, M., Mousavinik, S.M. and Golpayegani, A. 2011. "Plant growth promoting Rhizobacteria (PGPR) effect on physiological parameters and mineral uptake in Basil (*Ocimum basilicm* L.) under water stress". *ARPN Journal of Agricultural and biological Science*. 6(5): 6-11

Holt, J.G., Bergey, D.H. and Breed, 1993. Bergey's Manual of determinative bacteriology.

Ilyas, N., Bano, A., Iqbal, S. and Raja, N.I. 2012. "Physiological, biochemical and molecular characterization of Azospirillum spp. isolated from maize under water stress". *Pak. J. Bot*. 44: 71-80.

Joseph, B., Patra, R.R. and Lawrence, R. 2007. "Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer ariethium* L.)". *Int.J. Plant. Prod*. 2:141-152

Juniper, S. and Abott, L. 1993. "Vesicular-arbuscular mycorrhizas and soil salinity". *Mycorrhiza* 4:45-57. https://doi.org/10.1007/BF00204058e

Kataoka, R., Guneri, E., Turgay, O.C., Yaprak, A.E., Sevilir, B. and Baskose, I. 2017. "Sodium-resistant plant growth-promoting rhizobacteria isolated from a halophyte, Salsola grandis, in saline-alkaline soils of Turkey". *Eurasian J soil science*, 6(3): 216-225. https://doi.org/10.18393/ejss.289460

Kremmydas, G.F., Tampakaki, A.P. and Georgakopoulos, D.G. 2013. "Characterization of the biocontrol activity of Pseudomonas fluorescens strain X reveals novel genes regulated by glucose". *PloS one.* 8(4): 61808. https://doi.org/10.1371/journal.pone.0061808 PMid:23596526 PMCid:PMC3626644

Lorck, H. 1948. "Production of Hydrocyanic acid by bacteria". Plant. Physiol. 1:142-146. https://doi.org/10.1111/j.1399-3054.1948.tb07118.x

Majeed, A., Abbasi, M.K., Hameed, S., Imran, A. and Rahim, N. 2015. "Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion". *Front. Microbiol.* 6:198. https://doi.org/10.3389/fmicb.2015.00198

Naz, I., Bano, A. and Tamoor-Ul-Hassan. 2009. "Isolation of phtohormones producing plant growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine* max L". *African Journal of Microbiology Research.* 8 (21): 5762-5766 https://doi.org/10.5897/AJB09.1176

Paul, D. and Nair, S. 2008. "Stress adaptations in a plant growth promoting Rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils". *Journal of Basic Microbiology.* 48: 1–7.PMid:18702073 https://doi.org/10.1002/jobm.200700365

Priyanthi, L.K.T. 2007. *Studies on plant growth promoting rhizomicroorganisms of Tea [camellia sinensis L.* (o) Kuntze]. M.Sc. thesis. Submitted to Department of Agricultural Microbiology, College of Agriculture, University of Agricultural Sciences, Dharwad.pp239.

Reinhold, B., Hurek, T., Fendrik, I., Pot, B., Gillis, M., Kersters, K., Thielemans, S. and De, L. 1987. "Azospirillum halopraeferens sp. nov., a nitrogen fixing organism associated with roots of Kallar grass (Leptochloa fusca (L.) Kunth.)". *Int. J. Syst. Bacteriol*. 37: 43-51. https://doi.org/10.1099/00207713-37-1-43

Roohi, A., Ahmed, I., Iqbal, M. and Jamil, M. 2012. "Preliminary isolation and characterization of halotolerant and halophilic bacteria from salt Mines of Karak Pakistan". *Pak. J. Bot*. 44: 365-370.

Salantur, A. Ozturk, A. and Akten, S. 2006. "Growth and yield response of spring wheat (*Triticum aestivum* L.) to inoculation with rhizobacteria". *Plant Soil Environ*. 52: 111-118. https://doi.org/10.17221/3354-PSE

Samuel, S. and Muthukkaruppan, S.M. 2011. "Characterization of plant growth promoting rhizobacteria and fungi associated with rice, mangrove and effluent contaminated soil". *Current botany*. 2(3):22-25.

Schippers, Bakker, A.W., Bakker, R. and Van peer, R. 1990. "Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions". *Plant Soil*. 129:75-83. https://doi.org/10.1007/BF00011693

Somasegaran, P. and Hoben H. J. 1994. *Handbook for Rhizobia. Methods in Legume–Rhizobium Technology*. Heidelberg, NY: Springer. 10.1007/978-1-4613-8375-8 https://doi.org/10.1007/978-1-4613-8375-8 PMCid:PMC201998

Son, H.J., Park, G.T., Cha, M.S. and Heo, M.S. 2006. " Solubilization of insoluble inorganic phosphates by a novel salt- and pH tolerant *Pantoea agglomerans* R-42 isolated from Soyabean rhizosphere". *Bioresour. Technol*. 97:204-210 PMid:16171676 https://doi.org/10.1016/j.biortech.2005.02.021

Suresh, A., Pallavi, P., Srinivas, P., Kumar, V.P., Chandra, S.J. and Reddy, R. 2010. "Plant growth promoting activities of fluorescent Pseudomonads associated with some crop plants". *African Journal of Microbiology Research*. 4(14):1491-94.

Tank, N. and Saraf, M. 2010. "Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants". *Journal of plant interactions.* 5 (1): 51-58 https://doi.org/10.1080/17429140903125848

Tennakoon, P.L.K. 2007. *"Studies on plant growth promoting rhizomicroorganisms of tea [Camellia sinensis L. (o) Kuntze] plants"*. M.Sc. (Agri.) Thesis, Univ. Agric. Sci., Dharwad.

Ullah, S. and Bano, A. 2015. "Isolation of plant-growth-promoting rhizobacteria from rhizospheric soil of halophytes and their impact on maize (*Zea mays* L.) under induced soil salinity". *Canadian Journal of Microbiology*, 61(4): 307-313. https://doi.org/10.1139/cjm-2014-0668 PMid:25776270

Upadhyay, S.K., Singh, J.S., Saxena, A.K. and Singh, D.P. 2011. "Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions". *Plant Biol*. 14:605–611. https://doi.org/10.1111/j.1438-8677.2011.00533.x

Upadhyay, S.K., Maurya, S.K. and Singh, D.P. 2012. "Salinity tolerance in free living plant growth promoting rhizobacteria". *Indian J. Sci. Res.* 3(2): 73-78

Upadhyay, S.K., Singh, D.P. and Saika, R. 2009. "Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition". *Curr. Microbiol*. 59:489-496. https://doi.org/10.1007/s00284-009-9464-1 PMid:19701667

Usha, R.M., Arundathi and Reddy, G. 2012. "Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea". *A. J. Biotechnol.* 11(32):8085-8091. https://doi.org/10.5897/AJB10.2186

Ventosa, A. Ramose Cormenzana, A. and Kocur, M. 1983. "Moderately halophilic Gram-positive cocci from hypersaline environments". *Syst. App. Microbiol*. 4: 564-570. https://doi.org/10.1016/S0723-2020(83)80014-9

Verma, J.P., Yadav, J., Tiwari, K.N., Lavakush, and Singh, V. 2010. "Effect of plant growth promoting rhizobacteria on crop production". *International Journal of Agriculture Research*. 5: 954-983. https://doi.org/10.3923/ijar.2010.954.983

Wani, P.A., Khan, M.S. and Zaidi, A. 2007. "Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp (Vigna) on growth symbiosis, seed yield and metal uptake by green gram plants". *Chromosphere*. 70:36-45. PMid:17723236 https://doi.org/10.1016/j.chemosphere.2007.07.028

Yadav, S., Irfan, M., Ahmad, A. and Hayat, S. 2011. "Causes of salinity and plant manifestations to salt stress: A review". *Journal of Environmental Biology*. 32: 667-685

Zahid, M., Abbasi, M.K., Hameed, S. and Rahim, N. 2015. "Isolation and identification of indigenous plant growth promoting rhizobacteria from Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.)". *Front. Microbiol*. 6:207. PMid:25852667 PMCid:PMC4362393 https://doi.org/10.3389/fmicb.2015.00207

Zhao, Y. 2010. "Auxin biosynthesis and its role in plant development". *Annu. Rev. Plant Biol*. 61: 49-64. https://doi.org/10.1146/annurev-arplant-042809-112308 PMid:20192736 PMCid:PMC3070418