Biological Forum – An International Journal **15(3): 85-90(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Exploitation of Saline Tolerant Rhizobacteria for their Potential Saline Tolerant Traits and Plant Growth Promoting Activity under *in vitro* conditions

Lohith Kumar N.*, Arati, Ameer Pasha B., Abhinandana K.R. and Krishna Naik L.
Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru (Karnataka), India.

(Corresponding author: Lohith Kumar N.)*
(Received: 12 January 2023; Revised: 16 February 2023; Accepted: 27 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: One of the major obstacles to improving agricultural output and quality has been salty soil. The symbiotic heterogeneous bacteria known as plant growth-promoting rhizobacteria (PGPR) are critical to the recycling of plant nutrients through phytostimulation and phytoremediation. There is need of exploitation of saline tolerant rhizobacterial strains to overcome the salinity stress. In the present study eight potential saline tolerant rhizobacteria which were showed tolerance upto 23% NaCl were further screened for saline tolerant traits and plant growth promoting activity under *in-vitro* saline conditions. The results revealed that among the isolates, *Bacillus subtilis* GAN-4 and *Staphylococcus cohnii* MAN-3 exhibited higher nutrient uptake and biofilm formation, and also recorded higher siderophore production, Proline production and Total phenol production compare to other isolates under *in-vitro* conditions. Hence, these saline tolerant rhizobacteria isolated from saline soil can be used to counteract the negative effects of saline stress on plants, with beneficial effects of physiological functions of plants such as growth and yield.

Keywords: Salinity tolerance, Rhizobacteria, *in vitro* conditions.

INTRODUCTION

The world population is estimated approximately 7.8 billion. By 2050, this number is expected to reach 9.7 billion. The demand for food goods has increased as the world's population has grown (Mesa-Marín *et al.,* 2019; Arti *et al.,* 2020). Soil salinity has emerged as a serious issue for global food security. It is estimated that currently about 62 million hectares or 20 percent of the world's irrigated land is affected by salinity. The primary causes of the decreasing crop production include climate change, loss of soil structure, nutrient degradation, drought, and soil salinity. Plant development and metabolism are negatively impacted by high soil salt concentrations (El Ramady *et al.,* 2019). Additionally, the salinity stress may result in the production of free radicals like superoxide ions, hydrogen peroxide (H_2O_2), and singlet oxygen, as well as a drop in plant defence enzymes, an imbalance in sodium homeostasis, a reduction in iron uptake, and the absorption of phenols and other trace elements (Tully *et al.*, 2019).

Numerous strategies have been used recently to address the issue of soil acidity and salinity (Costa *et al.,* 2018; Acuna *et al.,* 2019). Phytoremediation and bioremediation are two other techniques for recovering soils damaged by salt. The heterogeneous bacteria known as plant growth-promoting rhizobacteria (PGPR) are widely known for their advantageous functions (Gangwar *et al.,* 2020). Various rhizobacteria, including the species *Alcaligenes, Pseudomonas, Azospirillum, Bacillus, Klebsiella, Azotobacter, Enterobacter, Burkholderia, Arthrobacter, and Serratia,* contribute to plant growth. The recycling of plant nutrients by these bacteria, which serve as biofertilizers, aids in phytostimulation and phytoremediation (Zhang *et al.,* 2018: Meena, 2019).

Based on the above points, in the present study, bacteria were isolated from saline patches of Karnataka and screened for salt tolerance and PGP activities. Osmolyte and siderophores production is increased by PGP bacteria, and they also modify harmful metals, solubilize phosphorus, change pH, and remove stress-relieving metabolites.

MATERIAL AND METHODS

Estimation of sodium, potassium calcium and magnesium uptake by the saline tolerant rhizobacterial isolates

Potential isolates growing luxuriantly in different saline conditions were screened for their ion uptake pattern. The isolates were grown overnight at 30 °C in minimal media containing $CaCl_2$, $MgSO_4$ 5 gL^{-1} and 23% NaCl 30 °C. After 24 h, cells were harvested by

centrifugation and the bacterial pellet was washed with sterilized distilled water to remove the traces of medium. The washed pellet was digested overnight with 0.1 N HCl at room temperature and the digested pellets were centrifuged and supernatant was taken for the estimation of sodium, potassium by using flame photometer and calcium, magnesium contents was measured by complexometric titration with standard ethylene diamine tetra acetic acid (EDTA) method.

Biofilm formation. Congo Red Agar (CRA) medium was prepared by mixing 0.8 g of Congo red and 36 g of sucrose to 37 g/L of Brain Heart Infusion agar in the absence and presence of 23% NaCl. After incubation period of 24 h at 37° C, the colour of the colonies was used to differentiate the biofilm producers. Black colonies with dry crystalline consistency were indication of biofilm formation, while colonies that retained the pink colour were negative for biofilm formation.

Proline accumulation. Proline content of saline tolerant bacterial cultures was estimated in TSA in the absence and presence of 23 % NaCl. The broth were inoculated with elite saline tolerant bacterial cultures and incubated for 48 h (Mishra *et al*., 2011). Two ml of culture was centrifuged at 10,000 rpm for 10 minutes. The cell pellets were boiled in 80% ethanol at 60 °C in water bath for 45 minutes. The ethanol added suspension was centrifuged at 8000 rpm for 15 minutes and 1ml of supernatant was collected. The supernatant was mixed with 1ml of acid ninhydrin and 1 ml of glacial acetic acid. The reaction mixture tube was kept in boiling water at 100 ℃ for 1 h and transferred to ice bath for cooling. Extraction of proline from the reaction mixture was done by adding 4 ml of toluene. The extracted proline appears pinkish to red color which was separated and transferred to new tubes and the absorbance was measured using a UV visible spectrophotometer (Thermo scientific, Biomate 3S, China) at 520 nm. A standard curve was prepared using the proline. The result was expressed as µg of proline ml^{-1} of bacterial culture (Ceylan *et al*., 2012).

Assay of total phenols. The total phenolic content of saline tolerant bacterial cultures was estimated in TSA broth in the absence and presence of 23 % NaCl. Folin-Ciocalteau method of Singleton and Rossi (1965) was followed to determine the total phenol. The broth was inoculated with elite saline tolerant bacterial cultures and incubated for 48 h (Mishra *et al*., 2011). Two ml of culture was centrifuged at 10,000 rpm for 10 minutes and 1 ml of the supernatant was transferred into new tubes. To each tube, 2 ml of 20 mM. Folin-Ciocalteau reagent was added and incubated at room temperature for 2 h. Catechol was used as a standard and the absorbance was measured at 760 nm a UV visible spectrophotometer (Thermo scientific, Biomate 3S, China). The results were expressed as µg g^{-1} of bacterial culture.

Quantification of siderophore production. The saline tolerant rhizobacterial isolates were tested for their ability to produce siderophore quantification in the absence and presence of 23 % NaCl. The supernatant was extracted from 3 days old culture grown in tryptic Soya Broth. One ml of culture was centrifuged at 10,000 rpm for 10 minutes and 100 µL of supernatant was inoculated in a micro centrifuge tubes to which 100 µl of universal Chrome Azurol S (CAS) reagent was added as described by Schwyn and Neilands (1987) to detect the siderophore production and kept under room temperature for 1 h. The absorbance was recorded at 630 nm by using a UV visible spectrophotometer (Thermo scientific, Biomate 3S, China) against a reference consisting of uninoculated broth. The percent siderophore units calculated by using formula below

$$\% \text{ Siderophore units} = (Ar_{630\ nm} - As_{630\ nm}) / Ar_{630\ nm} \times 100$$

Where, Ar = Absorbance of reference at 630 nm (CAS reagent)

As = Absorbance of sample at 630 nm.

RESULT AND DISCUSSION

Saline tolerant rhizobacterial isolates. Eight saline tolerant rhizobacterial strains were isolated from saline soils (rhizosphere) of Karnataka. They were identified as namely *Staphylococcus gallinarum* GAN-1(OM491215), *Staphylococcus xylosus* GAN-2(OM491216), *Bacillus subtilis* GAN-4(OM491217), *Staphylococcus simiae* GAN-6 (OM491218), *Staphylococcus arlettae* GAN-7(OM491219), *Staphylococcus cohnii* MAN-3(OM491220), *Staphylococcus succinus* MAN-5(OM491221) and *Staphylococcus saprophyticus* BEL-2 (OM491222) based on the sequencing of the 16S r RNA gene were used in this study (Kumar and Naik 2021).

Sodium, potassium, calcium and magnesium uptake by the saline tolerant rhizobacterial isolates. The elite rhizobacterial isolates were screened for their Na^+, K^+, Ca^+ and Mg^+ uptake. The highest sodium uptake was recorded by isolate *B. subtilis*GAN-4 (0.56 meq L^{-1}) whereas potassium uptake was highest in *B. subtilis*GAN-4 (0.11 meq L^{-1}) followed by *S. cohnii*MAN-3 (0.10 meq L^{-1}) (Table 1). The uptake of Ca^+ and Mg^+ salts were highest in the isolate *B. subtilis*GAN-4 followed by *S. cohnii* MAN-3 (Table 1). The results of above experiments are in accordance with the results obtained by Damodaran *et al.* (2013) who isolated sixteen rhizobacteria through natural selection from saline-sodic soils.

Table 1: Estimation of sodium, potassium, calcium and magnesium uptake by saline tolerant rhizobacteria.

Isolates	Na^+ (meq L^{-1})	K^+ (meq L^{-1})	Ca^+ (meq L^{-1})	Mg^+ (meq L^{-1})
S.gallinarum GAN-1	0.44[b]	0.08[d]	4.00[c]	3.00[d]
S. xylosus GAN-2	0.46[b]	0.06[f]	6.00[b]	4.00[c]
B. subtilis GAN-4	0.56[a]	0.11[a]	7.00[a]	6.00[a]
S. simiae GAN-6	0.51[a]	0.07[e]	6.00[b]	3.00[d]
S. arlettae GAN-7	0.52[a]	0.10[b]	3.00[d]	1.00[f]
S. cohnii MAN-3	0.55[a]	0.10[ab]	7.00[a]	5.00[b]
S. succinus MAN-5	0.54[a]	0.04[g]	6.00[b]	2.00[e]
S. saprophyticus BEL-2	0.52[a]	0.09[c]	6.00[b]	4.00[c]

Note: GAN- Gangavathi, MAN- Mandya and BEL-Bellary, N- normal condition, S-stress condition. Means with same superscript in a column do not differ significantly as per Duncan multiple Range Test (DMRT).

Biofilm formation. Biofilm is defined as a microbially derived sessile community of microorganisms, developed either from single or multiple species. Biofilm acts as a reservoir for bacteria, making extermination difficult. The ability of saline tolerant bacterial isolates to form biofilms was analyzed using Congo Red Agar (CRA) medium, all the isolates were able to form biofilm under normal conditions (Table 4.6). Under the saline stress conditions, *Bacillus subtilis* GAN-4 and *Staphylococcus cohnii* MAN-3 showed higher levels of biofilm formation. In our study an appreciable increase in the biofilm formation was observed by saline tolerant rhizobacterial isolates under both normal and saline stress conditions. Biofilm formation by these bacteria consists of extracellular matrix produced by cells, thereby protect plant from soil borne pathogens and also promote plant growth. The results are in agreement with results obtained by Hong *et al.* (2017) who observed that the *Micrococcus yunnanensis* RS222, possessed maximum biofilm formation among all the halotolerant strains at 1.5 M NaCl followed by *Bacillus licheniformis* RS656. *Staphylococcus epidermidis* ATCC 35894 produced more biofilm at 0.08 to 10% concentration of NaCl when compared to *Staphylococcus aureus* (Rode *et al*., 2007). Naves *et al.* (2008) observed biofilm formation intensity (0.78) in a clinical isolate of *Escherichia coli.*

Table 2: Qualitative estimation of biofilm formation and of saline tolerant rhizobacterial isolates under *in-vitro* conditions.

Saline tolerant rhizobacteria	Biofilm formation	
	N	S
S.gallinarum GAN-1	++	++
S. xylosus GAN-2	++	++
B. subtilis GAN-4	+++	++
S. simiae GAN-6	++	++
S. arlettae GAN-7	+	+
S. cohnii MAN-3	+	+++
S. succinus MAN-5	+	+++
S. saprophyticus BEL-2	+++	++

Note: GAN- Gangavathi, MAN- Mandya and BEL-Bellary, (-)- negative, (+)- good; (++)- very good, (+++) – excellent, N- normal condition, S-saline stress condition

Accumulation of proline. The highest proline accumulation was recorded by saline tolerant bacterial isolate *Bacillus subtilis* GAN-4 (3.79 µg ml^{-1}) and *Staphylococcus cohnii* MAN-3 (3.79 µg ml^{-1}) followed by *Staphylococcus arlettae* GAN-7 (3.53 µg ml^{-1}) in absence of NaCl, where as in presence of NaCl, the maximum proline production was observed in *Bacillus subtilis* GAN-4 (4.78 mg ml^{-1}) followed by *Staphylococcus cohnii* MAN-3 (4.53 µg ml^{-1}) and *Staphylococcus xylosus* GAN-2 (3.83 µg ml^{-1}). *Bacillus subtilis* GAN-4 and *Staphylococcus cohnii*MAN-3 isolates showed an increase in proline production, when they were subjected to 23 % NaCl stress (Table 3).

Total phenols. The highest total phenol content was recorded by saline tolerant bacterial isolates *Bacillus subtilis* GAN-4 (64.72µg ml^{-1}) and *Staphylococcus arlettae* GAN-7(49.35µg ml^{-1}) followed by *S. saprophyticus* BEL-2 (44.35µg ml^{-1}) in absence of NaCl, whereas in the presence of NaCl, the maximum phenol production was observed in *Bacillus subtilis* GAN-4 (64.72µg ml^{-1}) followed by *Staphylococcus arlettae* GAN-7 (49.35µg ml^{-1}) and *Staphylococcus xylosus* GAN-2 (134.88 µg ml^{-1}) (Table 2).

Table 2: Saline stress alleviation traits of the saline tolerant rhizobacterial isolates under *in vitro* conditions.

Saline tolerant rhizobacteria	Proline (µg ml^{-1})		Total phenols (µg ml^{-1})	
	N	S	N	S
S. gallinarum GAN-1	1.48^{e}	2.91^{c}	21.02^{e}	21.02^{e}
S. xylosus GAN-2	1.91^{de}	3.83^{b}	39.17^{d}	39.17^{d}
B. subtilis GAN-4	3.79^{a}	4.78^{a}	64.72^{a}	64.72^{a}
S. simiae GAN-6	2.7^{bc}	3.58^{b}	22.87^{e}	22.87^{e}
S. arlettae GAN-7	3.53^{a}	3.46^{b}	49.35^{b}	49.35^{b}
S. cohnii MAN-3	3.79^{a}	4.53^{a}	45.09^{c}	45.09^{c}
S. succinus MAN-5	2.29^{cd}	3.62^{b}	38.8^{d}	38.8^{d}
S. saprophyticus BEL-2	2.92^{b}	2.89^{c}	44.35^{c}	44.35^{c}

Note: GAN- Gangavathi, MAN- Mandya and BEL-Bellary, N- Normal condition, S- Saline stress condition. Means with same superscript in a column do not differ significantly as per Duncan multiple Range Test (DMRT).

Accumulation of compatible solutes in response to salinity is reported in all living groups to variable extents. Accumulation of osmolytes like proline and phenols in bacteria is an indicator of salt tolerance in response to salt stress (Gul and Khan 2008). These strategies confer salinity tolerance to bacteria in saline rhizospheres and results in their better growth and survival (Qurashi and Sabri 2011). In the current study, *Bacillus subtilis* GAN-4 recorded significantly higher proline and phenol content in the presence of 23% NaCl stress compared to other strains. The gradual increase in proline and phenol content under NaCl stress compared to normal condition. Results are similar with the results obtained by Upadhyay *et al.* (2011) who reported that the isolate SKU 8 recorded 7.5 µg and 14.8 µg of proline mg^{-1} protein at 0 and 60 g L^{-1} NaCl respectively). Upadhyay *et al*. (2012) observed that an increase in NaCl was associated with maximum proline production with the isolate SU8 (2.73 and 11.95 g/mg protein at 0 % and 10 % NaCl (w/v) respectively).

Siderophore production. Siderophores are high affinity iron chelating compounds secreted by microorganisms which help to chelate few elements essential for plants. It is one of the vital mechanisms for disease suppression and plant growth promotion. Saline tolerant bacterial isolates were tested for their siderophore production ability. Siderophore production was exhibited by all the isolates under normal and stressed conditions. Under saline stressed conditions the siderophore production was marginally impaired in some isolates. The quantitative assay revealed that the highest siderophore production was noticed in *Bacillus subtilis* GAN-4 (16.51 % units) followed by *Staphylococcus cohnii* MAN-3 (10.44% units) and *Staphylococcus simiae* GAN-6 (9.45 % units) in absence of NaCl. But in the presence of 23% NaCl the isolate *Bacillus subtilis* GAN-4 (11.37% units) exhibited maximum % siderophore units followed by *Staphylococcus cohnii* MAN-3 (9.48% units) and *Staphylococcus succinus* MAN-5 (4.05% units) (Table 3).

Several reports from the past have confirmed that siderophore producing bacteria significantly influence the uptake of various metals, including Fe, Zn, and Cu by plants (Gururani *et al.,* 2012). In the present study, though all strains of saline tolerant rhizobacteria could produce siderophore, but higher siderophore production was observed in *Bacillus subtilis* GAN-4 followed by *Staphylococcus cohnii* MAN-3 under salinity stress conditions. The comparable result was obtained by Masum *et al.* (2018) who reported that the halotolerant bacteria *Bacillus velezenis* NRRL B-41580 and *Bacillus siamensis* KCTC 13613 which produced 69 and 55 % siderophore units respectively. Khan *et al.* (2019) isolated halotolerant rhizospheric bacteria *Arthrobacter woluwensis* AK1, which formed orange clear halos in contrast to blue background. Color change in CAS agar plates confirmed siderophore production. Several other studies showed the production of siderophores by halophilic bacteria viz., a salt tolerant (12 %) *Serratia marcescens* KH1R produced siderophores (Vora *et al.,* 2014).

Table 3: Quantitative estimation of plant growth promotion activities of the saline tolerant rhizobacterial isolates under *in-vitro* conditions.

Saline tolerant rhizobacteria	Siderophore production (%)	
	N	S
S. gallinarum GAN-1	1.14^{h}	1.26^{e}
S. xylosus GAN-2	5.69^{f}	1.85^{e}
Bacillus subtilis GAN-4	16.51^{a}	11.37^{a}
S. simiae GAN-6	9.45^{c}	3.46^{cd}
S. arlettae GAN-7	8.72^{d}	3.32^{d}
S. cohnii MAN-3	10.44^{b}	9.48^{b}
S. succinus MAN-5	7.26^{e}	4.05^{c}
S. saprophyticus BEL-2	3.90^{g}	3.24^{d}

Note: GAN- Gangavathi, MAN- Mandya and BEL-Bellary, N- Normal condition, S- saline stress condition. Means with same superscript in a column do not differ significantly as per Duncan multiple Range Test (DMRT).

CONCLUSIONS

Rhizobacteria that support plant growth under saline tolerant environments have developed a number of mechanisms to deal with salinity stress, including efflux systems, the production and accumulation of compatible solutes for regulating external osmotic pressure, the production of ROS, as well as other methods. There is still much more has to be discovered about how the saline tolerant rhizobacteria support themselves and their symbiotic partner during salinity stress, which has numerous negative effects on the cell. The present study on saline tolerant rhizobacteria demonstrates their enormous potential for improving the productivity of agro-ecosystems with salt concerns. To develop specialised bioformulations for saline soil systems, which are becoming more prevalent all over the world on a daily basis, in-depth studies targeting gene level expression and functional characteristics of saline tolerant rhizobacteria involved in plant growth promotion under salinity stress must be conducted in the near future.

Acknowledgement. Authors wants to acknowledge Department of Agricultural Microbiology, University of Agricultural sciences, Bangalore, India, for the facility provided.

Conflict of Interest. None.

REFERENCES

Acuna, R. I. S., Hansen, H., Gallardo. J., Atala, C. and Molina-Montenegro, M. A. (2019). Antarctic extremophiles: biotechnological alternative to crop productivity in saline soils. *Frontiers Bioeng Biotechnology, 7*, 22.

Arti, D. K, Choudhary, M. and Sourirajan, A. (2020). Salt tolerant bacteria for crop improvement in saline agriculture fields: development, challenges and opportunities. *Plant Archives, 20*, 7139–7155.

Ceylan, S., Yilan, G., Akbulut, B. S., Poli, A. and Kazan, D. (2012). Interplay of adaptive capabilities of *Halomonas sp*. AAD12 under salt stress. *Journal of Bioscience and Bioengineering, 114*(1), 45-52.

Costa, S. F, Martins, D., Agacka-Mołdoch, M., Czubacka, A. and De Sousa, A. S. (2018). Strategies to alleviate salinity stress in plants. In: Salinity Responses and Tolerance in Plants. In: Salinity responses and tolerance in plants. Springer Nature: Berlin/Heidelberg, *Germany*, pp 307–337.

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

El-Ramady, H. Alshaal, T. Abdelrahman, H. and El-Hady, O. (2019). Future soil issues. In: The soils of Egypt. Springer, Cham, Germany, pp 215–224.

Gangwar, P., Singh, R., Trivedi, M. and Tiwari, R. K. (2020). Sodic soil: management and reclamation strategies. In: Environmental concerns and sustainable development. *Springer,* Singapore, pp 175–190.

Gul, B. And Khan, M. A. (2008). Effect of compatible osmotica and plant growth regulators in alleviating salinity stress on the seed germination of *Allenrolfea occidentalis*. *Pakistan journal of botany*, *40*(5), 1957-1964.

Gururani, C. P., Upadhyaya, V., Baskar, J., Venkatesh, A., Nookaraju, S. W. and Park (2012). Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *Journal of Plant Growth Regulation*, *10*, 654-657.

Hong, B. H., Joe, M. M., Selvakumar, G., Kim, K. Y., Choi, J. H. and Sa, T. M. (2017). Influence of salinity variations on exocellular polysaccharide production, biofilm formation and flocculation in halotolerant bacteria. J*ournal of environmental biology, 38*(4), 657.

Khan, Muhammad, A., Ihsan, U., Muhammad, W., Muhammad, H., Abdul, L. K., Sajjad, A., Sang-Mo, K., Kyung-Min, K., Rahmatullah, J. and In-Jung, L. (2019). Halo-tolerant rhizospheric *Arthrobacter woluwensis* AK1 mitigates salt stress and induces physio-hormonal changes and expression of GmST1 and GmLAX3 in soybean. *Symbiosis*., *77* (1), 9-21.

Kumar, L. N. and Naik, K. L. (2021). Isolation and characterization of saline tolerant rhizobacteria from saline tracts of Karnataka, *Mysore journal of agricultural sciences*, *55*(4), 175-180.

Masum, M. M. I., Liu, L., Yang, M., Hossain, M. M., Siddiqa, M. M., Supty, M. E., Ogunyemi, S. O., Hossain, A., An, Q. and Li, B. (2018). Halotolerant bacteria belonging to operational group *Bacillus amyloliquefaciens* in biocontrol of the rice brown stripe pathogen *Acidovorax oryzae*. *Journal of applied microbiology*, *125*(6), 1852-1867.

Mesa-Marín, J., Mateos-Naranjo, E., Rodríguez-Llorente, I. D., Pajuelo, E. and Redondo-Gómez, S. (2019). Synergic effects of rhizobacteria: increasing use of halophytes in a changing world. In *Halophytes and climate change: adaptive mechanisms and potential uses,* pp. 240-254.

Meena, V. S. (2019). Role of rhizospheric microbes in soil. Springer, Heidelberg Germany, Volume 1

Mishra, P. K., Bisht, S. C., Ruwari, P., Selvakumar, G., Joshi, G. K., Bisht, J. K., Bhatt, J. C. AND Gupta, H. S. (2011). Alleviation of cold stress in inoculated wheat (*Triticum aestivum* L.) seedlings with psychrotolerant Pseudomonads from NW Himalayas. *Archives of microbiology*, *193*(7), 497-513.

Naves, P., Del Prado, G., Huelves, L., Gracia, M., Ruiz, V., Blanco, J., Rodríguez-Cerrato, V., Ponte, M. C. and Soriano, F. (2008). Measurement of biofilm formation by clinical isolates of *Escherichia coli* is method-dependent. *Journal of Applied Microbiology,105*(2), 585-590.

Qurashi, A. W. and Sabri, A. N. (2011). Osmoadaptation and plant growth promotion by salt tolerant bacteria under salt stress. *African journal of microbiology research*, *5*(21), 3546–3554.

Rode, T. M., Langsrud, S., Holck, A. And Moretro, T. (2007). Different patterns of biofilm formation in *Staphylococcus aureus* under food-related stress conditions. *International Journal of Food Microbiology, 116*(3), 372-383.

Schwyn, B. and Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical biochemistry*, *160***,** 47-56.

Singleton,V. L. Anrossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, *16*(3), 144-158.

Tully, K. L., Weissman, D, Wyner, W. J, Miller J. and Jordan, T. (2019). Soils in transition: saltwater intrusion alters soil chemistry in agricultural fields. *Biogeochemistry, 142*, 339–356.

Upadhyay, S. K., Singh, J. S. and Singh, D. P. (2011). Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. *Pedosphere, 21*(2), 214–222.

Vora, J. U., Jain, N. K. and Modi, H. A. (2014). Extraction, characterization and application studies of red pigment of halophile Serratia marcescens H1R KM035849 isolated from Kharaghoda soil. *International journal of pure and applied bioscience*, *2*(6), 160-168.

Zhang, S., Fan, C., Wang, Y., Xia, Y., Xiao, W., Cui, X. (2018) Salt-tolerant and plant-growth-promoting bacteria isolated from high-yield paddy soil. *Canadian journal of microbiology, 64*, 968–978.

How to cite this article: Lohith Kumar N., Arati, Ameer Pasha B., Abhinandana K.R. and Krishna Naik L. (2023). Exploitation of Saline Tolerant Rhizobacteria for their Potential Saline Tolerant Traits and Plant Growth Promoting Activity under *in vitro* conditions. *Biological Forum – An International Journal, 15*(3): 85-90.