OPEN ACCESS

IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-16, Issue3, 2025, p-38-44

DOI: 10.5281/zenodo.16213460

Research Article

Efficiency of the Salt Tolerant Plant Growth Promoting Rhizobacteria Isolated from Rice Crop under Saline Condition

Suriya Sabarinath B [1], Santhana Bharathi S[2], Malarvizhi C[3] and *[4]Siva Kumar K

Article Info

[1]Faculty of Sciences, School of Agricultural Sciences, Takshashila University, India
[2]Thangapazham Agricultural College, Vasudevanallur, Tenkasi, India
[3]SRS Institute of Agriculture and Technology, Dindigul, Tamil Nadu, India
[4]Agricultural Microbiology, Horticultural Research Station, Tamilnadu, Agricultural University, Pechiparai, India

---

*Corresponding: **Siva Kumar K**

Received: 02/06/2025
Accepted: 19/07/2025
Published: 20/07/2025

DOI: 10.5281/zenodo.16213460

Publisher's Note: IJABR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that can help plants respond better to biotic and abiotic challenges. Eight salt-tolerant bacterial isolates were identified from rice rhizosphere in this research. These isolates were morphologically and biochemically described and identify at genus level. Plant growth-promoting properties such as IAA production, Gibberellic acid production, HCN generation, inorganic phosphate solubilization, and siderophore production were chosen among the isolates. And also, the different level of 1,5,10,15,20 & 25 % NaCl concentration was tested. Among the all tests the isolate STRB 5 was perform best in IAA production, Gibberellic acid production, HCN generation, inorganic phosphate solubilization, and siderophore production compare to other different isolates. All the isolates were tested for antifungal activity against plant pathogens *Machrophomina pasolina* and *Fusarium oxysporum*. STRB 5 exhibited the most effective antifungal activity against both test pathogens. In conclusion, the halotolerant PGPR strain STRB 5 described in this study might have great potential to under different salt stress conditions in invitro condition.

Keywords: rhizobacteria, salt stress conditions, Gibberellic acid, *Machrophomina pasolina* and *Fusarium oxysporum.*

Introduction

Drought, salt, high temperatures, and infections, among other biotic and abiotic conditions that might limit the growth and development of any specific crop, have a significant impact on agricultural productivity. Salinity is a negative factor impacting agricultural yield in arid and semi-arid regions across the world, where it accounts for a loss of 1–2% of arable land per year [1]. Plants' cellular metabolism is altered by salinity, resulting in a variety of physiological, morphological, biochemical, and molecular alterations [8]. The main effect of salinity on growth and development of plants is osmotic stress, which has an immediate influence on water availability, the buildup of harmful ions like Na^+ and Cl^- in cells, nutritional imbalances, and oxidative stress damage [11].

When compared to other cereals, high salt stress has a more dramatic effect on rice growth and development [12]. This is because to its inability to keep Na+ out of its tissues [16]. To reduce salt impacts on rice, several techniques were utilised, including proper soil practises and irrigation management [9], as well as conventional breeding and genetic engineering [9]. [12]. Microorganisms in the rhizosphere have an important role in reducing salt stress in plants, which leads to increased agricultural output [5]. Among them, bacteria known as plant growth promoting rhizobacteria (PGPR) may feature several plant growth promoting properties increases plant growth and yield, as well as being effective in reducing the effects of abiotic stressors both directly and indirectly.

The main objective of this study is to isolate salt tolerant PGP Rhizospheric bacteria from rice grown in saline soils in killlai near the pichavaram Sea side of Tamil Nadu, India. The isolates were partially identified by morphological and biochemical characterizations.

Collection and characteristics of soil of samples

Soil samples were collected aseptically in sterile plastic bags from agricultural fields in saline soils in killlai near the pichavaram Sea side of Tamil Nadu, India, on a regular basis and transported to the laboratory, where they were maintained at 40°C prior to processing. The majority of rhizospheric soil samples were taken from the roots of vegetable crops. Using typical laboratory procedures, many physical parameters of soil, such as temperature, pH, and moisture content, were investigated.

Isolation of salt tolerant PGPR

Rhizospheric soil samples were separated from roots of rice crop by gently in a Petridis. 10 grams of Rhizospheric soil was taken into a 100 ml of conical flask containing 90 ml of sterile distilled water and the flask was shaken for 10 minutes. Serial dilution technique was employed up to 10^{-6} dilutions. An aliquot (0.5 ml) of 10^{-5} and 10^{-6} diluted suspension was spread over the plates of Nutrient Agar Medium with 5% NaCl. Plates were incubated at 28 ± 2^0C to observe the colony of salt tolerant bacteria after 24 to 48 hrs. The single bacterial colony was selected and purified by quadrant steaking method. Store the culture in agar slants refrigerator at 4^0C for further studies.

Morphological Characterization of Isolates

Morphological characteristics of the colony of each isolate were examined on suitable media. All the isolates were streaked on Petri plates containing nutrient agar media. After three days of incubation, different characteristics of colonies such as shape, size, elevation, surface, margin, colour, pigmentation and gram reaction were recorded. In addition, cellular morphology, shape, gram staining and endospore formation were also examined [15].

Biochemical characterization

The different biochemical characterizations like Catalase, Urease, Glucose, Indole Production, V.P. Taste, Methyl Red, Citrate Utilization and Nitrate Reduction test was conducted for the genus level of identification.

Halotolerance Assay

Bacterial isolates were screened for halotolerance using NA media supplemented with various levels of NaCl (1, 5, 10, 15, 20, and 25%). The plates were inoculated with fixed volumes of starter inoculum (OD_{600} = 0.05) and the cultures were incubated for 7 days at 28^0C [14].

Functional characterization

The efficiency of isolates was studied by screening of their ability to Solubilizing phosphorus, IAA production, GA production and Siderophore production.

Determination of Indole Acetic Acid

Isolates were inoculated in 100 mL King's B broth with 0.1 mg/ml tryptophan and incubated at room temperature 24 hrs days. The supernatant was centrifuged, pH 2.5 acidified, and extracted with 10 mL ethyl acetate. The ethyl acetate fraction was evaporated under vacuum at 40°C, and the residue was suspended in 2 mL ethanol before being combined with $FeHClO_4$ reagent. After 25 minutes, the absorbance was measured at 530nm. [7].

Estimation of GA

Two milliliters of zinc acetate were added to 25 milliliters of culture filtrate in a test tube. After two minutes, 2 mL potassium ferrocyanide was added, and the mixture was centrifuged for 15 minutes at 1000 rpm. Five ml of this supernatant was mixed with five ml of 30% HCl and incubated at 20^0C for 75 minutes. The absorbance of the samples and the blank sample was measured at 254 nm in a UV-vis spectrophotometer after the blank sample was treated with 5% HCl. From the standard curve, the quantity of GA in the extract was determined and represented as g/ml of medium. IAA and GA standard curves were created using graduated concentrations of IAA and GA. [13].

Phosphate Solubilization

Spot inoculated active bacterial cultures on Pikovaskay's medium plate and incubated at 300°C for 5 days P solubilizers were chosen from isolates that showed a definite zone of solubilization surrounding the colony. The solubilization zone's diameter was measured and represented in cm.

Antifungal Activity

On a Potato Dextrose Agar plate, the fungal pathogen was grown. With the use of a sterile cork borer, a disc of fungal growth from this plate was removed and inserted in the middle of a new PDA plate. After that, a 24-hour-old bacterial culture was streaked on both sides of the fungal disc, 2-3 cm distant from the disc. The plates were incubated for 96 hours at 300°C. In compared to the control, fungal growth was inhibited after 96 hours of incubation. [6].

Result and discussion

Table 1 Morphological characteristics

Isolates	Colour	Size	Shape	Margin	Elevation	Opacity	Gram staining	Mot-ility	Pigme-ntation
RI 1	Whitish	0.5mm	Rods	Smooth	Convex	Opaque	+	+	None
RI 2	Whitish	0.4mm	Curved	Smooth	Flat	Translucent	-	+	None
RI 3	Milky	0.9mm	Rods	Rough	Raised	Translucent	-	+	None
RI 4	Yellowish	1.2mm	Rods	Rough	Raised	Translucent	-	+	Florescence
RI 5	Off-white	0.3mm	Rods	Rough	Flat	Translucent	-	+	None
RI 6	Brownish	1.4mm	Rods	Smooth	Convex	Translucent	-	+	None
RI 7	Whitish	0.5mm	Rods	Smooth	Convex	Opaque	+	+	None
RI 8	Yellowish	1.2mm	Curved	Rough	Raised	Translucent	-	+	None

Table 2 Biochemical characterizes

Isolates	Catalase	Urease	Glucose	Indole Production	V.P. Taste	Methyl Red	Citrate Utilization	Nitrate Reduction	Identify as
RI 1	+	+	+	-	+	-	+	+	*Bacillus sp.,*
RI 2	-	-	-	-	-	-	+	-	*Azosprillum sp.,*
RI 3	+	+	+	+	-	-	+	+	*Azotobacter sp.,*
RI 4	+	-	-	-	-	-	+	-	*Pseudomonas sp.,*
RI 5	+	-	+	-	-	-	-	-	*Pseudomonas sp.,*
RI 6	+	+	+	+	-	-	+	+	*Azotobacter sp.,*
RI 7	+	+	+	-	+	-	+	-	*Bacillus sp.,*
RI 8	-	-	-	-	-	-	+	+	*Azosprillum sp.,*

Table 3 PGP Efficiency of the different isolates

Isolate code	IAA Production	GA Production	Quantitative IAA Production (µg/ml)	Quantitative GA Production (µg/ml)	Phosphate solubilization
RI 1	+	+	80	59	+
RI 2	+	+	84	67	-
RI 3	+	+	72	51	-
RI 4	+	+	69	49	+
RI 5	+	+	92	72	+
RI 6	+	+	81	61	-
RI 7	+	+	62	47	+
RI 8	+	+	75	56	-

Characterization and isolation of in the rhizospheric soil of rice fields in Killlai near Pichavaram, Tamil Nadu, eight salt-tolerant bacterial strains were successfully identified. (Salt Tolerant Rice Bacteria) STRB 1, STRB 2, STRB 3, STRB 4, STRB 5, STRB 6, STRB 7, and STRB 8 were the isolates. The isolates were morphologically and biochemically characterised in order to identify four different species found in soil samples, like *Bacillus* sp., *Pseudomonas* sp., *Azosprillum* sp., and *Azotobacter* sp., (Table 1 &2).

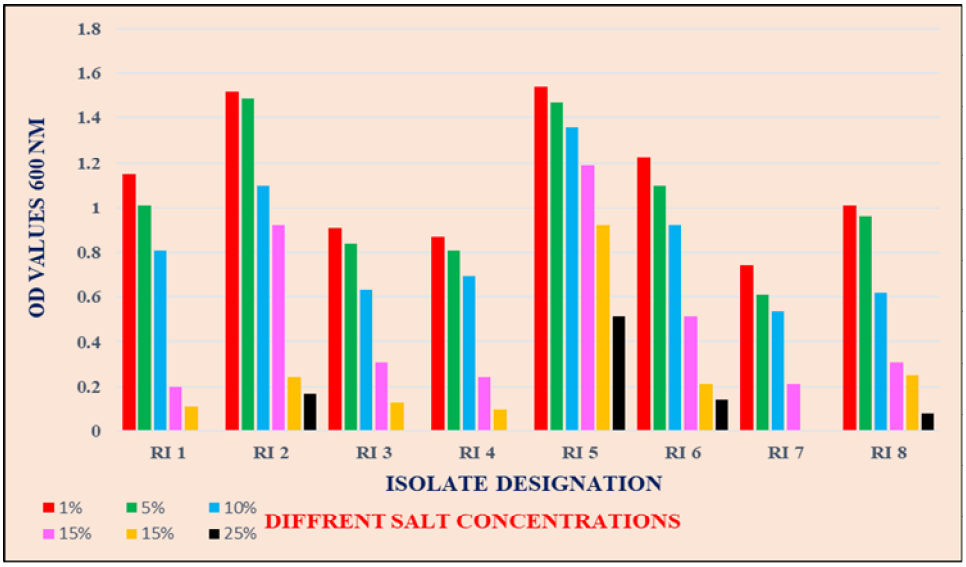
Production of IAA was discovered to be a common characteristic among all isolates. All isolates produced IAA, which resulted in the development of a pink colour with the addition of Salkowski reagent to a 48-hour-old broth (Table 3). IAA production varied amongst isolates, ranging from 67 to 92 g/ml. STRB 5 produced the highest amount of IAA (92 g/ml), followed by STRB 2, STRB 6, and STRB 1. STRB 8 (84, 81, 80, 75, and 72 g/ml) and STRB 3 (84, 81, 80, 75, and 72 g/ml) STRB 4 and STRB 7 had the lowest IAA levels (69 and 62 g/ml, respectively). IAA production by PGPR has been observed to vary between species and strains, as well as being influenced by culture conditions, growth state, and substrate availability. [17]

Phosphate is an essential nutrient for plants, and the majority of it is found in an insoluble form. Agricultural microbiologists are interested in the potential of isolated bacteria to solubilize mineral phosphate and make it accessible to plants, as this improves plant development. STRB 1, STRB 4, STRB 5, and STRB 7 are among the isolates that can solubilize the unavailable form of phosphate into the accessible form. (Table 3).

All the isolates were positive for GA production. GA production ranged from 47µg/ml to 72 µg/ml. STRB 5 was the highest producer of GA (72 µg/ml). Followed by STRB 2, STRB 6, STRB 1. STRB 8 and STRB 3 (67, 61, 59, 56 & 51µg/ml). The least GA producer STRB 4 and STRB 7 (49 & 47µg/ml) (Table 3).

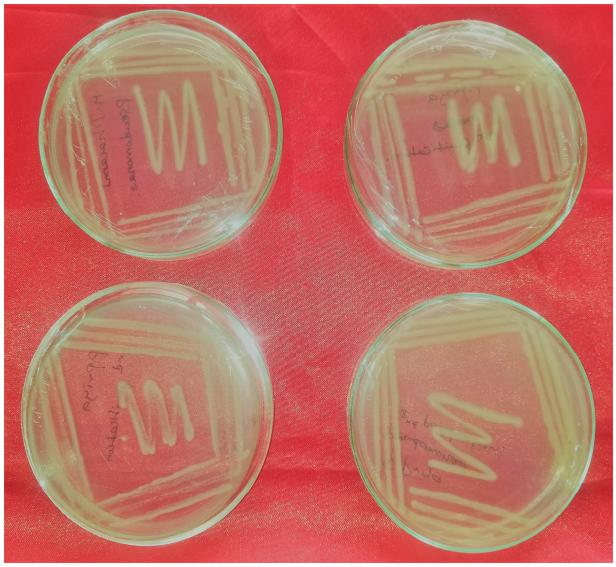
Isolate code	Mean zone of inhibition In (cm)		Siderophore Production	HCN Production
	Fungal plant pathogen			
	Machrophomina pasolina	*Fusarium oxysporum*		
RI 1	1.45	1.95	+	+
RI 2	-	-	-	+
RI 3	-	-	+	+
RI 4	3.74	3.10	+	+
RI 5	5.29	5.46	+	+
RI 6	-	-	-	+
RI 7	3.19	2.31	+	+
RI 8	-	-	-	+

Table 4 Effect of different isolates from various concentration of salt



Graph1: Different salt concentrations.

Figure 1 Pure culture of the Different Isolates



Antifungal activity of the different isolate was examined against *Machrophomina pasolina* and *Fusarium oxysporum*. Among the different isolates STRB 5 recorded maximum inhibition zone against *Machrophomina pasolina* and *Fusarium oxysporum*.at (5.29 mm & 5.46). Followed by STRB 4 (3.74 & 3.10), STRB 7 (3.19 & 2.31) and STRB 1 (1.45 & 1.95) was positive for antifungal activity. Other isolates where negative result was observer.

Plants and microorganisms both need iron to live. Siderophore-producing bacteria make it available to plants, and they also compete with soil-borne pathogens for iron, acting as a biocontrol agent [10]. Five of the eight isolates were positive for siderophore production, resulting in a bright orange zone surrounding the colony (STRB 1, STRB 3, STRB 4, STRB 5, & STRB 7). STRB 2, STRB 6, and STRB 8 were not produced by the other four isolates (Table 4). Siderophores are iron chelating compounds with a low molecular weight that play a significant function in plant growth promotion[2-4,18].

HCN production was observed was positive in all the isolates. HCN is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens [4].

All the different isolates were tested against various level sodium chloride concentrations at 1, 5, 10, 15, 20 & 25%. The isolate STRB 5 was recorded maximum various NaCl at 1.54 to 0.51 OD at 600nm. Followed by STRB 2, STRB 6 and STRB 8 were grown at up to 25% of NaCl concentration. STRB 7 was most susceptible to the NaCl concentrations up to 15%. They were presented Figure 2.

Acknowledgment

None Declared.

Conflict of interest

The author has not declared any conflict of interest

References

1. Shrivastava, P., and Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.* 22, 123–131.doi: 10.1016/j.sjbs.2014.12.001
2. Brick, J. M., Bostock, R. M., Silverstone, S. E. “Rapid in situ assay for IAA production by bacteria immobilized on nitrocellulose membrane”. Appl. Environmental Microbial 57: 535-538, 1994.
3. Cappuccino, J. G. and Sherman, N. “Biochemical activities of microorganisms. In: Microbiology, A Laboratory Manual”. The Benjamin / Cummings Pub- lishing Co.California, USA, 1992.
4. Defago, G., Berling, C. H., Borger, U., Keel, C. and Voisard, C., 1990. Suppression of black rot of tobacco by a *Pseudomonas* strain : Potential applications and mechanisms. In :*Biological Control Soil Borne Plant Pathogens*. Eds. D. Hornby, R. J. Cook and Y. Henis, CAB International, pp. 93-108
5. Etesami, H., and Beattie, G. A. (2018). Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. *Front. Microbiol.* 9:148.doi: 10.3389/fmicb.2018.00148
6. Ganesan, P., and Gnanamanickam, S.S. 1987. Biological control of Sclerotium rolfsii sacc in peanut by inoculation with *P. fluoresencs*. Soil Biological.Biochemistry, 19: 35–38.

7. Gordon S.A. and Weber R.P. 1951. Colorimetric estimation of indole- acetic acid. Plant Physiology. 26: 192-195.
8. Gupta, B., and Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genom.* 2014, 1–18.
9. Katerji, N., Mastrorilli, M., Van Hoorn, J. W., Lahmer, F. Z., Hamdy, A., and Oweis, T. (2009). Durum wheat and barley productivity in saline–drought environments. *Eur. J. Agron.* 31, 1–9.doi: 10.1016/j.eja.2009. 01.003
10. Kokalis – Burelle, N., Kloepper, J. W., Reddy, M. S. "PGPR as transplant amendments and their effect on indigenous rhizosphere micro-organisms". Appl. Soil. Ecology 31: 91-100, 2006.
11. Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*59, 651–681.doi: 10.1146/annurev.arplant.59.032607.092911
12. Munns, R., James, R. A., and Läuchli, A. (2006). Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* 57, 1025–1043.doi: 10.1093/jxb/erj100
13. Paleg LG, 1965. Physiological effect of gibberellins. Annual Review of Plant Physiology 16:291–322.
14. Ramadoss, D., Lakkineni, V. K., Bose, P., Ali, S., and Annapurna, K. (2013). Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. *SpringerPlus* 2:6.doi: 10.1186/2193-1801-2-6
15. Robinson, R. J., Fraaije, B. A., Clark, I. M., Jackson, R. W., Hirsch, P. R., and Mauchline, T. H. (2016). Endophytic bacterial community composition in wheat (*Triticum aestivum*) is determined by plant tissue type, developmental stage and soil nutrient availability. *Plant Soil* 405, 381–396.doi: 10.1007/s11104- 015-2495-4
16. Roy, S. J., Negrão, S., and Tester, M. (2014). Salt resistant crop plants. *Curr. Opin. Biotechnol.* 26, 115–124.doi: 10.1016/j.copbio.2013.12.004
17. Schwyn, B. and Neilands, J. B. "Universal chemical assay for the detection and determination of siderophores". Analytical Biochemistry 160: 47-56, 1987.
18. Stevenson, F. J. and Cole, M. A. "Cycles of soil: carbon, nitrogen, phosphorus, sulphur, micronutrients IInd ed.", Wiley and Sons Inc., Tronto, Canada, ISBN- 10 : 0471320714, 1999.