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# Evaluation of the Growth and Tolerance of Pseudomonades Isolates under Different pH and Salt Concentrations

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**ABSTRACT**

The study aimed to isolate pseudomonads from soil samples and evaluate their growth under varying pH levels and NaCl concentrations. The results showed that 46.66% of the 15 isolates showed high growth under 50 mM salinity, while 13.33% showed high growth at 100 mM salinity and 33.33% were able to tolerate up to 150 mM salinity with reduced growth. All isolates showed higher growth at pH 7 and 8, while 20% of isolated showed high growth even at pH 9 and 10. The study showed the potential of candidate isolates in pH and salt stress. These isolates having Plant growth-promoting rhizobacteria (PGPR) may be utilized to augment plant growth under pH and salt stress conditions.

***Key words:*** *Plant growth-promoting rhizobacteria (PGPR), Lentil (Lens culinaris M), Salt tolerant, pH, Soil salinity*

## Introduction

Salt stress is one of the major environmental factors limiting plant growth and productivity, particularly in coastal and arid regions. The cultivation of lentils (*Lens culinaris* M) in India can be negatively impacted by high pH levels and elevated salt concentrations in the soil (Singh *et al.,* 2022). These conditions can cause reduced growth and decreased yields for the crop. Studies have shown that high pH values can lead to toxicity of essential nutrients and ion imbalances that negatively affect plant growth and health (Kumar *et al.,* 2021). Additionally, high salt levels can cause soil salinization and increase the risk of drought stress for the lentil plants, resulting in reduced yields (Hossain *et al.,* 2017; Singh and Husen, 2019). To mitigate these negative effects, farmers in India can implement practices such as regular soil testing and management, irrigation management, and the use of salt-tolerant lentil varieties. To overcome this challenge, researchers have focused on the identification and characterization of plant growth-promoting rhizobacteria (PGPR) that are able to tolerate high salt concentrations (Kumawat *et al.,* 2022). PGPR are beneficial microorganisms that colonize the root system of plants and provide a range of benefits such as increased nutrient uptake, improved stress tolerance, and enhanced growth (Shultana *et al.,* 2022). In the context of soil salinity, the isolation and use of rhizobacteria that promote plant growth and can tolerate high salt levels could be a key factor in mitigating the harmful effects of soil salinity on plant growth and survival (Banerjee *et al.,* 2019). Recently, there has been a growing interest in the use of PGPR as biostimulants for crop improvement in salt-affected soils. However, the identification and characterization of salt-tolerant PGPR are crucial for the development of effective and sustainable biostimulants strategies. This information can be used to optimize the selection and use of PGPR for specific crops grown under different salinity conditions (Banjare *et al.,* 2023). In this context, recent studies have aimed to characterize salt-tolerant PGPR using a range of methods including physiological (different pH and salt concentration) characterization (Yañez *et al.,* 2021; Saleem *et al.,* 2021). These studies have provided valuable insights into the mechanisms of salt tolerance in PGPR under salt stress conditions. In present study, pseudomonads from various soil specimens were isolated and evaluated the influence of varying pH levels and NaCl concentrations on their growth.

**Materials and Methods Top of Form**

**Screening of bacterial isolates for different pH and salt concentration**

Based on their antagonistic activities, 15 bacterial isolates were screened for pH and salt stress tolerance activities such as different salt concentration; different pH ranges (Tsegaye *et al*., 2019)

**Effect of different pH values on growth of pseudomonades isolates**

The effect of different pH values of Kings' B broth medium (7, 8 9, 10, and 11pH) on growth of pseudomonades isolates in medium were estimated. Five treatments for each pH values of liquid medium were used for this analysis.

**Effect of different NaCl concentrations on growth of pseudomonades isolates**

The effect of different added dose of NaCl (50, 100 and 150 mM NaCl) in Kings' B broth on the growth of different isolates was performed. Isolates were inoculated in 10 ml medium containing different doses of NaCl and replicated thrice.

## Results and Discussion

Based on the antagonistic activity against *Fusarium oxysporum* f. sp. *lentis*. Fifteen (15) potential PGPR were selected i.e., PGPR18 (85.19), PGPR16 (84.81), PGPR22 (80.74), PGPR21(79.63), PGPR26 (75.56) PGPR24 (72.59), PGPR27 (67.41), PGPR9 (61.85), PGPR23 (61.48), PGPR30 (60.74), PGPR25 (59.63), PGPR14 (57.78), PGPR20 (55.56), PGPR29 (51.11) & PGPR13 (48.89) for screening of their tolerance at different salt concentrations and different pH ranges (Table 1).

Among these 15 isolates, 7 (46.66%) pseudomonad isolates *viz.* PGPR18, PGPR16, PGPR21, PGPR26, PGPR23, PGPR14 and PGPR20 were showed high growth (+++) and 8 (53.33%) pseudomonad isolates i.e., PGPR22, PGPR24, PGPR27, PGPR9, PGPR30, PGPR25, PGPR29 and PGPR13 were showed moderate growth (++) on 50 mM salinity, respectively.

Similarly, at 100 mM salinity, only two (13.33%) pseudomonad isolates (PGPR18 and PGPR16) were showed high growth (+++), 6 (40%) pseudomonad isolates (PGPR22, PGPR21, PGPR26, PGPR24, PGPR23 and PGPR14) were showed moderate growth (++) and seven isolates (46.66%) (PGPR27, PGPR9, PGPR30, PGPR25, PGPR20, PGPR29 and PGPR13) were showed less growth (+). Interestingly, five (33.33%) pseudomonad isolates (PGPR18, PGPR16, PGPR22, PGPR24 and PGPR23) were tolerated 150 mM salinity, however showed less growth (+) and other ten isolates (PGPR21, PGPR26, PGPR27, PGPR9, PGPR30, PGPR25, PGPR14, PGPR20, PGPR29 and PGPR13) were showed not any growth (-).

For pH tolerance test, all 15 (100%) pseudomonad isolates grew well and displayed high growth (+++) on pH-7 and pH-8 whereas, on pH-9 only 3 (20%) isolates PGPR18, PGPR16, PGPR22 were expressed high growth (+++) remaining 12 (80%) isolates showed moderate growth (++).

Similarly on pH-10 five (33.33%) isolates (PGPR18, PGPR16, PGPR22, PGPR21 and PGPR26) showed moderate growth (++) behavior and seven (46.66%) isolates showed less growth (+) while 3 (20%) isolates (PGPR20, PGPR29 and PGPR13) were displayed no growth (-) at pH-10 level.

At pH-11, 3 (20%) isolates (PGPR18, PGPR16 and PGPR22) grew moderately (++) and three (20%) isolates (PGPR21, PGPR26 and PGPR27) showed less growth (+) and there is no growth have been observed in 9 (60%) isolates (Table, Plate).

For saline prone areas, salt tolerant bacteria are a potential bioresource (Hayat *et al.,* 2013). There has been a substantial increase in soil salinization in recent years, which appears to be one of most common factors that limit photosynthetic capacity, protein synthesis, plant growth, energy (Parida and Das 2005), total nitrogen content and lipid metabolism (Rahman *et al.,* 2019; Tyerman *et al.,* 2019). An in-

**Table 1.** Bacterial isolates screened for different pH and salt concentration

| Sl. No. | Bacterial isolates | Salt. Conc of NaCl (mM) | | | pH | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 50 | 100 | 150 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 |
| 1 | PGPR18 | +++ | +++ | + | +++ | +++ | +++ | ++ | ++ |
| 2 | PGPR16 | +++ | +++ | + | +++ | +++ | +++ | ++ | ++ |
| 3 | PGPR22 | ++ | ++ | + | +++ | +++ | +++ | ++ | ++ |
| 4 | PGPR21 | +++ | ++ | - | +++ | +++ | ++ | ++ | + |
| 5 | PGPR26 | +++ | ++ | - | +++ | +++ | ++ | ++ | + |
| 6 | PGPR24 | ++ | ++ | + | +++ | +++ | ++ | + | - |
| 7 | PGPR27 | ++ | + | - | +++ | +++ | ++ | + | + |
| 8 | PGPR9 | ++ | + | - | +++ | +++ | ++ | + | - |
| 9 | PGPR23 | +++ | ++ | + | +++ | +++ | ++ | + | - |
| 10 | PGPR30 | ++ | + | - | +++ | +++ | + | + | - |
| 11 | PGPR25 | ++ | + | - | +++ | +++ | ++ | + | - |
| 12 | PGPR14 | +++ | ++ | - | +++ | +++ | ++ | + | - |
| 13 | PGPR20 | +++ | + | - | +++ | +++ | ++ | - | - |
| 14 | PGPR29 | ++ | + | - | +++ | +++ | ++ | - | - |
| 15 | PGPR13 | ++ | + | - | +++ | +++ | ++ | - | - |

*- = No growth, + = Less growth, ++ = Medium growth, +++ = High growth

crease in crop productivity can be attributed to the development and utilization of plant growth-promoting (PGP) bacteria that are tolerant to different abiotic stress including salt, pH and extreme temperatures. Our results showed that among these fifteen isolates, seven (46.66%) pseudomonad isolates grew well and were showed high growth (+++) and eight (53.33%) isolates were showed moderate growth (++) astolerated on 50 mM salinity respectively. Similarly, at 100 mM salinity tolerance, two (13.33%) pseudomonad isolates (PGPR18 and PGPR16) were tolerated and showed high growth (+++), six (40%) isolates were showed moderate growth (++) and seven isolates (46.66%) were showed less growth (+). Interestingly, five (33.33%) pseudomonad isolates were able to tolerate even in 150 mM salinity level but showed less growth (+) and ten (66.66%) isolates showed without any growth (-), thus are confirmed their ability of surviving in saline condition. A strain of *P. plecoglossicida*, strain Pp20, could reduce salt and aluminum damage to maize roots (Zerouk *et al.,* 2019). Further, it was discovered that strain Pp20 can survive in a range of salt concentrations, from 50 to 600 mM and found that the presence of salt had positive effect on seminal roots, lateral roots, root length and stem weight (Mahmood *et al.,* 2019). Similarly, the previous report showed that *P. libanensis* TR1 and *Helianthus annuus* exhibited high resistance to saline stress (8%) (Ma *et al.,* 2019). Another study showed that isolates UPMR2, UPMR7, UPMR17, and UPMR18 could tolerate high salt concentrations (up to 6%) which confirm their ability to survive in a saline environment (Habib *et al.,* 2013).

In pH tolerance test, all (100%) pseudomonad isolates grew well and displayed high growth (+++) on pH-7 and pH-8 whereas, on pH-9 only three (20%) isolates were expressed high growth (+++) remaining twelve (80%) isolates showed moderate growth (++). Similarly at pH-10 five (33.33%) isolates showed moderate growth (++) behavior and seven (46.66%) isolates showed less growth (+) while three (20%) isolates were displayed no growth (-) at pH-10 level. Whereas, at pH-11, three (20%) isolates grew moderately (++) and three (20%) isolates showed less growth (+) and there is no growth have been observed in nine (60%) isolates. The study found that the growth of selected bacterial isolates increases linearly under different range of stress conditions such as pH between 7 and 11 and salt concentration up to 150 mM. However, at a salt concentration of 150 mM, the bacterial growth was observed to decrease. The research indicates, bacterial strains isolated from acidic soils were able to convert insoluble phosphates into soluble forms under high salt and pH stress conditions (Chaiharn and Lumyong, 2009; Sharma *et al.,* 2021). Previous study showed that the isolate was able to tolerate salt concentrations up to 8% NaCl, but the optimum growth was observed at 4% NaCl (Singh *et al.,* 2019).

## Conclusion

Of the 15 pseudomonad isolates studied, 46.66% showed high growth (+++) under 50 mM salinity, while 53.33% showed moderate growth (++). At 100 mM salinity, 13.33% of the isolates (PGPR18 and PGPR16) displayed high growth (+++) while 40% showed moderate growth (++) and 46.66% showed less growth (+). 33.33% of the isolates were found to tolerate 150 mM salinity with less growth (+) while 66.66% did not grow (-), indicating their survival in saline conditions. All 100% of the pseudomonad isolates showed high growth (+++) at pH 7 and 8, while only 20% showed high growth (+++) at pH 9 and 80% showed moderate growth (++). At pH 10, 33.33% showed moderate growth (++) and 46.66% showed less growth (+), with 20% showing no growth (-). At pH 11, 20% grew moderately (++) and 20% showed less growth (+), while 60% did not grow at all. The study found that the growth of the bacterial isolates increased linearly under various stress conditions such as pH 7 to 11 and salt concentrations up to 150 mM. In conclusion, the characterization of salt-tolerant PGPR is important for the development of sustainable biostimulants strategies for the improvement of crop performance in salt-affected soils. Future research should focus on optimizing the selection and use of PGPR for specific crops and environmental conditions, leading to improved crop yields and increased food security.

## References

Banerjee, A., Sarkar, S., Cuadros-Orellana, S. and Bandopadhyay, R. 2019. Exopolysaccharides and biofilms in mitigating salinity stress: The biotechnological potential of halophilic and soil-inhabiting PGPR microorganisms. *Microorganisms in Saline Environments: Strategies and Functions.* 133-153.

Banjare, U., Patel, A.K. and Pandey, A.K. 2023. Biochemical and Molecular Evaluation of Rhizobium spp. and its Growth Promotion Studies with Lentil (*Lens culinaris* Medik. L.). *J Pure Appl Microbiol*.

Chaiharn, M. and Lumyong, S. 2009. Phosphate solubilization potential and stress tolerance of rhizobacteria from rice soil in Northern Thailand. *World Journal of Microbiology and Biotechnology*. 25(2): 305-314.

Habib, S.H., Kausar, H., Saud, H.M., Ismail, M.R. and Othman, R. 2016. Molecular characterization of stress tolerant plant growth promoting rhizobacteria (PGPR) for growth enhancement of rice. *Int. J. Agric. Biol.* 18 :184-191.

Hossain, M. S., Alam, M. U., Rahman, A., Hasanuzzaman, M., Nahar, K., Al Mahmud, J. and Fujita, M. 2017. Use of iso-osmotic solution to understand salt stress responses in lentil (*Lens culinaris* Medik.). *South African Journal of Botany.* 113: 346-354.

Kerbab, S., Silini, A., Chenari Bouket, A., Cherif-Silini, H., Eshelli, M., El Houda Rabhi, N. and Belbahri, L. 2021. Mitigation of NaCl stress in wheat by rhizosphere engineering using salt habitat adapted PGPR halotolerant bacteria. *Applied Sciences.* 11(3): 1034.

Kumar, A., Jha, M.N., Singh, D., Pathak, D. and Rajawat, M.V.S. 2021. Prospecting catabolic diversity of microbial strains for developing microbial consortia and their synergistic effect on Lentil (*Lens esculenta*) growth, yield and iron biofortification. *Archives of Microbiology.* 203(8): 4913-4928.

Kumawat, K.C., Nagpal, S. and Sharma, P. 2022. Potential of plant growth-promoting rhizobacteria-plant interactions in mitigating salt stress for sustainable agriculture: A review. *Pedosphere.* 32(2): 223-245.

Ma, Y., Rajkumar, M., Oliveira, R. S., Zhang, C. and Freitas, H. 2019. Potential of plant beneficial bacteria and arbuscular mycorrhizal fungi in phytoremediation of metal-contaminated saline soils. *Journal of Hazardous Materials.* 379: 120813.

Mahmood, A., Amaya, R., Turgay, O.C., Yaprak, A.E., Taniguchi, T. and Kataoka, R. 2019. High salt tolerant plant growth promoting rhizobacteria from the common ice-plant *Mesembryanthemum crystallinum* L. *Rhizosphere.* 9: 10-17.

Nathiya, S., Janani, R. and Kannan, V.R. 2020. Potential of plant growth promoting Rhizobacteria to overcome the exposure of pesticide in *Trigonella foenum-graecum* (fenugreek leaves). *Biocatalysis and Agricultural Biotechnology.* 23:101493.

Parida, A.K. and Das, A.B. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety.* 60(3): 324-349.

Rahman, M.M., Penny, G., Mondal, M.S., Zaman, M.H., Kryston, A., Salehin, M. and Müller, M.F. 2019. Salinization in large river deltas: Drivers, impacts and socio-hydrological feedbacks. *Water Security.* 6: 100024.

Saleem, S., Iqbal, A., Ahmed, F. and Ahmad, M. 2021. Phytobeneficial and salt stress mitigating efficacy of IAA producing salt tolerant strains in Gossypium hirsutum. *Saudi Journal of Biological Sciences*. 28(9): 5317-5324.

Sharma, A., Dev, K., Sourirajan, A. and Choudhary, M. 2021. Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology.* 19(1): 1-10.

Sharma, A., Dev, K., Sourirajan, A. and Choudhary, M. 2021. Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *Journal of Genetic Engineering and Biotechnology*. 19(1):1-10.

Shultana, R., Zuan, A.T.K., Naher, U. A., Islam, A.M., Rana, M.M., Rashid, M.H. and Hasan, A. K. 2022. The PGPR Mechanisms of Salt Stress Adaptation and Plant Growth Promotion. *Agronomy*. 12(10): 2266.

Singh, B., Padhy, A.K., Ambreen, H., Yadav, M., Bhardwaj, S., Singh, G. and Bhatia, S. 2022. Understanding Abiotic Stress Responses in Lentil under Changing Climate Regimes. In: *Developing Climate Resilient Grain and Forage Legumes* (pp. 179-204). Singapore: Springer Nature Singapore.

Singh, S. and Husen, A. 2019. Role of nanomaterials in the mitigation of abiotic stress in plants. *Nanomaterials and Plant Potential*. 441-471.

Tsegaye, Z., Assefa, F., Tefera, G., Alemu, T. and Gizaw, B. 2018. Characterization and identification of Tef (Eragrostis tef) seed endophytic bacterial species and evaluate their effect on plant growth promotion. *J Plant Pathol Microbiol*. 9(438): 2.

Tyerman, S.D., Munns, R., Fricke, W., Arsova, B., Barkla, B.J., Bose, J. and Wen, Z. 2019. Energy costs of salinity tolerance in crop plants.

Yañez-Yazlle, M.F., Romano-Armada, N., Rajal, V.B. and Irazusta, V.P. 2021. Amelioration of saline stress on chia (*Salvia hispanica* L.) seedlings inoculated with halotolerant plant growth-promoting bacteria isolated from hypersaline environments. *Frontiers in Agronomy*. 3: 665798.

Zerrouk, I.Z., Rahmoune, B., Khelifi, L., Mounir, K., Baluska, F. and Ludwig-Müller, J. 2019. Algerian Sahara PGPR confers maize root tolerance to salt and aluminum toxicity via ACC deaminase and IAA. *Acta Physiologiae Plantarum*. 41(6): 1-10.