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Analysis of salt tolerance capacity of phosphate solubilizing bacteria for sustainable agriculture

James Arockia John Paul[1], Balan Karunai Selvi[2]*, Ramachandran Ananthavalli[1], Pethanan Sivakumar[1] and Muniyandi Birunthа[3]

[1]Department of Zoology, Arumugam Pillai Seethai Ammal College, Tiruppattur-630 211, Tamil Nadu, India.
[2]*Department of Botany, V.V.Vanniaperumal College for Women, Virudhunagar-626 001, Tamil Nadu, India.
[3]Department of Animal Health and Management, Science Campus, Alagappa University, Karaikudi-630 003, Tamil Nadu, India
*Corresponding Author: Balan Karunai Selvi
karunaiselvi@vvvcollege.org, karunaiselvi@gmail.com

Abstract

Soil salinity is one of the major abiotic stresses limiting crop productivity worldwide, leading to nutrient imbalance, reduced microbial activity, and poor plant growth. Phosphateabundant in soils, often remains in insoluble forms, making it unavailable to plants under saline conditions. Phosphate solubilizing microorganisms (PSMs) play a significant role in mobilizing insoluble phosphates through the secretion of organic acids and enzymes, thereby enhancing phosphorus availability. This study focuses on the analysis of salt tolerance capacity of PSMs isolated from a leguminous plant. The ability of *Bacillus* sp. to solubilize tricalcium phosphate (TCP) under varying NaCl concentrations was assessed, revealing a significant reduction in phosphate solubilization as salt concentration increased from 0.02% to 0.04% during15 days of incubation. Between 0.04% to 1% NaCl, a gradual decline in solubilization was observed, although bacterial growth remained unaffected. This suggests that while *Bacillus* sp. can tolerate moderate salinity, its phosphate-solubilizing efficiency diminishes under higher salt concentrations. *Bacillus* sp. B1 with its ability to tolerate salinity and maintain phosphate solubilization activity, represents that the microorganism enhancing phosphorus availability in saline and alkaline soils, contributing to sustainable agricultural practices.

Keywords: Phosphate solubilizing microorganisms (PSMs), Salt tolerance, Sustainable agriculture, Soil salinity, *Bacillus* sp.

Introduction

Sustainable agriculture is an essential approach to meet the increasing global food demand while maintaining environmental integrity. Salinity is a major abiotic stress affecting approximately 20% of irrigated lands globally, posing a severe threat to agricultural productivity (Munns and Tester, 2008). High salt concentrations disrupt plant water uptake, nutrient balance, and enzymatic activities, leading to reduced growth and yield (Shrivastava and Kumar, 2015). Sustainable strategies to mitigate salinity stress are crucial for maintaining food security. One of the key challenges in sustainable farming is managing soil salinity, a significant abiotic stress that adversely affects crop productivity. Salinity hampers plant growth by inducing ionic and osmotic stress, disrupting nutrient uptake, and impairing metabolic processes (Munns and Tester, 2008; Shrivastava and Kumar, 2015). This global issue necessitates innovative solutions to improve crop resilience and maintain soil health in saline environments.

Phosphate is an essential macronutrient for plants, but its availability in soil is often limited due to its fixation in insoluble forms. Phosphate solubilizing microorganisms (PSMs), such as *Pseudomonas*, *Bacillus*, and *Aspergillus*, play a pivotal role in solubilizing these fixed phosphates by secreting organic acids and enzymes (Rodríguez and Fraga, 1999). PSMsare a promising biotechnological tool for enhancing plant growth under saline conditions. These beneficial microorganisms can convert insoluble forms of phosphate into soluble forms, making it bioavailable to plants (Rodríguez and Fraga, 1999;Khan et al., 2014). These microorganisms not only improve phosphate availability but also enhance plant growth by producing phytohormones like indole acetic acid (IAA) and gibberellins (Zaidi et al., 2009). PSMs exhibit diverse mechanisms to tolerate salinity, including the production of osmolytes (e.g., proline, trehalose), antioxidant enzymes, and exopolysaccharides (EPS) (Vardharajula et al., 2011).Furthermore, PSMs have shown potential in mitigating the adverse effects of salinity by improving nutrient uptake, producing growth-promoting substances, and enhancing the overall soil structure (Alori et al., 2017, Etesami andBeattie, 2018). Studies indicate that salinity-tolerant PSMs can enhance plant growth even under saline conditions by improving nutrient uptake and mitigating the effects of ionic and osmotic stress. Egamberdieva et al. (2011), reported that salinity-tolerant *Pseudomonas* strains significantly improved wheat growth under saline conditions. The ability of *Bacillus* species to solubilize phosphate and produce IAA in the presence of high salt concentrations, highlighting their dual role in nutrient mobilization and stress mitigation.

The role of PSMs in salt tolerance is increasingly being explored as an eco-friendly alternative to chemical fertilizers. Their capacity to thrive and function in saline soils makes them a valuable resource in sustainable agricultural practices (Sharma et al., 2013). By promoting plant growth and improving soil fertility under saline conditions, PSMs contribute to achieving agricultural sustainability while minimizing the environmental footprint (Rodriguez et al., 2008). The integration of PSMs into agricultural practices offers a sustainable solution to salinity stress. They reduce dependency on chemical fertilizers, improve soil health, and enhance crop resilience. Kaur and Reddy (2015), emphasized that PSMs could be used as bio-inoculants to promote sustainable farming in salt-affected soils. This study aims to analyze the salt tolerance capacity of phosphate solubilizing microorganisms under different salt stress condition. The findings could pave the way for developing microbial-based solutions to combat soil salinity and ensure food security in saline-prone regions.

Materials and Methods

Isolation of phosphate solubilizing microorganisms

Phosphate-solubilizing bacteria were isolated from the rhizosphere soil of a leguminous plant *Tephrosia purpurea*. *Tephrosia purpurea* belongs to the family Fabaceae (Papilionaceae) and is a perennial, much-branched herb or sub-shrub commonly found in India as a weed in wastelands, roadsides, and open fields. It is a hardy plant that tolerates dry and poor soils, producing small purple papilionaceous flowers arranged in axillary or terminal racemes, and linear, slightly curved legumes containing several seeds. The plant has nitrogen-fixing root nodules with *Rhizobium*, making it an important soil enricher. In addition to its ecological role, it holds great medicinal value in Ayurveda, being used as an antipyretic, expectorant, liver tonic, vermifuge, and for wound healing.

Serial dilutions of the rhizosphere soil samples of *Tephrosia purpurea* were prepared and spread onto Pikovskaya's Agar Medium (PVK), where phosphate source supplemented with Tricalcium Phosphate(Gaur, 1990). Colonies exhibiting clear halo zones around their growth were identified as potential phosphate solubilizers. These isolates were subsequently tested for their ability to solubilize phosphate under varying salt concentrations using a plate assay method.

Analysing the Salt Tolerance of Phosphate-Solubilizing Bacteria

The salt tolerance capacity of phosphate-solubilizing bacterial isolates were assessed by evaluating their phosphate solubilization activity under salt stress. Pikovskaya's solid medium, supplemented with tricalcium phosphate (TCP) as the insoluble phosphate source, was used with varying NaCl concentrations ranging from 0.02% to 15% (Kaur and Reddy (2015). Bacterial isolates were inoculated at the center of the plates and incubated at 28°C for 7 days. Observations were recorded for bacterial growth and the diameter of solubilization zones produced by the colonies. The best salt tolerant bacterial isolate wasidentified and tested for phosphate solubilization in a broth assay. In 100 ml of Pikovskaya's broth, 1 ml of a 24-hour-old bacterial broth culture (10^{-3} cells/ml) was inoculated. The cultures were incubated for 15 days at varying NaCl concentrations (0.02% to 15%). At regular intervals (3 days), the cultural filtrate was analyzed for pH changes and soluble phosphorus content, following the protocol described by Jackson (1973).

Results

Among the phosphate-solubilizing isolates obtained, seven *Bacillus* strains (B1–B7) showed varying degrees of growth and solubilization efficiency on Pikovskaya's agar medium (Table 1). The culture diameter ranged from 1.2 cm to 1.9 cm, while the solubilization zone varied between 0.5 cm and 1.0 cm. Bacillus sp. B2 and B4 exhibited the highest culture diameter of 1.9 cm with solubilization zones of 0.9 cm and 1.0 cm, respectively. Bacillus sp. B3 also showed good growth (1.8 cm) with a solubilization zone of 0.9 cm. In contrast, Bacillus sp. B5 and B6 recorded moderate culture diameters of 1.8 cm and 1.3 cm but had relatively smaller solubilization zones of 0.5 cm. The lowest growth was observed in Bacillus sp. B7 (1.2 cm) with a solubilization zone of 0.5 cm. Overall, Bacillus sp. B4 was found to be the most efficient phosphate solubilizer, showing both larger colony size and a prominent solubilization zone.

Table 1. Screening of phosphate solubilizing microorganisms for tricalcium phosphate solubilization in a plate assay method

Organism	Culture diameter (cm)	Solubilization zone (cm)	Solubilization activity
Bacillus sp. (B1)	1.3	0.8	+++
Bacillus sp. (B2)	1.9	0.9	++++
Bacillus sp. (B3)	1.8	0.9	++++
Bacillus sp. (B4)	1.9	1.0	++++
Bacillus sp. (B5)	1.8	0.5	++
Bacillus sp. (B6)	1.3	0.5	++
Bacillus sp. (B7)	1.2	0.5	++

The salt tolerance capacity of phosphate-solubilizing bacterial isolates was evaluated under varying NaCl concentrations ranging from 0.02% to 15%, as shown in Fig. 1. The bacterial isolates were exhibited tolerance up to 10% (w/v) NaCl during 7 days of incubation on Pikovskaya's agar plates. A decline in growth was noted beyond 0.1% NaCl, with colony diameters progressively reducing as the salt concentration increased. However, solubilization zones diminished at and above 8% NaCl, with most isolates failing to form zones at concentrations exceeding 6% NaCl. *Bacillus* sp. B1 demonstrated the highest salt tolerance, maintaining growth and phosphate solubilization up to 10% NaCl. Therefore, *Bacillus* sp. B1was selected for further testing of its phosphate solubilization capacity under salt stress condition in liquid Pikovskaya's medium.

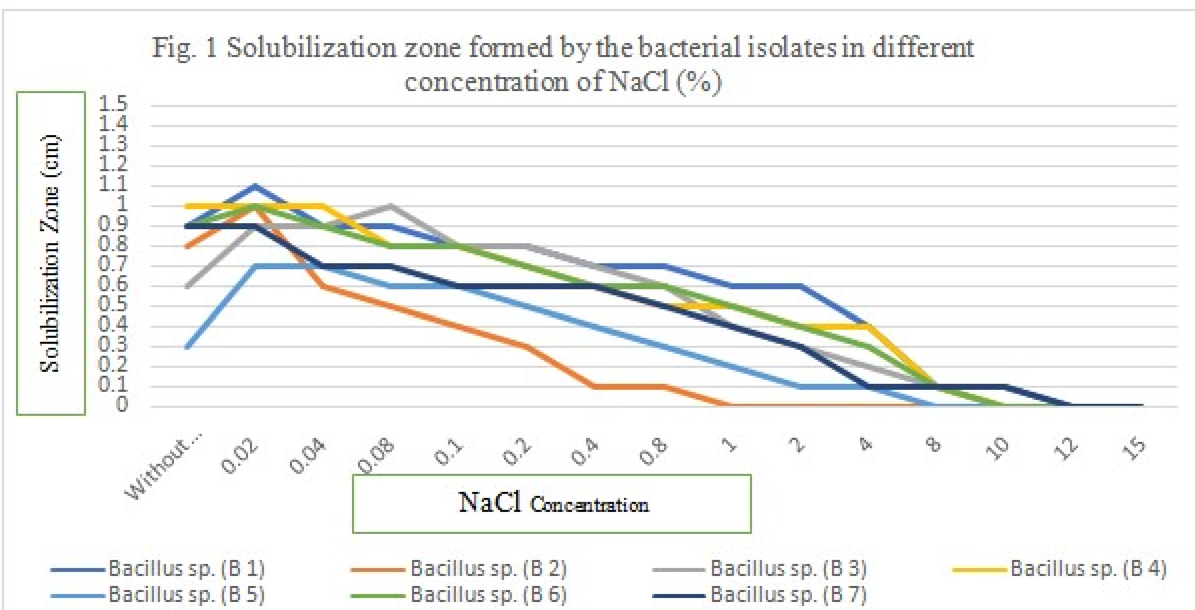
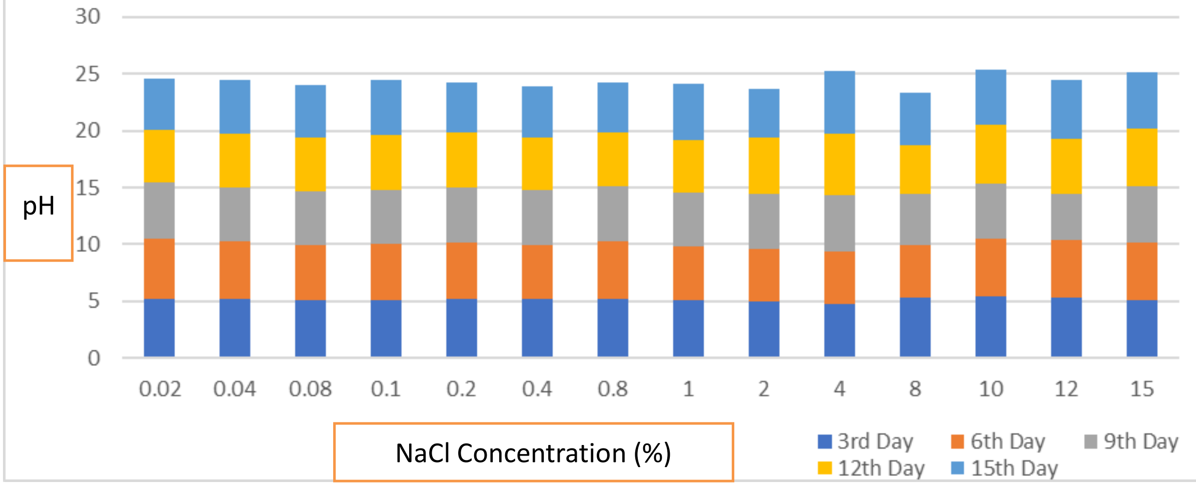


Fig. 1 Solubilization zone formed by the bacterial isolates in different concentration of NaCl (%)

In liquid assays, *Bacillus* sp. B1 tolerated NaCl concentrations as high as 15%, although phosphate solubilization declined with increasing salinity. The ability of *Bacillus* sp.B1 to solubilize tricalcium phosphate (TCP) and the associated pH changes in liquid Pikovskaya's broth at different NaCl concentrations is illustrated in Fig. 2. A significant reduction in phosphate solubilization was observed as salt concentration increased from 0.02% to 0.04% during 15 days of incubation. Between 0.04% and 1% NaCl, phosphate solubilization showed a gradual decline, although bacterial growth remained unaffected.The findings suggest that *Bacillus* sp. B1 can withstand moderate salinity and continue phosphate solubilization, though its efficiency declines under elevated salt concentrations.

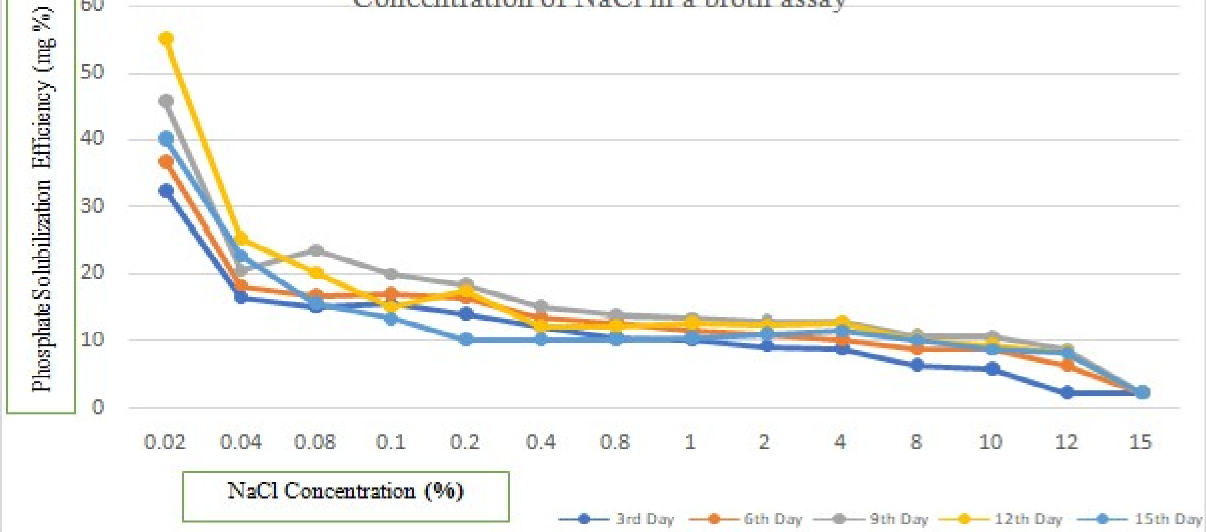
Fig. 2 Variation in pH of the bacterial isolate *Bacillus* sp. B1 in different concentration of NaCl



pH changes with different NaCl concentrations over time.

Overall, pH values remain around 5 on the 3rd day but generally decrease slightly by the 15th day Fig. 3.At lower NaCl concentrations (0.02–2%), pH gradually declines, showing more acidity over time. At higher concentrations (4–15%), pH fluctuates, with some values increasing slightly by the 12th–15th day indicating that higher salt levels may suppress microbial activity leading to variable pH changes. Overall, lower NaCl promotes a steady decline in pH, while higher NaCl disrupts this pattern.

Fig. 3 Phosphate Solubilization Efficiency of *Bacillus sp.* with different Concentration of NaCl in a broth assay



Discussion

The evaluation of salt tolerance capacity of phosphate-solubilizing bacterial (PSB) isolates under varying NaCl concentrations revealed that the bacterial isolates could tolerate up to 10% (w/v) NaCl on Pikovskaya's agar plates after 7 days of incubation. A notable decline in growth was observed at NaCl concentrations above 0.1%, as evidenced by the reduction in colony diameters. Similarly, the phosphate solubilization zones decreased progressively with increasing NaCl concentrations and were absent at concentrations exceeding 10%. These observations align with the findings of Narsian and Patel (1997), reported that a reduction in phosphate solubilization activity under high salinity, likely due to impaired organic acid production by bacteria under salt stress condition (Yadav and Yadav, 2003). This stress-induced limitation affects bacterial growth and their ability to solubilize phosphate.

The ability of bacterial strains to tolerate high salinity, pH, and temperature is critical for their survival, proliferation, and functionality in saline soils. Stress-tolerant bacteria are commonly found in environments subject to osmotic, pH, and temperature fluctuations, as highlighted by Nautiyal et al. (2000).Among the tested isolates, *Bacillus* sp. B1 demonstrated superior salt tolerance, maintaining both growth and phosphate solubilization up to 10% NaCl. *Bacillus* sp. B1 its ability to tolerate such high salinity makes it a promising candidate for agricultural applications in saline soils. This aligns with previous studies indicating that *Bacillus* spp. is well-adapted to saline conditions and exhibit astrong survival mechanism under osmotic stress (Nautiyal et al., 2000; Yadav and Yadav, 2003).

The decline in phosphate solubilization observed at higher NaCl concentrations could be attributed to reduced bacterial metabolic activity and the inhibition of acid production, which is essential for solubilizing insoluble phosphates. Yadav and Yadav (2003), suggested that the absence of solubilization zones at higher salt concentrations might result from the limitations in organic acid production due to osmotic stress. Proline and other osmolytes, such as glycine betaine, are known to support bacterial survival under salinity by stabilizing proteins and cellular structures (Shen et al., 1999). Proline plays a key role in enhancing bacterial tolerance to salinity by acting as an osmolyte. Organic solutes such as proline, glycine betaine, sugars, and sugar alcohols help bacteria mitigate osmotic stress. Proline accumulation has been shown to support bacterial survival in environments with up to 6% NaCl (Yadav andYadav, 2003).

This study observed a direct correlation between phosphate solubilization and medium acidity. Efficient phosphate solubilizers often decrease the pH of their environment during growth, as supported by findings from Nautiyal et al. (2000), and earlier studies (Swaby and Sperber, 1958; Louw andWebley, 1959; Ahmed andJha, 1968; Taha et al., 1969; Bajpai and Sundara Rao, 1971). The acidification of the medium facilitates the solubilization of insoluble phosphate, contributing to enhanced phosphorus availability.

Salinity, particularly chloride salinity, has been reported to inhibit phosphorus availability and uptake more than sulphate salinity, necessitating higher phosphorus inputs in such conditions. Saline soils are also characterized by low organic matter, which further impacts crop growth and nutrient dynamics (Narsian and Patel, 1997).

This study reveals that microorganisms capable of reducing medium pH are efficient phosphate solubilizers, as demonstrated in *Bacillus* sp. B1. Acidification of the growth medium is a critical factor in phosphate solubilization, as previously observed by Swaby and Sperber (1958), Louw and Webley (1959), and Nautiyal et al. (2000). Furthermore, salinity not only affects microbial activity but also impacts phosphorus availability in soils. Chloride salinity has been reported to inhibit phosphorus availability more than sulphate salinity, necessitating the use of salt-tolerant PSB in such environments (Narsian and Patel, 1997).

Conclusion

In this study, *Bacillus* sp. B1 demonstrated significant salt tolerance and phosphate solubilization activity under varying NaCl concentrations. The results show that *Bacillus*sp.B1 could tolerate up to 10% NaCl on solid media and maintained moderate phosphate solubilization activity in liquid Pikovskaya's broth, even under higher salt concentrations, though the efficiency decreased as salinity increased. The decline in phosphate solubilization with increasing NaCl concentrations suggests that while *Bacillus* sp. B1 can adapt to saline conditions, its ability to solubilize phosphate is compromised at higher salinity levels. These findings highlight the potential of *Bacillus* sp. B1 as a salt-tolerant phosphate-solubilizing bacterium, which could be beneficial for enhancing phosphorus availability in saline and alkaline soils. The ability of *Bacillus* sp. B1 to thrive in saline environments, coupled with its phosphate solubilization capacity, makes it a promising organism for sustainable agricultural practices aimed at improving soil fertility and crop productivity in saline-affected areas. Future studies could explore the mechanisms behind salt tolerance and phosphate solubilization in *Bacillus* sp. B1 as well as its potential in field applications.

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