Biological Forum – An International Journal **15(9): 476-484(2023)**

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

Unveiling the Multifaceted Abilities of Halotolerant Phosphate Solubilizing Bacteria Isolated from the Kutch Desert Ecosystem

Anurag Yadav[1*], Rahulkumar Chauhan[1], Kavin Soni[2] and Kusum Yadav[3]
[1]Department of Microbiology, College of Basic Science and Humanities,
Sardarkrushinagar Dantiwada Agricultural University, Sardrarkrushinagar, Dist. Banaskantha (Gujarat), India
[2]Bioscience Research Laboratory, Sardarkrushinagar Dantiwada Agricultural University,
Sardrarkrushinagar, Dist. Banaskantha (Gujarat), India
[3]Department of Biochemistry, University of Lucknow, Lucknow (Uttar Pradesh), India

(Corresponding author: Anurag Yadav)*
(Received: 20 June 2023; Revised: 24 July 2023; Accepted: 27 August 2023; Published: 15 September 2023)
(Published by Research Trend)

ABSTRACT: The study explored and characterized the functionalities of halotolerant phosphate solubilizing bacteria (PSB) isolated from soils of various locations in the Kutch desert. The isolates were screened for phosphate and zinc solubilization, and their growth patterns were analyzed under different NaCl concentrations. The three screened isolates—PH27, PH28, and PH30—were characterized biochemically and identified as *Enterobacter bugandensis* PH27, *Psychrobacter faecalis* PH28, and *Bacillus amyloliquefaciens* PH30 through 16S rRNA gene sequencing. While the study is comprehensive, it inherently faces several challenges. The complexity of microbial enzymology and the interactions between environmental variables and microbial functionality pose challenges in data interpretation. A temporal growth analysis of isolates revealed phosphorus solubilization capabilities and pH modulation in Pikovskaya's broth cultures. Enzymatic activities of acid and alkaline phosphatases revealed unique profiles for each isolate. PH27 and PH28 excelled in phosphate solubilization and medium acidification, while PH30 showed significant pH alteration despite lower phosphorus solubilization. The study makes several pivotal contributions to the field. Firstly, it adds to the existing body of knowledge by identifying and characterizing three unique halotolerant PSB isolates, thereby enriching our understanding of microbial diversity in saline environments. Secondly, it provides valuable insights into their phosphorus and zinc solubilizing capabilities, which affect nutrient cycling in soil ecosystems. Thirdly, the study explores the enzymatic activities of these isolates, laying the groundwork for future research in microbial enzymology. Lastly, the study examines the rate of phosphate solubilization and its corresponding impact on pH levels, offering targeted insights with broader environmental implications.

Keywords: phosphate solubilizing bacteria, halotolerant bacteria, phosphatase, phosphorous bioavailability.

INTRODUCTION

The Kutch desert, located in the western part of Gujarat, India, is a unique and harsh ecosystem characterized by extreme aridity, high salinity, and fluctuating temperatures (Raji *et al.*, 2019; Thumar *et al.*, 2010). This unique environment provides a niche for the growth and adaptation of halotolerant bacteria, which are capable of thriving in high salt concentrations (Jiang *et al.*, 2021; Kothe *et al.*, 2020; Najafi Zilaie *et al.*, 2022; Parada-Pinilla *et al.*, 2021; Pathak and Mukherjee, 2020).

Halotolerant bacteria have attracted significant attention due to their multifaceted traits and potential applications in agriculture and environmental remediation (Orhan, 2016; Raddadi *et al.*, 2015). Their ability to adapt to saline conditions has been the subject of extensive research, revealing unique mechanisms of osmoregulation, ion transport, and metabolic adaptations. These bacteria have developed various mechanisms to tolerate and utilize the abundant salt in their surroundings (Kothe *et al.*, 2020; Najafi Zilaie *et al.*, 2022; Pathak and Mukherjee, 2020).

In addition, phosphorus is an essential nutrient for plant growth, but its availability in soil is often limited due to its presence in insoluble forms (Elhaissoufi *et al.*, 2022). Phosphate solubilizing bacteria (PSB) can convert insoluble phosphates into soluble forms, making this accessible to plants (Elhaissoufi *et al.*, 2022; Yadav *et al.*, 2022; Yuan *et al.*, 2012). Phosphate solubilization is a crucial process in the desert ecosystem, as the availability of this element is often limited in saline soils (Dey *et al.*, 2021; Shah and Saraf, 2019). Several reports show the unique ability of halotolerant PSB to solubilize insoluble forms of phosphate, making it accessible to plants (Bhatt *et al.*, 2018; Dey *et al.*, 2021; Jiang *et al.*, 2021, Shah and Saraf 2019). This ability is of great importance in the Kutch desert, where plants face challenges in nutrient

uptake due to the high salinity levels (Dey *et al.*, 2021; Shah and Saraf, 2019).

Multitrait microorganisms, such as halotolerant PSB, possess several beneficial characteristics that can be harnessed for various applications (Orhan, 2016). In agriculture, they can enhance soil fertility, promote plant growth, and increase crop yield, especially in saline soils. Furthermore, halotolerant PSBs have been found to alleviate salt stress in plants, making them potential alternatives for salt-sensitive crops (Orhan, 2016).

The isolation of multitrait, halotolerant PSB from the Kutch desert of Gujarat represents a significant step in exploring the untapped microbial diversity of extreme environments (Raddadi *et al.*, 2015).

This research paves the way for further studies on the ecological roles, molecular mechanisms, and potential applications of such bacteria in various fields, including agriculture, environmental science, and biotechnology (Orhan, 2016).

MATERIAL AND METHODS

The study was conducted in the Department of Microbiology, College of Basic Science and Humanities, Sardarkrushinagar Dantiwada Agricultural University, India. The desert soil of Kutch from various locations was collected in plastic vials. The vials were transported to the laboratory within 24 hours and microbiologically processed.

A. Screening soil bacteria for phosphate solubilization

The PSBs were isolated from soil samples by serial dilution and plate count method using Pikovskaya's medium (RI, 1948), a selective medium for P solubilizers. One gram of soil was dissolved in 10 mL sterilized distilled water in the test tubes and serially diluted (Ranjan *et al.*, 2013). The diluted samples were spread onto Pikovskaya's agar medium (RI, 1948) and incubated. All the bacterial colonies displaying clear halo around were picked and pure cultured. The phosphate solubilization index (PSI) of isolates was determined using the following formula (Nautiyal, 1999).

$$\text{Phosphate Solubilizing Index} = \frac{\text{Solubilizing zone diameter}}{\text{Bacterial colony diameter}}$$

B. Screening PSB isolates for halotolerance and additional traits

A quantitative growth assay was conducted to investigate halotolerance among the isolated PSB. Aliquot of 0.1 mL from the overnight grown culture was inoculated into separate tubes of nutrient broth, each amended with 2.5%, 5%, and 7.5% (w/v) concentrations of NaCl. These tubes were subsequently incubated at a controlled temperature of 30 ± 1°C for 48 hours. Each culture's optical density (OD) was measured spectrophotometrically following the incubation period to assess the growth and halotolerance.

The three isolates were further screened for potassium solubilization on Aleksandrow Agar (Raji and Thangavelu, 2021). Also, a standardized approach was employed to evaluate the zinc solubilizing ability of the bacterial strains under investigation, as described by Fasim *et al.* (2002). Specifically, tris-minimal agar medium was prepared and supplemented with D-glucose and various insoluble zinc compounds. The selected zinc compounds included ZnO (1.244 g L^{-1}, equivalent to 15.23 mM), $Zn_3(PO_4)_2$ (1.9882 g L^{-1}, equivalent to 5.0 mM), and $ZnCO_3$ (1.728 g L^{-1}, equal to 5.2 mM).

C. Morphological and biochemical characterization of halotolerant PSB isolates

The biochemical characterization was initiated using a HiAssorted™ Biochemical Test Kit (HiMedia Laboratories Pvt. Ltd., Mumbai, India) containing ten distinct tests: utilization of citrate, lysine, and ornithine; production of urease, H_2S, and indole; hydrolysis of starch and gelatin; nitrate reduction; and phenylalanine deamination. The carbohydrate fermentation test was performed on 21 sugars, namely, adonitol, arabinose, cellobiose, dextrose, dulcitol, fructose, galactose, inositol, inulin, lactose, melibiose, maltose, mannitol, mannose, raffinose, rhamnose, sorbitol, sucrose, trehalose, xylulose, and glucose. The 24 hour old cultures of halotolerant PSB were carefully streaked with an inoculating loop on every section of the HiAssorted™ test kit, followed by incubation at a controlled temperature of 30±1°C for 24 hours. Subsequently, the results of the kit strips were compared with the catalog.

In parallel, Petri plates containing phenol red agar medium were prepared and streaked with PSB isolates through spread plating (Mazhar *et al.*, 2016). Different sugar disks were pressed onto the surface of the plates and incubated at 30°C for a period ranging from 24 to 48 hours. Upon the incubation, sugar utilization by the isolates was observed and marked positive with a color change from red to yellow around the disk, indicating successful sugar fermentation (Table 1).

Furthermore, plates containing the Mueller-Hinton agar medium were prepared to assess the antibiotic effect on bacterial growth (Saffari *et al.*, 2016). The test organisms were streaked thrice onto the entire agar surface of the plate with a sterile cotton swab. Thereafter, the hexa discs of antibiotics were pressed onto plates, followed by incubation at 30°C for 24 hours. The results were recorded, contributing to the comprehensive characterization.

D. Measuring PSB mediated bioavailability of inorganic phosphate in growth medium

Soluble phosphate was estimated from the supernatant of bacteria inoculated and incubated in Pikovskaya's broth following the Fiske-Subbarow method (Fiske and Subbarow, 1925). This procedure used double distilled water, devoid of phosphorus, for all purposes. A standard phosphorus solution was prepared by dissolving 340 mg of KH_2PO_4 in 100 mL of water, then increasing the volume to 250 mL with distilled water, resulting in a solution containing 10 μ moles mL^{-1}. The 10 N sulphuric acid was prepared by adding 200 mL of 36 N concentrated H_2SO_4 to a flask containing 520 mL of distilled water. A 2.5% molybdate solution was made by dissolving 25 g of ammonium molybdate in 200 mL

of distilled water, transferring it to a flask containing 520 mL of 10 N H_2SO_4, and bringing the final volume to 1 L with distilled water; this solution was stored in a brown bottle. The 1-amino-2-napthol-4-sulfonic acid (ANSA) reagent was made through a series of steps involving the dissolution of bisulfite and sodium sulfite, mixing with ANSA powder, and storing in a brown bottle in the cold.

Additionally, 10% trichloroacetic acid (TCA) was prepared by dissolving 10 g salt in 100 mL of distilled water. Aliquots of the standard phosphorus solution were pipetted into a series of test tubes and made up to 1 mL. To each tube, including the blank, the following solutions were added in order: 0.4 mL of 10% TCA, 0.4 mL of ammonium molybdate solution, 0.2 mL of ANSA reagent, and 4 mL of double-distilled water. After thorough mixing, the tubes were left for 5 minutes to observe the blue color development in the solution. The solution was filled into cuvettes, and the OD was measured at 640 nm.

E. Enzymatic assay of acid and alkaline phosphatase from halotolerant PSB isolates

Acid and alkaline phosphatase were assayed using 10 ml of sterilized Pikovskaya's broth in a 20 ml sterilized test tube. The tubes were inoculated with target bacterial isolates and incubated at 37°C for up to 96 hours. The samples were withdrawn every 24 hours and centrifuged at 5000 rpm for 10 min at 4°C. The cell-free supernatant was assayed for crude acid and alkaline phosphatase activity according to the method outlined by Bergmeyer *et al.* (1974). A reaction mixture comprising 0.5 mL of 100 mM acetate buffer at pH 4.8, supplemented with 0.01M $MgCl_2$ and 0.1 mL of the enzyme source, was prepared for the acid phosphatase assay. Conversely, the alkaline phosphatase assay employed 0.5 mL of 100 mM glycine-NaOH buffer at pH 8.0, fortified with 0.01M $MgCl_2$ and 0.1 mL of the enzyme source. These mixtures were allocated into three distinct tubes labeled as test, control, and reagent blank and subsequently subjected to pre-equilibration in a water bath stabilized at 37°C. Following pre-equilibration, 0.5 mL of 15.2 mM p-nitrophenyl phosphate (pNPP) substrate, also pre-equilibrated at 37°C, was introduced into the tubes labeled "test". The enzymatic reaction was allowed to proceed for 10 minutes before being quenched with 4 mL of 0.1N NaOH. In parallel, a zero-minute control was established by adding the substrate after the quenching agent in the control tube. The reagent blank was processed identically to the test, albeit with substituting the respective buffer in place of the pNPP substrate. Spectrophotometric measurements were conducted at a wavelength of 410 nm and calibrated to 100% transmission using the reagent blank. The resultant absorbance values for the control and test tubes were recorded, and the concentration of p-nitrophenol generated during the 10 minute incubation was quantified utilizing a millimolar extinction coefficient ($\varepsilon\lambda_{max}$) of 18.3 mM^{-1} cm^{-1}.

The acid/alkaline phosphatase activity is calculated as:

$$Enzyme\ Units\ L^{-1} = \frac{(A_{410\ nm}\ Test - A_{410\ nm}\ Control)\ x\ assay\ volume\ x\ d.f.}{\varepsilon_{410\ nm}\ x\ enzyme\ volume\ x\ incubation\ time}$$

Where ε_{410nm} = millimolar extinction coefficient of pNPP
d.f. = dilution factor

RESULTS AND DISCUSSION

A. Screening of potent PSB isolates for halotolerance and additional traits

In the study, we obtained 98 PSB isolates, each with varying abilities to solubilize phosphate. These isolates were then incubated in a nutrient broth containing up to 7.5% NaCl to assess their salt tolerance. Among them, three isolates—PH27, PH28, and PH30—demonstrated a notable ability to tolerate 7.5% NaCl concentration. Their respective phosphate solubilizing indices were 2.56, 2.86, and 1.37.

Overall, an increase in NaCl concentration corresponded to a decrease in optical density in the nutrient broth, indicative of reduced bacterial growth (Fig. 1). The isolate P27 had profuse growth till 7.5% NaCl. In contrast, P28 and P30 had moderate growth. Isolate PH27 demonstrated a relatively higher tolerance to salt, with a gradual decrease in optical density from 2.5% to 7.5% of NaCl. This pattern suggests a moderate sensitivity to increasing salt concentrations, potentially indicative of specific osmoregulatory mechanisms.

Conversely, isolate PH28 had a more pronounced decrease in optical density, mainly between 5% and 7.5% NaCl. This sharp decline may signify a critical threshold of salt tolerance for this isolate, warranting further investigation into its physiological response to hyperosmotic stress. The optical density values for PH30 were relatively consistent between 2.5% and 5% NaCl, followed by a decrease at 7.5% NaCl. This unique pattern may reflect a specific adaptation to moderate salt concentrations, possibly mediated by specialized salt transporters or other cellular adaptations (Plemenitaš *et al.*, 2014). Interestingly, PH28 also exhibited the ability to solubilize zinc, with a solubilizing index of 2.44. None of the isolate solubilized potassium in the growth medium.

Multiple studies have reported the isolation of halotolerant PSBs from various geographical regions, each demonstrating varying degrees of salt tolerance. Specifically, one investigation in the Yellow River Delta of China isolated ten halotolerant PSB strains capable of surviving in an environment with an 8.8% NaCl concentration (Jiang *et al.*, 2018). Similarly, a study from Tamil Nadu, India, reported the isolation of eight halotolerant PSB strains that exhibited resilience in a 7% NaCl medium (Mohan *et al.*, 2017). Further, a distinct study focused on the rhizospheric soil of the coastal region of the Bay of Bengal identified 12 PSB strains that could tolerate up to 3.51% NaCl in the growth medium (Sourav and Chayanika, 2016). Additionally, research conducted on the rhizospheric soils of Karnataka revealed 12 PSB isolates capable of surviving in a 2.28% NaCl environment (Srinivasan *et al.*, 2012).

B. Characterization and identification of halotolerant PSB isolates

The isolates PH27 and PH28 were characterized as Gram-negative, capsulated short rods, while PH30 was identified as a Gram-positive, non-capsulated short rod.

The colonies of all three isolates were translucent, convex, medium round, and exhibited entire margins. The biochemical characterization of halotolerant PSB isolates performed through HiAssorted™ biochemical test kit is detailed in Table 1. Each isolate displayed a unique biochemical profile. Specifically, PH27 and PH28 used citrate, whereas PH30 exhibited nitrate reduction.

Further characterization of the three halotolerant PSB isolates was conducted based on the hydrolysis of 21 sugars. Isolate PH27 demonstrated the ability to utilize all 21 sugars. In contrast, PH30 metabolized 11 specific sugars: glucose, arabinose, cellobiose, dextrose, dulcitol, inositol, inulin, melibiose, raffinose, sorbitose, and sucrose. Isolate PH28 hydrolyzed 19 sugars but was negative for adonitol and dulcitol utilization, as displayed in Table 1.

The study also revealed distinct antibiotic sensitivity patterns across the three bacterial isolates. Notably, levofloxacin was highly effective against PH 27 and PH30, while imipenem showed strong inhibition against PH28 and PH30. On the other hand, aztreonam was the least effective across all isolates, particularly against PH27 and PH 28. The isolate PH30 demonstrated high sensitivity to multiple antibiotics, making it the most susceptible.

C. Measuring PSB mediated bioavailability of inorganic phosphate in growth medium

We conducted a temporal analysis of phosphorus solubilization and pH modulation in nutrient broth inoculated with three bacterial isolates: PH27, PH28, and PH30 (Fig. 2). Over 96 hours, PH27 and PH28 demonstrated superior phosphorus solubilization capabilities, with soluble phosphorus concentrations escalating to 2.3 and 2.6 μ moles mL^{-1}. Conversely, PH30 exhibited a modest increase from an initial concentration of 1 μ moles mL^{-1} to 1.5 μ moles mL^{-1}, indicating a relatively lower efficiency in phosphorus solubilization.

Simultaneously, all isolates manifested a decline in pH levels, a characteristic trait of PSB. Specifically, PH27 initiated at a pH of 7.02 and plummeted to 4.46, while PH28 commenced at 7.05 and descended to 4.98. Intriguingly, PH30 registered the most substantial pH reduction despite its lower solubilization efficiency, plummeting from an initial pH of 7.2 to 4.24.

The data suggest a direct correlation between phosphorus solubilization and medium acidification for isolates PH27 and PH28. However, despite its lower phosphorus solubilization, the pronounced pH alteration exhibited by PH30 alludes to the potential involvement of alternative metabolic pathways or mechanisms that contribute to growth medium acidification.

Overall, while PH27 and PH28 excel in both phosphorus solubilization and medium acidification, the unique behavior of PH30 in significantly altering pH, despite lower phosphorus solubilization, warrants further investigation. These findings lay the groundwork for subsequent research to elucidate the metabolic pathways and mechanisms underlying these observed phenomena, thereby contributing to the broader understanding of microbial phosphorus solubilization and its environmental implications.

D. Acid and alkaline phosphatase activity of the halotolerant PSB isolates

The study also delved into the temporal dynamics of acid and alkaline phosphatases activities of three PSB isolates: PH27, PH28, and PH30 (Fig. 3). The study elucidates bacterial metabolic versatility and ecological significance in nutrient cycling.

Starting with isolate PH27, a remarkable escalation in acid phosphatase activity was observed, from a modest 4.9 Units L^{-1} at the 24 hour mark to an impressive 27.7 Units L^{-1} by the 96th hour. This trend was mirrored, albeit more subtly, in alkaline phosphatase activity, hinting at a nuanced regulation of these enzymes within the bacterial physiology. In contrast, isolate PH30 began with a lower initial acid phosphatase activity of 1.9 Units L^{-1} but astonishingly surpassed PH27 at the 48 hour juncture. This isolate sustained elevated acid phosphatase levels throughout the experimental period while its alkaline phosphatase activity remained relatively stable, underscoring its unique enzymatic equilibrium. Isolate PH28 presented a distinct yet analogous enzymatic profile. The acid and alkaline phosphatase activity exhibited an upward trajectory over the 96 hours. However, the acid phosphatase activity was particularly noteworthy between 48 and 96 hours, suggesting a potential metabolic shift or adaptation.

When the enzymatic activities within each isolate were juxtaposed, it was evident that acid phosphatase activities in PH27 and PH28 were consistently higher than their alkaline counterparts. This disparity, however, narrowed at the 96 hour time point for PH28. For PH30, acid phosphatase activity was dominant, particularly after the 48 hour milestone. Comparative analysis across the isolates revealed that PH28 and PH30 exhibited higher acid phosphatase activities than PH27, especially after the 48 hour mark. PH28 consistently outperformed its counterparts in alkaline phosphatase, indicating a unique enzymatic proclivity. The study findings resonate with existing literature. Acid phosphatases, specialized enzymes that catalyze the hydrolysis of phosphorus esters in acidic environments, are notably expressed in soil bacteria from genera such as *Rhizobium*, *Enterobacter*, and *Pseudomonas*, among others (Araújo *et al.*, 2008). These enzymes, although not directly acting on inorganic phosphorus, can alter the pH of the culture medium through dephosphorylation, thereby generating organic acids (Araújo *et al.*, 2008). Previous research corroborates these observations, documenting varying levels of acid phosphatase activity in different bacterial isolates (Chen and Liu, 2019; Park *et al.*, 2011). Furthermore, the observed heterogeneity in alkaline phosphatase production can be attributed to the pH milieu or the accumulation of specific secondary metabolites, which also delineates a direct relationship between alkaline phosphatase activity and pH decrements (Fraser *et al.*, 2015; Zheng *et al.*, 2016). In a broader context, the study aligns with previous research, which found a preponderance of alkaline over

acid phosphatase activity in *Enterobacter* sp (Park *et al.*, 2011).

Conversely, an inverse relationship was reported in *Bacillus* species (Ibarra-Galeana *et al.*, 2017). Our study comprehensively explains the multifactorial determinants influencing enzymatic activities in bacterial isolates. It augments current knowledge and lays a robust foundation for future research endeavors in microbial enzymology. The implications of these findings extend well beyond mere academic interest, offering valuable insights into the complex interplay between environmental variables and microbial enzymatic functionality.

E. Molecular identification of halotolerant PSB isolates

Through 16S rRNA gene sequencing, the bacterial isolates designated as PH27, PH28, and PH30 were taxonomically identified as *Enterobacter bugandensis, Psychrobacter faecalis,* and *Bacillus amyloliquefaciens*, respectively. The National Center for Biotechnology Information (NCBI) assigned unique accession numbers to the PSB isolates: MW857285 for *Enterobacter bugandensis* PH27, MW857286 for *Psychrobacter faecalis* PH28, and MW857287 for *Bacillus amyloliquefaciens* PH30.

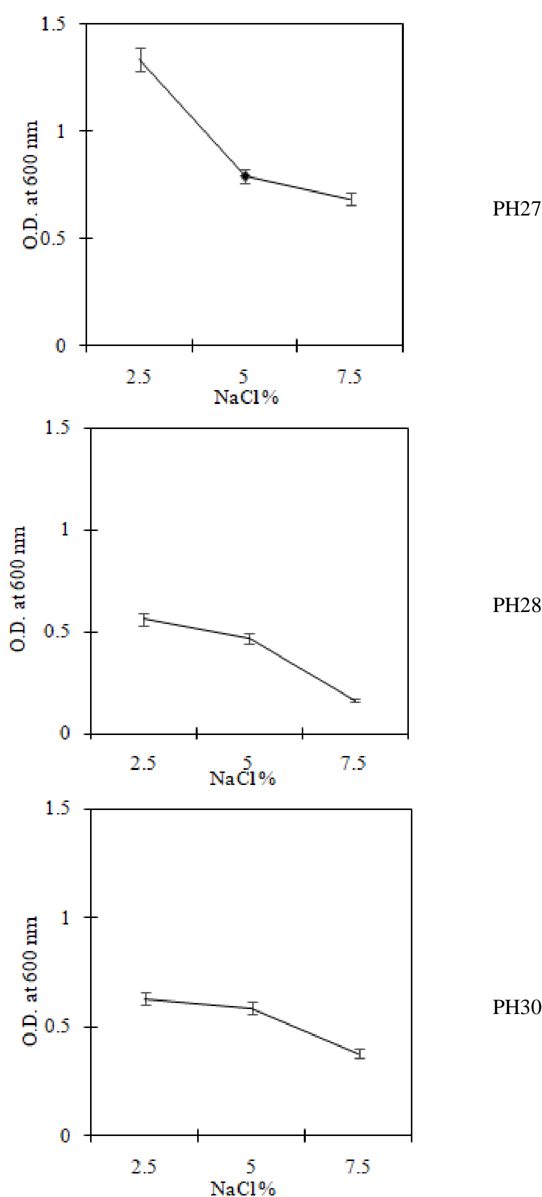


Fig. 1. Growth of halotolerant PSB isolates on nutrient broth subjected to three distinct concentrations of NaCl * ά

*– Each value is the mean of three replicates
ά – Vertical bars represent SEm

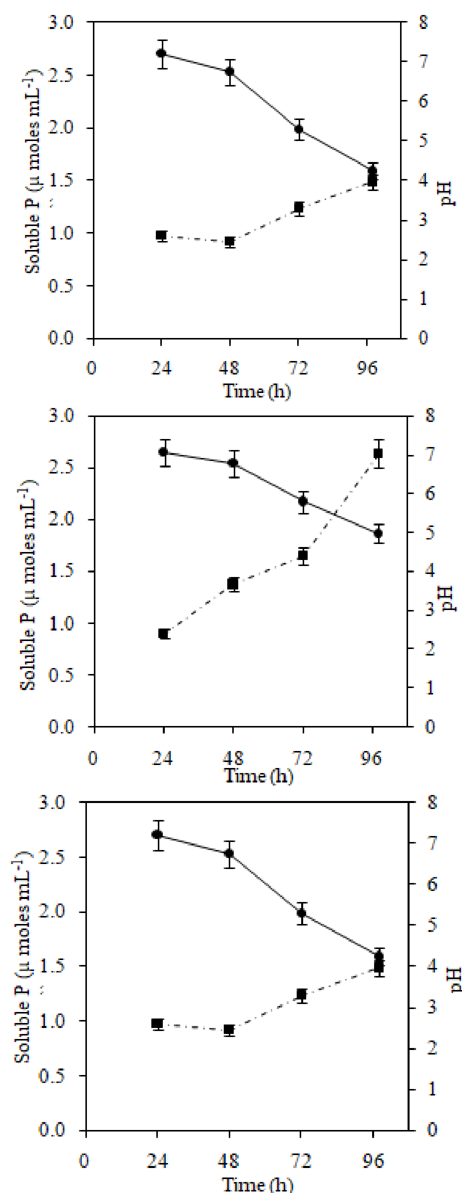
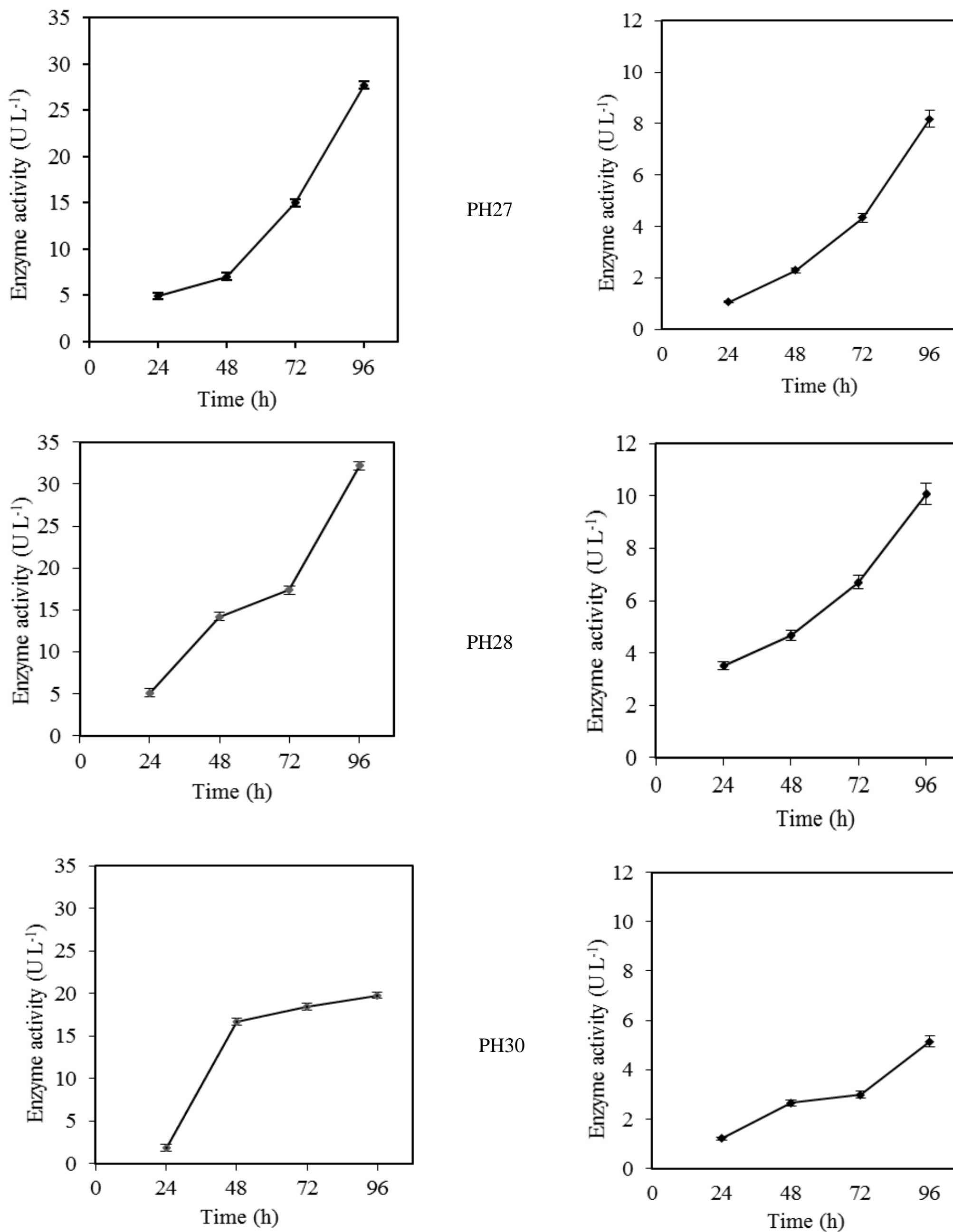


Fig. 2. Released phosphorus and pH change in Pikovskaya's broth by halotolerant PSB isolates* ά

*– Each value is the mean of three replicates
ά – Vertical bars represent SEm
(Dotted lines — Soluble P, continuous lines — pH)



Acid phosphatase activity   Alkaline phosphatase activity

Fig. 3. Acid and alkaline phosphate activity of halotolerant PSB isolates* ά

*– Each value is the mean of three replicates

ά – Vertical bars represent SEm

Table 1: The biochemical characterization of three halotolerant PSB isolates.

Characteristics	Sr. No.	Biochemical test	Isolate		
			PH27	PH28	PH30
Biochemical tests through Hi Assorted™ kit	1.	citrate utilization	+	+	-
	2.	lysine utilization	-	-	-
	3.	ornithine utilization	-	-	-
	4.	urease detection	-	-	-
	5.	phenylalanine deamination	-	-	-
	6.	nitrate reduction	-	-	+
	7.	H_2S production	-	-	-
	8.	starch hydrolysis	-	-	-
	9.	gelatin hydrolysis	-	-	-
	10.	indole production	-	-	-
Sugars	11.	adonitol	+	-	-

utilization tests	12.	arabinose	+	+	+
	13.	cellobiose	+	+	+
	14.	dextrose	+	+	+
	15.	dulcitol	+	-	+
	16.	fructose	+	+	-
	17.	galactose	+	+	-
	18.	inositol	+	+	+
	19.	inulin	+	+	+
	20.	lactose	+	+	-
	21.	melibiose	+	+	+
	22.	maltose	+	+	-
	23.	mannitol	+	+	-
	24.	mannose	+	+	-
	25.	raffinose	+	+	+
	26.	rhamnose	+	+	-
	27.	sorbitol	+	+	+
	28.	sucrose	+	+	+
	29.	trehalose	+	+	-
	30.	xylulose	+	+	-
	31.	glucose	+	+	+
Antibiotic sensitivity tests		Antibiotics with concentrations	Inhibition zones(mm)		
	32.	amikacin 30 µg mL^{-1}	22	20	24
	33.	imipenem 10 µg mL^{-1}	16	24	25
	34.	ceftazidime 30 µg mL^{-1}	15	22	28
	35.	cefotaxime 30 µg mL^{-1}	24	15	24
	36.	levofloxacin 5 µg mL^{-1}	32	20	29
	37.	aztreonam 30 µg mL^{-1}	12	10	28

CONCLUSIONS

The study comprehensively examined halotolerant PSB isolates, PH27, PH28, and PH30, across multiple dimensions, which include phosphate solubilization, halotolerance, enzymatic activities, and molecular identification. The research biochemically, physiologically, and molecularly characterized the isolates using various methodologies, from screening and identification assays to 16S rRNA gene sequencing. The isolates demonstrated distinct phosphate and zinc solubilization capabilities, especially PH27 and PH28 showed superior phosphorus solubilization abilities. Halotolerance profiles revealed a complex relationship between NaCl concentration and bacterial growth. Enzymatic assays further indicated unique acid and alkaline phosphatase activities among the isolates, highlighting their metabolic versatility.

Molecular characterization identified the isolates as *Enterobacter bugandensis* (PH27), *Psychrobacter faecalis* (PH28), and *Bacillus amyloliquefaciens* (PH30), providing a taxonomic framework for future research. The study also found intriguing patterns in pH modulation and phosphorus solubilization, particularly with PH30, which exhibited significant medium acidification despite lower phosphorus solubilization efficiency.

Overall, the study contributes to the existing knowledge on PSBs and opens up new avenues for future research. It lays a robust foundation for understanding the complex interplay between halotolerance, phosphorus solubilization, and enzymatic activities in PSBs, offering valuable insights into microbial contributions to nutrient cycling in saline environments.

FUTURE SCOPE

This research provides a foundational understanding of the halotolerant phosphate solubilizing bacterial isolates *Enterobacter bugandensis* MW857285, *Psychrobacter faecalis* MW857286, and *Bacillus amyloliquefaciens* MW857287, opening several avenues for future exploration. Future work could explore the metabolic pathways involved in phosphorus solubilization and pH modulation using techniques like transcriptomics and metabolomics. Whole-genome sequencing could offer genetic insights into the isolates' unique capabilities. Field trials are essential to validate the practical applications of these isolates in agriculture, particularly in saline conditions. Further studies could also focus on the enzymatic kinetics of acid and alkaline phosphatases, the symbiotic relationships between the bacterial strains and various plant species, and their long-term environmental impact. Comparative analyses with other known PSB strains could identify unique or superior features, while research in adaptive mechanisms to varying salt concentrations could offer new perspectives. Finally, once optimized, the commercial viability of these isolates for use in biofertilizers or bio-remediation in saline environments could be explored. The future scope could build upon the current findings to develop more effective and sustainable nutrient management strategies in saline agricultural systems.

Acknowledgment. The authors acknowledge the Principal College of Basic Science and Humanities, Sardrkrushinagar Dantiwada Agricultural University, Gujarat, for providing facilities for this research.

Conflict of Interest. None.

REFERENCES

Araújo, A. P., Plassard, C., & Drevon, J. J. (2008). Phosphatase and phytase activities in nodules of common bean genotypes at different levels of phosphorus supply. *Plant and Soil, 312*(1-2), 129-138.

Bergmeyer, H. U., Bernt, E., & Lachenicht, R. (1974). Alkaline phosphatase in serum determination with automatic analysers, Methods of enzymatic analysis. Elsevier, pp. 864-868.

Bhatt, H. B., Gohel, S. D., & Singh, S. P. (2018). Phylogeny, novel bacterial lineage and enzymatic potential of haloalkaliphilic bacteria from the saline coastal desert of Little Rann of Kutch, Gujarat, India. *3 Biotech, 8*(1), 1-12.

Chen, Q., & Liu, S. (2019). Identification and characterization of the phosphate-solubilizing bacterium *Pantoea* sp. S32 in reclamation soil in Shanxi, China. *Frontiers in microbiology, 10.* [Article 2171].

Dey, G., Banerjee, P., Sharma, R. K., Maity, J. P., Etesami, H., Shaw, A. K., Huang, Y. H., Huang, H. B., & Chen, C. Y. (2021). Management of phosphorus in salinity-stressed agriculture for sustainable crop production by salt-tolerant phosphate-solubilizing bacteria—A Review. *Agronomy, 11*(8): [Article 1552].

Elhaissoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y., & Bargaz, A. (2022). Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. *Journal of Advanced Research, 38,* 13-28.

Fasim, F., Ahmed, N., Parsons, R., & Gadd, G. M. (2002). Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. *FEMS Microbiol Lett,* 213(1), 1-6.

Fiske, C. H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *J. Biol. Chem, 66*(2), 375-400.

Fraser, T., Lynch, D. H., Entz, M. H., & Dunfield, K. E. (2015). Linking alkaline phosphatase activity with bacterial phoD gene abundance in soil from a long-term management trial. *Geoderma, 257* 115-122.

Ibarra-Galeana, J. A., Castro-Martínez, C., Fierro-Coronado, R. A., Armenta-Bojórquez, A. D., & Maldonado-Mendoza, I. E. (2017). Characterization of phosphate-solubilizing bacteria exhibiting the potential for growth promotion and phosphorus nutrition improvement in maize (*Zea mays* L.) in calcareous soils of Sinaloa, Mexico. *Annals of Microbiology, 67,* 801-811.

Jiang, H., Li, S., Wang, T., Chi, X., Qi, P., & Chen, G. (2021). Interaction between halotolerant phosphate-solubilizing bacteria (*Providencia rettgeri* strain TPM23) and rock phosphate improves soil biochemical properties and peanut growth in saline soil. *Front Microbiol, 12.* [Article 777351].

Jiang, H., Qi, P., Wang, T., Wang, M., Chen, M., Chen, N., Pan, L., & Chi, X. (2018). Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils. *3 Biotech, 8*(11). 461.

Kothe, C.I., Bolotin, A., Kraïem, B. F., Dridi, B., Team, F., & Renault, P. (2020). Unraveling the world of halophilic and halotolerant bacteria in cheese by combining cultural, genomic and metagenomic approaches. *International Journal of Food Microbiology, 358.* [Article 109312].

Mazhar, H., Abbas, N., Hussain, Z., Sohail, A., & Ali, S. (2016). Extracellular lipase production from *Bacillus subtilis* using agro-industrial waste and fruit peels. *Punjab Univ. J. Zool, 31*(2). 261-267.

Mohan, V., Devi, K. S., Anushya, A., Revathy, G., Kuzhalvaimozhi, G.V., & Vijayalakshmi, K. (2017). Screening of salt tolerant and growth promotion efficacy of phosphate solubilizing bacteria. *J Acad Ind Res, 5*(12), 168-172.

Najafi Zilaie, M., Mosleh Arani, A., Etesami, H., & Dinarvand, M. (2022). Improved salinity and dust stress tolerance in the desert halophyte *Haloxylon aphyllum* by halotolerant plant growth-promoting rhizobacteria. *Front Plant Sci, 13*. [Article 2625].

Nautiyal, C. S. (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Lett, 170*(1), 265-270.

Orhan, F. (2016). Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). *Braz J Microbiol, 47*(3), 621-627.

Parada-Pinilla, M. P., Ferreira, M. A., Roncallo, J. C., Santos, S.N., Melo, I. S., Assef, A. N. B., Wilke, D. V., Silva, L. F., Garrido, L. M., Araujo, W. L., & Padilla, G. (2021). Biopolymer production by halotolerant bacteria isolated from Caatinga biome. *Braz J Microbiol, 52*(2), 547-559.

Park, J. H., Bolan, N., Megharaj, M., & Naidu, R. (2011). Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. *Journal of Hazardous Materials, 185*(2-3), 829-836.

Pathak, S., & Mukherjee, S. (2020). Entrepreneurial ecosystem and social entrepreneurship: case studies of community-based craft from Kutch, India. *Journal of Enterprising Communities: People and Places in the Global Economy, 15*(3), 350-374.

Plemenitaš, A., Lenassi, M., Konte, T., Kejžar, A., Zajc, J., Gostinčar, C., & Gunde-Cimerman, N. (2014). Adaptation to high salt concentrations in halotolerant/halophilic fungi: a molecular perspective. *Frontiers in Microbiology,* 5: [Articlel 199].

Raddadi, N., Cherif, A., Daffonchio, D., Neifar, M., & Fava, F. (2015). Biotechnological applications of extremophiles, extremozymes and extremolytes. *Appl Microbiol Biotechnol, 99*(19), 7907-7913.

Raji, M., & Thangavelu, M. (2021). Isolation and screening of potassium solubilizing bacteria from saxicolous habitat and their impact on tomato growth in different soil types. *Archives of Microbiology,* 203(6): 3147-3161.

Raji, R.O., Oyewole, O.A., Ibrahim, O. H., Tijani, Y. N., & Gana, M. (2019). Microbial communities and activities in caves. *Brazilian Journal of Biological Sciences, 6*(14), 557-564.

Ranjan, A., Mahalakshmi, M.R., & Sridevi, M. (2013). Isolation and characterization of phosphate-solubilizing bacterial species from different crop fields of Salem, Tamil Nadu, India. *International Journal of Nutrition, Pharmacology, Neurological Diseases, 3*(1), 29-33.

RI, P. (1948). Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiologiya, 17*, 362-370.

Saffari, N., Salmanzadeh-Ahrabi, S., Abdi-Ali, A., & Rezaei-Hemami, M. (2016). A comparison of antibiotic disks from different sources on Quicolor and Mueller-Hinton agar media in evaluation of antibacterial susceptibility testing. *Iran J Microbiol, 8*(5), 307-311.

Shah, R. K., & Saraf, M. (2019). Exploring inorganic phosphate solubilizing trait of halotolerant rhizobacteria isolated from *Cuminum cyminum*.

International Journal of Research in Advent Technology, 7(5), 308-315.
Sourav, S., & Chayanika, P. (2016). In vitro phosphate solubilization by halotolerant *Pseudomonas aeruginosa* PHSD5 isolated from paddy rhizosphere. *Research Journal of Chemistry and Environment, 20*(5), 14-19.
Srinivasan, R., Yandigeri, M. S., Kashyap, S., & Alagawadi, A.R. (2012). Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. *Saudi J Biol Sci, 19*(4), 427-434.
Thumar, J. T., Dhulia, K., & Singh, S. P. (2010). Isolation and partial purification of an antimicrobial agent from halotolerant alkaliphilic *Streptomyces aburaviensis* strain Kut-8. *World Journal of Microbiology and Biotechnology, 26*(11), 2081-2087.
Yadav, A., Hinaben, G., Yadav, K., & Ahmad, R. (2022). Physicochemical properties of four phosphate solubilizing bacteria isolated from the semi-arid regions of North Gujarat, India: Physicochemical properties of phosphate solubilizing bacteria. *Journal of AgriSearch,* 9(3): 236-243.
Yuan, M., Chen, M., Zhang, W., Lu, W., Wang, J., Yang, M., Zhao, P., Tang, R., Li, X., Hao, Y., Zhou, Z., Zhan, Y., Yu, H., Teng, C., Yan, Y., Ping, S., Wang, Y., & Lin, M. (2012). Genome sequence and transcriptome analysis of the radioresistant bacterium *Deinococcus gobiensis*: insights into the extreme environmental adaptations. *Plos One, 7*(3), e34458.
Zheng, Z., Tang, A., Guan, Y., Chen, L., Wang, F., Chen, P., Wang, W., Luo, Y., Tian, Y., & Liang, G. (2016). Nanocomputed tomography imaging of bacterial alkaline phosphatase activity with an iodinated hydrogelator. *Analytical chemistry, 88*(24), 11982-11985.

How to cite this article: Anurag Yadav, Rahulkumar Chauhan, Kavin Soni and Kusum Yadav (2023). ISSN No. (Online): 2249-3239. Unveiling the Multifaceted Abilities of Halotolerant Phosphate Solubilizing Bacteria Isolated from the Kutch Desert Ecosystem. *Biological Forum – An International Journal, 15*(9): 476-484.