Effect of halotolerant phosphate solubilizing bacterial treatments on the growth of mung bean, pea and wheat plants

Abstract

Sustainable agriculture necessitates innovative solutions that enhance crop yield while minimizing environmental impact. This study evaluates the efficacy of three halotolerant phosphate solubilizing bacterial (PSB) strains viz., *Enterobacter bugandensis* PH27, *Psychrobacter faecalis* PH28, *and Bacillus amyloliquefaciens* PH30 across growth stages of pea GDF-1, wheat GW-451, and mung bean GM-4 varieties. Our analysis reveals that *B. amyloliquefaciens* PH30 consistently outperformed other isolates, significantly enhancing seed germination rates and key growth metrics such as plant height, dry weight, and leaf area. Additionally, PH30 improved soil fertility by elevating levels of soluble phosphorus. The data suggest a multi-pronged mechanism of action for PH30 involving phytohormone production, nutrient chelation, and phosphate solubilization.

Keywords: phosphate solubilizing bacteria, PGPR, halotolerant bacteria, phosphorous bioavailability

1. Introduction

In the quest for sustainable agriculture, halotolerant phosphate-solubilizing bacteria (PSB) have emerged as pivotal players in enhancing plant growth and nutrient bioavailability, particularly in saline soils.

Halotolerant PSBs are biological marvels capable of thriving in high-salinity environments, offering a promising agricultural solution in salt-affected terrains. Previous studies have substantiated their growth promoting attributes, revealing their capacity to solubilize phosphorus and produce plant growth regulators [1, 2]. Moreover, halotolerant PSBs are biological alleviators of salt stress, a pervasive issue plaguing global agriculture. These bacteria enhance plant resilience by producing growth regulators like indole-3-acetic acid (IAA) and making phosphorus more accessible in saline soils [3, 4]. Their unique osmoregulatory and metabolic adaptations have been the subject of extensive research, further solidifying their role in sustainable agriculture.

The solubilization of phosphorus, an essential but often limiting nutrient, enhances its bioavailability, catalyzing plant growth and yield [5]. PSB inoculation strategies were shown to amplify the growth and yield of crops, including mung bean and wheat, through enhanced nutrient uptake and seed yield [6, 7].

Our study offers a granular analysis across growth stages—from seed germination to young plant metrics like height, dry weight, and leaf area. We also evaluated the soil fertility impact of PSB inoculation on post-harvest soluble phosphorus levels. Notably, *B. amyloliquefaciens* PH30 emerged as a standout performer. This comprehensive study delves into the multifaceted impact of three distinct halotolerant PSB strains—*Enterobacter bugandensis PH27, Psychrobacter faecalis PH28, and Bacillus amyloliquefaciens PH30*—on the growth dynamics of mung bean, pea, and wheat crops.

2. Materials and methods

The study was conducted in the Department of Microbiology, College of Basic Science and Humanities, Sardarkrushinagar Dantiwada Agricultural University, India. Seeds of different crop varieties, viz., pea GDF-1, wheat GW-451, and mung bean GM-4, were obtained from certified suppliers. Three halotolerant PSB—*E. bugandensis* PH27, *P. faecalis* PH28, and *B. amyloliquefaciens* PH30—used in the study were isolated by authors in a previous study [8].

2.1 Seed germination assay

The seeds underwent a rigorous sterilization process to ensure a sterile experimental environment. They were submerged in a 6% hydrogen peroxide (H_2O_2) solution for 15 minutes in a 100 mL beaker. Subsequently, the seeds were rinsed quintuple times using distilled water to remove any residual sterilizing agent. Following sterilization, the seeds were primed for 30 minutes with halotolerant PSB isolates in nutrient broth tubes maintained at 10^{12} CFU mL^{-1}. Post-priming, the seeds were carefully transferred to water agar plates for the germination assay. The seed plates were incubated at 25°C in a growth chamber with a 16/8 hour light/dark cycle. Germination was observed after seven days, and germination percentage was calculated.

2.2 Pot study of germinated seeds treated with halotolerant PSB isolates

A pot experiment was initiated with complete random design (CRD) during the first week of January 2021 to investigate the influence of halotolerant PSB isolate treatments on three plant types. Before the pot experiment, *E. bugandensis* PH27, *P. faecalis* PH28, and *B. amyloliquefaciens* PH30 were incubated in nutrient broth culture tubes for 24 hours. The culture broths contained a population density of no less than log $_{12}$ CFU mL^{-1}. To achieve a target microbial density of 10^9 colony forming units (CFU) per gram of soil, 10 mL of bacterial broth containing 10^{12} CFU mL^{-1} was uniformly incorporated into each pot containing 10 kg of soil. Subsequently, germinated seedlings were transplanted into the microbiologically treated pots. The experimental details are described in Table 1. These pots were subjected to a consistent watering regimen. Concurrently, any adventitious grasses that emerged were promptly eradicated to minimize competition for nutrients and space.

The plants were uprooted from pots after 30 days of growth, and an exhaustive evaluation was conducted, encompassing five key growth parameters. These included plant height, dry weight, collar diameter, leaf count, and leaf surface area. The metrics were rigorously measured to provide a holistic understanding of plant growth dynamics. The plant dry weights were ascertained through an overnight desiccation process, employing a hot air oven set at 80°C. The acquired data were articulated as mean values. The statistical tool for agricultural research (STAR) was utilized for a robust statistical interpretation.

Table 1: Experimental details of the pot study

i. Location and agro-climatic zone: Sardarkrushinagar, Gujarat; semi-arid

ii. Experimental Details:

   a. Bacterial treatment: *E. bugandensis* PH27, *P. faecalis* PH28, and *B. amyloliquefaciens* PH30 with GenBank® accession numbers MW857285, MW857286, and MW857287.
   b. Design: CRD
   c. Replications: 05
   d. Crops and varieties: Pea GDF-1, wheat GW-451, and mung bean GM-4
   e. Seed rate: Five per pot
   f. Manuring and fertilizers: Nil

iii. Cultural details:
   a. Date of transplanting: 03/01/2021
   b. No. of wateringS: 05
   c. No. of weddings: As required
   d. Date of harvesting: 03/02/2021

iv. Initial soil fertility status:
   a. Total available phosphorus: 1.20 mg Kg^{-1} soil
   b. Total carbon content: 0.45 %

v. General environmental conditions:
   a. Plant stand: Direct sunlight
   b. Season: Winter
   c. Rainfall (mm) and rainy days: Nil

2.3 Soil phosphorus analysis

Soil samples were collected from each pot post-harvest and analyzed for soluble phosphorus. Soluble phosphorus was estimated using the Fiske-Subbarow method [9]. All experiments were conducted in five replicates, and data were analyzed using one-way ANOVA followed by Fisher's LSD test for multiple comparisons. The pot experiment results are presented as mean.

3. Results and Discussion

3.1 Effects of halotolerant PSB treatment on seed germination

Table 2 presents data on the germination percentages of three varieties. In the control group, pea GDF-1 seeds exhibited the highest germination percentage at 87.8%, setting a baseline for evaluating the effects of bacterial treatments. Wheat GW-451 seeds, with a germination rate of 67.2%, had the lowest percentage in the control group. Mung bean GM-4 seeds sit in between with a germination rate of 77.2%.

When treated with *E. bugandensis* PH27, mung bean GM-4 seeds showed a notable increase in germination percentage to 86.6%, suggesting a positive interaction between this bacterial strain and mung bean seeds. Wheat GW-451 seeds also show a modest improvement to 70.4%, but less so than mung bean.

Turning our attention to *P. faecalis* PH28, wheat GW-451, and mung bean GM-4 seeds exhibited a slight uptick in germination percentages to 70.8% and 79.8%, respectively, compared to their respective controls. While the increase is not as dramatic as the previous bacterial strain, it is still noteworthy.

However, the most striking results were observed with *B. amyloliquefaciens* PH30. All plant varieties treated with this bacterial strain showed a significant increase in germination percentage. Seeds of pea GDF-1 reached 92.2%, wheat GW-451 climbed to 80.6%, and mung bean GM-4 stood out with the most dramatic improvement to 94.6%.

Table 2: Germination percentage of three different plant varieties treated with three halotolerant PSB isolates

Plant varieties	Control	*E. bugandensis* PH27	*P. faecalis* PH28	*B. amyloliquefaciens* PH30
Mung bean GM-4	77.2±4.49	86.6±2.69	79.8±2.22	94.6±1.36
Pea GDF-1	87.8±2.82	87.2±2.8	87.6±2.71	92.2±1.46
Wheat GW-451	67.2±3.54	70.4±3.5	70.8±2.99	80.6±2.54

*Each value is a mean of five replicates ± SEm

3.2 Effects of halotolerant PSB treatment on plant growth

The plant growth experiment showed that *B. amyloliquefaciens* PH30 consistently positively impacted multiple growth parameters (**Table 3**). In mung bean, the height increased significantly to 19.74 cm when treated with *B. amyloliquefaciens* PH30, compared to a control (10.68 cm). Similarly, the dry weight was highest at 2.28 g for the same treatment, in contrast to the control (1.7 g). The collar diameter, leaf number, and leaf area also showed marked improvements, with values of 4.3 mm, 8.2, and 2.88 cm^2, respectively.

The trend of *B. amyloliquefaciens* PH30 enhancing growth parameters was also observed in pea and wheat plants. In the pea plant, the height reached 26.18 cm, with a dry weight of 19.74 g, both highest among the treatments and significantly higher than the control values of 13.14 cm and 10.68 g, respectively. The collar diameter, leaf number, and leaf area followed suit, with values of 1.74 mm, 19.4, and 7.22 cm^2, respectively.

In wheat plants, the height, dry weight, collar diameter, leaf number, and leaf area were highest when treated with *B. amyloliquefaciens* PH30, with values of 20.28 cm, 2.088 g, 1.6 mm, 16.4, and 2.58 cm^2, respectively, compared to the control. When comparing the growth of all three plants, it is noteworthy that the effect of *B. amyloliquefaciens* PH30 was most pronounced in pea plants, followed by mung bean and wheat. These observations imply that *B. amyloliquefaciens* PH30 holds considerable promise as a versatile bio-stimulant for augmenting plant growth across various species, although species-specific factors may modulate its effectiveness.

Table 3: Growth parameters of plants treated with halotolerant PSB isolates*

a) Mung bean GM-4

Bacterial treatments	Height	Dry wt. (g)	Collar dia. (mm)	Leaf number	Leaf area (cm^2)
Control	10.68^{d}	1.70^{c}	1.32^{b}	5.00^{b}	2.56^{ab}
E. bugandensis PH27	13.12^{c}	1.88^{bc}	1.90^{b}	7.20^{a}	2.66^{ab}
P. faecalis PH28	16.14^{b}	2.12^{ab}	3.30^{a}	7.20^{a}	2.42^{b}
B. amyloliquefaciens PH30	19.74^{a}	2.28^{a}	4.30^{a}	8.20^{a}	2.88^{a}
R^2	0.85	0.54	0.70	0.39	0.36
MSE	2.48	0.05	0.74	2.65	0.06
F	30.90	6.38	12.24	3.45	3.06
CV%	25.23	15.47	53.12	27.78	10.83

b) Pea GDF-1

Bacterial treatments	Height	Dry wt. (g)	Collar dia. (mm)	Leaf number	Leaf area (cm^2)
Control	13.14^{d}	10.68^{d}	0.86^{c}	14.80^{c}	2.64^{b}
E. bugandensis PH27	22.56^{b}	13.12^{c}	1.44^{ab}	16.80^{bc}	7.06^{a}
P. faecalis PH28	16.96^{c}	16.14^{b}	1.26^{bc}	17.20^{b}	7.24^{a}
B. amyloliquefaciens PH30	26.18^{a}	19.74^{a}	1.74^{a}	19.40^{a}	7.22^{a}
R^2	0.82	0.82	0.54	0.59	0.91
MSE	6.90	0.10	0.11	2.35	0.48
F	24.31	24.58	6.36	7.57	53.28
CV%	28.84	31.18	33.46	12.83	35.00

c) Wheat GW-451

Bacterial treatments	Height	Dry wt. (g)	Collar dia. (mm)	Leaf number	Leaf area (cm^2)
Control	15.8^{b}	1.26^{c}	0.96^{b}	12.20^{b}	1.40^{b}
E. bugandensis PH27	18.08^{ab}	1.51^{b}	1.36^{a}	13.60^{ab}	1.66^{b}
P. faecalis PH28	16.78^{b}	1.67^{b}	1.34^{a}	10.60^{b}	1.74^{b}
B. amyloliquefaciens PH30	20.28^{a}	2.09^{a}	1.60^{a}	16.40^{a}	2.58^{a}
R^2	0.43	0.86	0.59	0.48	0.79
MSE	4.61	0.02	0.05	6.15	0.07
F	4.07	34.12	7.61	4.92	19.86
CV%	14.75	20.22	23.31	23.91	27.72

*The F-value for each treatment was significant at the 5% ANOVA

3.3 Effect of halotolerant PSB treatment on the phosphorus availability of potting soil

Table 4 describes the quantity of available phosphorus (mg Kg^{-1} soil) in the potting soils after the plant harvest. In the control group for mungbean, the soluble phosphorus level was 1.29 mg Kg^{-1} soil. When treated with *E. bugandensis* PH27, the level increased to 1.83; with *P. faecalis* PH28, it goes up to 1.51. However, the highest increase was observed with *B. amyloliquefaciens* PH30, reaching 2.03.

The control group showed a soluble phosphorus level of 1.39 mg Kg^{-1} for pea plant pot soil. Treatment with *E. bugandensis* PH27 and *P. faecalis* PH28 resulted in levels of 1.96 and 1.66, respectively. Again, *B. amyloliquefaciens* PH30 showed the highest level at 2. Similarly, in the case of wheat, the control group has a level of 1.15. The levels increased to 1.60, 1.48, and 1.98 mg Kg^{-1} soil when treated with *E. bugandensis* PH27, *P. faecalis* PH28, and *B. amyloliquefaciens* PH30, respectively.

Table 4: Soluble phosphorus (mg Kg^{-1} soil) in pot soil of various treatments post harvest

Plant	Bacterial treatments			
	Control	*E. bugandensis* PH27	*P. faecalis* PH28	*B. amyloliquefaciens* PH30
Mungbean GM-4	1.29±0.09	1.83±0.11	1.51±0.14	2.03±0.10
Pea GDF-1	1.39±0.09	1.96±0.07	1.66±0.22	2.00±0.19
Wheat GW-451	1.15±0.10	1.60±0.15	1.48±0.20	1.98±0.13

* Each value is a mean of three replicates ± SEm

B. amyloliquefaciens PH30 consistently outperformed other halotolerant PSB isolates across multiple growth stages and parameters. Initially, it significantly boosted seed germination rates, laying a foundation for subsequent growth stages. The initial advantage amplified as the plant matured, with PH30-treated plants showing superior metrics in height, dry weight, collar diameter, leaf number, and leaf area.

Concurrently, PH30 also elevated soil soluble phosphorus levels, an essential nutrient for plant development. It is proposed that this dual action—enhancing plant and soil health—created a virtuous cycle leading to robust, high-biomass plants. In summary, *B. amyloliquefaciens* PH30 emerged as a comprehensive growth promoter, offering a multifaceted approach to sustainable agriculture. Its consistent high performance across growth stages makes it a promising candidate for further research and agricultural applications.

4. Conclusions

Our analysis reveals halotolerant PSB *B. amyloliquefaciens* PH30 as a potent growth promoter, demonstrating superior efficacy across multiple growth stages and parameters in three plant varieties. This bacterial strain significantly enhanced seed germination rates, providing an initial advantage carried through subsequent growth stages of plants. PH30-treated plants consistently outperformed controls and other bacterial treatments in crucial growth metrics, including height, dry weight, and leaf area as plants grew. The data suggest a multi-pronged mechanism of action for PH30 involving various strategies like phytohormone production, nutrient chelation, and phosphate solubilization. *B. amyloliquefaciens* PH30 treatment offers a holistic approach to sustainable agriculture, acting as a comprehensive growth promoter. Its consistently high performance across growth stages and impact on soil fertility make it a promising candidate for further research and practical agricultural applications.

5. Future study

The current study establishes *B. amyloliquefaciens* PH30 as a potent biofertilizer with consistently high performance across multiple plant species and growth parameters. However, further research is needed to elucidate the molecular mechanisms underlying its efficacy. Future studies could focus on genomic and transcriptomic analyses to identify the specific genes and metabolic pathways involved in phytohormone production, nutrient chelation, and phosphate solubilization. Understanding the molecular basis of such interactions will validate the proposed mechanisms and offer opportunities for strain improvement through genetic engineering.

Another promising avenue for future research is field trials to assess the scalability of such isolates in real-world applicability. While controlled experiments provide valuable insights, field trials will offer a more comprehensive understanding of how *B. amyloliquefaciens* PH30 performs under varying environmental conditions, including different soil types, climates, and cropping systems. Such trials could also evaluate the long-term impact of PH30 on soil health and fertility, providing data crucial for its commercial application in sustainable agriculture.

Lastly, future studies should explore the potential synergistic effects of combining *B. amyloliquefaciens* PH30 with other beneficial microorganisms or organic amendments. Such combinations could enhance its efficacy as a biofertilizer and broaden its applicability across various crops and soil conditions. Additionally, economic analyses could be conducted to assess the cost-effectiveness of implementing this bacterial strain in large-scale agricultural operations, thereby providing a comprehensive evaluation of its potential as a sustainable alternative to chemical fertilizers.

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