



Screening and Application of IAA-producing Bacteria for Seed Priming to Enhance Rice Germination and Salinity Stress Tolerance

Uthika Baenlee¹, Jiraporn Inthasan², Vassana Viroonrat², Jakkrapong Kangsopa^{1,3}

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ABSTRACT

Background: Rice is a staple crop that plays an important role in global food security, but seed germination and early seedling establishment are often limited by salinity stress. Salinity reduces water uptake, disrupts metabolic activity and impairs early seedling growth through osmotic and ionic stress. Seed priming with plant growth-promoting bacteria, particularly indole-3-acetic acid (IAA)-producing bacteria, may improve germination performance and salinity tolerance during early growth.

Methods: Bacterial isolates were obtained from saline soils in northeastern Thailand and screened for IAA production and salinity tolerance. Four selected isolates were identified and applied to rice seeds of the cultivar Khao Dawk Mali 105 through seed soaking and seed priming. Seed quality was evaluated under laboratory and greenhouse conditions and the most promising isolate was further tested under salinity stress using NaCl concentrations from 0 to 100 mM.

Result: The selected isolates were identified as *Bacillus megaterium* isolates 1, 2 and 3 and *Microbacterium proteolyticum* isolate 4. *B. megaterium* (isolate 1) produced the highest IAA concentration, but *M. proteolyticum* (isolate 4) showed the best overall seed performance. Under laboratory conditions, seeds soaked with *M. proteolyticum* (isolate 4) had the highest germination percentage of 97%, the highest speed of germination of 9.41 plants/day, the shortest mean germination time of 2.95 days and the greatest root length of 15.63 mm. Under greenhouse conditions, the same treatment produced the highest germination percentage of 99%, the highest speed of germination of 9.89 plants/day, the shortest mean germination time of 4.76 days and the greatest shoot length of 6.62 cm. Under salinity stress, *M. proteolyticum* priming improved germination performance under selected NaCl levels, particularly by maintaining higher germination percentage at 40 and 60 mM NaCl and higher germination potential and germination index under severe salinity. These results indicate that effective bacterial seed priming depends not only on IAA production but also on the stress-adaptive capacity of the bacterial isolate.

Key words: PGPR, Rice germination, Salinity stress, Seed enhancement, Seed priming.

INTRODUCTION

Rice, a staple crop feeding more than half of the global population, faces several constraints during early growth, including non-uniform germination, seed quality deterioration and poor seedling establishment, which directly affect crop establishment and yield potential (Bewley *et al.*, 2013). These constraints are intensified under salinity, one of the most serious abiotic stresses in agricultural systems. About 20% of cultivated land and up to 50% of irrigated land worldwide are affected by soil salinity and this problem continues to expand due to climate change and improper water management (Munns and Tester, 2008). Salinity impairs seed germination through osmotic stress, which restricts water uptake and ionic toxicity, in which excessive Na⁺ and Cl⁻ disrupt ion homeostasis and cellular metabolism (Shrivastava and Kumar, 2015; Verma and Solanki, 2022). These effects reduce the activity of hydrolytic enzymes involved in reserve mobilization, decrease mitochondrial respiration and energy production and delay embryonic development and radicle elongation (Bewley *et al.*, 2013). Salinity also induces reactive oxygen species accumulation, leading to lipid peroxidation, membrane damage, electrolyte leakage and reduced seed viability and vigor (Gill and Tuteja, 2010).

¹Division of Agronomy, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand.

²Division of Soil Resources and Environment, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand.

³Modern Seed Technology Research Center, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand.

Corresponding Author: Jakkrapong Kangsopa, Division of Agronomy, Faculty of Agricultural Production, Maejo University, Chiang Mai 50290, Thailand. Email: jakkrapong_ks@mju.ac.th

ORCID: <https://orcid.org/0009-0001-1967-4932>, <https://orcid.org/0000-0003-3609-0338>

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Seed enhancement technologies, particularly seed priming, have been developed to mitigate these adverse effects. Seed priming is a controlled hydration treatment

that activates pre-germinative metabolism without allowing radicle protrusion (Paparella *et al.*, 2015). This treatment enhances reserve mobilization, DNA repair, membrane restoration and antioxidant defense, thereby improving early germination under stress. However, conventional priming methods using synthetic chemicals may increase production costs and raise environmental concerns (Bailly *et al.*, 2000; Tounekti *et al.*, 2020). Plant growth-promoting rhizobacteria (PGPR), particularly indole-3-acetic acid (IAA)-producing strains, have emerged as a sustainable alternative for seed treatment. IAA promotes cell division, cell elongation and root system development, thereby improving water and nutrient uptake and enhancing seedling vigor (Ganesh *et al.*, 2024). PGPR can also alleviate salinity stress through ACC deaminase activity, osmoprotectant accumulation, hormonal regulation and antioxidant defense, which help maintain membrane integrity and cellular homeostasis under stress (Yang *et al.*, 2009; Agnihotri and Waoo, 2024).

Despite these advances, important knowledge gaps remain. Most studies have focused on PGPR application at the seedling stage or in soil systems, whereas seed-based applications, particularly microbial seed priming, are still less explored. In addition, few studies have selected microbial strains from naturally saline environments for direct use in seed priming and the link between microbial IAA production and salinity stress responses during germination remains unclear. Therefore, salinity-tolerant, IAA-producing bacteria from saline soils may provide a promising strategy for developing bio-based seed enhancement technologies.

Accordingly, this study aimed to isolate and screen IAA-producing bacteria from saline soils and evaluate their potential use in seed priming to improve germination performance, seedling vigor and salinity tolerance in rice.

MATERIALS AND METHODS

The present experiment was conducted at the Division of Agronomy, Faculty of Agricultural Production, Maejo University, Chiang Mai, from November 2025 to January 2026. Rice (*Oryza sativa* L.) seeds of the cultivar Khao Dawk Mali 105, with an initial germination percentage of 95%, were used in this study. The seeds were first rinsed with distilled water to remove surface debris. Surface sterilization was then performed by immersing the seeds in a 1% (v/v) sodium hypochlorite (NaOCl) solution for 1 min to eliminate potential surface contaminants. After sterilization, the seeds were thoroughly rinsed with sterile distilled water 3-5 times to remove any residual disinfectant. Finally, the seeds were air-dried to a moisture content of approximately 14% prior to further experimental procedures.

Soil samples for bacterial isolation were collected from salt-affected rice fields in Nong Mek, Nong Song Hong District, Khon Kaen Province and Non Daeng District, Nakhon Ratchasima Province, northeastern Thailand.

These areas represent saline agroecosystems that favor halotolerant and stress-adapted microorganisms. Samples were randomly collected from rice fields and visibly salt-encrusted patches at a depth of 0-15 cm (Somasegaran and Hoben, 1994). The samples were air-dried, homogenized and used for bacterial isolation. For isolation, 10 g of homogenized soil were suspended in 90 mL of sterile distilled water and serially diluted from 10^{-1} to 10^{-8} . Aliquots of 0.1 mL from appropriate dilutions were spread onto nutrient agar plates and incubated at 35°C for 24 h. Colonies with distinct morphology, including differences in shape, size and pigmentation, were selected and purified using the streak plate method. Purified isolates were maintained on nutrient agar slants at 4°C for further characterization and subsequent experiments (El Ahmadi *et al.*, 2025).

Screening for indole-3-acetic acid (IAA) production was performed using the van Urk-Salkowski colorimetric method. A standard curve was prepared from IAA standards at concentrations of 0, 20, 40, 100, 200, 300 and 400 µM in 50% methanol (Lebrazi *et al.*, 2020). For each isolate, 1 mL of cell-free culture supernatant was mixed with 2 mL of van Urk-Salkowski reagent, vortexed and incubated in the dark for 30 min. Absorbance was measured at 530 nm and IAA concentration was calculated from the standard curve (Ehmann, 1977). Among 25 bacterial isolates, four isolates with high IAA production, stable growth and distinct colony morphology were selected and designated as isolates 1-4. Molecular identification was performed by comparing 16S rRNA gene sequences with reference sequences in the GenBank database using BLAST. Isolates 1, 2 and 3 were identified as *Bacillus megaterium*, with 99.65-99.73% sequence similarity, while isolate 4 was identified as *Microbacterium proteolyticum*, with 99.15% sequence similarity. For bacterial seed soaking and priming, the four selected isolates were individually cultured in 50 mL of nutrient broth in 125 mL Erlenmeyer flasks at 30°C and 125 rpm for 7 days. Before seed treatment, bacterial cultures were adjusted to OD₆₀₀ of approximately 1.0, equivalent to about 10^8 CFU mL⁻¹. Surface-sterilized rice seeds, 15 g per treatment, were soaked in each bacterial suspension for 24 h at 25°C. For seed soaking treatment, treated seeds were used directly for seed quality evaluation. For seed priming treatment, soaked seeds were air-dried at room temperature to a moisture content of approximately 14±2% before seed quality assessment (Paparella *et al.*, 2015).

Laboratory germination and seedling growth were evaluated under controlled conditions using the between-paper (BP) method. Seeds from each treatment were placed on moistened germination paper, covered with another sheet and rolled carefully to maintain uniform contact and moisture. Four replications of 50 seeds were used for each treatment. The rolls were placed vertically in a germination incubator at 25°C and 80% relative humidity under continuous light at 180 µE. Standard germination

percentage (GE) was determined from the number of normal seedlings, with the first count recorded on day 5 and the final count on day 14 after sowing (ISTA, 2023). Germination percentage was calculated as the number of normal seedlings at the final count divided by the total number of seeds tested and expressed as a percentage. Daily germination counts were recorded from day 1 to day 14 after sowing and seeds with radicle protrusion of at least 2 mm were considered germinated. Speed of germination (SG) was calculated as (Maguire, 1962):

$$SG = \frac{\sum(ni)}{di}$$

Where,

Number of newly germinated seeds on day *i* and *di* is the number of days after sowing. Mean germination time (MGT) was calculated as (Ellis and Roberts, 1980):

$$MGT = \frac{\sum(ni \times di)}{\sum ni}$$

Where,

Number of seeds germinated on day *i* and *di* is the number of days after sowing.

At day 14, ten normal seedlings from each replication were randomly selected for seedling growth measurement. Root length was measured from the seed attachment point to the primary root tip, while shoot length was measured from the seedling base to the tip of the longest leaf and both were expressed in millimeters (Baki and Anderson, 1973).

Greenhouse germination and seedling growth were evaluated using a seedling tray assay. Seeds from each treatment were sown in peat moss substrate (Klasmann-Deilmann GmbH, Germany) with four replications of 50 seeds per treatment. Germination percentage, speed of germination and mean germination time were determined using the same definitions and calculation procedures as described for the laboratory test, with daily counts recorded up to 14 days after sowing. At day 14, ten seedlings from each replication were randomly selected, shoots were cut at the substrate surface and shoot length was measured from the cutting point to the tip of the longest leaf and expressed in centimeters (Baki and Anderson, 1973).

Seed quality under salinity stress was evaluated using sodium chloride (NaCl), which is the predominant soluble salt in saline soils of arid and semi-arid regions (Tobe *et al.*, 2001). NaCl solutions were prepared at concentrations of

0, 20, 40, 60, 80 and 100 mM. For each salinity level, germination tests were conducted in 90 × 15 mm Petri dishes lined with three layers of germination paper. Fifty seeds per dish were evenly spaced and four replications were prepared for each treatment. The dishes were placed in a germination incubator maintained at 25°C and 80% relative humidity, under continuous light at an intensity of 180 µE. Germination was monitored daily and a seed was considered germinated when the radicle reached a length of at least 2 mm. At the end of the test, germination percentage, germination potential and germination index were calculated according to Yang and Li (2014).

Germination experiments conducted under both laboratory and greenhouse conditions were arranged in a completely randomized design (CRD) and data were subjected to analysis of variance (ANOVA) using statistical software. Percentage data were arcsine-transformed prior to analysis and values of 0% were transformed using the square-root transformation $\sqrt{x+0.5}$ to stabilize variance (Ahrens *et al.*, 1990). Treatment means were compared using Duncan's new multiple range test (DMRT) at the 5% probability level ($p \leq 0.05$). For germination traits evaluated under salinity stress, data were analyzed using factorial ANOVA in a completely randomized design to assess the main effects and interaction effects of salinity levels and seed treatments. When significant differences were detected, mean separation was performed using DMRT at $p \leq 0.05$.

RESULTS AND DISCUSSION

Screening and characterization of IAA-producing bacteria under temperature and salinity stress

Based on 16S rDNA sequence analysis, isolates 1, 2 and 3 were identified as *Bacillus megaterium*, whereas isolate 4 was identified as *Microbacterium proteolyticum* (Table 1). All isolates were obtained from saline rice-growing soils in northeastern Thailand, where halotolerant and stress-adapted microorganisms are likely to be selected. The high sequence similarity values of more than 99% supported species-level identification, while the observed functional and morphological differences among isolates were likely related to strain-level variation rather than species-level differences (Poretsky *et al.*, 2014). The three *B. megaterium* isolates showed slight differences in colony size, shape and surface texture, whereas *M. proteolyticum* isolate 4 showed clearly distinct colony morphology

Table 1: Bacterial identification based on 16S rDNA sequence analysis.

Isolate number	Identified bacteria	% Similarity	Accession number	District
Isolate 1	<i>Bacillus megaterium</i>	99.65	CP009920.1	Nong Song Hong District, Khon Kaen
Isolate 2	<i>Bacillus megaterium</i>	99.72	CP009920.1	Nong Song Hong District, Khon Kaen
Isolate 3	<i>Bacillus megaterium</i>	99.73	CP009920.1	Non Daeng District, Nakhon Ratchasima
Isolate 4	<i>Microbacterium proteolyticum</i>	99.15	NR_135869.1	Non Daeng District, Nakhon Ratchasima

(Fig 1). The selected isolates differed in their growth responses to temperature stress (Table 2). *B. megaterium* isolates 1, 2 and 3 grew well at both 30 and 40°C, whereas *M. proteolyticum* (isolate 4) grew well at 30°C but did not grow at 40°C, indicating lower thermotolerance than the *B. megaterium* isolates. None of the isolates grew at 50°C, suggesting that this temperature exceeded the tolerance threshold of all tested strains. These temperature responses may be associated with differences in heat-shock and chaperone systems that protect bacterial cells from heat-induced protein damage (Nakamoto and Vigh, 2007).

Under salinity stress, all selected isolates maintained high growth across 0-150 mM NaCl, indicating good salinity tolerance under the tested conditions (Table 2). *B. megaterium* isolates 1, 2 and 3 and *M. proteolyticum* (isolate 4) all showed consistently strong growth at all NaCl concentrations, suggesting that these isolates were well adapted to saline conditions. These responses may involve

compatible solutes such as proline, glycine betaine and trehalose, as well as Na⁺/H⁺ antiporters and K⁺ uptake systems that reduce ionic toxicity and osmotic imbalance (Miao *et al.*, 2025). IAA production differed markedly among the selected isolates (Fig 2). *B. megaterium* isolate 1 produced the highest IAA concentration of 150.10 µg mL⁻¹, followed by *B. megaterium* (isolate 3) with 117.77 µg mL⁻¹, *M. proteolyticum* (isolate 4) with 94.43 µg mL⁻¹ and *B. megaterium* (isolate 2) with 80.77 µg mL⁻¹. These differences indicate isolate-specific variation in tryptophan-dependent IAA biosynthesis (Idris *et al.*, 2007). However, the isolate with the highest IAA production was not necessarily the most effective candidate for seed application. Since all isolates failed to grow at 50°C, this temperature response could not explain the lower seed-based effectiveness of *B. megaterium* (isolate 1). The favorable seed-based performance of *M. proteolyticum* (isolate 4) suggests that bacterial effectiveness may depend not only on IAA production but also on other strain-specific traits

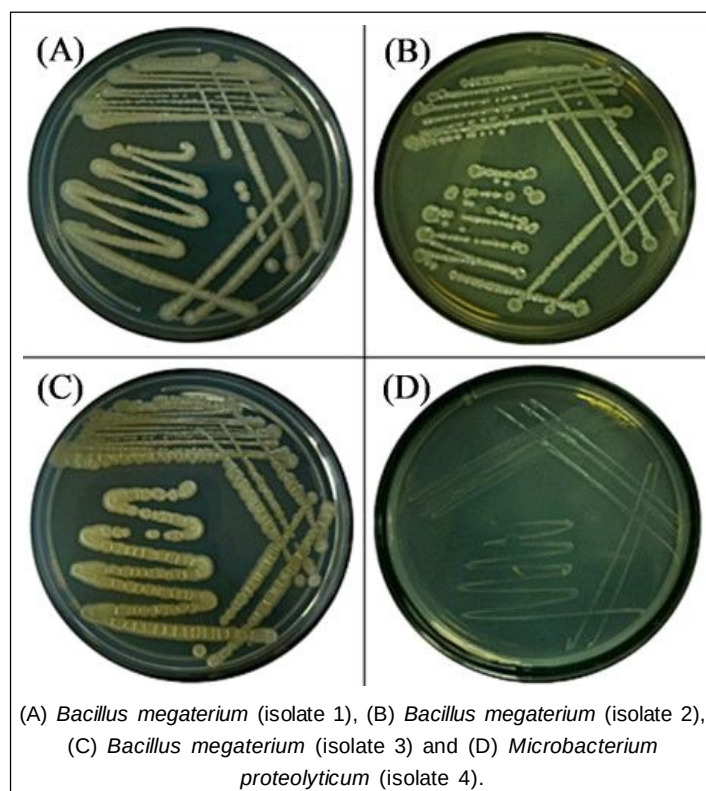


Fig 1: Colony morphology of selected IAA-producing bacterial isolates grown on agar medium.

Table 2: Growth performance of bacterial isolates under temperature and salinity stress conditions.

Isolate number	Temperature stress			Salt stress			
	30°C	40°C	50°C	0 mM	50 mM	100 mM	150 mM
Isolate 1	+++	+++	-	+++	+++	+++	+++
Isolate 2	+++	+++	-	+++	+++	+++	+++
Isolate 3	+++	+++	-	+++	+++	+++	+++
Isolate 4	+++	-	-	+++	+++	+++	+++

Notes: "+" = Low; "++" = Moderate; "+++" = High; "-" = Negative.

related to seed priming compatibility and functional activity during early germination. Therefore, an appropriate balance between phytohormone production and seed-associated performance may be more important than maximum IAA production alone. Excessive auxin may enhance ethylene-associated inhibition of primary root elongation and auxin-ethylene crosstalk is an important regulator of root growth under stress (Li *et al.*, 2015).

Functional screening of bacterial isolates for improving seed germination and seedling vigor under laboratory and greenhouse conditions

Seed soaking with bacterial isolates significantly affected germination and seedling growth under laboratory conditions (Table 3). Seeds soaked with *M. proteolyticum* (isolate 4) gave the highest germination percentage of 97% and the highest speed of germination of 9.41 plants/day, whereas seeds soaked with distilled water showed the lowest values for these parameters. Seeds soaked with *M. proteolyticum* (isolate 4) also had the shortest mean germination time of 2.95 days, indicating faster germination

than the other treatments. Root length was greatest in seeds soaked with *M. proteolyticum* (isolate 4), with a value of 15.63 mm. Shoot length was highest in seeds soaked

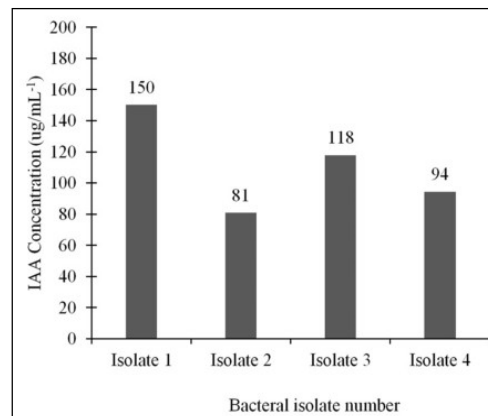


Fig 2: Screening of indole-3-acetic acid (IAA) producing strains isolated from saline soils in the northeastern region of Thailand.

Table 3: Germination percentage (GE), speed of germination (SG), mean germination time (MGT), root length (ROL) and shoot length (SHL) of rice seeds after soaking with bacteria tested under laboratory conditions.

Treatment	Laboratory condition				
	GE (%)	SG (plant/day)	MGT (day)	ROL (mm)	SHL (mm)
Unsoaked seeds	95 ab	9.23 a	4.08 a	11.89 c	10.20 b
Seeds soaked+distilled water	88 b	8.53 b	3.71 ab	13.23 bc	10.30 b
Soaked+ <i>B. megaterium</i> (isolate 1)	95 ab	9.26 a	3.98 a	14.54 ab	11.51 ab
Soaked+ <i>B. megaterium</i> (isolate 2)	94 ab	9.13 a	3.44 b	14.96 ab	11.54 ab
Soaked+ <i>B. megaterium</i> (isolate 3)	92 ab	9.13 a	3.78 ab	14.62 ab	12.42 a
Soaked+ <i>M. proteolyticum</i> (isolate 4)	97 a	9.41 a	2.95 c	15.63 a	12.36 a
F-test	**	**	**	**	**
C.V. (%)	6.22	4.16	7.34	9.30	10.72

** : Significant differences at $P \leq 0.01$.

¹Germination percentage data were arcsine-transformed prior to statistical analysis.

²Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

Table 4: Germination percentage (GE), speed of germination (SG), mean germination time (MGT) and shoot length (SHL) of rice seeds after soaking with bacteria tested under greenhouse conditions.

Treatment	Greenhouse condition			
	GE (%)	SG (plant/day)	MGT (day)	SHL (cm)
Unsoaked seeds	95 b	9.22 b	5.02 a	5.69 c
Seeds soaked+distilled water	96 ab	9.25 b	5.06 a	5.86 bc
Soaked+ <i>B. megaterium</i> (isolate 1)	95 ab	9.73 ab	4.95 ab	6.33 ab
Soaked+ <i>B. megaterium</i> (isolate 2)	97 ab	9.74 ab	4.89 ab	6.46 ab
Soaked+ <i>B. megaterium</i> (isolate 3)	97 ab	9.68 ab	4.85 ab	6.30 ab
Soaked+ <i>M. proteolyticum</i> (isolate 4)	99 a	9.89 a	4.76 b	6.62 a
F-test	**	**	**	**
C.V. (%)	2.15	2.32	2.42	6.82

** : Significant differences at $P \leq 0.01$.

¹Germination percentage data were arcsine-transformed prior to statistical analysis.

²Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ by DMRT.

with *B. megaterium* (isolate 3), with a value of 12.42 mm and was statistically comparable with seeds soaked with *M. proteolyticum* (isolate 4), which had a shoot length of 12.36 mm. Under greenhouse conditions, *M. proteolyticum* (isolate 4) produced the best overall response among the bacterial treatments (Table 4). Seeds soaked with *M. proteolyticum* (isolate 4) had the highest germination percentage of 99%, the highest speed of germination of 9.89 plants/day, the shortest mean germination time of 4.76 days and the greatest shoot length of 6.62 cm. These results indicate that seed soaking with *M. proteolyticum* (isolate 4) improved germination rate, germination uniformity and early seedling growth under both laboratory and greenhouse conditions. The positive effects may be associated with bacterial IAA production and the activation of early germination-related metabolic processes (Bewley *et al.*, 2013; Spaepen *et al.*, 2007).

In addition, *M. proteolyticum* (isolate 4) produced indole 3 acetic acid at $94.43 \mu\text{g mL}^{-1}$, promoting cell elongation and division in root tissues, thereby enhancing root system development and increasing root and shoot

length, consistent with previous reports that microbially derived IAA supports root growth and nutrient uptake (Kangsopa *et al.*, 2025). The reduced mean germination time may also reflect stimulation of antioxidant defense systems during imbibition, which preserves membrane and organelle integrity by limiting reactive oxygen species damage and thus enables faster, more efficient germination (Gill and Tuteja, 2010; Hasanuzzaman *et al.*, 2020). The consistency of these effects in greenhouse trials indicates that the benefits of bacterial seed soaking extend beyond controlled laboratory conditions, likely because the bacteria maintain activity in situ, including ACC deaminase production to lower stress induced ethylene and stimulation of osmoprotectant accumulation that supports cellular homeostasis during early seedling growth (Glick, 2014).

Effects of seed priming on rice germination under salinity stress

Following the screening of bacteria from saline soils, four isolates with high indole 3 acetic acid (IAA) production and strong salinity tolerance were selected. Although most

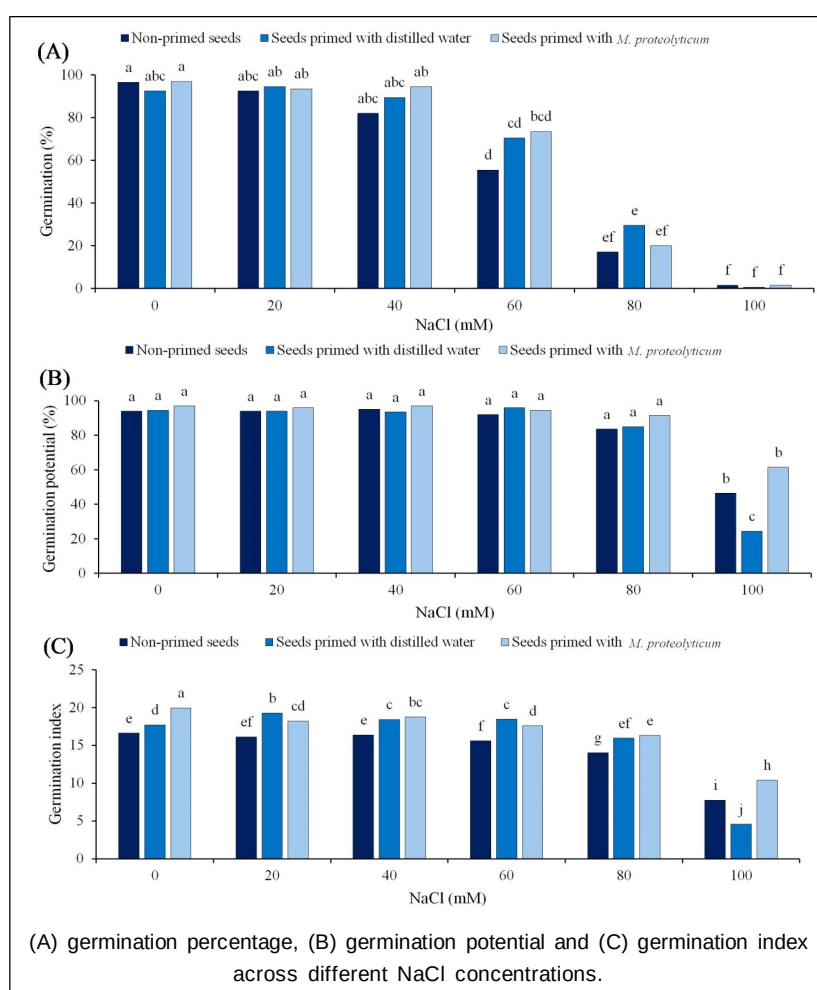


Fig 3: Effects of seed priming treatments on rice germination under salinity stress.

isolates grew well at 30-40°C, *M. proteolyticum* (isolate 4) showed a distinct thermotolerance pattern, indicating isolate specific stress adaptability. These isolates were then tested *via* seed soaking and consideration of both screening and seed quality data showed that *M. proteolyticum* (isolate 4) consistently provided the most favorable responses. Consequently, this isolate was chosen for further evaluation as a seed priming agent under salinity stress.

Salinity strongly inhibited rice seed germination as NaCl concentration increased from 0 to 100 mM. Germination percentage, germination potential and germination index declined with increasing salinity in all treatments, reflecting the effects of osmotic and ionic stress on water uptake, metabolic activity, reserve mobilization and energy production during germination (Fig 3) (Bewley *et al.*, 2013). Seeds primed with *M. proteolyticum* showed better germination performance than non-primed and hydro-primed seeds under selected salinity levels. For germination percentage, *M. proteolyticum*-primed seeds maintained higher values at 40 and 60 mM NaCl, while germination was strongly reduced in all treatments at 80 and 100 mM NaCl (Fig 3A). For germination potential, *M. proteolyticum*-primed seeds maintained high values from 0 to 80 mM NaCl and showed a clear advantage at 100 mM NaCl compared with hydro-primed seeds (Fig 3B). Germination index showed clearer treatment differences, with *M. proteolyticum*-primed seeds giving the highest values at 0 and 40 mM NaCl and maintaining higher values than non-primed seeds at 80 and 100 mM NaCl (Fig 3C). These results indicate that *M. proteolyticum* priming improved germination rate and uniformity under salinity stress, although its effect on final germination percentage was limited under severe salinity.

The improved performance of *M. proteolyticum*-primed seeds may be associated with the activation of pre-germinative metabolism, stimulation of hydrolytic enzymes such as α -amylase and protease, regulation of hormonal balance through IAA-mediated cell elongation and induction of antioxidant defenses that reduce salinity-induced oxidative damage (Ashraf and Foolad, 2005). Plant growth-promoting rhizobacteria may also enhance salinity tolerance through ACC deaminase activity, reduced stress-induced ethylene and accumulation of osmoprotectants such as proline and soluble sugars, which help maintain cellular osmotic balance (Yang *et al.*, 2009). Hydro-priming alone showed a weaker overall effect than bacterial priming, indicating that hydration without microbial functions such as phytohormone synthesis and antioxidant activation may be insufficient to improve germination performance under salinity stress (Paparella *et al.*, 2015). Notably, *B. megaterium* (isolate 1), despite producing the highest IAA level, did not provide the best seed performance under salinity, suggesting that an optimal balance between IAA production and stress-adaptive traits may be more important than maximum IAA production alone (Fig 2) (Lam *et al.*, 2023).

Thus, seed priming with *M. proteolyticum* is an effective strategy to enhance rice germination under salinity stress. This treatment improves rate, uniformity and overall germination potential, which are critical for crop establishment in adverse environments and shows strong promise for application to other crops exposed to abiotic stress.

CONCLUSION

The results of this study indicate that the selection of IAA-producing bacteria from saline soils is useful for improving rice seed germination and early seedling growth. Although *B. megaterium* (isolate 1) produced the highest IAA level, it did not provide the best seed performance. In contrast, *M. proteolyticum* (isolate 4), which produced a moderate IAA level and showed favorable performance under saline conditions, gave the best overall response in seed-based evaluations. Seed soaking with *M. proteolyticum* (isolate 4) improved germination percentage, speed of germination, mean germination time, root length and shoot length under laboratory conditions and also enhanced germination and shoot growth under greenhouse conditions. Under salinity stress, seed priming with *M. proteolyticum* improved germination performance under selected NaCl levels, particularly by maintaining higher germination percentage at 40 and 60 mM NaCl and improving germination potential and germination index under severe salinity. These findings suggest that the effectiveness of bacterial seed priming depends not only on IAA production but also on the stress-adaptive capacity of the bacterial isolate.

Therefore, *M. proteolyticum* (isolate 4) represents a promising bacterial candidate for seed priming to improve rice germination and early seedling establishment under saline conditions.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the official views of their affiliated institutions. The authors are responsible for the accuracy, integrity and completeness of the information presented in this manuscript.

Informed consent

This study did not involve human participants or experimental animals. Therefore, informed consent and animal ethical approval were not required. The research

was conducted using seed materials and bacterial isolates under standard laboratory and greenhouse conditions in accordance with institutional research practices.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article. The authors further confirm that no financial or personal relationships have inappropriately influenced the work reported in this manuscript.

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