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Enhancement of Rice Seed Performance Using Rhizobacterial Agents and Molecular Characterization of Isolates from Paddy Rhizosphere

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ABSTRACT

Study aims to evaluate the effect of two rhizobacterial isolates on the germination and vigor of rice seeds and to identify the strains through molecular characterization. A factorial completely randomized design was used, involving concentrations of rhizobacterial (0%, 25%, 50%, 75%, and 100%) and soaking durations (1 and 4 hours), with three replications, totaling 30 experimental units. Variables included seed viability and seed vigor. Diversity is assessed by Shannon and Simpson Diversity Index. Molecular identification used 16S rRNA gene sequences. Phylogenetic trees were constructed using the NeighborJoining method, and evolutionary distances were calculated with the Kimura 2-parameter model. The results showed that soaking duration of 4 hours significantly enhanced seed vigor, the average vigor index from 76.80% to 92.07%, and growth rate from 51.88% to 72.53%, regardless of rhizobacterial concentration. The highest maximum growth potential (100%) was recorded 25% concentration and 4-hour soaking. Shannon Diversity Index for two species is: 0.693 and Simpson Diversity Index 0,5. Molecular analysis identified Isolate 1 related to Bacillus cereus strain ATCC 14579 with 95% identity. Isolate 2 showed 88% similarity, suggesting potential novelty. These findings support rhizobacteria as bioenhancement, increasing ecologically important diversity and strengthening their relevance in sustainable rice cultivation.

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KEY WORDS: Rhizobacteria, seed bioenhancement, *Bacillus cereus*, microbial diversity, rice

1. INTRODUCTION

Seed quality is a critical factor in ensuring optimal plant development. One emerging innovation to improve seed quality is the use Plant Growth-Promoting Rhizobacteria (PGPR), that not only improve seed quality through techniques such as biopriming but also significantly contribute to soil restoration, reduction of greenhouse emissions, and phytohormone production. According to PGPR can produce significant amounts of IAA, which positively influences plant growth and development (Homthong et al. 2022). These organisms facilitate nutrient acquisition by enhancing the solubility of phosphates and minerals in the soil (Wahyudi et al. 2021). Specifically, bacteria like *Pseudomonas* spp. and *Bacillus* spp. are known to promote plant growth by stimulating physiological and biochemical processes in plants (Marwan et al. 2020). Moreover, rhizobacteria in agroecosystems enriches the biodiversity of the soil, enhances system resilience against biotic and abiotic stresses, and facilitates beneficial plant–microbe interactions.

Microorganisms, such as Bacillus, act as growth promoters and biocontrol agents that activate the plant's physiological processes, offering a more sustainable alternative to chemical fertilizers (Kaushik. 2024). Bacillus subtilis has been documented to improve seed germination rates in various crops, reflecting the potential of these techniques across species (Miljaković et al. 2022). In rice, rhizobacterial inoculation promotes germination and reduces disease severity caused by Rhizoctonia solani (Kanjanasopa et al. 2021). However, germination is a complex process that begins with rapid water uptake and a marked increase in oxygen consumption rate from initiation of aerobic respiration. (Rodrigues. 2023).

Beneficial microorganisms colonize the seed surface, improving germination through mechanisms like enhanced membrane permeability and better management of cellular water potential (Forti et al., 2020). Utilization Indigenous rhizobacteria from the rice rhizospher increasing population and biodiversity, enhance seed germination while also serving as biostimulants by producing phytohormones such as auxins and gibberellins (Verma et al. 2022) .This is notable as colonization of seeds prior to germination increase resilience against biotic stresses caused by pathogens, thereby reducing disease severity and ultimately contributing to better yield (Savvas et al. 2024).

Acording to Fu et al (2024) when techniques osmopriming or hydropriming are utilized, responses vary significantly based on seed lot quality and environmental conditions (Fu et al. 2024). Furthermore, recent studies emphasize the importance of utilizing the right concentration of biostimulants and soaking duration during the priming process. Different concentrations of rhizobacteria applied to rice seeds can yield varying effects on seed vigor and subsequent seedling growth (Mhada et al. 2021). For example, conditioning rice seeds with optimal microbial concentrations alongside appropriate soaking times could significantly enhance seedling emergence and growth parameters, including root and shoot development (Moreno et al. 2020).

Indigenous rhizobacteria isolated in previous research was tested in this study, with the aims of their potential as biopriming, molecular identification used 16S rRNA gene sequences. and diversity index. The analysis of PCR (Polymerase Chain Reaction) serves as a fundamental molecular technique for amplifying specific DNA sequences, which subsequently enables the identification and classification of these microorganisms. More specifically, the 16S rRNA gene has emerged as a critical marker in bacterial phylogeny due to its conserved nature and the insight it provides into the evolutionary patterns of bacteria, making it a widely accepted method for molecular identification across various environments (Alyousif. 2022; Suriani et al. 2023; Zulaika et al. 2022).

II. METHODS

The experiment employed a Completely Randomized Design (CRD) arranged in a two-factor factorial pattern with: Factor 1 (R): Rhizobacterial concentration at 5 levels: R0 = 0% (control), R1 = 25%, R2 = 50%, R3 = 75%, and R4 = 100%. Factor 2 (L): Soaking duration at 2 levels: L1 = 1 hour, L2 = 4 hours. Each treatment combination was replicated three times, resulting in a total of 30 experimental units (5 × 2 × 3). The assignment of treatments to experimental units was randomized using a random number generator to minimize positional or environmental bias. All seed treatments and measurements were conducted under controlled laboratory conditions, ensuring consistent temperature, lighting, and aseptic technique throughout all procedures.

Procedures

1. *Preparation of Culture Media*

Rice washing water was filtered and collected in a 500 mL Erlenmeyer flask. Then, 5% molasses was added, mixed thoroughly, and sterilized at 121°C for 15 minutes.

2. *Culturization*

The rhizobacterial biological agent was inoculated onto the culture medium (Al-Azmiya et al. 2021) and shaker at 200 rpm for 48 hours. The resulting bacterial culture was stored as stock for future use.

3. *Seed Preparation*

Prior to soaking, seeds were surface-sterilized using a 1% sodium hypochlorite solution for 2–3 minutes, followed by rinsing thoroughly with sterile distilled water. This step was performed to eliminate surface contaminants and ensure that any microbial effects observed were due to the inoculated rhizobacteria ratr than native seed-borne microorganisms.

4. *Inoculation*

To inoculate the seeds with rhizobacteria, 50 seeds were soaked in 50 mL of rhizobacterial suspension in a Petri dish, according to the concentration specified for each treatment. The seeds were placed in a laminar airflow cabinet and incubated for the soaking duration determined by the treatment.

5. *Rice Seed Viability and Vigor Testing*

Seed viability and vigor were assessed by germinating the seeds on a Petri dish lined with filter paper, which was then covered and incubated. All procedures were conducted aseptically.

6. *Molecular Characterization via 16S rRNA Sequencing*

Molecular identification of rhizobacterial isolates was conducted through amplification and sequencing of the 16S rRNA gene, a widely accepted marker for bacterial phylogenetic analysis. Genomic DNA was extracted using a standard phenolchloroform protocol. Polymerase Chain Reaction (PCR) was carried out using universal bacterial primers: Forward primer (63f): 5'-CAG GCC TAA CAC ATG CAA GTC-3'. Reverse primer (1387r): 5'-GGG CGG GTA CAA GGC-3'

PCR amplification was performed in a 25 µL reaction volume containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer, 1 U Taq DNA polymerase, and ~50 ng of template DNA. The thermal cycling profile was as follows: initial

denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 7 minutes.
Amplicons (~1300 bp) were confirmed via agarose gel electrophoresis and sequenced using the Sanger method. Sequencing reads were quality-checked, and depth of sequencing was sufficient to cover the entire 16S variable region V1–V9. Sequence alignment and taxonomic identification were conducted using NCBI's BLASTN tool against the GenBank database.

Parameters

1. Seed Viability

Germination Rate (%)

The germination rate is determined by counting the number of seeds that develop into normal sinews by the 8th day after sowing. It is calculated using the following formula:

$$Germination\ power = \frac{\Sigma\ Normal\ sprouts}{\Sigma\ Samples\ of\ seeds\ tested}\ x\ 100\%$$

Maximum Growth Potential (%)

This parameter measures the proportion of seeds that successfully produce sinews either normal or abnormal by 8 days after planting (DAP). It is calculated as follows:

$$Maximum\ growth\ potential = \frac{\Sigma\ \text{growing sprouts}}{\Sigma\ \text{samples of seeds tested}}\ x\ 100\%$$

2. Seed Vigor

Vigor Index (%)

The Vigor Index is calculated based on the number of normal sinews observed on the 4th and 8th day after planting. The formula used is:

$$Vigor\ index = \frac{\sum KN\ 4}{\sum Planted\ seeds}\ x\ 100\%$$

Note:

KN 4 = Number of normal sinews on the 4th day of observation.

Growth synchrony (%)

Growth synchrony is expressed as the percentage of robust normal sinews observed on the 6th day, between the first (4 DAP) and second (8 DAP) observation points. The calculation follows formula:

$$Growth\ synchrony = \frac{\sum Normal\ sprouts\ are\ strong}{\sum Total\ seeds\ planted}\ x\ 100\%$$

Growth Rate (%)

Growth rate is determined based on the daily percentage of normal sinews from planting until the 7th day. The formula is:

$$Growth\ Rate = \frac{N1}{W1} + \frac{N2}{W2} + \ldots \frac{Nn}{Wn}$$

Note:

N1–Nn : Percentage of germinated sinews on day n

W1–Wn : Day of observation (n = 1, 2, 3, ..., 7)

Sprout Dry Weight

Sprout dry weight is measured by oven-drying sinews harvested on the 8th day after planting at 65°C for 48 hours, followed by weighing.

3. Microbial Diversity

To assess the biodiversity of rhizobacterial, two ecological diversity indices were calculated based on colony morphology and genotypic grouping base on 16S rRNA analysis:

1. ***Shannon Diversity Index (H):***

$$H = -\Sigma p_i \times ln(p_i)$$

where:

***ln*:** Natural log

p_i**:** The proportion of the entire community made up of species *i*

2. **Simpson Diversity Index (D):**

$$D = 1 - \left(\frac{\Sigma\, n(n-1)}{N(N-1)} \right)$$

Where:

n : The total number of organisms a particular species

N : The total number of organisms of all species

Data Analysis

Statistical Analysis of Seed Performance. Quantitative data for seed viability and vigor (germination rate, maximum growth potential, vigor index, growth synchrony, growth rate, and sprout dry weight) were analyzed using Analysis of Variance (ANOVA) at significance levels of 5% and 1%. When significant differences were found, Least Significant Difference (LSD) tests were applied for mean comparison among treatments.

Phylogenetic Tree Construction and Molecular Characterization. For molecular identification, 16S rRNA gene sequences obtained from Sanger sequencing were aligned using ClustalW in MEGA X software. Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method, and evolutionary distances were calculated with the Kimura 2-parameter model.

To assess the robustness and reliability of the phylogenetic groupings, bootstrap analysis with 1000 replicates was performed. Bootstrap values were presented as percentages on the tree branches, with values above 70% considered to indicate strong branch support.The resulting phylogenetic tree was compared against reference strains obtained from NCBI Gen Bank,

III. RESULTS

Seed Viability (Germination Rate and Maximum Growth Potential)

Table 1 presents the analysis of variance indicated that neither the concentration of the biological agent nor the soaking duration had a significant effect on the germination rate of seeds ($F = 0.62$ and $F = 0.01$, respectively; $p > 0.05$). Additionally, the interaction between concentration and soaking duration was also not significant ($F = 0.13$; $p > 0.05$). In contrast, soaking duration showed a highly significant effect ($F = 12.23$; $p < 0.01$) on the maximum growth potential of seeds. Meanwhile, the concentration of biological agents did not significantly affect maximum growth potential ($F = 0.67$; $p > 0.05$), nor did the interaction between concentration and soaking duration ($F = 0.75$; $p > 0.05$).

Table 1. The Effects of Concentration and Soaking Duration on Germination Rate and Maximum Growth Potential

Treatment	Degree Freedom	F. Count		F. Tabel	
		Germination rate	Max. growth	0.05	0.01
Treatment	9	0.34^{ns}	01.99^{ns}	2.39	3.46
Concentration	4	0.62^{ns}	00.67^{ns}	4.43	4.43
Soaking Duration	1	0.01^{ns}	12.23**	4.35	8.10
Interaction (C×S)	4	0.13^{ns}	00.75^{ns}	2.87	4.43
Error	20	—	—	—	—
Total	29	—	—	—	—

Note: ns: Not significant

**: Highly significant at 1% level

Table 2 presents the results of the Least Significant Difference (LSD) The average maximum growth potential at 1-hour soaking (L0) was 92.67, whereas at 4-hour soaking (L1) it was 97.73. The difference between these means is 5.06, which is greater than the LSD value of 3.38. This indicates a statistically significant difference at the 5% level, demonstrating that a longer soaking duration (4 hours) significantly improves the seed's growth potential compared to a shorter duration (1 hour).

Tabel 2. The Least Significant Difference showing the average maximum growth potential

Treatment	Soaking duration	
	L0 : 1 hour	**L1 : 4 hours**
R0 : Control	91.33	98.67
R1 : Concentration of rhizobacterial agents 25%	91.33	100.00
R2 : Concentration of rhizobacterial agents 50%	92.00	94.00
R3 : Concentration of rhizobacterial agents 75%	94.00	98.00
R4 : Concentration of rhizobacterial agents 100%	94.67	98.00
Average	92.67[a]	97.73[b]
Comparative value of LSD :	3.38	

Note: Along Average, values having different superscript letters vary significantly

Seed Vigor (Vigor index, growth synchrony, growth Rate, and sprout dry weight)

Table 3 presents the effect of rhizobacterial concentration and soaking duration on seed vigor, that soaking time notably influences the vigor index of sinews (F = 8.48 > F table 0.05 = 4.35), whie concentrationand intreaction did not significantly affect. On Growth Synchrony, the treatments did not influence how synchronized the senews emergence and growth were. On Growth Rate, Soaking duration showing that seed soaking significantly accelerates senews growth (F = 20.17 > F table 0.01 = 8.10).On Sprout Dry Weight. The interaction between concentration and soaking duration was significant at 5% level (F = 2.97 > F table 0.05 = 2.87). Main effects (concentration and soaking duration) were not significant individually.

Table 3. The effect of rhizobacterial agent concentration and soaking duration on, vigor index, growth synchrony, growth rate and sprout dry weight.

Treament		F. Count				F. Table	
	Degrees freedom	**Vigor index**	**growth synchrony**	**Growth rate**	**Sporout dry weight**	**0.05**	**0.01**
Treatment	9	1.68[ns]	1.13[ns]	3.17[ns]	0.82[ns]	2.39	3.46
Concentration	4	0.65[ns]	0.15[ns]	1.75[ns]	1.16[ns]	4.43	4.43
Soaking duration	1	8.48*	3.19[ns]	20.17**	0.23[ns]	4.35	8.10
Interaction	4	1.01[ns]	1.59[ns]	0.34[ns]	2.97*	2.87	4.43
Error	20						
Total	29						

Note: ns: Not significant

**: Highly significant at 1% level

Table 4 presents the results of the Least Significant Difference (LSD) test comparing the effects of soaking duration and rhizobacterial concentration on vigor Index, growth rate and sporout. The mean vigor index was higher in the 4-hour soaking treatment (92.07) compared to the 1-hour treatment (76.80), but the difference was not statistically significant, since it did not exceed the LSD value of 24.44. Soaking for 4 hours tended to improve vigor, but not significantly at the 5% level.

Table 4. The Least Significant Difference showing the average vigor Index, growth rate, and sprout dry weight

Treatment	Vigor index		Growth rate		Sporout dry weigh t	
	1hours (L1)	**4 hours (L1)**	**1 hours (L0)**	**4 hours (L1)**	**1 hour (L0)**	**4 hours (L1)**
R0 : Control	62.00	93.67	39.32	64.53	1.04[ax]	0.99[ax]
R1 : Rhizobacteria 25%	70.00	93.33	44.19	72.87	1.22[ax]	1.29[ax]

R2 : Rhizobacteria 50%	83.33	88.67	59.41	74.92	0.96^{ax}	1.26^{ax}
R3 : Rhizobacteria 75%	81.33	92.00	58.04	76.67	1.31^{ay}	1.14^{ax}
R4 : Rhizobacteria 100%	87.33	92.67	58.41	73.69	0.96^{axz}	1.03^{ax}
Average	76.80^{a}	92.07^{a}	51.88^{a}	72.53^{a}		
LSD (5%)	24.44		21.45		0.32	

Note: Along the same column and row, values having different superscript letters vary significantly ($p < 0.05$).

The growth rate increased consistently from 1-hour to 4-hour soaking in all treatments. The overall mean increased from 51.88 to 72.53, a difference of 20.65, which is just below the LSD value of 21.45, meaning this improvement was not statistically significant. While soaking time clearly enhanced growth rate trends, the differences were not statistically significant.
Sprout Dry Weight. Based on the LSD value of 0.32, the only statistically significant difference was found between. R3L0 (1.31^{ay}) and R4L0 (0.96^{axz}), and possibly R2L0 (0.96^{ax}), suggesting that 75% rhizobacterial concentration with 1-hour soaking resulted in the highest dry weight. The rest of the values fall into the same statistical grouping (marked with "a x"), meaning most treatments were not significantly different from each other. Only one combination (R3L0) showed significantly higher sprout biomass, suggesting an interaction effect between concentration and short soaking duration.

Diversity Index

Table 5 presence The Shannon-Wiener Index (H'), The theoretical maximum value of H' for two species is: 0.693 thus indicates maximum diversity possible for two equally represented isolates, meaning that the two morphotypes were evenly distributed in proportion (50%-50%). The Simpson Index (D) also measures species diversity but gives more weight to the dominance of species. A value of 0.5 indicates moderate diversity, consistent with a situation in which two species are equally abundant. In this case, the value further confirms that no single isolate dominated the microbial populatio.

Table. 5. Diversity index summary

No	Parameter	Value
1	Number of Isolate	2
2	Observed colony morphotypes	2 (different)
3	Shannon-Wiener Index (H')	0.693
4	Simpson Index (D)	0.500

Phylogenetic Tree Construction and Molecular Characterization

Phylogenetic Tree Interpretation

Figure 1. shows that isolate 1 is closely related to *Bacillus cereus* ATCC 14579 (95% similarity), supporting its identification as part of the *B. cereus* sensu lato group. Isolate 2 falls into a separate clade but remains proximal to the *Bacillus sp.* cluster, with approximately 88% similarity, suggesting the possibility of a local variant or a novel strain.The longer evolutionary branch length of Isolate 2 indicates greater genetic divergence, reinforcing the importance of further exploration of indigenous microbial resources.

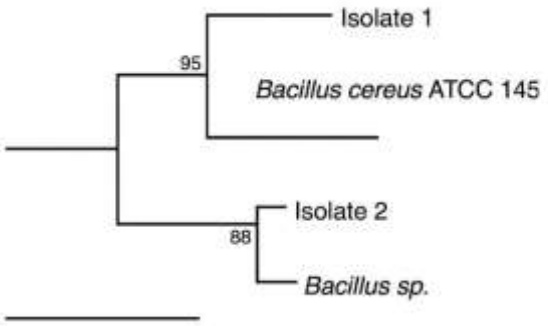

Figure 1. Phylogenetic Tree Structure (based on 16S rRNA)

Molecular Characterization

Both isolates 1 and 2 produced clear, strong DNA bands around 1300 bp (Figure 2).This corresponds to the typical size of the bacterial 16S rRNA gene, which usually ranges from 1300–1500 bp depending on the primer design. Both samples show a single, distinct band, indicating that the amplification was specific with no contamination or non-specific products. No double bands or smears were observed, which suggests no degradation or contamination. 1 kb DNA Marker, used to verify that the band size is accurate (approximately 1300 bp).The bands in the marker lane appear complete and can be used as a reliable size reference. PCR using 16S rRNA primers successfully amplified the target gene in both isolates 1 and 2. The PCR product is approximately 1300 bp in size, consistent with the 16S rRNA gene fragment, indicating that both isolates are likely bacterial. The size of 16S rDNA generally reaches 1500 bp. The base length of the 16S rDNA gene is 1500-1550 bp (Dong ert al., 2023).

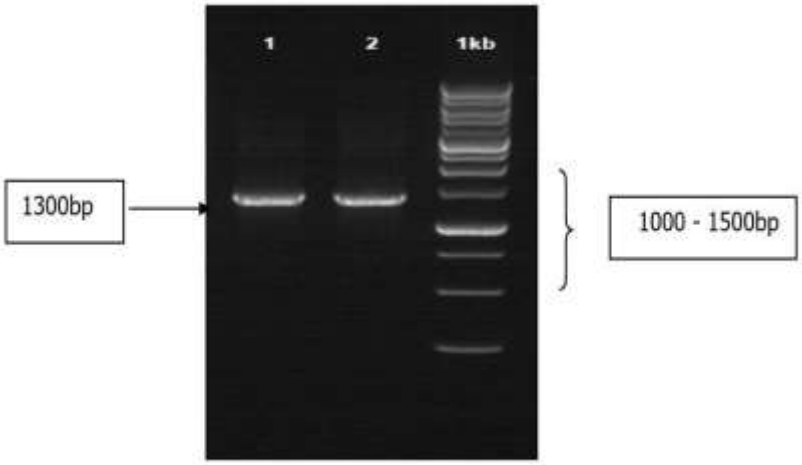

Figure 2. Visualization of PCR results using 16S rRNA primers
Description: No. 1 = Isolate 1, No. 2 = Isolate 2, Marker = 1 kb

Table 6 shows the BLASTN result shows a maximum alignment score of 1932, indicating a highly significant match between the query sequence and the reference database. The query coverage reaches 98%, and the percent identity is 95% with several strains of *Bacillus cereus*, including ATCC 14579, JCM 2152, CCM 2010, and NBRC 15305. These consistent values across multiple entries suggest that Isolate 1 is most closely related to the *Bacillus cereus* species group. However, because the percent identity is below 97%, which is commonly used as a threshold for confident species-level identification using 16S rRNA, it is possible that this isolate represents a distinct strain or a closely related species within the *B. cereus* complex.

Table 6. Interpretation of BLASTN Results for isolate 1

No	Description	Max Score	Query Cover	Max Ident	Accesion
1	*Bacillus cereus* strain ATCC 14579	1932	98%	95%	NR 074540.1
2	*Bacillus cereus* strain JCM 2152	1932	98%	95%	NR 113266.1
3	*Bacillus cereus* strain CCM 2010	1932	98%	95%	NR 115714.1
4	*Bacillus cereus* strain NBRC 15305	1932	98%	95%	NR 112630.1
5	*Bacillus cereus* strain ATCC 14579	1932	98%	95%	NR 114582.1

Table 7 shows the BLASTN results for Isolate 2, indicating that the closest matches are strains of *Bacillus cereus*, including ATCC 14579, JCM 2152, CCM 2010, and NBRC 15305. The maximum alignment score recorded is 1393, with a query coverage ranging from 93% to 98%, and percent identity consistently at 88%. These values suggest a lower degree of similarity between Isolate Gowa 2 and the reference *Bacillus cereus* strains compared to Isolate 1. The percent identity of 88% is below the commonly accepted threshold for species-level identification based on 16S rRNA gene sequences, which is typically 97%. This indicates that Isolate 2 may not belong to *Bacillus cereus* sensu stricto, but rather to a more distantly related species within the *Bacillus cereus* group or possibly to a different but related species within the genus *Bacillus*. The relatively low identity also raises the possibility that Isolate2 could represent a novel taxon or an environmental strain with distinct genetic characteristics.

Table 7. Interpretation of BLASTN Results for isolate 2

No	Description	Max Score	Query Cover	Max Ident	Accesion
1	*Bacillus cereus* strain ATCC 14579	1393	93%	88%	NR 074540.1
2	*Bacillus cereus* strain JCM 2152	1393	93%	88%	NR 113266.1
3	*Bacillus cereus* strain CCM 2010	1393	93%	88%	NR 115714.1
4	*Bacillus cereus* strain NBRC 15305	1393	98%	88%	NR 112630.1
5	*Bacillus cereus* strain ATCC 14579	1393	98%	88%	NR 114582.1

IV. DISCUSSION

The rice seeds used in this research came from the Inpari 50 Marem variety, with a viability rate of 75%, with these data it shows that the seeds have experienced a decline in quality, as optimal seeds typically yield germination rates exceeding 90%, which ensures robust growth even under suboptimal conditions (Ilangathilaka et al. 2021). The implications of this decline necessitate interventions such as seed invigoration techniques, which can stimulate metabolic activities to enhance germination performance and growth potential.

The results from the current investigation align well with findings from the latest literature, confirming the complex interplay between soaking duration and seed performance. Several studies have underscored the relationship between soaking duration and germination efficiency. For instance, Nithyadevi et al (2022) found that increased soaking times initially enhanced germination rates, peaking around 16 hours. Thus, the current findings align with the literature indicating that soaking duration can significantly influence senews growth potential, even if not necessarily germination rates. In the study published by Xie et al (2022), findings highlight that a nuanced understanding of treatment interactions and specific biochemical responses during soaking could clarify the differences in results between germination rates and growth potential.

The literature illustrates that priming treatments including hydropriming, consistently result in improved seed vigor and growth potential. Tania et al (2019) demonstrated that hydropriming stimulated physiological changes essential for faster emergence and stronger seedlings. This is evident in the current research findings showing significant growth enhancement with extended soaking time, as indicated in the results. Moreover, studies by Chiboub et al (2024) corroborate the suggestion that seed soaking, when properly timed, can enhance metabolic activities contributing to overall vigor and growth. This synergistic effect between soaking duration and maximum growth potential may provide avenues for optimizing seed treatment protocols in agricultural practices aimed at enhancing crop yields.

Most significant effects were driven by soaking duration rather than rhizobacterial concentration, suggesting that hydration time had a greater influence on seed performance. Moreover, bacterial viability and colonization were not confirmed at each concentration, making it unclear whether observed effects were due to microbial activity. Future studies should verify colonization and include microbial quantification to clarify the role of rhizobacteria in seed enhancement.

This study assumes that seed soaking led to effective rhizobacterial colonization, yet no direct confirmation was conducted. This represents a key limitation, as the effectiveness of microbial seed priming depends on successful colonization and persistence. Future work should include scanning electron microscopy to confirm colonization patterns and viability. Such data would provide stronger evidence for the biological role of the rhizobacteria and support the reliability of seed inoculation strategies.

Soaking is known to trigger key metabolic events that initiate germination.seed imbibition can enhance membrane integrity recovery, leading to better water and solute uptake. Extended soaking can also activate hydrolytic enzymes such as α-amylase, which mobilize stored reserves for embryonic growth. Moreover, prolonged soaking may stimulate early signaling pathways involving reactive oxygen species (ROS), calcium fluxes, and phytohormones like gibberellins and auxins, which are essential for breaking dormancy and promoting radicle emergence. These cellular changes help explain the observed improvements in germination vigor and early senews development.

The vigor index was observed to increase with prolonged soaking duration and higher concentrations of rhizobacterial agents. Data indicated a rise in average maximum growth potential from the control value (62.00) to 90.00 in treatments with the highest concentration following an extended soaking period. Recent literature supports these findings, suggesting that rhizobacterial amendments can enhance seed vigor by improving physiological processes. Nivetha et al. found that Bacillus spp. strains mitigate osmotic stress, promoting both seed germination and seedling vigor, which is consistent with the observed growth benefits in this study Nivetha et al (2024). Furthermore, provided insights into germination and vigor mechanisms influenced by

environmental factors, indicating that these dynamics could favorably affect growth rates in response to biological treatments (Yan et al. 2024).

The data indicated no significant influence of treatment on growth synchrony, suggesting variability in how these seeds activate their growth phases. Despite this study's findings of non-significant outcomes, previous research highlights that plant genetics play a crucial role in germination timing and uniformity. Initial seed vigor and sowing densities affect growth parameters significantly, emphasizing the varied responses of plants from different vigor backgrounds (Cardoso et al. 2021). This suggests that while biological interventions like rhizobacterial application are beneficial, genetic factors can complicate uniformity in growth.

Analysis of sprout dry weight results demonstrated that while treatments individually did not show significance, interactions between soaking duration and rhizobacterial concentration were noteworthy. This aligns with findings by Mushtaq et al (2025) who reported that auxin-producing plant growth-promoting rhizobacteria can enhance biomass accumulation in crops, hence improving yield . The enhanced dry weight observed corresponds with broader trends in seed vigor research, where effective seed treatments typically lead to improved biomassa. This study was limited to controlled laboratory conditions with only 30 experimental units, without validation in greenhouse or field settings. Such limitations reduce the applicability of the findings to real-world agricultural environments, where multiple factors can influence the performance of rhizobacterial inoculants. Future research should include pot and field trials across diverse agroecological zones to assess the consistency of these results. Field validation will help evaluate interactions with native soil microbes and environmental factors, ensuring the practical relevance and potential for wider adoption of these isolates in sustainable rice farming.

Although several parameters such as germination rate and vigor index did not show statistically significant differences. However statistical non-significance does not necessarily imply a lack of effect; it may reflect limitations such as small sample size or high variability.

The rhizobacterial isolates identified in this study particularly Isolate 1, which showed 95% similarity to *Bacillus cereus* ATCC 14579—represent promising candidates for seed bioenhancement and plant growth promotion. *Bacillus cereus* is a Gram-positive bacterium distinguished by its large, rod-shaped morphology, typically measuring between 3–5 µm in length and approximately 1 µm in width. It is capable of forming elliptical spores under aerobic conditions, reflecting its facultative anaerobic physiology. As a mesophilic microorganism, *B. cereus* thrives optimally at temperatures between 30 –35°C and exhibits motility through multiple peritrichous flagella (Bhadani et al., 2024). Its mobility, which contributes significantly to its environmental adaptability, is modulated by various factors such as salinity (Bhadani et al., 2024).

The *Bacillus cereus* species is known for several beneficial including: IAA (Indole-3-Acetic Acid) Production, This auxin hormone enhances root elongation, root hair formation, and nutrient uptake. P many Bacillus isolates, including *B. cereus*, can solubilize insoluble inorganic phosphate into plant-available forms (e.g., $H_2PO_4^-$ and HPO_4^{2-}) through the release of organic acids. Siderophores production, siderophores are iron-chelating compounds that enhance iron bioavailability, particularly in iron-deficient soils. Root Colonization and Biofilm Formation, many *Bacillus* strains are known to form biofilms on root surfaces, enhancing microbial adhesion, persistence, and local delivery of bioactive compounds in the rhizosphere. Its inoculation into the rhizosphere has been shown to enhance plant growth and yield, such as in cherry tomato crops, through beneficial modulation of the microbial community in the root zone (Dong et al., 2023; Sui et al., 2023). Furthermore, *B. cereus* functions as a biostimulant by producing phytohormones including auxins (3.375 ppm), gibberellins (3.960 ppm), and siderophores (2.910 ppm) within five days of incubation (Nontji et al., 2023). Among these, gibberellins play a key role in seed germination, particularly in rice, by breaking seed dormancy, stimulating the synthesis of hydrolytic enzymes for starch degradation, and promoting embryo elongation and development

Base on Uniqueness and Potential of Local, Isolate 2, which exhibited only 88% genetic similarity to known *Bacillus* species, suggests the possible presence of a novel local strain with unique genetic and functional characteristics. Its phylogenetic distance indicates that it may not yet be characterized in global databases, offering opportunities to explore previously unknown beneficial traits. Such uniqueness may include: Production of site-specific secondary metabolites that support microbial adaptation to tropical agroecosystems, Biosynthetic gene clusters (BGCs), responsible for the production of these secondary metabolites, are readily identifiable on bacterial genome sequences. partial deletion of BGCs and frameshift mutations in selected biosynthetic genes are conserved within phylogenetically related isolates bacillus (Stinke, etal. 2021). Specialized symbiotic interactions with local rice cultivars not found in common strains. Potential roles in bioprotection or bioremediation, given that many *Bacillus* species produce antimicrobial compounds and detoxifying enzymes. Some of the isolated *Bacillus* have the ability to produce lipase, protease, and cellulase that potential to be used in biotechnology processes (Sulistiyani et al, 2021)

The presence of rhizobacteria with such functional traits reinforces the concept that local microbial diversity is a valuable biological resource for sustainable agricultural management. The mutualistic relationships established between plants and microbes enhance seedling performance and crop growth while simultaneously restoring soil ecosystem functions, improving input efficiency, and reducing reliance on chemical fertilizers and pesticides.

This study highlights that isolating and characterizing native rhizobacteria from rice agroecosystems—such as those in Gowa Regency, South Sulawesi—contributes to the documentation of Indonesia's microbial biodiversity and lays the foundation for developing locally adapted bioinoculants that align with ecological principles.The ability of *B. cereus* to act not only as a plant growth promoter but also as a mediator of plant–microbe interactions suggests its ecological importance (Du et al., 2023). Certain strains have also demonstrated potential in enhancing phytoremediation, allowing plants to tolerate and grow in contaminated soils (Akhtar et al., 2021).

The discovery of a potentially novel strain (Isolate 2) further highlights the untapped diversity within indigenous microbial populations. This microbial novelty not only enriches our understanding of rhizosphere ecology but also supports the global effort to conserve microbial genetic resources, which are rapidly being threatened by monoculture practices, agrochemical overuse, and land degradation.

Furthermore, integrating native PGPR into organic farming systems enhances nutrient use efficiency, supports soil health regeneration, and builds resilience against climate variability. These functions are increasingly recognized as critical components of climate-smart agriculture and agroecological intensification.

In conclusion, insights gained from 16S rRNA gene amplification and phylogenetic studies reveal the genetic diversity and functional potential of *B. cereus* as a beneficial soil microorganism. Its dual role as a growth-promoting agent and a biocontrol agent offers promising applications in sustainable agriculture. Further research is needed to develop optimized strategies for its use in field conditions and integrated crop management systems.

V. CONCLUSION

This study confirms that soaking duration particularly 4 hours is a significant factor in enhancing the vigor of rice seeds. Although the direct effect of rhizobacterial concentration was statistically variable, treatments involving native isolates showed promising trends in improving senews performance. Molecular characterization revealed that Isolate 1 shares high similarity with *Bacillus cereus* ATCC 14579, while Isolate 2 appears to be a potentially novel strain with distinct phylogenetic positioning. These findings highlight the functional importance of native rhizobacteria as key components of soil microbial biodiversity, offering real potential for biological seed enhancement (biofortification) strategies. The use of local PGPR isolates, especially those with traits such as IAA production and phosphate solubilization, aligns with sustainable agriculture goals by reducing chemical input dependency and enhancing resource efficiency from the seed stage. Furthermore, the integration of such native PGPRs into seed priming and seedling production systems could serve as a scalable and ecologically sound approach to improve early-stage plant establishment in rice cultivation. Given their adaptability to local soil and climate conditions. These local microbes have great potential in implementing sustainable organic farming system, especially increasing biodiversity, therefore this study not only contributes to the functional characterization of native PGPRs but also emphasizes their role in biodiversity conservation, and scalable innovations for resilient and eco-friendly agriculture.

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VII. DISCLOSURE

Conflicts Of Interest: All authors declare no conflict of interest

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