

Tolerance of rhizospheric and endophytic microbes to pesticide residues and their potential for rice growth

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Manuscript received: 21 April 2025. Revision accepted: 17 October 2025.

Abstract. Rangkuti EE, Akhdiya A, Munif A, Siregar IZ, Anwar S. 2025. Tolerance of rhizospheric and endophytic microbes to pesticide residues and their potential for rice growth. *Biodiversitas* 26: 5267-5276. Rhizospheric and endophytic bacteria are recognized for their ability to enhance plant growth by producing hormones and acting as biocontrol agents. Therefore, this study aimed to identify potential rhizosphere and endophytic bacteria tolerant to three pesticide-active substances. Based on the antagonistic activity against *Rhizoctonia solani*, *Bacillus* sp. and *Bacillus siamensis* isolates achieved the highest inhibitory activities of 81.13% and 78.55%, respectively. *Bacillus* sp., *B. siamensis*, *Acinetobacter radioresistens*, and *Providencia vermicola* were able to solubilize phosphate. Production of Indole-3-Acetic Acid (IAA) was confirmed in *Bacillus* sp., *Bacillus toyonensis*, and *Acinetobacter radioresistens*, and all isolates also possessed the ability to fix nitrogen (N). Based on these parameters, *Bacillus* sp. and *B. siamensis* were selected for further analysis. The results showed that *Bacillus* sp. isolates supported rice seed tolerance to herbicides and insecticides in the range of 5-10 ppm and improved growth in the presence of difenoconazole and glyphosate at 10 ppm. Meanwhile, *B. siamensis* was tolerant to fungicides in the 5-10 ppm range. None of the selected isolates showed reduced growth after 24 h of incubation in the tolerance test for pesticide contamination. Observably, *Bacillus* sp. isolate treated with fipronil (5 ppm) was better than the control. It also matched the performance of the control treated with difenoconazole at 5 ppm. These suggested that the selected isolates improved rice growth and tolerance to the active ingredients of the pesticides, contributing to sustainable cultivation practices.

Keywords: Beneficial bacteria, biocontrol agents, pesticides-active substances, plant growth hormone, rice seedlings

INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial nutritional source for approximately half of the global population and plays a central role in worldwide food security (FAO 2022). Its significance lies in the nutritional, farming, economic, and adaptability to environments (Fukagawa and Ziska 2019; Hussain et al. 2020). In many impoverished nations, rice accounts for more than 70% of the daily caloric intake for a large portion of the population (Kumar et al. 2023). Consequently, any disruption in production can have significant effects on the prices and availability, particularly in vulnerable communities (Raihan 2023). To ensure sustainable rice production with maximum yield and minimal input dependency, it is essential to prioritize the mechanization of farming operations in cultivation practices (Cassman and Grassini 2020; Yuan et al. 2021). The main challenge faced by farmers is pests and diseases, specifically rice sheath blight, which is caused by *Rhizoctonia solani*, a soil-borne fungus. Based on observation, the fungus presents a major challenge to rice cultivation in Indonesia, since it can cause 20 to 69% yield loss (Rasool et al. 2025).

To overcome the problem, farmers continue to apply chemical pesticides to control pests and diseases in rice and other cultivated plants. These chemicals effectively manage crop pests, but also pose environmental risks (Nath and Puzari 2022). Previous studies have reported that pesticides can cause soil contamination and air pollution due to direct application and volatilization of active substances, respectively. The use of certain pesticides over a long period can lead to resistance to plant disease pathogens (Qiu et al. 2022), strengthening protective structures and increasing the possibility of re-infection (Thind 2022).

To mitigate farmers reliance on pesticides, the introduction of alternative solutions, such as beneficial bacteria, can offer significant advantages for rice plants and the surrounding ecosystem. Endophytic and rhizosphere microbes offer various benefits, functioning not only as biocontrol agents against pathogens but also as growth promoters (Rana et al. 2020). Microorganisms in both groups produce mucolytic enzymes, siderophores, antibiotics, and volatile compounds that can inhibit pathogen growth through multiple mechanisms (Dimkić et al. 2022; Amoo et al. 2023), and these microbes possess the capability to produce chitinolytic enzymes with a chitin-based structural composition that

can lyse the cell walls of pathogenic fungi (Munif and Asmoro 2021). These microbes can also produce phytohormones and function as Phosphate-Solubilizing Microorganisms (PSM) with a mechanism that involves the secretion of organic acids, such as citric acid, to facilitate the release of phosphate. According to Lu et al. (2025), employing a mechanism that involves the incorporation of Phosphate-Solubilizing Microorganisms (PSM) can decrease the reliance on chemical phosphate fertilizers by over 30%. These beneficial microbes help plants adapt to a range of environmental challenges and simultaneously decrease the reliance on chemical fertilizers as well as limit environmental degradation (Subiramani et al. 2020). Rhizobacteria and endophytes offer a viable alternative to the combined use of biological agents and chemical pesticides.

The present study has several limitations, notably the challenges associated with bacterial cultivation due to difficulties in cell proliferation. Consequently, further research is warranted to develop microbial-based liquid or solid fertilizer products that can be optimally integrated with pesticide dosages to enhance their practical application by farmers. A novel aspect of this study is the identification of the potential of rhizosphere and endophytic bacterial isolates, which exhibited significant plant growth-promoting properties and demonstrated efficacy as biocontrol agents against *R. solani*. These isolates also showed resilience under pesticide stress, and the combination of microbial and pesticide treatments enhanced the growth of rice seedlings. The research gap addressed in this study involves examining the synergistic interaction between pesticides and microbes, which can enhance plant growth without compromising either component. Therefore, the objective of this study was to evaluate the plant growth-promoting and multi-trait biocontrol potential of selected rhizosphere bacteria and endophytes as well as their tolerance and capacity to mitigate the adverse effects of difenoconazole, fipronil, and glyphosate on rice seedling growth.

MATERIALS AND METHODS

This study was conducted between October 2023 and May 2024 at the Nematode Laboratory, Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor, Bogor, Indonesia. The procedures included (i) evaluation of bacterial potential as plant growth promoters, (ii) assessment of microbial effectiveness as biocontrol agents, (iii) examination of microbial growth on pesticide-contaminated agar media, (iv) analysis of rice seed viability in the presence of pesticide active ingredients and microbes, and (v) observation of microbial growth in liquid media contaminated with pesticides.

Source of rhizospheric and endophytic bacterial isolates

Endophytic bacteria obtained from the Plant Protection Department, Institut Pertanian Bogor, consisted of *Bacillus siamensis* (APE35), *Providencia vermicola* (LCA19), *Bacillus toyonensis* (BAT 27), *Acinetobacter resistens* (EAP10), *Bacillus toyonensis* (AC112), and while the endophytic

bacteria from BRIN is *Micrococcus endophyticus* (G053). Rhizosphere microbial isolates from the BRIN collection comprised *Bacillus aryabhatai* (TKF2), *Bacillus* sp. (S12), and *Bacillus* sp. (SP1), presented in Table 1.

Isolation from previous study was done through surface sterilization method. The bacterial isolates preserved from the previous study were recultured on 100% Tryptic Soy Agar (TSA) media, and incubated for 24 to 48 hours at room temperature (25-27°C). Subsequently, the resulting bacterial colonies were subjected to further purification to obtain single isolates for subsequent experimental analysis.

The potential of bacteria as biocontrol agents against the pathogenic fungus *Rhizoctonia solani*

Tests were conducted using the dual culture method based on Wonglom et al. (2019). Each of studied bacterial isolates with suspension concentration of 10^8 CFU/mL, was first grown on Potato Dextrose Agar (PDA) media by zig zag scratching on two sides of the edge of a 9 cm diameter petri dish and incubated for two days at 25°C. Then, a pathogenic isolate of *Rhizoctonia solani* (also collection by Plant Protection Department, Institut Pertanian Bogor) with a diameter of 5 mm was grown on the same petri dish on the center side between the bacterial isolates and incubated for eight days at 25°C. The control was performed by growing *R. solani* without bacterial isolates. Observations were made by measuring the diameter of the inhibited *R. solani*. The percentage inhibition was calculated using the following equation:

$$P = \frac{(PK1 - PK2)}{PK1} \times 100\%$$

Where:

P : Inhibition (%)

PK1 : Colony growth of *Rhizoctonia solani* in control

PK2 : Colony growth of *Rhizoctonia solani* in bacterial isolate treatment

Test of bacterial ability to dissolve phosphate

Bacterial isolates were cultured on Pikovskaya agar medium containing 10 g glucose, 5 g $\text{Ca}_3(\text{PO}_4)_2$, 0.5 g $(\text{NH}_4)_2\text{SO}_4$, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g yeast extract, 0.2 g KCl, 0.2 g NaCl, 0.002 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 g $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, and 1.5% Bacto agar. The assay was incubated for eight days at 25°C with a petri dish diameter of 9 mm. Qualitative observations were made daily on the formation of a clear zone around the bacterial colonies, indicating phosphate dissolving activity or a positive (+) test result. Negative test results (-) were characterized by the absence of clear zones formed around the bacterial colonies (Budi et al. 2023). The positive control was carried out using *Serratia surfactantfaciens* isolates, which have been known to have potential as phosphate solvents (Amaria et al. 2024). The solubilization index was measured for up to 8 days of incubation using the following equation:

$$\text{Phosphate solubilization index} = \frac{\text{total diameters(mm)} - \text{colony diameters (mm)}}{\text{colony diameters (mm)}} \times 100\%$$

Table 1. Isolate code, origin, and the original isolation paper

Isolate code	Species	Origin	References
APE35	<i>Bacillus siamensis</i>	Fern plant (<i>Pteris ensiformis</i> Burm.)	Munif and Asmoro (2021)
BAT27	<i>Bacillus toyonensis</i>	Mangrove plant (<i>Avicennia</i> sp.)	Oktafiyanto et al. (2017)
AC112	<i>Bacillus toyonensis</i>	Pinang (<i>Areca catechu</i> L.)	Anggita et al. (2020)
LCA19	<i>Providencia vermicola</i>	Tembelekan (<i>Lantana camara</i> L.)	Zulaiha et al. (2022)
EAP10	<i>Acinetobacter radioresistens</i>	Porang (<i>Amorphophallus muelleri</i> Blume)	Maghfiroh et al. (2022)
G053	<i>Micrococcus endophyticus</i>	Potatoes plant (<i>Solanum tuberosum</i> L.)	Akhdiya et al. (2014)
SP1	<i>Bacillus</i> sp.	Rhizosphere plant	Unpublished
S12	<i>Bacillus</i> sp.	Rhizosphere of potatoes (<i>Solanum tuberosum</i> L.)	Unpublished
TKF2	<i>Bacillus aryabhatai</i>	Rhizosphere of black-eyed pea (<i>Vigna unguiculata</i> (L.) Walp.)	Satwika et al. (2017)

N fixation test

Tests were conducted using semi-solid Jensen Agar medium containing Bromothymol Blue (BTB) (Sulistiyan and Meliah 2017). The media composition consisted of 0.05 g FeSO₄·7H₂O, 4 g KOH, 5 g malic acid, 0.01 g MgSO₄·7H₂O, and 0.01 g MnSO₄·H₂O, 0.5 g K₂HPO₄, 0.002 g NaCl, 0.01 g CaCl₂, 0.002 g Na₂MO₄·2H₂O, 1.75 g Bacto agar, 2 mL bromthymol blue 0.5% and 1 L distilled water. Bacterial isolates were grown in sterile Jensen's medium in test tubes. The cells were incubated for seven days at 25°C. Observations were made daily for pellicles formed on the surface of the medium, indicating nitrogen-fixing activity or positive results. Pellicles formed were categorized into three groups: thin pellicles (+), medium pellicles (++) , and thick pellicles (+++). Bacterial isolates that did not form pellicles showed negative results (-). A positive control was conducted using bacterial isolates known to have potential as nitrogen-fixers.

Determination of indole-3-acetic acid (IAA) production

The test was carried out by growing bacterial isolates into 5 mL of sterile Tryptic Soy Broth (TSB) media that had been supplemented with 0.001 g/100 mL L-tryptophan based on Athfin et al. (2023). The culture was incubated for two days at 25°C using a rotary shaker. The suspension was centrifuged at 4000 rpm for 15 min using a Joanlab MC-7Pro mini centrifuge. One milliliter of the supernatant was mixed with 2 mL of Salkowski reagent (1 mL of 0.5 FeCl₃ + 50 mL of 50% HClO₄). The test was incubated in a dark room for one hour. Positive test results of IAA production activity qualitatively characterized by the presence of pink color formed in the supernatant solution (Budi et al. 2023). The pink color formed was categorized into three groups: pink was not clear (+), pink was clearly formed (++) , and pink was clearly formed like the positive control (+++). A pink color that is not formed or similar to the negative control indicates a negative test result (-). Positive control was done by using bacterial isolates that have been known to produce IAA, and negative control was done by not giving supernatant.

Bacterial growth test on media contaminated with pesticide-active ingredients

Bacterial isolates capable of degrading or growth tolerance for pesticide-active ingredients were first cultured on NA media contaminated with the active ingredients of

difenoconazole, fipronil, and glyphosate pesticides at concentrations of 5, 7, and 10 ppm, respectively. The procedure followed the method outlined by Akhdiya et al. (2020), with modifications. The TSA media contamination method with agar wells was carried out using a cork borer diameter of 8 mm. Subsequently, a pesticide solution of a specified concentration was introduced into the media hole using a micropipette, with a volume of 0.1 mL applied to the media previously inoculated with bacterial isolates. The samples were then incubated for 3-7 days at room temperature (25°C). Observations were conducted daily to assess bacterial growth inhibition by specific active pesticide ingredients, as indicated by the presence of a clear zone surrounding bacterial colonies.

Rice seed inter-paper test with added pesticide active ingredient and bacterial suspension

The inter-paper method was conducted as outlined by de Mattos Sorana et al. (2019). In this procedure, bacteria were cultured in TSB media and incubated for 48 hours. Inpari 32 rice seeds underwent surface sterilization in a 1% NaOCl solution for 1 minute, followed by rinsing with sterile water. Subsequently, the seeds were immersed in a bacterial suspension with a concentration of 10⁸ CFU/mL, prepared from the nine isolates, for 24 hours at room temperature (25°C). The filter paper was treated with pesticide solutions containing three active ingredients at concentrations of 5, 7, and 10 ppm in 500 mL of sterile water until the final observation. Each treatment was replicated three times, with each replicate comprising 30 seeds. The experimental setup was incubated in a well-lit room at 25°C for one week. The variables observed included radicle and plumule lengths in both the treatment (bacteria + pesticide + seeds) and control (bacteria + seeds) groups, measured using a ruler. Data analysis was performed using SPSS version 24 to assess the effect of each treatment. Further analysis was conducted using Tukey's test at a 5% significance level.

Bacterial growth curve measurements on liquid media contaminated with pesticides

Based on previous screening results, three selected bacterial isolates (APE35, S12, and TKF2) were grown in TSB broth medium containing pesticide contaminants and incubated at 25-37°C for 24 h until the exponential growth phase was reached. At this point, the bacterial isolates were

re-cultured and diluted to a concentration of 10^8 CFU/mL. Following the adsorption period, the culture was centrifuged at 10,000 rpm for 10 min using a Joanlab MC-7Pro mini centrifuge to collect the bacterial cells as a pellet. The supernatant was carefully discarded, and the bacterial pellet was resuspended in fresh TSB broth to continue the experiment. Samples (100 μ L) were observed every 2 h by measuring the Optical Density (OD) at $\lambda = 640$ nm using spectrophotometer (Ye et al. 2023).

Data analysis

The data obtained were analyzed descriptively using Analysis of Variance (ANOVA) statistical test with a completely randomized design. In cases where significant differences were observed, further analysis was conducted using Tukey Test at a 5% error level.

RESULTS AND DISCUSSION

The potential of bacteria as biocontrol agents against the pathogenic fungus *Rhizoctonia solani*

The nine bacterial isolates tested showed inhibition in the growth of the pathogenic fungus *R. solani*, at levels ranging from 13.59% to 81.13%. Inhibition above 50% was obtained for LCA19 (78.47%), AC112 (78.03%), BAT27 (76.52%), TKF2 (81.13%), and APE 35 (78.55%), as detailed in Table 2. These isolates significantly inhibited the growth of *R. solani* colonies compared with the others. The results showed that the mycelia of the test fungi were lysed and could not develop in the zone of inhibition, as presented in Figure 1.

Test of phosphate solubilizing ability of bacteria

Among the tested isolates, S12 and TKF2 showed phosphate-solubilizing activity. Isolate TKF 2 featured more intensive colony growth, but the dissolution index was

greater in S12. It was important to acknowledge that no activity was observed in G053 and SP1 showed no phosphate-solubilizing activity. In previous studies, isolates APE35 (Munif and Asmoro 2021), EAP10 (Maghfiroh et al. 2022), and LCA19 (Zulaiha et al. 2022) had phosphate-solubilizing activity, while BAT27 (Zulaiha et al. 2022) and AC112 (Anggita et al. 2020) were inactive. During the experimental procedures, *Serratia surfactantfaciens* was used as a positive control. The clear zone around bacterial colonies grown on Pikovskaya medium signified the ability of bacteria to dissolve phosphate (Pi) (Figure 2).

N fixation test

All tested isolates were confirmed positive for N-fixing ability, as characterized by bacterial growth on NFB media and the formation of ring-like pellicles below the surface. Isolate SP1 showed nitrogen-fixing activity, marked by a colour change in the media from blue to clear, as detailed in Figure 3. This was different from the other isolates and the positive control, where the color remained blue.

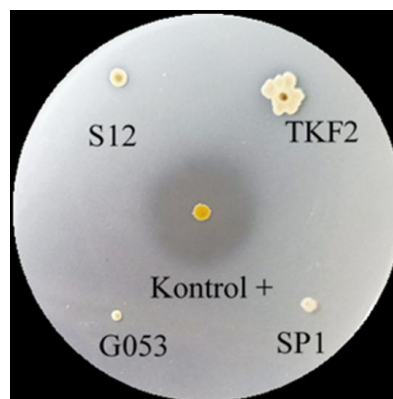


Figure 2. Phosphate solubilization test results of rhizospheric and endophytic bacteria with control + (*Serratia surfactantfaciens*)

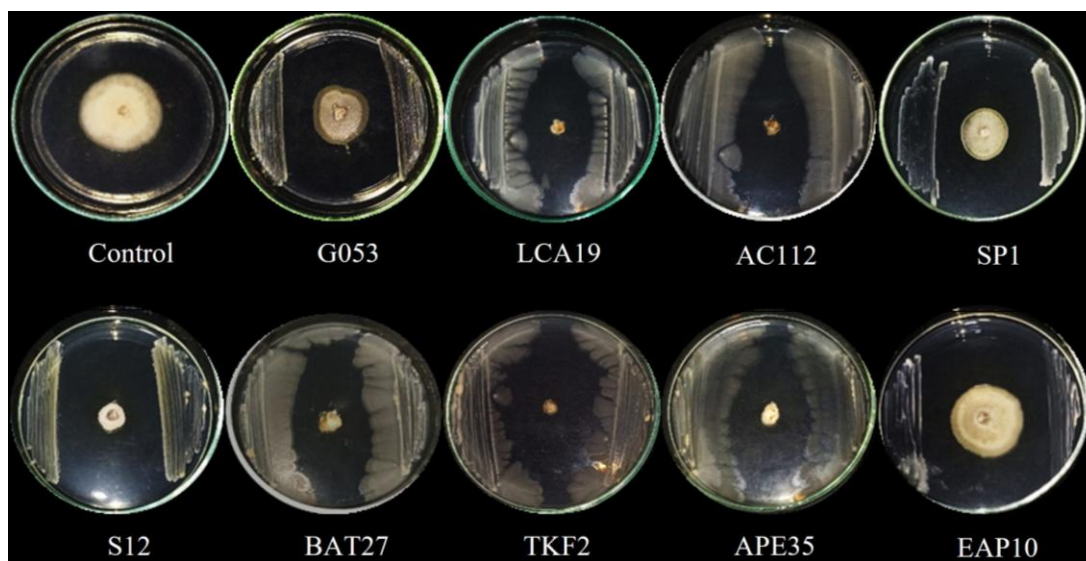


Figure 1. Test results of the effectiveness of bacterial isolates as biocontrols for the growth of the fungal pathogen *Rhizoctonia solani*, causing sheath blight disease

Determination of IAA production

Isolate G053, APE35, S12, and TKF2 had IAA-producing activity, signified by a color change in the media from yellow to pink, while the other showed no visible color change. Previously tested BAT27 (Oktafiyanto et al. 2017) and EAP10 (Maghfiroh et al. 2022) were also reported to produce IAA, as presented in Figure 4.

Bacterial growth test on media contaminated with pesticide-active ingredients

Based on bacterial growth tests on TSA medium containing the three active ingredients, nine isolates were able to grow and survive under pesticide stress. There were no signs of growth inhibition or cell death, and no zone of inhibition was observed in the bacterial colonies. The diameters of the bacterial colonies could not be measured because bacteria were applied by scratching the medium. The concentrations that have been determined in this study have passed preliminary tests with experiments of various concentrations, so that the best concentration of bacterial growth on TSA media can be obtained, as detailed in Table 3.

Rice seed inter-paper test with the addition of pesticide active ingredient and bacterial suspension

The inter-paper test was conducted to assess the effect of pesticide treatments on rice seedling growth, using three

active ingredients at concentrations of 5, 7, and 10 ppm. These concentrations were selected based on prior screening to ensure bacterial viability. ANOVA showed significant differences in radicle length among the treatment groups, supported by a p-value below 0.05. This signified that pesticide exposure significantly influenced radicle development. Post-hoc analysis confirmed shorter radicles in all pesticide-treated groups compared to the untreated control. The mean radicle lengths in all pesticide treatments were consistently lower than those in the control group (Tables 4 and 5).

Measurement of bacterial growth curves in liquid media contaminated with pesticides

The level of tolerance to difenoconazole and fipronil was assessed by monitoring 24 hrs. Isolate APE35 showed that exposure to these compounds at a concentration of 5 ppm did not affect microbial growth decline. However, bacterial growth increased when the media was contaminated with fipronil at 5 ppm, matching the control at 9 hours. For isolates with code S12, a higher bacterial growth was observed during contamination with 10 ppm difenoconazole compared to the control at 12 h, as detailed in. In isolate TKF2, bacterial growth when contaminated by fipronil at a concentration of 5 ppm in 12 h was in line with the control, all isolate activity as presented in Figure 5.

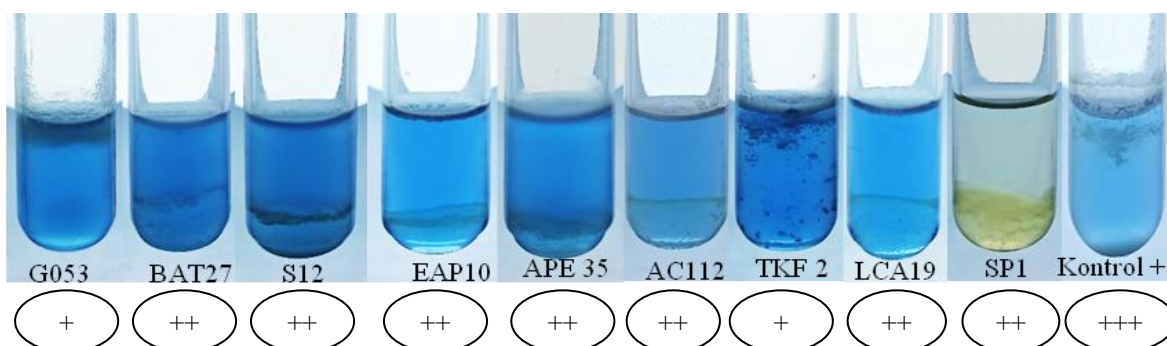


Figure 3. Nitrogen-fixing activity test results of rhizospheric and endophytic bacteria. The pellicles formed were categorized into three groups: thin pellicles (+), medium pellicles (++) , and thick pellicles (+++). Bacterial isolates that did not form pellicles showed negative test results (-)

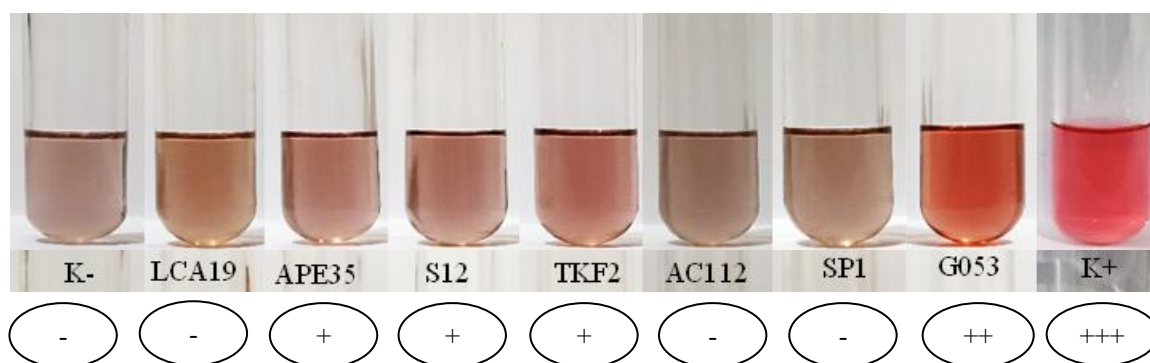


Figure 4. IAA production activity test results of rhizospheric and endophytic bacteria, the pink color formed was categorized into three groups: pink was not clear (+), pink was clearly formed (++) , and pink was clearly formed like the positive control (+++). A pink color that is not formed or similar to the negative control indicates a negative test result (-)

Table 2. Test results of biocontrol agents and their potential as plant growth promoters

Isolate code	Solubilization phosphate	Nitrogen fixation		IAA production		Inhibited growth of <i>Rhizoctonia solani</i> (%)
		Pellicle formed	Medium color changes	Medium color changes		
BAT27	-	++	+			13.6d±6.74
APE35	+	++	+			20.9cd±3.69
AC112	-	++	-			78.5a±0.35
EAP10	+	++	+			78a±1.24
G053	-	+	++			29.5c±6.39
LCA19	+	++	-			62.8b±2.55
SP1	-	++	-			76.5a ±4.38
S12	+	++	+			81.1a±1.55
TKF2	+	+	+			78.5a±2.29

Note: The numbers in each treatment followed by the same letter are not significantly different based on Tukey's test at the 5% level.
 Note: Solubilization phosphate test: + (average±s.tdv): Positive (measurement clear zones), +: Positive (not measurement clear zones in previous studies), -: Negative. Pellicles categories of fixation N test: +: Thin, ++: Medium, color changes categories of IAA production test: +: Pink was not clear, ++: Pink was clearly formed, -: No color changes

Table 3. Bacterial growth on pesticides-contaminated agar media

Isolate code	Grown on media (+), did not grow on media (-)								
	TSA (Tryptic Soy Agar) media								
	Difenoconazole (ppm)			Glyphosate (ppm)			Fipronil (ppm)		
	5	7	10	5	7	10	5	7	10
BAT27	+	+	+	+	+	+	+	+	+
APE35	+	+	+	+	+	+	+	+	+
AC112	+	+	+	+	+	+	+	+	+
EAP10	+	+	+	+	+	+	+	+	+
G053	+	+	+	+	+	+	+	+	+
LCA19	+	+	+	+	+	+	+	+	+
SP1	+	+	+	+	+	+	+	+	+
S12	+	+	+	+	+	+	+	+	+
TKF2	+	+	+	+	+	+	+	+	+

Table 4. Radicle length based on the effect of pesticides and bacterial treatment

Isolate code	Control (seed + bacteria)	Radicle length (cm)								
		Difenoconazole (ppm)			Glyphosate (ppm)			Fipronil (ppm)		
		5	7	10	5	7	10	5	7	10
BAT27	14.8 de	10.1 b	10.26 b	10.2 b	9.77 ab	10.3 b	10.9 bc	14.6 de	13.3 bcd	13.7 d
APE35	19.7 h	13.1 bcd	13.5 bcd	11.2 bc	12.6 bcd	12.8 bcd	12.4 bcd	13.7 d	11.5 bc	11.6 bc
AC112	14.7 de	11.4 bc	10.3 b	11.8 bc	13.4 bcd	11.7 bc	11.7 bc	11.1 bc	11 bc	11.1 bc
EAP10	19 h	11.4 bc	11 bc	10.4 bc	14.9 de	11.7 bc	11.8 bc	11 bc	11 bc	11.1 bc
G053	14.7 de	8.27 ab	10.3 b	10.3 b	14.5 d	12.4 bcd	12.7 bcd	11.5 bc	11.5 bc	11.8 bc
LCA19	15.6 f	10.2 b	10.5 bc	10.3 b	14.1 d	11.7 bc	11.6 bc	11.5 bc	11.5 bc	11.4 bc
SP1	19.2 h	7.86 a	11.1 bc	10.7 bc	16.2 e	15.2 de	13.8 d	14.4 de	12.4 bcd	12.5 bcd
S12	13.1 bcd	9.76 ab	9.60 ab	9.57 ab	13.7 cd	13 bcd	12.7 bcd	12.4 bcd	11.6 bc	11.6 bc
TKF2	16.7 g	10.6 bc	11.3 bc	11.5 bc	14.7 de	13.2 bcd	14.6 de	14 d	11.6 bc	11.7 bc

Note: The numbers in each treatment followed by the same letter indicate no significant difference based on Tukey's test at the 5% level

Table 5. Analysis of plumule length based on the effect of pesticide and bacterial treatments

Isolate code	Control (seed + bacteria)	Plumule length (cm)								
		Difenoconazole (ppm)			Glyphosate (ppm)			Fipronil (ppm)		
		5	7	10	5	7	10	5	7	10
BAT27	7.60 a	11 b	11 b	11.2 b	11.3 b	11.5 b	11.4 b	10.4 ab	10.2 ab	12 b
APE35	12.8 b	8.53 a	10.5 ab	8.68 ab	10.5 ab	10.5 ab	10.7 ab	14.6 c	14.4 c	14 c
AC112	12.2 b	9.6 ab	11 b	10.49 ab	10.7 ab	10.6 ab	10.4 ab	11.5 b	11.7 b	11.4 b
EAP10	11.7 b	10.5 ab	10.2 ab	10.2 ab	10.1 ab	11.5 b	10.3 ab	10.9 ab	10.2 ab	12.4 b
G053	12.9 bc	10.9 ab	11.6 b	13.1 bc	10.8 ab	10.9 ab	12 b	11.5 b	10.8 ab	11.8 b
LCA19	12 b	9.4 ab	9.67 ab	9.21 ab	11.8 b	10.6 ab	10.6 ab	10.8 ab	10.6 ab	13.1 bc
SP1	14.1 c	11.6 b	11.6 b	9.54 ab	17.3 d	13.9 bc	11.4 b	13.4 bc	12.5 b	11.4 b
S12	11.2 bc	11 b	10.9 ab	12.1 b	8.31 a	9.2 ab	10.4 ab	11.2 b	12.1 b	11.4 b
TKF2	13.8 bc	12.3 b	12 b	10.7 ab	14.7 cd	12.2 b	10.9 ab	13 bc	12.4 b	10.9 b

Note: The numbers in each treatment followed by the same letter indicate no significant difference based on Tukey's test at the 5% level

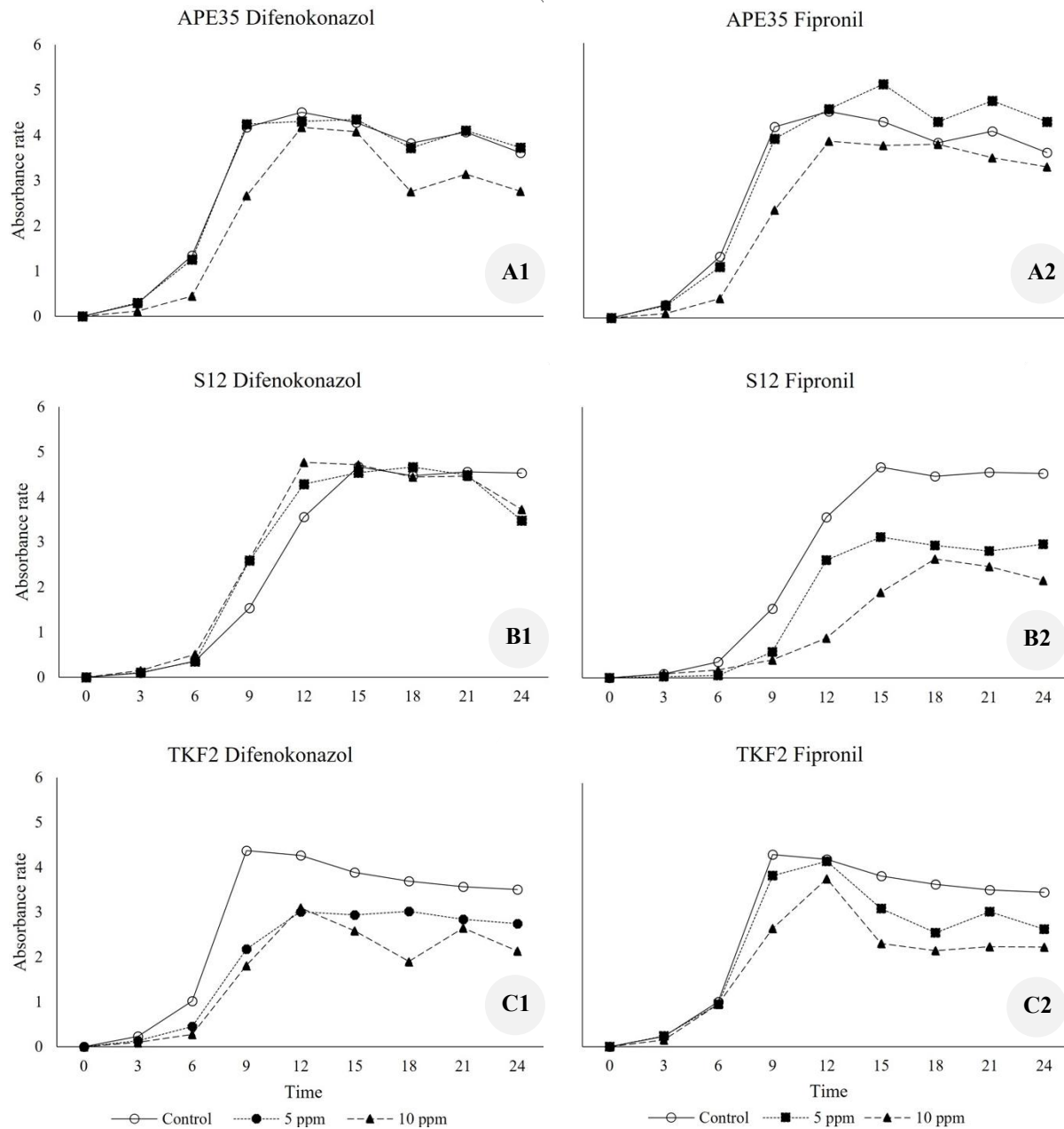


Figure 5. Growth curve of *B. siamensis* (APE35) with the addition of active ingredients difenoconazole (A1) and fipronil (A2), *Bacillus* spp. (S12) with the addition of active ingredients difenoconazole (B1) and fipronil (B2), *Bacillus* spp. (TKF2) with the addition of difenoconazole (C1) and fipronil (C2). The most significant growth was observed for APE35 difenoconazole, APE35 fipronil, and S12 difenoconazole compared with control

Discussion

The endophytic and rhizosphere bacteria tested in this study have potential as biocontrol agents. Our results showed that *B. aryabhattai* (TKF2) (81.13%) and *B. siamensis* (APE35) (78.55%) showed the highest inhibition of *R. solani*, consistent with the studies conducted by Sharma et al. (2021) and Hussain et al. (2025) regarding the role of *Bacillus* species, namely *B. subtilis* strain Sh-17 and *B. siamensis* strain NKIT9, which can produce lipopeptides such as surfactin and bacillomycin compounds that can inhibit the growth activity of *R. solani*. *Bacillus*

velezensis HC6 has also been reported to produce other lipopeptide compounds such as iturin, fengycin, and surfactin, three types of LPs that also have antimicrobial activity against pathogenic bacteria and fungi (Liu et al. 2020). Physical damage to pathogenic fungi can be observed from various inhibitory mechanisms, such as fungal cell damage, including mycelial lysis, which is caused by hydrolytic enzymes released by antagonistic bacteria. According to Riseh et al. (2024), these enzymes not only facilitate pathogen cell wall degradation but also promote the attachment, proliferation, and parasitic activity

of biocontrol agents. Bhunia and Meshram (2022) reported that various microorganisms, including *Pseudomonas* and *Bacillus*, produce enzymes capable of breaking down the cell walls of pathogens. Marianah et al. (2025) reported that endophytic actinomycetes in Liliaceae plants could suppress the growth of *F. oxysporum* f. sp. cepae, with 11 out of 24 isolates achieving inhibition rates above 50% and a maximum of 63.49%.

In this study, several bacteria have been reported to be able to dissolve phosphate semi-quantitatively, namely APE35, EAP10, and LCA19 (reported in the previous study), but not measured directly, while isolates S12 and TKF2, which are in the *Bacillus* group, have been widely reported to be able to produce gluconic acid and lactic acid in their phosphate dissolution mechanism. In line with what was reported by Zhao et al. (2025) in their study, *B. velezensis* lacks genes for phytate mineralization and inorganic phosphorus dissolution, which may prevent it from utilizing additional phosphorus sources. Further studies analyzing the mechanism of bacteria in dissolving phosphate are very important as a future research direction. Phosphate-solubilizing bacteria have also been reported by Chi et al. (2021) that can affect the increase in production and degradation of pesticides, namely an indigenous effective Organophosphorus Pesticides (OPP)-degrading bacterial strain has been isolated from soil samples of a contaminated tea farming site in Northern Vietnam with chlorpyrifos degradability and plant growth-promoting characteristics. The bacterial isolate was determined to belong to the genus *Ensifer* (syn. *Sinorhizobium*); exhibiting remarkable chlorpyrifos degradability in liquid culture and test soil with 94.75% and 76.27% of substrate removal after 14 days of inoculation, respectively. Besides, *Ensifer* sp. CNN3 appeared as a promising growth-promoting bacteria with IAA excretion and phosphate-solubilizing properties. The results open a prospect of applying the dual-effective bacterial strain in agriculture practices, either to reduce the use of chemical fertilizer or to remediate OPP contaminated soils.

All isolates were able to fix N, but there were differences in the category of pellicles formed in the results. From the nine isolates tested, two formed thin pellicles. The results of this study are similar to those of Arsita et al. (2020) in that bacteria that form pellicles are accompanied by a change in media color to blue, and some become clear. The blue discoloration of BTB contained in Jensen's media is due to an increase in media pH caused by bacterial nitrogenase activity.

Nine bacterial isolates were obtained that were able to produce IAA, three of which namely the bacterial species *B. toyonensis* (AC112), *P. vermicola* (LCA19), and *Bacillus* sp. (TKF2) were unable to produce IAA. Although there are groups of bacteria that are able to produce IAA and those that are not able to produce the same species, their ability as PGPB can be different. This can be caused by the influence of bacterial genetics and the bacterial environment Amin et al. (2021). Bacterial communities are ubiquitous and are found in natural ecosystems, such as soil, and within living organisms, such as the human microbiome. The dynamics of these communities in diverse

environments depend on factors such as the spatial features of the microbial niche, biochemical kinetics, and inorganic processes. Endophytic and rhizospheric bacteria enhance plant development through symbiotic relationships. Typically, the introduction of rhizobacteria can boost plant growth through various mechanisms, including the production of siderophores, IAA, ACC deaminase activity, nitrogen fixation, and other growth-enhancing traits (Zaveri and Dasgupta 2023). The genus *Bacillus* is a group of rhizobacteria commonly found in plant roots (Liu et al. 2022a; Shi et al. 2022). In the results of Nunes et al. (2022), species such as *B. licheniformis*, *B. subtilis*, and *B. altitudinis* (Elfira et al. 2020; Zhang et al. 2021), and their combination can improve tomato growth and act as an IAA producer.

All bacterial isolates demonstrated the ability to proliferate in media stressed with pesticides at concentrations of 5, 7, and 10 ppm in the stock media solution. The production of phytohormones alters the architecture of plant roots, thereby enhancing the uptake and retention of water and nutrients. Agunbiade et al. (2024) highlighted the potential of PGPR to alleviate abiotic environmental stressors, affirming the diverse metabolic and physiological changes induced in host plants. In addition to in vitro growth assessments, greenhouse experiments revealed that the growth characteristics of rhizobacterial strains A1-2 and C7_8 significantly improved the seed germination index compared to that of the control.

In the context of the extensive application of bacteria at the field scale, it is essential to preliminarily assess the growth of Inpari32 rice seeds under conditions of bacterial addition and pesticide stress. Generally, rice growth is significantly enhanced by the incorporation of bacteria. However, it is noteworthy that certain bacteria can maintain their functionality even under stress conditions, suggesting that these bacteria may utilize pesticide-active compounds as a carbon source in their metabolic processes. Yeon et al. (2022) reported that bacteria isolated from pesticide-contaminated soil, specifically *B. aryabhatai*, could tolerate pesticide exposure and promote plant growth.

Following the evaluation of bacterial isolates as biocontrol agents and their potential to enhance the growth of Inpari32 rice varieties, three promising isolates were identified for further testing in liquid media. The results indicated that the isolates APE35 difenoconazole, APE35 fipronil, and S12 difenoconazole exhibited significantly greater growth than the control group. Key abiotic factors include temperature, salinity, chemicals, drought, and heavy metals. These abiotic stressors can limit crop yields, cause difficulties for farming in certain areas, and generally lead to unfavorable conditions. The extent of biotic stress is significantly affected by abiotic stress. Collectively, these stressors significantly affect the physiology, metabolic composition, and gene expression of plants. Abiotic stressors are detrimental environmental factors that alter the physicochemical properties of soil and reduce the functional diversity of microorganisms, leading to significant decreases in crop yield (Goswami and Deka 2020). The complex effects of climate change on abiotic stress outcomes present an immediate threat to the sustainability and productivity of agricultural systems. The decline in agricultural production

is mainly attributed to several abiotic factors, including drought, salinity, heavy metals, flooding, and extreme temperatures. To ensure environmental sustainability, safeguard ecosystems, and enhance agricultural yields, it is crucial to remove heavy metals in an eco-friendly manner. As a result, PGPR facilitates plant growth and alleviate the harmful impacts of heavy metals on plants (Sachdev et al. 2021). According to Liu et al. (2022b), *Bacillus* groups such as *Bacillus subtilis* and *Bacillus* spp. are the most widely reported PGPR groups that can withstand abiotic stress, including pesticide chemicals and heavy metals in rice. Several studies have shown that some bacteria tolerates and degrade various types of pesticides from various groups. According to a study by Monica et al. (2016), *Acinetobacter* sp. isolated from agricultural land exposed to pesticides in Ecuador can degrade mankozeb, propined, sihalofop butyl, deltamethrin, difenoconazole, lamda sihalotrin, chlorpyronyl, acetode dodemorf, prokloraz, and teboconazole by 90-98% for 60 days. Jabeen et al. (2017) also reported that *Acinetobacter* sp. isolated from the rhizosphere of *Pennisetum pedicellatum* plants degraded the insecticide cipermethrin. In addition, Kafilzadeh et al. (2015) stated that *Acinetobacter* degraded endosulfan insecticides.

In conclusion, in a study examining the use of bacteria as biocontrol agents, growth promoters, and pesticide-tolerant organisms, all bacterial isolates demonstrated efficacy as biocontrol agents against *R. solani*. They exhibit the ability to grow on agar media under pesticide stress and fix nitrogen for utilization by rice plants. Notably, five isolates were capable of solubilizing phosphate in the soil, facilitating direct absorption by plants, whereas six isolates produced Indole-3-Acetic Acid (IAA), which is associated with the stimulation of plant hormones. Certain bacteria, when combined with pesticide active ingredients, enhanced the growth of the radicle and plumule in Inpari32 rice varieties. From the screening results, the three most effective isolates were identified as APE35, S12, and TKF2, which exhibited superior growth performance when combined with the active pesticide ingredients. Consequently, the diversity of these bacterial species offers an alternative to chemical fertilizers and demonstrates resistance to pesticides, thereby supporting sustainable rice cultivation through integrated pest- and disease-management practices.

ACKNOWLEDGEMENTS

The authors are grateful to Ministry of Research, Technology and Higher Education, Republic of Indonesia, for funding this study through a BPPDN scholarship with contract number B/276/D3.2/KD.02.00/2019.

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