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HALOTOLERANT ENDOPHYTIC BACTERIA ISOLATED FROM *ACROSTICHUM AUREUM* L. AS POTENTIAL BIOFERTILIZERS FOR RICE GROWTH PROMOTION

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SUMMARY

Rice cultivation constraints on peat soils often result from low nutrient availability, highlighting the need for sustainable biofertilizer alternatives. *Acrostichum aureum* L., a naturally growing fern in coastal environments, could harbor beneficial endophytic bacteria with plant growth-promoting properties. This study aimed to isolate and characterize endophytic bacteria from *A. aureum* L. and evaluate their potential as biofertilizers for rice cultivation on peat soil. Seventeen endophytic bacterial isolates, screened for plant growth-promoting traits, emerged with three promising isolates selected for further evaluation. Growing of rice seedlings in peat soil used a completely randomized design for 14 days. Inoculated seedlings generally exhibited higher mean values for germination and growth parameters than the control. The isolate A.T 4 (*Acinetobacter* sp.) achieved the highest germination percentage (80%), plant height (25.78 cm), and root length (7.48 cm). The topmost mean leaf number (3.00) and dry weight (24.36 mg) recording appeared in seedlings treated with the isolate B.A 3 (*Bacillus* sp.). Overall, the selected isolates improved rice seedling performance and demonstrated potential as biofertilizer candidates for peat soil cultivation. Further greenhouse and field studies are essential to confirm their effectiveness.

Keywords: Endophytic bacteria, *A. aureum* L., rice, biofertilizer, halotolerant, salinity stress, sustainable agriculture

Key findings: Endophytic bacteria procured from the sea fern (*A. aureum* L.) revealed the robust plant growth-promoting activities and tolerance to salinity and acidic soils, which highlighted its potential as promising biofertilizer for sustainable rice cultivation on marginal lands.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops worldwide and plays a crucial role in food security, particularly in Asian countries. However, rice productivity frequently faces constraints from poor soil fertility, especially in peatland ecosystems characteristic of high acidity, low nutrient availability, and poor nutrient retention. These limitations often reduce plant growth and yield while increasing dependence on chemical fertilizers. The long-term use of excessive mineral fertilizers can further degrade soil quality and adversely affect beneficial soil microbial communities. Therefore, environmentally sustainable approaches are necessary to improve nutrient availability and support rice production in peatland agricultural systems. Beneficial microorganisms, particularly plant growth-promoting bacteria (PGPB), have attracted considerable attention because of their ability to enhance nutrient availability, improve soil quality, and stimulate plant growth through various biological mechanisms (Danapriatna *et al.*, 2023; Godínez *et al.*, 2023).

Endophytic bacteria are microorganisms that colonize internal plant tissues without causing disease and establish beneficial associations with their host plants. These bacteria contribute to plant growth through multiple mechanisms, including phytohormone production, biological nitrogen fixation, phosphate and potassium solubilization, and improved tolerance to environmental stresses (Pala *et al.*, 2022; Sharma *et al.*, 2023). Several studies have demonstrated the potential of endophytic bacteria as biofertilizers for agricultural crops. For example, endophytic bacteria isolated from rice and other plant species have had reports to produce indole-3-acetic acid (IAA), solubilize phosphate, and promote plant growth under greenhouse conditions (Ningsih *et al.*, 2022; Linda *et al.*, 2024a). These characteristics make endophytic bacteria promising candidates for sustainable agricultural applications.

Acrostichum aureum L. is a halophytic fern commonly found in coastal and mangrove ecosystems. Given that this species naturally survives under environmentally challenging conditions, it could harbor endophytic microorganisms with unique physiological and metabolic capabilities. Previous studies on *A. aureum* L.-associated endophytes have primarily focused on their bioactive and enzymatic properties. Reports on endophytic bacteria isolated from *A. aureum* L. detailed them to possess antibacterial and antidiabetic activities (Linda *et al.*, 2022 and 2023), while other studies demonstrated their capacity to produce hydrolytic enzymes, such as cellulase, amylase, and lipase (Linda *et al.*, 2024b). These enzymatic activities showed an association with organic matter decomposition and nutrient cycling, suggesting that *A. aureum* L.-associated endophytes may also possess traits beneficial for plant growth promotion.

The consequent study focused on halotolerant endophytic bacteria because microorganisms adapted to stressful environments often possess physiological characteristics that enable survival and functional activity under diverse environmental constraints. Although peat soils are not saline, they represent a challenging habitat due to their low nutrient status and acidic conditions. Several reports stated halotolerant bacteria maintain metabolic activity under osmotic stress and frequently exhibit plant growth-promoting traits, including nutrient solubilization, nitrogen fixation, and phytohormone production (Zahir *et al.*, 2019; Kaur and Kanrnwal, 2023). Therefore, halotolerant endophytic bacteria from *A. aureum* L. may represent a valuable source of beneficial microorganisms for biofertilizer development in peatland agriculture.

Despite the ecological importance of *A. aureum* L., studies investigating the agricultural potential of its endophytic bacteria remain limited. Previous research largely gave emphasis on their enzymatic activities and bioactive compounds (Linda *et al.*, 2022, 2023, and 2024b), whereas information regarding

their plant growth-promoting characteristics and application as biofertilizers is still scarce. Furthermore, the potential of halotolerant endophytic bacteria isolated from *A. aureum* L. to enhance rice growth in peat soils has inadequate investigations. Therefore, this study aimed to isolate and characterize halotolerant endophytic bacteria from *A. aureum* L. and evaluate their plant growth-promoting traits and potential as biofertilizers for rice cultivation on peat soil.

MATERIALS AND METHODS

Preparation of media and growth substrates

Nutrient agar (NA) and nutrient broth (NB) preparations proceeded by dissolving 28 and 13 g of commercial NA and NB powders in one liter of distilled water, respectively. Nitrogen-free bromothymol blue (NFB) medium, Pikovskaya medium, and Alexandrov medium entailed preparations following standard protocols. Physiological saline (0.85% NaCl) preparation continued by dissolving 0.85 g NaCl in 100 mL distilled water before sterilization via autoclaving. The use of peat soil (pH 5–6) served as a growth substrate, sterilized by tyndallization at 100 °C for one hour over three consecutive days and proceeded to be placed in sterilized pot trays for rice cultivation.

Revitalization of endophytic bacterial isolates

The obtained endophytic bacteria totaled 17 from *A. aureum* L., consisting of nine root isolates and eight stem isolates collected from Meranti Island, Riau, Indonesia. The isolates previously remained on nutrient agar slants at 4 °C. Before experimentation, each isolate incurred revitalization by streaking onto fresh nutrient agar plates and incubation at room temperature for 24–48 h. A single colony reached subsequent transfer into the nutrient broth before incubation for 24 h to obtain actively growing cultures for further analyses.

Qualitative tests for growth-promoting traits

Nitrogen fixation: One mL of NB-grown culture sustained inoculation into 9 mL of NFB semi-solid medium and then received incubation at room temperature for three days. A color change from yellow to blue indicated nitrogen fixation (Ekowati *et al.*, 2021).

Phosphate solubilization: Isolates underwent spot inoculation on Pikovskaya agar and entailed incubation for seven days. The formation of a clear halo surrounding the colony indicated phosphate solubilization. The phosphate solubilization index (PSI), as calculated, used the following equation (Mohamed *et al.*, 2018):

$$\text{PSI} = (\text{Colony diameter} + \text{Halo zone diameter}) / \text{Colony diameter}$$

Potassium solubilization: Isolates inoculated on Alexandrov agar reached incubation for seven days. Potassium solubilization indication exhibited the formation of a clear halo around the colony. The potassium solubilization index (KSI) calculation was as follows (Sukmadewi *et al.*, 2017):

$$\text{KSI} = (\text{Colony diameter} + \text{Halo zone diameter}) / \text{Colony diameter}$$

All phosphate and potassium solubilization assays ensued in triplicate ($n = 3$).

Salinity tolerance and pathogenicity tests

Endophytic isolate inoculation into NB containing NaCl concentrations of 8% continued incubation for 24 h (Kaur and Kanrnwal, 2023). The concentration of 8% NaCl was the selected stringent screening level for salt tolerance. Bacteria capable of growth at this salinity tended generally to be halotolerant because they can survive elevated salt concentrations without requiring salt for growth. Visible turbidity proved evidence of bacterial growth and tolerance to salinity.

Isolates underwent testing for pathogenicity on two-month-old tobacco (*Nicotiana tabacum*) leaves. The use of tobacco (*Nicotiana tabacum*) leaves was common in hypersensitivity assays because they rapidly express visible necrotic lesions in response to pathogenic bacteria possessing virulence determinants. The hypersensitive response typically develops within 24–72 h and therefore serves as a rapid screening method for identifying potentially pathogenic isolates before biofertilizer development. The 0.5 mL of bacterial suspension (10^8 CFU/mL) bore infiltration, with necrotic symptoms observed after two days. The sterile NB served as a negative control.

Rice seed and seedling tests

The rice variety used in this study was Sigudang (*Oryza sativa* L.). Soaking rice seeds in bacterial suspension for 48 h occurred before sowing. After planting, the application of 10 mL bacterial suspension proceeded to each experimental unit at seven days after sowing to reinforce bacterial establishment in the rhizosphere and root tissues during early seedling development. Growth observations continued on day 14, recording the parameters, such as leaf number, plant height, root length, and biomass at 14 days (Andesmora *et al.*, 2023). The study transpired using a completely randomized design comprising four treatments, namely, a control and three selected bacterial isolates: isolate A.T 4, isolate A.A 4 from the stem, and isolate B.A 3 from the stem. Each treatment consisted of five biological replicates. These isolates were selected samples because they exhibited multiple plant growth-promoting traits, including nitrogen fixation and phosphate and potassium solubilization; showed tolerance to 8% NaCl; and did not induce hypersensitive reactions. The selected isolates received tentative assignments to *Acinetobacter* sp. and *Bacillus* sp. based on preliminary phenotypic observations. Seedling dry biomass determination occurred after oven drying at 70 °C for 48 h until constant weight.

Statistical analysis

Statistical analyses, performed in the study, used IBM SPSS Statistics version 25. All the recorded qualitative data (N fixation, P/K solubilization, pathogenicity, and germination) had descriptive presentations. Quantitative agronomic data, as analyzed, employed an analysis of variance (ANOVA), with significant differences determined using Duncan's multiple range test (DMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

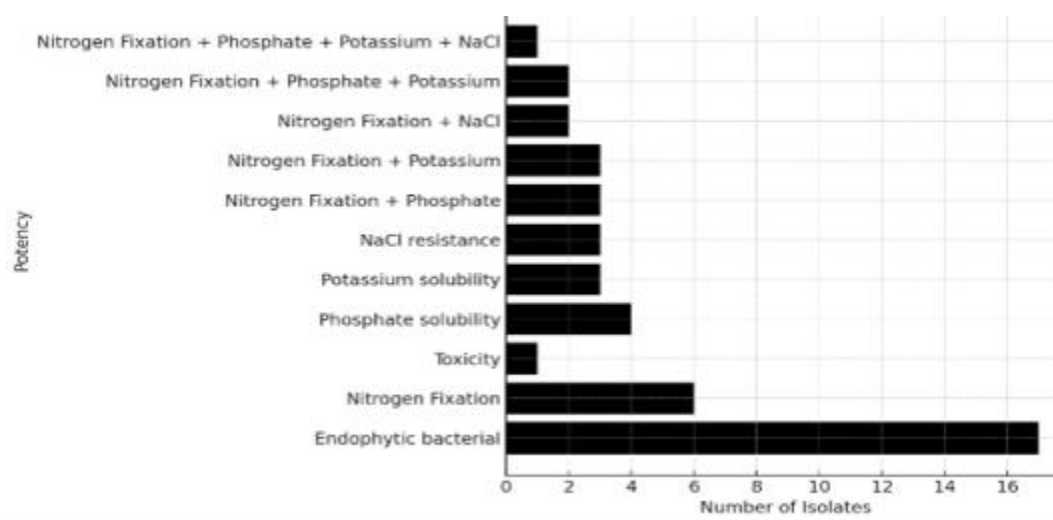
Seventeen halotolerant endophytic bacterial isolates obtained from the sea fern (*A. aureum* L.) received evaluation for plant growth-promoting traits, including nitrogen fixation, phosphate and potassium solubilization, and pathogenicity. Overall, three bacterial isolates showed the highest potential, also reaching confirmation as non-pathogenic. Previous studies reported that halotolerant endophytic bacteria associated with halophytic plants mostly possess multiple plant growth-promoting traits that support plant adaptation under saline stress conditions in marginal lands (Zahir *et al.*, 2019).

Selection of endophytic bacteria with nitrogen-fixing potential

The selected endophytic bacteria collected from sea ferns originating from Meranti Island, Indonesia, appeared to be capable of nitrogen fixation, which resulted in six different isolates: A.T 1, A.T 2, A.T 3, A.A 3, A.A 4 and B.A 3 (Table 1). Among the different isolates, the occurrence of various plant growth-promoting features appears in Figure 1. The figure illustrates the distribution of plant growth-promoting traits among the 17 endophytic bacterial isolates. Phosphate solubilization was evident in four isolates, potassium solubilization in three isolates, and nitrogen fixation in six isolates. The isolate B.A 3 exhibited multiple beneficial traits, including nitrogen fixation and phosphate and potassium

Table 1. Selection of endophytic bacteria from sea ferns from Meranti using NFB medium, phosphate solubility index, and potassium solubility index.

Isolates	Nitrogen fixation	Phosphate solubilization index	Potassium solubilization index
A.T 1	+	-	2.24±0.12
A.T 2	+	2.45±0.13	11.54±3.83
A.T 3	+	-	-
A.T 4	-	2.37±0.11	-
A.B 2	-	-	-
A.A 1	-	-	-
A.A 3	+	-	-
A.A 4	+	2.41±0.03	-
A.A 5	-	-	-
B.B 1	-	-	-
B.B 2	-	-	-
B.B 3	-	-	-
B.B 4	-	-	-
B.A 1	-	-	-
B.A 2	-	-	-
B.A 3	+	2.23±0.04	1.50±1.30
B.A 4	-	-	-

**Figure 1.** Occurrence of various plant growth-promoting features among different isolates.

solubilization, suggesting its potential as a multifunctional biofertilizer candidate. The variation observed among isolates indicates functional diversity of endophytic bacteria associated with *A. aureum* L.

The color change reveals the bacteria can grow on media lacking a nitrogen source (Ekowati *et al.*, 2021). The NFB medium contains the indicator compound bromothymol blue, which changes the color from yellow to blue under alkaline conditions (Figure 2). The

ability of selected isolates to grow and induce color changes in NFB medium signified their potential nitrogen-fixing activity. Similar observations have gained wide usage as preliminary indicators of biological nitrogen fixation in plant growth-promoting bacteria (Ekowati *et al.*, 2021; Dewi and Trimulyono, 2024).

Six of the 17 endophytic bacterial isolates exhibited nitrogen-fixing activity, indicating their potential role as diazotrophs.

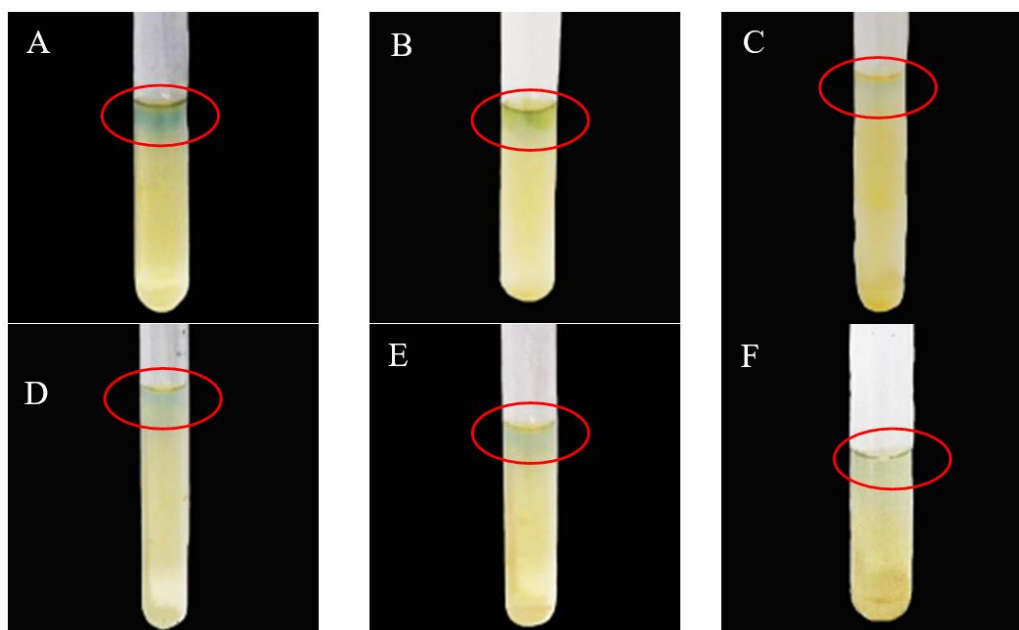


Figure 2. Results of the selection of sea fern endophytic bacteria on NFB medium: A) A.T 1, B) A.T 2, C) A.T 3, D) A.A 3, E) A.A 4, and F) B.A 3.

Endophytic diazotrophic bacteria can colonize internal plant tissues and provide a direct source of biologically fixed nitrogen to the host, which may enhance plant nutrition and growth, particularly under nitrogen-limited conditions (Mandon *et al.*, 2021). The endophytic sea fern bacteria obtained from Meranti Island, Indonesia, proved capable of nitrogen fixation and displayed bacilli-shaped cells. Specifically, the isolate A.T1 emerged as gram-positive with a bacillus shape, A.T2 was gram-negative with a short bacillus, A.T3 was gram-positive and bacillus-shaped, A.A3 was gram-negative and bacillus-shaped, A.A4 was gram-positive and also bacillus-shaped, and B.A3 was gram-negative with a short bacillus shape.

Selection of phosphate-solubilizing endophytic bacteria

The endophytic sea fern bacterial selection was capable of solubilizing phosphate using the Pikovskaya medium at pH 7 and phosphorus sourced from $\text{Ca}_3(\text{PO}_4)_2$ (Table 1). Four bacterial isolates exhibited the phosphate-solubilizing activity, incurring classification as

A.T 2, A.T 4, and A.A 4 isolates from roots and B.A 3 isolate from the stem. The phosphate solubilization index (PSI) of isolate A.T 2 was 2.45 ± 0.13 , A.T 4 (2.37 ± 0.11), A.A 4 (2.41 ± 0.03), and B.A 3 was 2.23 ± 0.04 . According to Matos *et al.* (2017), the PSI values reached categorization into four groups, viz., very low (< 1.00 mm), low (1.00–2.00 mm), medium (2.00–3.00 mm), and high (> 3.00 mm).

For the isolate A.T 2, the clear zone appeared after one day of incubation, whereas three other isolates, A.T 4, A.A 4, and B.A 3, formed obvious zones after seven days of incubation. Ningsih *et al.* (2022) reported seven endophytic bacteria from cashew tree roots were capable to solubilize phosphate within a 5-day incubation period using the Pikovskaya medium with phosphorus sourced from $\text{Ca}_3(\text{PO}_4)_2$ and exhibited low phosphate solubilization indices (< 2.00).

The phosphate solubilization mechanism occurs through acidification by phosphate-solubilizing microbes, which secrete organic and inorganic acids capable of breaking the chelation bonds between phosphate and cations, such as calcium (Ca), thereby lowering the pH (Mardyansyah and Trimulyo, 2021).

Phosphate solubilization observed in the selected isolates indicates their potential to increase phosphorus availability for plants. Similar phosphate-solubilizing activity has surfaced in reports on plant growth-promoting bacteria isolated from various environments. The formation of clear halos around bacterial colonies suggests the production of organic compounds capable of mobilizing insoluble phosphate sources (Umar *et al.* 2024; Kumar *et al.* 2025).

Selection of endophytic potassium-solubilizing bacteria

Among the 17 endophytic bacterial isolates obtained from sea fern, only three isolates (A.T 1, A.T 2, and B.A 3) were able to solubilize potassium containing feldspar as the potassium source. The isolates exhibited different potassium solubilization indices (KSI), indicating variation in their ability to release potassium from insoluble minerals. The isolate A.T 2 showed the highest KSI (11.54 ± 3.83), followed by A.T 1 (2.24 ± 0.12), whereas B.A 3 displayed the lowest KSI (1.50 ± 1.30). Such variation among isolates is commonly referring to differences in microbial metabolic activities involved in mineral weathering and nutrient mobilization.

The potassium-solubilizing activity observed in this study was comparable to or higher than that reported in previous studies. The recorded highest KSI for the isolate A.T 2 (11.54 ± 3.83) was substantially greater than the values reported for potassium-solubilizing bacteria isolated from rice rhizosphere, which generally ranged from 1.4 to 5.3 (Istiyova and Santosa, 2023). These findings suggest that the isolate A.T 2 possesses a strong capacity to mobilize potassium from feldspar under tested conditions.

Differences in potassium-solubilizing ability among bacterial isolates may acquire influences from variations in the production of organic acids and other metabolites involved in the dissolution of potassium-bearing minerals. The exceptionally high KSI, as exhibited by the isolate A.T 2, therefore, highlights its potential for further investigation as a candidate biofertilizer for improving potassium availability

in agricultural soils. Additional studies are essential to quantify the potassium release under liquid culture conditions and evaluate its effectiveness in promoting plant growth.

Bacterial endophyte hypersensitivity in tobacco plants

The results of the pathogenicity test, called the hypersensitivity test on tobacco plants from bacterial isolates, attained successful rejuvenation and revealed the potential to fix N and dissolve P and K emerged in isolates, A.T 1, A.T 2, A.T 3, A.T 4, A.A 3, A.A 4, and B.A 3 (Table 2). Three isolates (A.T 4, A.A 4, and B.A 3) were able to grow in the nutrient broth supplemented with 8% NaCl, indicating substantial salinity tolerance. The ability to survive under elevated salt concentrations suggests that these isolates possess adaptive mechanisms that enable growth under osmotic stress. Such traits could contribute to their suitability for application in marginal environments.

Based on Table 2, one can observe that in seven tested isolates on tobacco plants, one isolate (A.T 2) caused necrosis symptoms on tobacco leaves. Tobacco plants serve as an indicator due to their rapid response to pathogenic bacteria in leaf tissues. Previous findings also revealed that non-pathogenic isolates can reach safe development into biofertilizers (Herdiyantoro *et al.*, 2022). Necrotic symptoms appeared as localized brown to dark-brown lesions surrounding the infiltration site. The lesions were clearly visible within 48 h after inoculation and were absent in the negative control. The severity of the hypersensitive response received a qualitative assessment based on the presence or absence of tissue necrosis.

The endophytic isolate A.T 2 can fix nitrogen and solubilize phosphate and potassium; however, it cannot apply as a biofertilizer agent due to its pathogenicity. Nugraheni and Mawaddah (2023) reported that endophytic bacteria may possess virulence factors against plant defenses, meaning that endophytes can exhibit hidden pathogenic traits, which require specific stimulation to manifest pathogenic responses in crop plants.

Table 2 Results of endophytic bacteria selection in fixing N, dissolving P and K, NaCl resistance, and pathogenicity.

Isolate	N	P	K	NaCl resistance (8%)	Toxicity test
A.T 1	+	-	+	-	-
A.T 2	+	+	+	-	+
A.T 3	+	-	-	-	-
A.T 4	-	+	-	+	-
A.A 3	+	-	-	-	-
A.A 4	+	+	-	+	-
B.A 3	+	+	+	+	-

**Figure 3.** Growth of Sigudang rice plants with various treatments: a) Control, b) A.T 4, c) A.A 4, and d) B.A 3.

In hypersensitivity tests, the severity of the bacterial isolates proved to have an association with the extent of necrotic lesions formed.

Rice plant growth

The growth of Sigudang rice seedlings underwent evaluation in peat soil over 14 days. The rice seedlings at 14 days after sowing may still partially rely on seed nutrient reserves. Therefore, the observed responses should entail interpretation as early growth promotion effects. Longer-term experiments are crucial to fully evaluate the contribution of endophytic bacteria to nutrient acquisition and plant development. Among the 17 endophytic bacterial isolates obtained, three isolates (A.T

4, A.A 4, and B.A 3) were the options for seedling inoculation experiments because they exhibited the most promising combination of plant growth-promoting traits, including nitrogen fixation, phosphate and potassium solubilization, NaCl resistance, and the absence of hypersensitive reactions.

The control treatment showed the lowest germination percentage (28%), indicating that peat soil conditions alone were suboptimal for rice seedling establishment. Peat soils have features typically of acidic pH, low nutrient availability, and poor phosphorus accessibility, which can constrain seed germination and early plant growth. These conditions could explain the generally poor performance observed in the control treatment

(Figure 3). Therefore, bacterial traits, such as nitrogen fixation, phosphate solubilization, and potassium solubilization are particularly relevant for improving nutrient availability in peat-based agricultural systems. The evaluation of these traits sought to identify isolates with biofertilizer potential under nutrient-limited conditions commonly encountered in peatlands.

The application of the three endophytic bacterial inoculums considerably influenced the rice plants' growth in terms of germination, plant height, and dry biomass over 14 days compared with the control. Rice seeds treated with the isolate A.T 4 expressed a higher germination rate and plant stature than the control and the other three bacterial isolate treatments. The isolate A.T 4 appeared to be capable of solubilizing phosphate. Maulidan and Putra (2024) reported that phosphate can stimulate root growth in young rice seedlings, and accelerate tillering, flowering, and the ripening of grains.

Although isolates A.T 4 and B.A 3 produced higher mean values for several growth parameters; the Duncan's multiple range test indicated that differences among treatments were generally not statistically significant. Therefore, the interpretation of these findings should be on preliminary growth trends rather than confirmed treatment effects. The application of endophytic bacterial isolates resulted in variation in rice seedling growth parameters (Table 3). The isolate A.T 4 produced the highest mean germination percentage (80%), plant height (25.78 cm), and root length (7.48 cm), while the isolate

B.A 3 produced the maximum mean leaf number (3.00) and dry weight (24.36 mg). These results suggest that the tested isolates showed positive growth trends during the early seedling stage, although clear treatment effects could not yet have statistical confirmation within the 14-day observation period. The dry weight, plant height, chlorophyll content, and tiller number received influence from nitrogen availability in rice (Jiaying *et al.*, 2022).

The positive growth trends observed in seedlings inoculated with isolates A.T 4 and B.A 3 may have an association with mechanisms commonly reported in plant growth-promoting endophytic bacteria. The isolate A.T 4, identified as *Acinetobacter* sp., produced the highest mean values for seed germination and plant height, whereas the isolate B.A 3, identified as *Bacillus* sp., produced the ultimate mean leaf number and dry biomass. Members of *Acinetobacter* and *Bacillus* have had reports to produce phytohormones, such as indole-3-acetic acid (IAA), cytokinins, ACC deaminase, and organic acids that enhance nutrient availability and stimulate root development (Olanrewaju *et al.*, 2017). However, these traits sustain no direct evaluation in this latest study. Therefore, the mechanisms responsible for the observed responses remain speculative and require further investigation. The findings highlight halophytic ferns, such as *A. aureum* L., as a relatively underexplored source of beneficial microorganisms for sustainable agriculture, particularly in nutrient-limited peat and other marginal soils.

Table 3. Agronomy of Sigudang rice variety growth with the application of various endophytic bacterial isolates using DMRT analysis for 14 days on peat soil.

Isolates	Germination (%)	Plant height (cm)	Root length (cm)	Number of leaves	Wet weight (mg)	Dry weight (mg)
Control	28.00±1.67	23.13±6.27	6.37±1.46	3.00±0.00	73.83±26.30	20.43±6.02
A.T 4	80.00±1.00	25.78±1.60	7.48±3.29	2.40±0.55	60.10±6.94	21.12±2.86
A.A 4	64.00±1.10	20.42±2.57	5.08±0.95	2.20±0.45	48.60±9.67	15.34±1.22
B.A 3	68.00± 1.67	17.60±2.21	4.74±0.57	3.00±0.00	61.58±8.68	24.36±8.44

Values are presented as mean ± SD (n = 5). Means followed by the same letter within a column are not significantly different according to Duncan's multiple range test (DMRT) at P < 0.05.

CONCLUSIONS

Based on the screening of sea fern (*Acrostichum aureum* L.) endophytic bacteria from Meranti Island, Indonesia, isolates A.T 4 and B.A 3 were the identified promising candidates for further biofertilizer development in rice cultivation on peat soil. The isolate A.T 4 (*Acinetobacter* sp.) produced the highest germination percentage and plant height, whereas the isolate B.A 3 (*Bacillus* sp.) produced the topmost mean leaf number and dry biomass. Although not all growth parameters differed significantly among treatments, both isolates exhibited multiple plant growth-promoting traits, including nutrient solubilization and nitrogen fixation. These findings highlight *A. aureum* L. as a relatively underexplored source of beneficial endophytic bacteria for sustainable agriculture in peat and other marginal soils. Future studies involving longer growth periods and field-scale validation are essential to confirm their effectiveness.

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