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Potential of an indigenous bacterial consortium from tidal lowlands to improve rice growth, yield components, and nutrient uptake under different saturation regimes

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Abstract: Type B tidal lowlands are characterized by low nutrient availability and high iron (Fe) concentrations, which constrain rice growth and productivity. This study aimed to evaluate the potential of an indigenous bacterial consortium as a liquid biofertilizer to improve rice growth, yield components, and nutrient uptake under tidal lowland conditions. Bacterial isolates were obtained from rice rhizosphere and root samples, screened based on biosafety, nitrogen fixation ability, and siderophore production, and identified using 16S rRNA gene analysis. Four selected isolates were formulated into a liquid biofertilizer and evaluated in a field experiment under different saturation regimes and inorganic fertilizer levels. The results showed that biofertilizer application enhanced plant growth, improved several yield components, increased nitrogen uptake, and reduced Fe uptake compared with the non-biofertilizer treatment. Saturated soil management also contributed to improved plant performance and nutrient acquisition. Overall, the indigenous bacterial consortium demonstrated potential as a biofertilizer component for supporting rice cultivation in tidal lowlands. Further studies are required to evaluate its long-term effectiveness under broader field conditions.

Keywords: biofertilizer; nitrogen fixation; nutrient use efficiency; siderophore production; water regime.
Abbreviations: Atomic Absorption Spectrophotometry (AAS), Basic Local Alignment Search Tool (BLAST), Chrome Azurol S (CAS), Deoxyribonucleic Acid (DNA), iron (Fe), indole-3-acetic acid (IAA), potassium chloride (KCl), Molecular Evolutionary Genetics Analysis (MEGA), Nutrient Agar (NA), Nutrient Broth (NB), National Center for Biotechnology Information (NCBI), Nitrogen-Free Bromothymol Blue (NFB), nitrogen (N), phosphorus (P), Plant Growth-Promoting Rhizobacteria (PGPR), Polymerase Chain Reaction (PCR), revolutions per minute (rpm), Total Plate Count (TPC), and 16S ribosomal ribonucleic acid (16S rRNA).

Introduction

The expansion of rice cultivation into tidal swamp areas constitutes an important strategy to maintain the sustainability of national rice production in line with the increasing food demand due to population growth (Zahra et al., 2024). Rice is a staple food source for the majority of Indonesia's population and also a major global staple crop (Junaedi *et al.* 2025; Aswidinnoor et al., 2019). Indonesia has approximately 33.4 million ha of swamp land, of which ±20.11 million ha are tidal swamp areas distributed across Sumatra, Kalimantan, Sulawesi, and Papua (Hairani et al., 2024). In Jambi Province, the area of tidal swamp land reaches 684,000 ha, with approximately 246,481 ha having the potential to be developed for agriculture (Jambi, 2023). This potential makes tidal lowlands a strategic source for the expansion of rice production, although they are classified as sub-optimal land.

The main constraints in tidal lowlands are related to soil acidity, fluctuations in redox conditions, and high concentrations of soluble iron. Under flooded conditions, the oxidation–reduction of pyrite (FeS_2) causes the transformation of Fe^{3+} into Fe^{2+}, which is more soluble and readily absorbed by plants; however, in excessive amounts, it can cause iron toxicity, inhibit root growth, reduce photosynthesis, and decrease rice yield (Ghulamahdi et al., 2024). These problems become increasingly complex due to climate change, which affects rainfall patterns and the stability of water management in tidal lowlands (Ratmini et al., 2021).

Water management through the saturated soil culture system (BJA) has been reported to suppress pyrite oxidation, stabilize soil pH, reduce the solubility of Al and Fe, and increase nutrient availability for rice plants (Ghulamahdi et al., 2024; Pujiwati et al., 2016). In addition, biological approaches involving biofertilizers based on Plant Growth-Promoting Rhizobacteria (PGPR) have attracted increasing attention as sustainable alternatives for supporting crop growth and nutrient acquisition while reducing dependence on chemical inputs (Ju et al., 2020).
Indigenous bacteria from tidal lowlands possess adaptive advantages because they have evolved under extreme environmental conditions, including high acidity, fluctuating redox status, and elevated iron concentrations. Several bacterial genera, such as *Sphingobacterium, Paenibacillus, and Burkholderia*, have been reported to possess plant growth-promoting characteristics, including nitrogen fixation, siderophore production, and phytohormone synthesis, which may contribute to plant growth and stress tolerance (Ali et al., 2021; Chang et al., 2024; Igiehon et al., 2024; Moon & Ali, 2022). Likewise, siderophore-producing rhizobacteria have been associated with improved iron acquisition and nutrient availability in soils with unstable redox conditions (Gureeva & Gureev, 2023; Singh et al., 2022).
Although various studies have reported the beneficial effects of PGPR on rice cultivation, information regarding the potential contribution of indigenous bacterial consortia from tidal lowlands to rice growth, yield performance, and nutrient uptake under different saturation regimes remains limited. Therefore, this study aimed to evaluate the potential role of indigenous bacteria derived from tidal lowlands as a liquid biofertilizer in improving rice growth, yield components, nutrient content, and nutrient uptake under different saturation regimes. The results of this study are expected to provide a scientific basis for the development of sustainable and site-specific biofertilizer technologies for rice cultivation in tidal swamp agroecosystems.

Results and Discussion

Macroscopic colony morphology of bacterial isolates

Macroscopic morphological observations revealed substantial variation among the bacterial isolates, particularly in colony color, size, and appearance, although most isolates exhibited circular colonies with smooth margins and convex elevation (Figure 1; Table 1). This phenotypic diversity reflects the presence of taxonomically diverse bacterial populations and their adaptive capacity to the dynamic environmental conditions of tidal lowlands. Molecular identification of selected isolates further revealed the presence of different bacterial taxa, including *Sphingobacterium multivorum, Paenibacillus chitinolyticus, Paenibacillus amylolyticus,* and *Burkholderia plantarii.*
The variation in colony morphology observed among the isolates is consistent with previous studies reporting that rhizosphere bacterial communities inhabiting periodically flooded environments exhibit diverse adaptive traits that support survival under fluctuating redox conditions and enhance their ability to colonize plant roots (López et al., 2023; Zapata et al., 2021). Morphological diversity among bacterial isolates may also indicate differences in physiological and ecological characteristics that contribute to plant–microbe interactions and support their potential role as Plant Growth-Promoting Rhizobacteria (PGPR) (Ajijah et al., 2023; Santoyo et al., 2021).
These morphological characteristics provided preliminary information for differentiating bacterial isolates and served as the basis for selecting candidate isolates for further characterization, including nitrogen fixation, indole-3-acetic acid (IAA) production, siderophore production, hypersensitivity testing, and molecular identification.
Uji hipersensitivitas (hypersensitive response/hr).
The hypersensitive response assay was used as a preliminary screening method to assess the potential pathogenicity of the bacterial isolates prior to consortium development. Although positive and negative controls were not included in the assay, all tested isolates were evaluated under the same experimental conditions.
The results of the hypersensitivity assay on tobacco leaves showed that none of the tested isolates induced necrosis, chlorosis, or other visible hypersensitive symptoms at the infiltration sites (Figure 2). All treated leaf tissues remained healthy throughout the observation period, indicating the absence of detectable pathogenic activity under the conditions tested. Notably, isolate TRF5, which showed the highest sequence similarity to *Burkholderia plantarii* based on 16S rRNA analysis, also produced a negative hypersensitive response.
The hypersensitive reaction is generally characterized by localized cell death as a plant defense response against pathogen infection, functioning to restrict the spread of pathogenic microorganisms (Balint-Kurti, 2019). The absence of such a reaction suggests that the tested isolates did not trigger plant defense responses typically associated with pathogenic bacteria.
Although *Burkholderia plantarii* has been reported as the causal agent of bacterial seedling blight in rice Kang et al, (2024) no necrotic or chlorotic symptoms were observed for isolate TRF5 in the present assay. Therefore, pathogenicity of the isolate could not be confirmed under the conditions evaluated in this study, and further pathogenicity testing on rice plants is required before practical application.
Overall, the results suggest that the tested isolates have potential for further evaluation as candidate Plant Growth-Promoting Rhizobacteria (PGPR). These findings are consistent with previous studies reporting that bacterial isolates showing negative hypersensitive reactions may establish beneficial plant–microbe interactions without causing visible tissue damage under the conditions tested (Zhu et al., 2022).
Nitrogen Fixation Production
Nitrogen fixation is an important biological process carried out by diazotrophic bacteria through the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), which can be utilized by plants. This activity is catalyzed by the nitrogenase

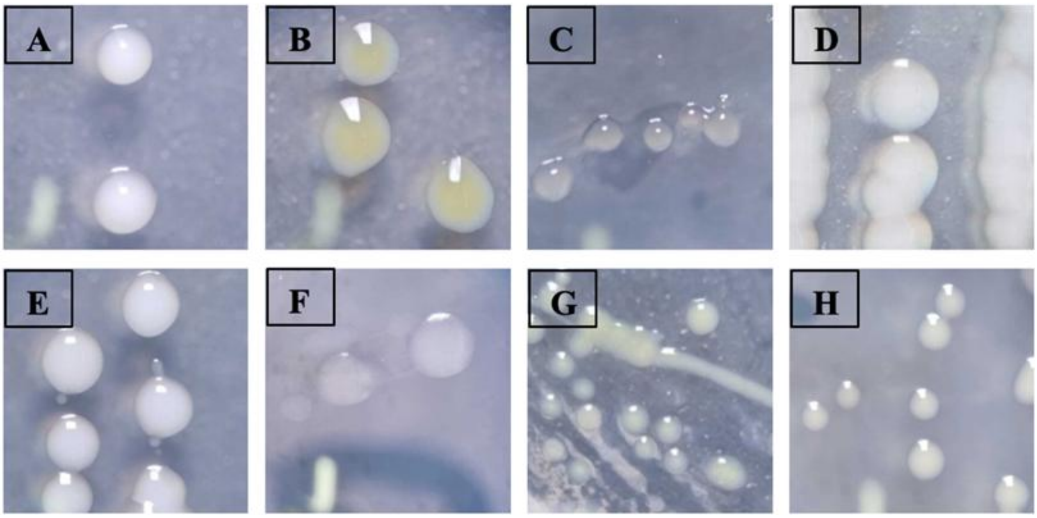

Fig 1. Macroscopic morphology of eight bacterial isolates, Note : A:TRF 1, B:TRF2, C:TRF 3, D:TRF4, E:TRF5, F:TRF6, G:TRF7, H:TRF8.

Table 1. Macroscopic colony morphology of bacterial isolates.

Isolate Code	Colony Shape	Margin	Elevation	Colony Color	Colony Size
TRF 1	Circular	Smooth	Convex	White	Medium
TRF 2	Circular	Smooth	Convex	Yellow	Medium
TRF 3	Circular	Smooth	Convex	Transparent	Small
TRF 4	Circular	Smooth	Convex	White	Medium
TRF 5	Circular	Smooth	Convex	White	Medium
TRF 6	Circular	Smooth	Flat	Translucent	Medium
TRF 7	Circular	Smooth	Convex	Yellow	Punctiform
TRF 8	Circular	Smooth	Convex	White	Small

Fig 2. Hypersensitive response assay of bacterial isolates on tobacco leaves. No necrotic lesions or chlorotic symptoms were observed at the infiltration sites, indicating a negative hypersensitive response for all tested isolates.

enzyme and generally proceeds optimally under microaerobic conditions, as nitrogenase is sensitive to oxygen (Alleman et al., 2021).

In this study, all isolates demonstrated nitrogen fixation ability with varying levels of activity (Figure 3). This difference reflects variation in the physiological capacity among isolates to express and regulate the nitrogenase system, which is influenced by medium conditions, the availability of fixed nitrogen, and cellular metabolic regulation. At the molecular level, the N_2 fixation process is controlled by nif genes, which encode three main types of nitrogenase, namely molybdenum (Mo)-based, vanadium (V)-based, and iron (Fe)-based nitrogenase, with Mo-nitrogenase being the most common and biologically efficient form (Alleman et al., 2021; Zhang et al., 2023).

The ability of the isolates to produce ammonia indicates their functional potential as plant growth-promoting bacteria, particularly in enhancing nitrogen availability in tidal lowlands, which are generally deficient in available nitrogen.

Table 2. Identification of indigenous bacterial isolates from tidal lowland soils through 16S rRNA gene sequencing analysis.

Isolate Label	Source of Isolation	Homologous Microorganism	% Identity
TRF 3	Rhizosphere	*Paenibacillus chitinolyticus*	99.93 %
TRF 4	Rhizosphere	*Sphingobacterium multivorum*	100 %
TRF 5	Rhizosphere	*Burkholderia plantarii*	100 %
TRF 8	Rhizosphere	*Paenibacillus amylolyticus*	100 %

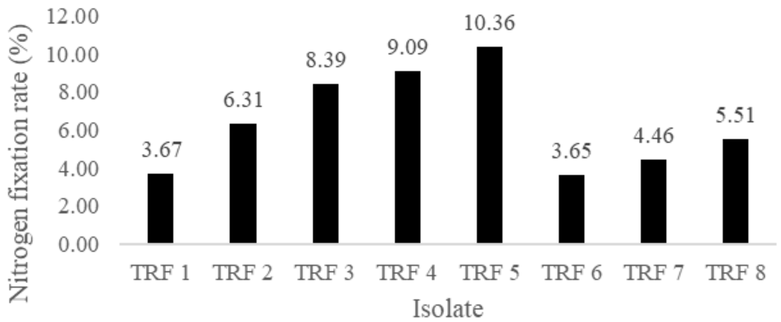

Fig 3. Nitrogen-fixing production.

Table 3. Results of initial soil chemical content analysis.

Criteria	Result	Status
pH H_2O	4.49	Very acidic
pH KCl	3.85	-
Sand (%)	0.88	-
Silt (%)	33.57	-
Clay (%)	65.55	Clay texture
C-organic (%)	8.13	Very high
N-total (%)	0.46	Moderate
P-Bray (ppm)	16.28	Moderate
CEC (cmol(+)/kg)	16.50	Moderate
Mg-dd (cmol(+)/kg)	0.20	Very low
Ca-dd (cmol(+)/kg)	2.15	Moderate
K-dd (cmol(+)/kg)	0.13	Low
Na-dd (cmol(+)/kg)	0.18	Low
Al-dd (cmol(+)/kg)	3.15	Very high
K (mg/100 g)	12.04	Low
Fe (ppm)	19,021	High
P (mg/100 g)	44.01	High

Criteria: Soil fertility status was classified according to Agricultural Research and Development Agency (2012).

Siderophore production

The test results showed that all bacterial isolates were capable of producing siderophores at varying levels among isolates (Figure 4). The highest production was exhibited by isolate TRF4 (82.14%), followed by TRF3 (73.33%), whereas the lowest production was observed in TRF8 (18.26%). This variation reflects differences in physiological capacity and microbial adaptive strategies in responding to iron availability in the growth medium.

Tidal lowlands are characterized by fluctuations in redox conditions that cause changes in the chemical form of iron, from Fe^{3+} to Fe^{2+} during the anaerobic phase and conversely under oxidative conditions. Although the total Fe content in tidal lowlands is relatively high, Fe availability to plants is often unstable due to precipitation and changes in iron valence (Ghulamahdi et al., 2024). Under these conditions, siderophore production by rhizosphere bacteria plays a role as an adaptive mechanism to regulate Fe acquisition and mobilization in the root microenvironment (Liu et al., 2022).

The high siderophore production in isolates TRF4 and TRF3 indicates a strong adaptive capacity to environments with extreme Fe dynamics. In addition to functioning in Fe acquisition, siderophores also contribute to reducing the potential toxicity of Fe by complexing Fe^{3+}, thereby decreasing excessive Fe^{2+} accumulation that can impair plant physiology (Ghulamahdi et al., 2024).

Identification and Selection of Several Isolates

Partial 16S rRNA gene sequence analysis revealed that the selected isolates belonged to three major bacterial genera, namely *Paenibacillus*, *Sphingobacterium*, and *Burkholderia* (Table 2). Isolate TRF3 exhibited 99.93% sequence similarity with Paenibacillus chitinolyticus, while isolates TRF4, TRF5, and TRF8 showed 100% sequence similarity with

Table 4. Results of observations on the vegetative growth of rice plants under saturation treatment, optimal fertilizer, and liquid biofertilizer.

Treatment	Parameter			
	Plant Height	Number of productive tillers	Root Length	Root Volume
Saturation				
P1	73.65±11.89 b	21.88±3.27 b	21.12±4.73 b	41.16±4.73 b
P2	78.76±4.90 ab	24.15±5.07 ab	25.62±5.81 ab	44.93±5.05 ab
P3	79.93±15.71 a	26.22±4.74 a	28.28±5.47 a	47.28±4.16 a
Inorganic Fertilizer Dose				
O1	75.81±7.15 ns	23.51±4.57 ns	25.12±6.76 ns	44.01±5.20 ns
O2	79.09±9.33 ns	24.66±4.81 ns	24.89±5.35 ns	44.87±5.33 ns
Liquid Biofertilizer				
A0	73.09±8.96 b	21.87±3.25 b	22.22±6.71 b	42.28±3.54 b
A1	81.81±4.85 a	26.30±4.87 a	27.79±3.58 a	46.60±5.78 a

Note: P1 = one-month saturation treatment; P2 = two-month saturation treatment; P3 = continuous saturation treatment. O1 and O2 represent 50% and 100% of the recommended inorganic fertilizer dose, respectively. A0 = without liquid biofertilizer (control), and A1 = with liquid biofertilizer application. Values are presented as mean ± standard deviation. Means followed by different letters within the same column indicate significant differences according to Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

Table 5. Results of the calculation of rice plant production under saturation treatment, optimal fertilizer, and liquid biofertilizer.

Treatment	Parameter				
	Panicle Length	Filled Grains	Unfilled Grains	Weight of 1000 Grains	Grain yield
Saturation					
P1	21.65±3.11 b	102.25±8.65 b	23.93±5.14 b	21.88±3.27 b	3.51±0.60 b
P2	23.63±2.56 b	106.31±4.78 b	27.40±6.13 ab	24.16±5.07 ab	3.83±0.80 b
P3	26.33±4.46 a	116.64±12.19 a	30.82±4.63 a	26.22±4.74 a	4.39±0.87 a
Inorganic Fertilizer Dose					
O1	23.52±3.67 ns	107.93±11.35 ns	26.75±6.19 ns	23.51±4.57 ns	3.85±0.90 ns
O2	24.22±4.18 ns	108.87±10.39 ns	28.01±5.74 ns	24.66±4.81 ns	3.97±0.77 ns
Liquid Biofertilizer					
A0	22.12±2.82 b	105.46±9.42 b	24.83±4.65 b	21.87±3.25 b	3.59±0.79 b
A1	25.62±4.10 a	111.34±11.40 a	29.94±6.05 a	26.30±4.87 a	4.24±0.75 a

Note: P1 = one-month saturation treatment; P2 = two-month saturation treatment; P3 = continuous saturation treatment. O1 and O2 represent 50% and 100% of the recommended inorganic fertilizer dose, respectively. A0 = without liquid biofertilizer (control), and A1 = with liquid biofertilizer application. Values are presented as mean ± standard deviation. Means followed by different letters within the same column indicate significant differences according to Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

Sphingobacterium multivorum, *Burkholderia plantarii*, and *Paenibacillus amylolyticus*, respectively. These results indicate a close genetic relationship between the selected isolates and their corresponding reference strains.

Phylogenetic analysis based on 16S rRNA gene sequences further supported the taxonomic placement of the isolates (Figure 5). The phylogenetic tree showed that TRF3 clustered with reference sequences of *Paenibacillus chitinolyticus*, TRF4 grouped with *Sphingobacterium multivorum*, TRF8 clustered with *Paenibacillus amylolyticus*, and TRF5 was positioned within the *Burkholderia plantarii* clade. The clustering patterns were consistent with the sequence similarity results and confirmed the close evolutionary relationships between the isolates and their respective reference strains.

Although 16S rRNA gene sequencing is widely used for bacterial identification, species-level assignments based solely on this marker should be interpreted cautiously because closely related taxa may share highly similar 16S rRNA sequences. Therefore, additional molecular approaches, such as multilocus sequence analysis or whole-genome sequencing, are recommended for more robust taxonomic confirmation (Ishaq et al., 2025).

Soil chemical analysis

Initial soil chemical analysis results showed that the soil was under very acidic conditions (pH H_2O 4.49; pH KCl 3.85), reflecting the dominance of Al^{3+} and H^+ ions in the exchange complex (Table 3). This condition is commonly found in humid tropical soils and tidal lowlands and has direct implications for aluminum and iron toxicity to plants (Zhu & Shen, 2024).

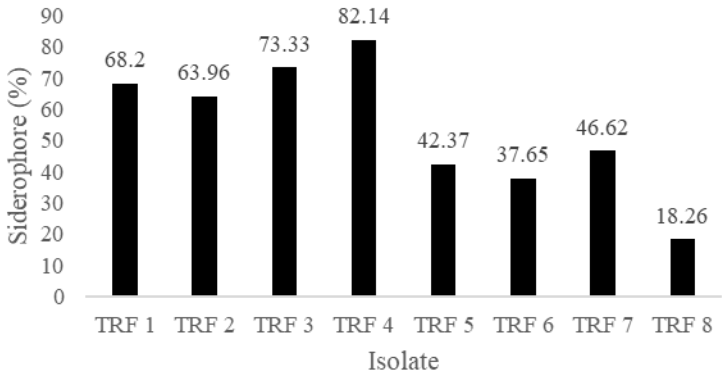

Fig 4. Siderophore production by bacteria from the rhizosphere of rice plants in tidal lowland areas.

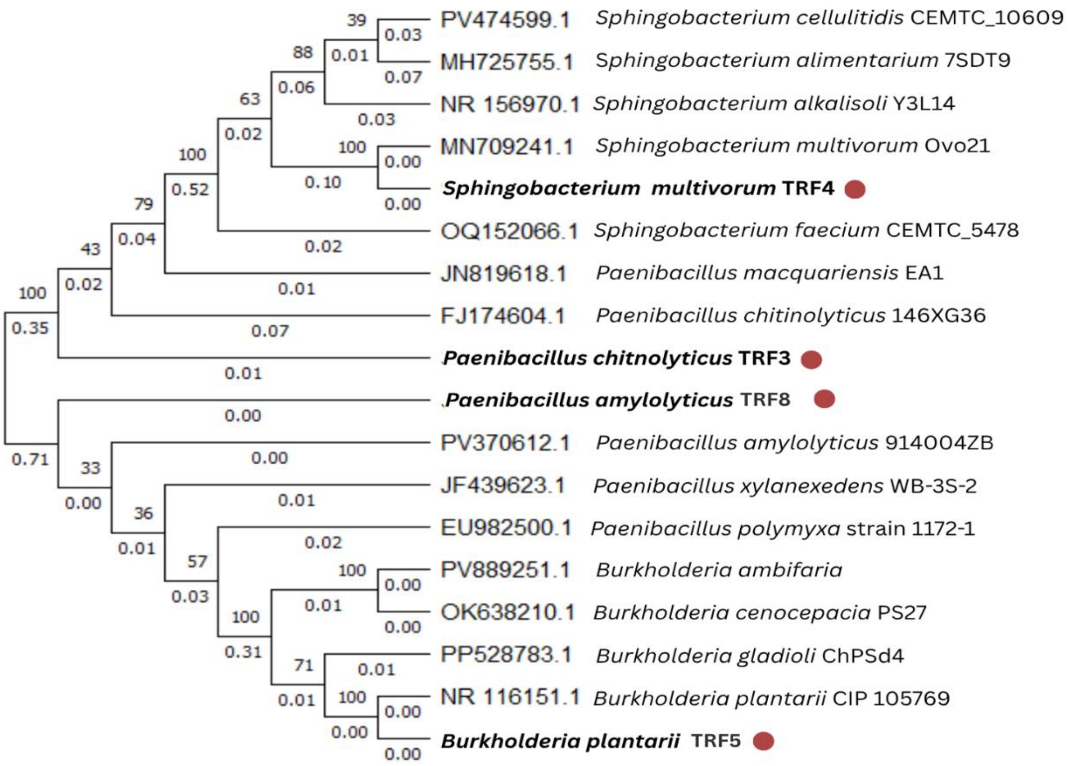

Fig 5. Phylogenetic tree of selected isolates.

The high levels of exchangeable Al (3.15 cmol/kg) and soluble Fe (19,021 ppm) have the potential to suppress root growth and inhibit nutrient uptake, particularly phosphorus through the mechanism of phosphate fixation by Fe and Al oxides.
The soil texture, dominated by the clay fraction (65.55%), indicates a relatively good adsorption capacity, as reflected by the moderate CEC value (16.5 cmol/kg). The very high organic C content (8.13%) indicates intensive accumulation of organic matter, which plays an important role in improving soil structure and reducing free Al activity through the formation of organo-Al complexes (Gerke, 2021). However, an imbalance of base cations is clearly observed, with very high exchangeable Ca, whereas exchangeable Mg and exchangeable K are classified as low, which may cause plant nutrient disorders.

Vegetative plant growth
Soil saturation significantly increased plant height, number of productive tillers root length, and root volume, with the highest response observed under treatment P3 (Table 4). This increase is associated with the improvement of redox conditions in acidic soils, which reduces the solubility of Al^{3+} and Fe^{2+}, thereby supporting root system development and nutrient uptake efficiency (Ghulamahdi et al., 2024; Pujiwati et al., 2016). A more developed root system directly contributes to enhanced shoot growth through increased water and nitrogen supply (Xia et al., 2024).
The optimal fertilizer treatment did not show significant differences among levels, indicating that the response to mineral fertilizer in acidic soils is limited by soil chemical factors, particularly Al saturation and low effective nutrient availability (Tkaczyk et al., 2020). In contrast, the application of liquid biofertilizer significantly increased plant height, number of productive tillers and root parameters, which may be associated with the plant growth-promoting characteristics of the bacterial consortium identified during isolate screening.

Table 6. Results of the analysis of nutrient content in the shoot of rice plants under liquid biofertilizer treatment and water regime.

Treatment	Parameter		
	Fe	N	P
Saturation			
P1	6199±32.76 a	1.37±0.30 ns	0.18±0.04 ns
P2	2547±21.76 b	1.46±0.31 ns	0.19±0.03 ns
P3	2035±10.53 b	1.47±0.24 ns	0.23±0.12 ns
Optimal Fertilizer			
O1	3652±29.97 ns	1.47±0.25 ns	0.20±0.04 ns
O2	3535±30.09 ns	1.40±0.31 ns	0.19±0.10 ns
Liquid Biofertilizer			
A0	4579±34.83 a	1.31±0.27 b	0.17±0.04 ns
A1	2609±19.48 b	1.57±0.22 a	0.23±0.09 ns

Note: P1 = one-month saturation treatment; P2 = two-month saturation treatment; P3 = continuous saturation treatment. O1 and O2 represent 50% and 100% of the recommended inorganic fertilizer dose, respectively. A0 = without liquid biofertilizer (control), and A1 = with liquid biofertilizer application. Values are presented as mean ± standard deviation. Means followed by different letters within the same column indicate significant differences according to Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

Crop production

The results of the analysis showed that the saturation treatment and liquid biofertilizer had a significant effect on panicle length, filled grains, unfilled grains, and 1000-grain weight, whereas the optimal fertilizer did not provide a significant response (Table 5). The improvement in yield components under the highest saturation treatment (P3) indicates that water management plays an important role in supporting grain filling and crop productivity under tidal lowland conditions. Improved water availability and a more favorable soil environment during the reproductive stage may contribute to better grain development and reduced yield losses (Mosharrof et al., 2021).

The application of liquid biofertilizer significantly increased panicle length and 1000-grain weight and reduced unfilled grains. These responses may be associated with the plant growth-promoting characteristics of the bacterial consortium identified during the screening stage. However, the present study did not directly quantify the physiological or biochemical mechanisms underlying these responses under field conditions; therefore, the observed effects should be interpreted as potential contributions of the bacterial consortium rather than directly demonstrated mechanisms (Mosharrof et al., 2021).

Shoot Nutrient Content (N, P, and Fe)

Water regime and the application of liquid biofertilizer had a significant effect on shoot Fe content, whereas their interaction was not significant (Table 6). The reduction in shoot Fe under optimal saturation and liquid biofertilizer treatments suggests that these treatments may have contributed to limiting excessive Fe accumulation in plant tissues. This response is particularly important in tidal lowland soils, where elevated Fe availability may increase the risk of Fe toxicity and adversely affect plant growth (Carmona et al., 2021; Weinand et al., 2023).

Shoot N content was influenced by water regime, whereas the effect of biofertilizer was not significant. This result suggests that water management played a more important role than biofertilizer application in determining shoot N concentration under the conditions of this study (Flynn et al., 2023; Oldroyd & Dixon, 2014). Shoot P content was relatively stable across treatments, although a slight increasing trend was observed under biofertilizer application. This finding indicates that the response of P accumulation in plant tissues was limited and may depend on soil conditions and plant nutrient demand (Zhang et al., 2025).

N, P, and Fe uptake

N uptake increased under optimal saturation treatments (P2–P3) and the application of liquid biofertilizer (A1) (Table 7). These responses suggest that appropriate water-management practices and biofertilizer application may have contributed to improved plant nutritional status under tidal lowland conditions. Controlled flooded conditions have been reported to influence N availability and facilitate nutrient acquisition by rice plants (Marlina et al., 2025; Midya et al., 2021).

The observed increase in N uptake may be associated with the plant growth-promoting characteristics of the bacterial consortium identified during the screening stage, including nitrogen-fixing and siderophore-producing capabilities. However, the present study did not directly quantify biological nitrogen fixation or other underlying physiological mechanisms under field conditions. Therefore, these explanations should be interpreted as potential mechanisms rather than directly demonstrated processes (Flynn et al., 2023; Oldroyd & Dixon, 2014).

In contrast, Fe uptake tended to decrease under treatments that promoted greater N uptake, suggesting that water-management and biofertilizer treatments may have contributed to reducing excessive Fe accumulation in plant tissues. This response is important in tidal lowland soils, where elevated Fe availability may increase the risk of Fe toxicity and disrupt plant nutrient balance (Aung & Masuda, 2020; Wairich et al., 2024).

P uptake showed a relatively limited response compared with N uptake. This finding indicates that the effects of water-management and biofertilizer treatments on nutrient acquisition may vary among nutrient elements and depend on soil chemical conditions and plant demand. Overall, the combination of optimal saturation management and liquid biofertilizer

Table 7. N, P, and Fe uptake.

Treatment	Parameter		
	N	P	Fe
Saturation			
P1	53.75±7.21 b	32.73±1.40 ns	451.13±267.20 b
P2	63.53±11.88 a	33.08±1.45 ns	253.73±233.04 ab
P3	70.25±10.59 a	33.27±1.38 ns	160.29±249.27 a
Optimal Fertilizer			
O1	62.43±12.65 ns	33.20±1.48 ns	272.27±272.36 ns
O2	62.59±11.60 ns	32.85±1.29 ns	304.50±277.23 ns
Liquid Biofertilizer			
A0	59.16±10.31 b	32.87±1.30 ns	365.56±269.75 b
A1	65.86±12.83 a	33.19±1.48 ns	211.21±256.59 a

Note: P1 = one-month saturation treatment; P2 = two-month saturation treatment; P3 = continuous saturation treatment. O1 and O2 represent 50% and 100% of the recommended inorganic fertilizer dose, respectively. A0 = without liquid biofertilizer (control), and A1 = with liquid biofertilizer application. Values are presented as mean ± standard deviation. Means followed by different letters within the same column indicate significant differences according to Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

was associated with improved nutrient uptake patterns and lower Fe accumulation, thereby supporting favorable rice growth under tidal lowland conditions.

Materials and Methods

Study site and experimental period

The laboratory research was conducted at the Biotechnology Center Laboratory IPB and the Soil Biotechnology Laboratory IPB from January 2024 to July 2025. The field experiment was carried out in a type B tidal lowland in Karya Bhakti Village, Rantau Rasau Subdistrict, Tanjung Jabung Timur Regency, Jambi Province, from August 2024 to March 2025.

Sample Collection and Bacterial Isolation

Rhizosphere and rice root samples were collected from six observation points grouped into two main areas and then composited. Bacterial isolation was performed using the serial dilution method. Soil suspensions were serially diluted in 0.85% physiological NaCl solution, and aliquots of the diluted suspensions were spread onto Nutrient Agar (NA) medium followed by incubation at 30 °C for 24–48 h. Colonies exhibiting distinct morphological characteristics were selected and purified by repeated streaking on fresh NA medium to obtain pure cultures. Nitrogen-fixing bacteria were subsequently isolated through serial dilution and selection on semi-solid Nitrogen-Free Bromothymol Blue (NFB) medium. Isolates forming a white pellicle were selected and purified on solid NFB medium until pure cultures were obtained.

Safety, morphological, and functional characterization

Isolate safety was evaluated through a hypersensitivity test on tobacco leaves and a hemolysis test on 5% blood agar medium to ensure that the isolates were non-pathogenic (Gilligan, 2013; Nøhr-Meldgaard et al., 2026). Morphological characterization was performed based on macroscopic colony characteristics, including colony shape, margin, elevation, color, and colony size observed on Nutrient Agar (NA) medium. Nitrogen fixation ability was quantitatively analyzed using the Nessler method after incubation in Ashby medium, whereas siderophore production was qualitatively tested using Chrome Azurol S (CAS) medium, indicated by the formation of an orange halo zone around the colonies (Mir et al., 2022). Selected isolates were subsequently subjected to molecular identification based on 16S rRNA gene sequencing.

Biofertilizer consortium and molecular identification

Four isolates with the best plant growth-promoting characteristics were selected and formulated as a liquid biofertilizer consortium using Nutrient Broth supplemented with molasses as a carrier medium. The production of liquid biofertilizer is carried out with a ratio of 2:1 where the bacteria are first grown in nutrient broth and 5% molasses media. The bacterial population is 10^8 CFU/ml, molasses is added with a concentration of 2% and 5% bacterial isolates and fermented for 14 days.The selected isolates were chosen based on their performance in nitrogen fixation, siderophore production, and molecular identification analyses. Molecular identification was performed through genomic DNA extraction, amplification of the 16S rRNA gene using universal primers 63F and 1389R, followed by BLASTn analysis against sequences available in the NCBI database. Phylogenetic relationships were further evaluated using the neighbor-joining method with 1,000 bootstrap replicates in MEGA version 12 software.

Prior to consortium formulation, compatibility among the selected isolates was evaluated through an in vitro synergism assay on Nutrient Agar medium. No inhibition zones were observed between isolates after incubation, indicating compatibility among the bacterial strains and supporting their use as a consortium. The consortium was subsequently propagated in Nutrient Broth supplemented with molasses and incubated until a bacterial density of approximately 10^8 CFU mL^{-1} was achieved.

Experimental design and treatments

A field experiment was conducted to evaluate the effectiveness of a liquid biofertilizer consortium derived from indigenous tidal lowland bacteria under different water-management and fertilizer regimes. The experiment was arranged in a split-split plot design with three replications. Water-management regime was assigned as the main plot factor, inorganic fertilizer level as the subplot factor, and biofertilizer application as the sub-subplot factor. In total, 12 treatment combinations were evaluated in each replication.

The main plot factor consisted of three water-management regimes: P1, P2, and P3. Saturated soil conditions were maintained by controlling the water table at approximately 5 cm below the soil surface. In P1, soil saturation was maintained from transplanting until 30 days after transplanting (DAT), after which the plots were managed under conventional flooded conditions until harvest. In P2, soil saturation was maintained until 60 DAT and subsequently converted to flooded conditions until harvest. In P3, saturated soil conditions were maintained continuously from transplanting until harvest.

The subplot factor consisted of two inorganic fertilizer levels. O1 represented 50% of the recommended fertilizer rate, whereas O2 represented 100% of the recommended fertilizer rate. The recommended fertilizer rate consisted of 200 kg ha^{-1} urea, 499 kg ha^{-1} Fertiphos, and 167 kg ha^{-1} KCl. Accordingly, O1 received 100 kg ha^{-1} urea, 249.5 kg ha^{-1} Fertiphos, and 83.5 kg ha^{-1} KCl, while O2 received 200 kg ha^{-1} urea, 499 kg ha^{-1} Fertiphos, and 167 kg ha^{-1} KCl. Fertiphos was used as the phosphorus source in place of SP-36.

The sub-subplot factor consisted of two biofertilizer treatments: A0 (without biofertilizer application) and A1 (with biofertilizer application). The liquid biofertilizer was formulated as a consortium of four indigenous bacterial isolates obtained from tidal lowland rice rhizospheres. These isolates were previously selected based on their plant growth-promoting characteristics, including nitrogen fixation, siderophore production, and molecular identification. The bacterial population density in the liquid biofertilizer was maintained at approximately 10^8 CFU mL^{-1} in accordance with the Indonesian Ministry of Agriculture standards for liquid biofertilizer products.

Biofertilizer application was conducted through three stages. First, rice seeds assigned to the biofertilizer treatment were subjected to biopriming by soaking them in the liquid biofertilizer suspension for 12 h prior to sowing. Second, rice seedlings were root-dipped in the biofertilizer suspension immediately before transplanting. Third, field application was performed at 7 days after transplanting by applying the biofertilizer around the plant base at a rate of 5 mL $plant^{-1}$. Each experimental plot measured 3 m × 1 m.

The split-split plot structure was maintained throughout the experiment, with water-management regime assigned to the main plots, fertilizer level to the subplots, and biofertilizer application to the sub-subplots. Statistical analyses were conducted according to this experimental structure.

Field experiment and crop management

Initial soil analysis was conducted on composite samples, including pH, organic C, total N, available P, K_2O, and Fe. Rice seeds of the Inpari 16 Pasundan variety were treated with liquid biofertilizer and transplanted at 18 days after sowing. The application of liquid biofertilizer was carried out at the seed soaking stage, at transplanting, and one month after planting. Basal fertilization included dolomite at 2 ton ha^{-1} and rice husk charcoal at 0.5 ton ha^{-1}, whereas inorganic fertilizers were applied according to recommendations at 50% and 100% doses. Pest control was implemented in an integrated manner through mechanical and chemical methods according to field requirements (Dhakal & Poudel, 2020).

Observed parameters and statistical analysis

Growth parameters included plant height, number of productive tillers, root length, and root volume. Production parameters included panicle length, number of filled and unfilled grains, 1,000-grain weight, and grain yield. Shoot N, P, and Fe contents were analyzed using standard laboratory methods, whereas nutrient uptake was calculated based on plant dry weight (Shruti et al., 2024; Ugile et al., 2024).

Data were analyzed using analysis of variance (ANOVA) appropriate for a split-split plot design with three replications. Water-management regime was treated as the main plot factor, inorganic fertilizer level as the subplot factor, and biofertilizer application as the sub-subplot factor. Mean comparisons were performed using Duncan's Multiple Range Test (DMRT) at the 5% significance level when treatment effects were significant. Nitrogen fixation and siderophore production assays were used as preliminary screening parameters for isolate selection and were therefore presented descriptively.

Conclusion

The indigenous bacterial consortium derived from tidal lowlands showed potential as a liquid biofertilizer for rice cultivation in type B tidal lowlands. The application of liquid biofertilizer and saturated soil management improved several rice growth variables, yield components, and nutrient uptake characteristics. Biofertilizer application was associated with increased N uptake and reduced Fe accumulation in plant tissues, whereas saturated soil conditions contributed to a more favorable environment for rice growth under tidal lowland conditions. These findings indicate that the integration of indigenous bacterial biofertilizer and appropriate water management may support rice cultivation in tidal lowlands and warrants further evaluation under broader field conditions.

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The entries most clearly requiring verification are **Aswidinnoor et al. (2019)** and **Junaedi et al. (2025)** because the journal/source name is missing, **Jambi B (2023)** because it is incomplete as an institutional reference, and **Santoyo et al. (2021)** because the supplied DOI is incomplete (10.3390/biology does not identify a specific article).