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Endophytic bacterial isolate diversity in suboptimal field rice and their potential in sheath blight control

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Abstract. *Prihatiningsih N, Rahayuniati RF, Djatmiko HA, Lestari P, Wulansari NK, Widnyana IK, Sutanto KD. 2024. Endophytic bacterial isolate diversity in suboptimal field rice and their potential in sheath blight control. Biodiversitas 25: 3359-3366.* Rice root endophytic bacteria from suboptimal fields rice are beneficial for controlling plant pathogens. The present research aimed to characterize morphology and biochemical properties, identify secondary metabolites of rice root endophytic bacteria and evaluate their potential as biocontrol for sheath blight caused by *Rhizoctonia solani*. The present study focused on five endophytic bacteria, specifically *Bacillus* sp., with white colonies, rod-shaped cells, Gram-positive characteristics, and catalase-positive. These bacteria were selected from rice root samples due to their potential as antagonists against the pathogenic fungus *R. solani*. The inhibition potential of endophytes against *R. solani* was determined by measuring the radius of the fungal colony in the presence of the endophytic bacteria isolates. Among them, NP KR4 showed the highest inhibition percentage of 46.00 ± 6.25%, which was attributed to the antibiosis mechanism causing hyphal swelling. A molecular test using the 16S rRNA gene was conducted to determine the isolates' genetic identity. Comparing the gene sequences with those in the genetic database, it was found that NP A5, NP KR4, and NP KR7 isolates were similar to *B. subtilis* strain 2JKP676166, although with a few base pair differences. NP A6 exhibited a close relation to *B. subtilis* strain YT2 HQ143571, while NP SB3 was identified as *B. subtilis* subsp. *stercoris* strain EG1265MN704551. Further analysis of the endophytic bacteria's secondary metabolites through Gas Chromatography and Mass Spectroscopy revealed the presence of alkaloids, phenols, alcohols, and fatty acids. Among the isolates, NP KR4 demonstrated the highest biocontrol effectiveness against sheath blight, with an efficacy of 58.74%. Overall, the results indicated that the rice root endophytic bacteria isolated from suboptimal fields possess multiple contemporary traits, including antifungal activity and strain-specific differences as *Bacillus subtilis*. These findings suggest promising prospects for developing bio-fungicide formulations to promote sustainable rice production.

Keywords: Characteristics, endophytic bacteria, molecular, *Rhizoctonia solani*, rice

INTRODUCTION

Excessive use of pesticides can lead to residues that are harmful to both plants and the environment. To minimize such negative impacts, it is important to emphasize the use of biological control, especially microbial antagonists, including endophytic bacteria, which provide a more sustainable and environmentally friendly approach to disease control. Endophytic bacteria are beneficial bacteria that can live and interact with plants without causing disease. These bacteria have been shown to enhance plant growth by producing growth-promoting substances, fixing nitrogen, mobilizing phosphate, and improving overall plant health. In addition, endophytic bacteria are believed to enhance the plant's defense system against disease and produce compounds that induce plant resistance, such as antimicrobial compounds, enzymes, salicylic acid, and ethylene (Santos et al. 2018; Oukala et al. 2021; Liu et al. 2022).

The rice root endophytic bacteria found in suboptimal paddy fields have the potential to effectively control both fungal and bacterial plant pathogens, which are major hurdles in rice production. The suboptimal land is generally soils that have several problems, including high soil acidity and toxicity of Fe and Al. The nutrient deficiency, namely N, P, K, Ca, and Mg, is one of the characteristics of suboptimal land. Therefore, this condition needs to be improved by biology such as endophytic bacteria and chemical techniques. The suboptimal land has a reason to explore the endophytic bacteria because the endophytic bacteria the same as the other microbe were tolerance to extreme environments, and they have the potential to produce some compound to increase land productivity (Marlina et al. 2014; Sutariati et al. 2019). Suboptimal lands are grouped into four land typologies: acid climate, dry climate, tidal swamp, lowland swamp, and peat land (Arista et al. 2023).

A total of twenty-two isolates of endophytic bacteria were collected from rice roots in suboptimal areas of Petanahan, Kebumen Regency, as well as Karangwangkal and Sumbang, Banyumas Regency, Central Java, Indonesia are included in the dry climate, dry land type with the characteristics of a low rainfall, neutral pH, and low fertility

(Fuqara and Tanjung 2023). These bacteria stimulate rice growth by producing plant growth-promoting components such as siderophore, hydrogen cyanide (HCN), indole acetic acid (IAA), and phosphate solubility. The five selected isolates tested are capable of controlling *Xanthomonas oryzae* pv. *oryzae* in vitro, these isolates are A5, A6, KR4, KR7 and SB3. In particular, the KR4 isolate shows strong siderophore production, HCN, IAA, phosphate solubility, and hydrolytic enzymes such as chitinase. Chitinase is an enzyme that degrades chitin to N-acetylglucosamine, which can be used as a biocontrol agent against plant diseases caused by phytopathogenic fungi Prihatiningsih et al. (2022). KR4 isolate is the isolate code resulting from the isolation from rice root endophytes in the Karangwangkal Banyumas (Prihatiningsih et al. 2022), while the NP KR4 is the code after this isolate was molecularly analyzed and registered in GenBank. *Bacillus subtilis* B298 is an example of a bacterium that produces chitinase, with an optimum temperature of 40°C, pH of 5.0, and an activity level of 6.813 U/mL (Lestari et al. 2017). This enzyme is classified as a metalloenzyme. Due to their potential, microbial antagonists are widely used as biocontrol for plant diseases caused by phytopathogenic fungi.

Several genera of endophytic bacteria have significant potential as biocontrol agents and plant growth promoters (Resti et al. 2018). Some specific strains such as *B. subtilis* HB1310 and *Stenotrophomonas maltophilia* RR-10, as well as species such as *B. aryabhattai* and *B. megaterium*, have shown promising results when found in plant roots and leaves. These endophytes can produce a wide range of bioactive secondary metabolites, including alkaloids, quinones, flavonoids, phenolic acids, terpenoids, steroids, tetralones, and xanthones (Yadav and Yadav 2021).

The original research focuses on isolating and identifying endophytic bacteria from rice roots with the potential to control phytopathogenic fungi and stimulate plant growth. Moreover, the novelty of this research lies in the molecular selection of these endophytic bacteria. This substitution of synthetic pesticides is more environmentally friendly and safer for food and supports sustainable agriculture. This research aimed to select rice root endophytic bacteria with antagonistic potential against *Rhizoctonia solani*, characterize their morphology, perform biochemical and molecular assays, identify secondary metabolites, and evaluate their potential as biocontrol agents against sheath blight in the screen house.

MATERIALS AND METHODS

Preparation and selection of rice root endophytic bacteria

The endophytic bacteria used in this study were isolated from fresh rice roots taken from suboptimal fields in Petanahan, Kebumen Regency, Karangwangkal and Sumbang, Banyumas Regency, Central Java, Indonesia. These bacteria were selected to investigate their potential as biological control agents. Out of the twenty-two isolates found, five of them showed compatible growth and similar morphological characteristics and exhibited preliminary antagonistic behavior. These isolates were named NP A5, NP A6, NP KR4, NP KR7, and NP SB3, and demonstrated antagonism against *Xanthomonas oryzae* pv. *oryzae* (Prihatiningsih et al. 2022).

These five isolates were prepared on a Nutrient Agar (NA) medium (Merck, USA) and preserved as work cultures for further testing. This research mainly focused on assessing the antagonistic activity of these endophytic bacteria against fungal rice pathogens, specifically *R. solani*. Dual culture method was employed to evaluate the inhibitory effects of the endophytic bacteria on *R. solani*. was used. A mycelial plug with a diameter of 5 mm, consisting of *R. solani*, was placed in the center of a 9 cm diameter petri dish containing 10 mL of Potato Dextrose Agar (PDA) (Merck, USA) and incubated at room temperature for a day. After incubation, paper discs (diameter 6 mm) containing 10 µL of the endophytic bacteria (10^8 cfu/mL), which had been pre-dripped onto Whatman No. 4 filter paper (Merck, USA), were placed on both sides of the petri dish, 3 cm away from the edges, and then incubated at room temperature for 5 days.

The growth of the *R. solani* colony was measured after 5 days in the presence of endophytic bacteria. The inhibition percentage was calculated using the formula $I= \{(R1-R2) / R1\} \times 100\%$, as described by (Wang et al. 2013) and (Muthukumar and Venkatesh 2013). *I*: inhibition (%); *R1*: growth of radius colony of *R. solani* opposite with the paper disc of endophytic bacteria; *R2*: growth of radius colony of *R. solani* toward the paper disc of endophytic bacteria. The mechanism of inhibition observes the malformation of the mycelium or hyphae cell wall as lysing, vacuolating, swelling or deformation, and distortion (Herdyastuti et al. 2010; Yadav and Yadav 2021; Khairah et al. 2023).

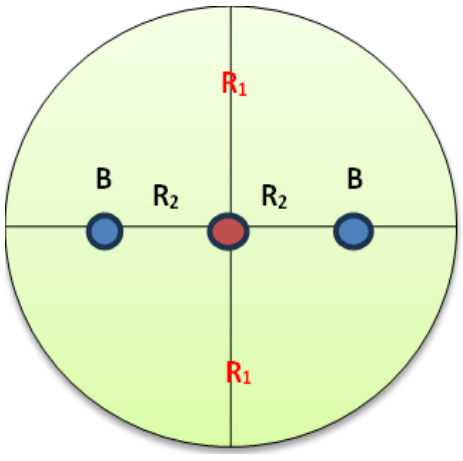

Figure 1. The illustration demonstrates the inhibition assay of endophytic bacteria on *Rhizoctonia solani* in a PDA medium. A. shows the plug of the *R. solani* colony, while in B. a paper disc was treated with 10 µL of the endophytic bacteria. R1 reveals the growth of the *R. solani* colony's radius opposite the paper disc of the endophytic bacteria. At the same time, R2 demonstrates the growth of the *R. solani* colony's radius towards the paper disc of the endophytic bacteria

Characteristics of rice root endophytic bacteria selected based on morphology and biochemical test

The endophytic bacteria were thoroughly examined for their morphological characteristics, including pigment, shape, margin, and texture of the colony, as well as the shape of the cell and endospore. These characteristics were compared to reputable libraries such as Bergey's Manual Bacteriology to ensure accuracy. Furthermore, the biochemical properties of the endophytic bacteria were assessed through Gram staining, the KOH 3% test, the catalase test using hydrogen peroxide 10%, and the chitinase test, as described by (Lestari et al. 2017; Prihatiningsih et al. 2020).

Molecular characteristics of rice root endophytic bacteria

Molecular identification

The bacterial endophytes were identified by partially sequencing the 16S, 27F, and 1492R DNA genes. The endophytic bacterial isolates were cultivated in a nutrient broth medium (NB) at 30°C for 20 hours. The total DNA was extracted using the standard procedures of the Quick-DNA Fungal/Bacterial Miniprep Kit from Zymo Research D6005 (Merck, USA), following the manufacturer's instructions (Sambrook et al. 2001).

The DNA was amplified by PCR using universal 16S eubacterial primers 27F (5'- AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'TACGGYTACCTTGTTACGACTT-3'). Amplification reactions were performed using MyTaq ™ HS Red Mix (Bioline, BIO-25048). About 1 µL (200 ng) DNA template and 1 µL of primers (200mM each) were added to 12.5 µL MyTaq HS Red Mix and Water (dH2O) up to 25 µL. The reactions were carried out in a thermocycler with the initial denaturation of 95°C for 1 minute. The PCR amplification was set for 35 cycles of 96°C for 15s, annealing for the 30s at 52°C, and extension for 45s at 72°C. Reaction products were checked for size and purity on 1% (w/v) agarose gels by electrophoresis (Figure 3). A total of 2.5 µL of 1 Kb ladder was used as a sample identification marker. PCR products were sent to First Base Laboratory, Selangor, Malaysia for Bi-directional sequencing methods.

Phylogenetic analysis

The chromatograms obtained from 1st BASE Malaysia were analyzed using the Applied Biosystem. We employed the MEGA X software (Kumar et al. 2018) with the maximum likelihood method to construct the phylogenetic trees using the Tamura 3 parameter model. Additionally, we utilized 1,000 bootstrap replicates to ensure the reliability of the trees. Next, to compare the complete nucleotide sequences from DNA isolates NP A5, NP A6, NP SB3, NP KR4, and NP KR7, we referred to the available nucleotide sequences of *Bacillus* sp. in GenBank. As an outgroup, we utilized *Vibrio parahaemolyticus* strain VC018 (MT534025.1). The DNA sequences have been deposited in GenBank with accession numbers as specified in Table 2.

The secondary metabolite of rice root endophytic bacteria by GC-MS

Secondary metabolite analysis was carried out using Gas Chromatography and Mass Spectroscopy (GC-MS) Shimadzu Type QP-2010 SE (Merck, USA), with MS SH-Rxi5Sil column, length 30 m, inner diameter 0.25 mm, with initial column temperature operating conditions of 80°C and final temperature of 300°C, injector temperature 128°C, detector temperature 280°C, Helium carrier gas, ionizing type EI (Electron Impact), sample volume injected was 0.1 µL. Computer-assisted compound identification was performed by Wiley Library 229, NIST 12, and NIST 62 software. GC-MS mass spectrum interpretation was performed using the National Institute of Standards and Technology (NIST) database having more than 62,000 patterns. The unknown components' mass spectra were compared to those of known components stored in the NIST library (Nas et al. 2021).

The potential of rice root endophytic bacteria to control sheath blight of rice in the screen house

The endophytic bacterial assay was used to control sheath blight in the screen house. It was arranged using a Randomized Completely Block Design (RCBD), 6 treatments, 4 replications, and 3 polybags per unit. The treatment is as follows: Control: without endophytic bacteria, only inoculated with *R. solani*; NP A5: inoculated with *R. solani* + NP A5 of endophytic bacteria application; NP A6: *R. solani* + NP A6 isolate; NP KR4: *R. solani* + NP KR4 isolate; NP KR7: *R. solani* + NP KR7 isolate; NP SB3: *R. solani* + NB SB3 isolate. *R. solani* on the PDA is crushed, then with a syringe, it is inoculated into the sheath 28 days after planting (Park et al. 2008). The endophytic bacteria were applied four times, i.e., in seed soaking and spraying on rice sheath at 20, 30, and 40 days after planting at the density of population 10^8 cfu/mL. The observed variables were incubation period, disease intensity, infection rate, and effectiveness of biocontrol. The incubation period was observed from the inoculation of *R. solani* until the first symptom appeared. The first symptom was marked by the presence of spots, especially on the colored sheath, reddish brown to white-gray with brown edges. The disease intensity was observed at 5 days interval with asses the percentage of sheath infections and then was calculated with the formula according to (Asmaliyah et al. 2016).

$$DI = \frac{\Sigma (n_i . v_i)}{N.V} \times 100\%$$

Where: *DI*: disease intensity; n_i: number of plants with the certain symptom; v_i: the certain scale of symptom category; *N*: number of plants; *V*: the highest scale of the symptom category. The symptom category based on a percentage of sheath infection 0: no symptom; 1: 1-20% of sheath infected; 2: 21-41% of sheath infected; 3: 41-60% of sheath infected; 4: 61-80% of sheath infected, and 5: 81-100% of sheath infected.

The infection rate was calculated based on the conversion of disease intensity to X as a proportion of the infected plant tissue according to Van der Plank (1963) with the formula:

$$r = \frac{2,3}{t}\left\{\log\frac{1}{(1-X_t)} - \log\frac{1}{(1-X_0)}\right\}$$

Where: The infection rate (r), the time interval of observation (t), the proportion of infected plant tissue at early times (Xo), different times (Xt), and the effectiveness of treatments on sheath blight disease intensity were calculated based on disease intensity compared to the control treatment.

The data was analyzed using Analysis of Variance (ANOVA), and significant results will be followed by Duncan's Multiple Range Test (DMRT) at a 5% error level.

RESULTS AND DISCUSSION

The present study revealed that among 22 isolates of rice root endophytic bacteria from suboptimal land, five isolates, namely NP A5, NP A6, NP KR4, NP KR7, and NP SB3, showed an inhibitory reaction to *R. solani* on PDA medium due to an antibiosis mechanism (Table 1; Figure 2).

Molecular characteristics of rice root endophytic bacteria

Figure 3 presents the visualization of DNA banding for endophytic bacteria NP A5, NP A6, NP SB3, NP KR4, and NB KR7. Gel electrophoresis demonstrated that all five samples produced single bands at sizes above 1,000 bp, with similar banding patterns indicating successful amplification. Sequence assembly of the PCR products revealed that all endophytic bacteria have around 1,435 - 1,445 bp lengths. Blast analysis showed that the isolates have over 90% similarity with Bacillus strains in GenBank. All identified endophytic bacteria were submitted to GenBank for accession numbers (see Table 2).

Figure 4 interprets the relationship between the five endophytic *Bacillus* isolates and the *Bacillus* in GenBank. *Bacillus* isolates NP KR4 MZ 440886 and NP KR 7 MZ 44087 are in the same clade. This means that they are similar. Both, together with *Bacillus* isolate NP A5 MZ 440883, are in the same group, Bacterium strain BS 0464 MK 823652, with 92% similarity. They differ from *Bacillus* isolate NP A6 MX 440884, which has similarities with *Bacillus subtilis* strain YT2 HQ 143571. Meanwhile, *Bacillus* isolate NP SB3 MZ440885 showed high similarity with *B. subtilis* subsp. *stercoris* EG 1265 MN704551.

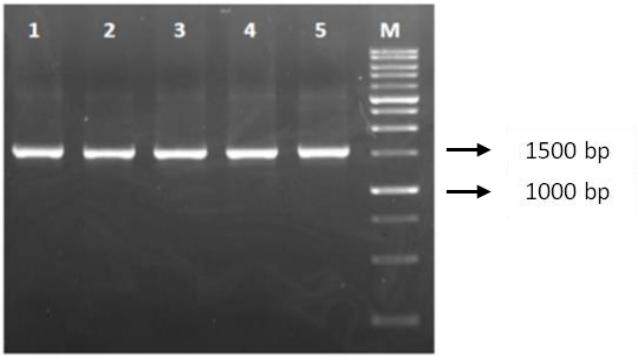

Figure 3. The DNA banding visualization of endophytic bacteria (NP A5, NP A6, NP SB3, NP KR4, NP KR7) exhibited amplification above 1,000 bp. The PCR products were evaluated via electrophoresis using 0.8% TBE agarose. The 1 Kb ladder (loaded 2.5 uL) revealed isolate NP A5 as band number 1, isolate NP A6 as band number 2, isolate NP SB3 as band number 3, isolate NP KR4 as band number 4, and isolate NP KR7 as band number 5

Table 1. Assessment of endophytic bacteria antagonism against *Rhizoctonia solani* rice pathogens

Treatments	Inhibition (%)	Mechanism of inhibition	Malformation of the mycelium
Control	0	-	-
NP A5	36.04±6.30 c	Antibiosis	Lysis
NP A6	39.66±9.22 ab	Antibiosis	Lysis
NP KR4	46.00±6.25 a	Antibiosis	Swelling
NP KR7	35.69±5.75 c	Antibiosis	Swelling
NP SB3	39.92±4.89 ab	Antibiosis	Swelling

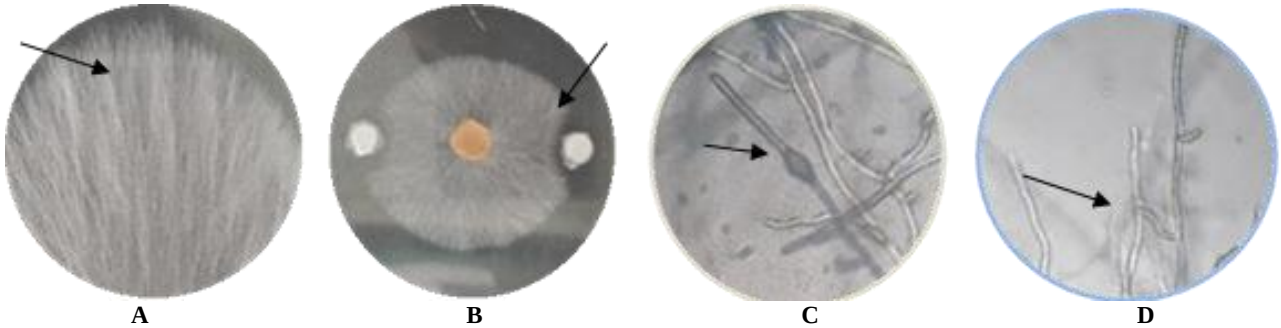

Figure 2. The inhibition mechanism of endophytic bacteria against *Rhizoctonia solani*: A. Impeding the growth of mycelium normally; B. Conducting an inhibition assay; C. Inducing the swelling of hyphae; D. Prompting the lysis of hyphae

Table 2. Accession numbers for the identified endophytic bacteria

Endophytic bacteria isolates	Identified bacteria and accession number
NP A5	*Bacillus* isolate NP A5 MZ440883
NP A6	*Bacillus* isolate NP A6 MZ440884
NP SB3	*Bacillus* isolate NP SB3 MZ440885
NP KR4	*Bacillus* isolate NP KR4 MZ440886
NP KR7	*Bacillus* isolate NP KR7 MZ440887

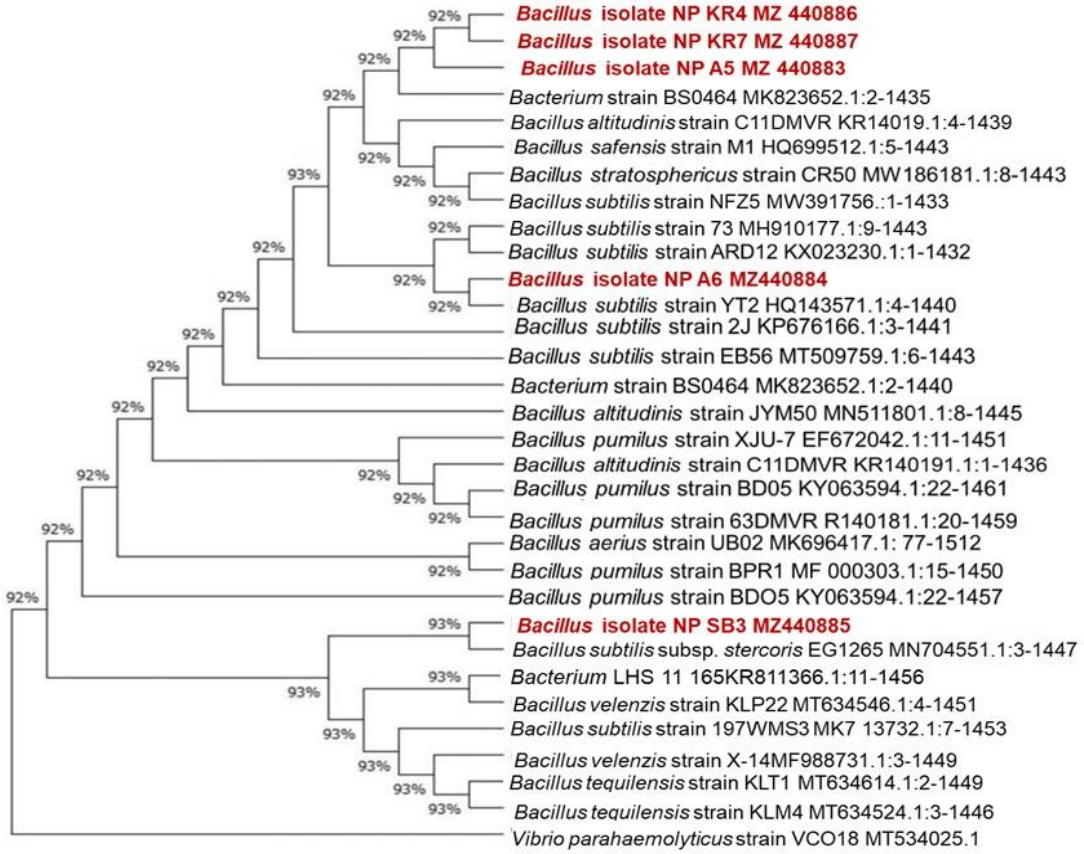

Figure 4. A phylogenetic tree was constructed to analyze the relationship between five endophytic *Bacillus* targets: *Bacillus isolates* NP A5, NP A6, NP SB3, NP KR4, and NP KR7. The Maximum Likelihood method and Tamura 3-parameter model were utilized to infer the evolutionary history. The tree branches display the percentage of trees in which the associated taxa clustered together, providing valuable insights into their evolutionary relationships. The evolutionary analyses were performed using the powerful software MEGA X (Kumar et al. 2018)

The secondary metabolite of rice root endophytic bacteria by GC-MS

The secondary metabolites of five endophytic bacteria were analyzed, revealing varying amounts of compounds detected for each bacterium. NP A5 yielded 312 compounds, NP A6 yielded 301 compounds, NP KR4 yielded 310 compounds, NP KR7 yielded 301 compounds, and NP SB3 yielded 295 compounds. The key antimicrobial compounds from each endophytic bacterium were chosen based on their peak area percentages. The GC-MS analysis results for several compounds are presented in Table 3.

The potential of rice root endophytic bacteria to control sheath blight of rice in the screen house

The control of rice sheath blight showed effectiveness by endophytic bacteria isolates. It means the endophytic bacteria can penetrates in plant to induce the resistance against pathogen infection. The effectiveness more than 50% and NP KR4 is the high effective at 58.74%. The NP KR4 isolate, which belongs to the same clade as isolate *Bacterium* strain BS0464 MK823652 and *B. altitudinis* strain C11DMVR KR14019, proved to be highly effective in controlling sheath blight disease in rice. The results showed a remarkable effectiveness of 58.74% (Table 4, Figure 5).

Table 3. Analysis of secondary metabolites from endophytic bacteria NP A5, NP A6, NP KR4, NP KR7, and NP SB3 isolates

Isolates	Retention time	Name of the compound	Peak area (%)	Number of compound	Compound class
NP A5	2.75	4-Acetoxy-1-methoxycarbonyl-2-methyl-3-phenylamino-1,2,3,4-tetrahydropyridine	3.47	4	Alkaloid
	3.81	Propionic acid, 2-[(2-dicyclohexyl carbamoyl cyclohexane carbonyl) (2-hydroxyethyl) amino] ethyl ester	3.65		Fatty acids
	4.47	ethyl E-4-decenoate	3.96		Fatty acids
	4.80	(4-Chloro-Phenyl)-Pyrrolidin-1-Yl-Methanethione	4.81		Alkaloid
	8.76	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)	3.19		Alcohol
	9.13	Phenol, 2,6-di(t-butyl)-4-(cyclohexanylidene)amino-	4.70		Fenol
	11.19	Cyclohexene-4,5-dicarboxylic acid, 1-[4,5-bis(ethoxycarbonyl)-2-methylene-1-pentyl]-	4.91		Fatty acids
	11.25	Tetrahydroaraucarolone	3.69		Alcohol
NP A6	2.72	Lyxitol, 1-thiooctyl-	9.14	2	Alcohol
	6.39	Estra-1,3,5(10)-triene-3,17-diol, 2-bromo-1-methyl-, bis(trifluoroacetate), (17.beta.)- (CAS)	8.72		Alcohol
	7.76	beta.-L-Altropyranoside, phenylmethyl 2,4-bis(acetylamino)-2,4,6-trideoxy-,3-acetate (CAS)	12.23		Fatty acids
	10.87	Methyl (E)-4-bromo-3-methoxy-2-butenoate	8.60		Fatty acids
NP KR4	4.13	Butanedioic acid, 2-benzyl-2-carboxyl-, triethyl ester	7.34	2	Fatty acids
	4.37	.Beta.-Cis-Methyl-Decenoate	9.15		Fatty acids
	5.65	2(1H)-Pyrimidinone, 4-amino-1-(4,5-dihydroxy-3-(hydroxymethyl)-2-cyclopenten-1-yl)-,(1R-(1-.alpha.,4-.beta.,5-.beta.))-	7.47		Alkaloid
	6.80	Octadecanoic acid, docosyl ester (CAS)	18.71		Fatty acids
NP KR7	4.02	2-[3-(4-Fluoro-Phenoxy)-5-Nitro-Phenylamino]-6-Hydroxymethyl-Tetrahydro-Pyran-3,4,5-Triol	7.03	2	Alcohol
	6.23	2H-Pyran-2-methanol, 3,4-dihydro-2,5-dimethyl- (CAS)	8.00		Alcohol
	10.19	Camptothecin	6.44		Alkaloid
NP SB3	10.82	Dimethyl 4-Oxoundecanedioate	9.13	2	Fatty acids
	11.56	4-(2-Amino-1,3-Thiazol-4-Yl)-1,2-Benzenediol	10.67		Alcohol
	12.68	Methyl 8,8-Dideuteriooctadecanoate	8.55		Fatty acids

Table 4. Control of rice sheath blight by rice root endophytic bacteria

Treatments	Incubation period (DAI)	Disease intensity (%)	Control effectiveness (%)	Infection rate (unit/day)
Control	2	32.62d	-	0.025
NP A5	2	16.86c	48.31	0.022
NP A6	2	15.67ab	51.96	0.021
NP KR4	2	13.46b	58.74	0.022
NP KR7	2	15.54ab	52.36	0.023
NP SB3	2	14.78ab	54.69	0.018

Note: DAI: days after inoculation

A **B**

Figure 5. A. The symptoms of sheath blight on untreated plants; B. Plants treated with endophytic bacteria

Discussion

The antibiosis mechanism of endophytic bacteria is shown by their produce secondary metabolites such as siderophore and chitinase, and the consortium of these endophytic bacteria can produce protease. Chitinase enzyme plays a crucial role in altering the mycelial structure, inhibiting five isolates of endophytic bacteria with an antibiosis mechanism. The malformation of mycelium in this research is swelling and lysis because the antibiosis effect of endophytic bacteria can alter the mycelial of *R. solani* morphology. The transformation of mycelium morphology in *Fusarium proliferatum* was observed in a study conducted by Khairah et al. (2023). Over seven days, crude enzyme and precipitate chitinase induced significant changes in the morphology of *F. proliferatum* mycelium. The crude enzyme effectively lysed the mycelium, while the precipitated chitinase caused vacuolization, swelling, and distortion of the *F. proliferatum* mycelium. The chitinase enzyme in bacteria also provides nutrition and parasitism mechanisms (Veliz et al. 2017).

The diversity of the five isolates was low, indicating no significant change in species due to environmental factors among the endophytic bacteria isolates from rice roots. It was showed by the Bacillus isolate NP A5 MZ440883 that was taken from different region with isolates NP KR4 MZ440886, and NP KR7 MZ440887, but showed the same molecular characteristics. The different type of soil, microclimate and other environment factors were not influence to those characters. But, the isolate NP A6 MZ440884 from the same region of NP A5 MZ440883 showed different character, it might be because of the differences rice varieties. This is in accordance to Ding and Melcher (2016) who stated that the characteristics of the plant host species are the primary determinant of endophytic bacterial populations. The isolate NP SB3 MZ440885, also exhibited highly different molecular properties from other isolates. This lends even more credence to the idea that variations in variety impact character differences.

GCMS analysis results revealed that endophytic bacteria contain alkaloids, phenols, alcohols, and fatty acids as their primary secondary metabolites. Microbial activity that produces flavonoids is known to cause leakage of cell membranes, interference in biofilm growth, and suppression of pathogens growth (Hochma et al. 2021). These compounds possess antimicrobial properties, supporting the antibiosis mechanism of endophytic bacteria, and if they are applied to plants, they increase plant resistance to biotic and abiotic stress environments. This phenomenon is supported by Kushalappa et al. (2016) research that can induce regulatory and resistance metabolite and protein biosynthetic genes (R) to reduce pathogen advancement through their antimicrobial and cell wall enforcement properties. The properties of endophytic bacteria and other microbes range from an improved defense against disease and antimicrobial pathogens to antioxidant, anti-inflammatory, and insecticidal, to the promotion of plant growth through improved nutrient acquisition and stress tolerance (Watts et al. 2023). In the control of sheath blight, the application of an antagonist *Trichoderma harzianum* UBSTH-501 and *Pseudomonas fluorescens* PF-08 as biopriming the rice plant growth and induce the resistance to *R. solani* (Singh et al. 2016). *In vitro* studies demonstrated that the NP KR4 isolate exhibited an impressive inhibition rate of 46.00 ± 6.25% against rice sheath blight disease, making it the top antagonist (Table 1). NP KR4's isolate may be particularly effective at suppressing bacterial or fungal pathogens due to production of antimicrobial compounds, competitive exclusion. this can be due to faster growth rates, more efficient resource utilization, or the ability to colonize and establish in a niche more effectively than pathogens. The isolate might produce specific antimicrobial compounds such as antibiotics, bacteriocins, or lytic enzymes that directly inhibit or kill other pathogenic bacteria. Furthermore, NP KR4's production of siderophores surpassed that of other isolates, indicating its potent activity (Prihatiningsih et al. 2021; Djatmiko et al. 2023). Siderophore production positively influences plants by increasing iron uptake and encouraging rhizobacteria to colonize plant roots (Shanmugaiah et al. 2015). The compound of alkaloids, phenols, alcohol, and fatty acids supports plant resistance to infecting pathogens so that the plant is prevented from pathogen attack and becomes resistant. Additionally, studies by Jamali et al. (2020) highlighted the diverse antifungal properties of *Bacillus* sp., including bacylisin, surfacin, subtilin, bacyllomicin, iturin, and fengycin. Through their ability to obstruct fungal penetration in plant tissues, endophytic bacteria significantly alter disease symptoms compared to control plants (Marwan 2017). The potential of these five root endophytic bacteria of paddy isolates as bio-fungicide formulations holds great promise for sustainable rice production (Kumar and Dara 2021; Morales-Cedeño et al. 2021; Rana et al. 2023).

In conclusion, the *in vitro* study of NP KR4 isolate revealed an impressive 46.00 ± 6.25% inhibition while standing on the same clade as the highly effective isolate *B. altitudinis* strain C11DMVR KR14019, showing a 58.74% control of sheath blight disease. The five endophytic bacteria, identified as *Bacillus* sp., exhibit promising characteristics such as white colony, rod-shaped cell, Gram-positive, and catalase-positive. Notably, NP A5, NP KR4, and NP KR7 isolates also resemble *B. subtilis* strain 2JKP676166, with a few base pair differences. At the same time, NP A6 is closely related to *B. subtilis* strain YT2HQ143571, and NP SB3 is identified as *B. subtilis* subsp. *stercoris* strain EG1265MN704551. These endophytic bacteria can secret diverse secondary metabolites, such as alkaloids, phenols, alcohols, and fatty acids.

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