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# Potential of Endophytic Bacteria from Rambusa Plant as Disease Control and Growth Promoters of Samarinda White Pepper (*Piper nigrum* L.)

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Abstract. Stem base rot (SBR) disease caused by Phytophthora capsici Leonian is associated with significant yield loss. Meanwhile, wild passion fruit (Passiflora foetida L.) interacts with bacteria that have potential to control SBR disease. Therefore, this research aimed to examine potential of endophytic bacteria from wild passion fruit to control SBR disease in pepper plant and the role as growth promoters. A total of 12 endophytic bacteria isolates were tested for potential to promote growth and biocontrol, focusing on the ability to produce the phytohormone IAA, dissolve phosphate, fix nitrogen, as well as produce cellulase and protease enzymes. The three best isolates were then tested on pepper cuttings (Var. Malonan I). The results showed that the highest disease severity was found in control at 40.74% (moderately susceptible), followed by isolate KSA 01 at 37.03%, and SIB 01 at 33.33% (resistant). Disease severity in isolate KPA 03 was significantly lower at 7.41% (very resistant). The highest disease incidence occurred in control and KSA 03 at 44.44%, while the lowest occurred in KPA 03 and SIB 02 at 33.33%. Isolate KPA 03 produced the highest plant height increase (39.87 cm) and the highest number of leaves (5.78), although it was not significantly different from isolate SIB 02. This research showed that endophytic bacteria from wild passion fruit could effectively control SBR disease and improve pepper plant growth. Keywords: Pepper; wild passion fruit; endophytic bacteria; stem base rot.

Type of the Paper: Regular Article.

# 1. Introduction

Pepper is a world spice crop with a high export value according to current reports [1]. East Kalimantan is recognized as a white pepper (*Piper nigrum* L.) production center. However, this plant, also known as Malonan I, is easily attacked by stem base rot (SBR) disease [2]. *Phytophthora capsici* fungus is the most dangerous disease that causes stem rot disease in East Kalimantan [3]. Disease causes death in a relatively short time and spreads to other surrounding plant between one to two months [4]. The use of copper-based fungicides is commonly recommended for SBR control [5], but synthetic fungicides can pollute the environment and the residues left behind are permanently harmful to health [6].

The use of biological agents is an environmentally friendly method to control fungal populations, with endophytic bacteria being an effective option [7]. Endophytic bacteria are found

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in plant [8], playing a crucial role as a pathogen controller and growth promoter, thereby increasing production [7,9,10]

According to [11], wild passion fruit can grow around rivers, under dry forest canopies, on roadsides, and around houses in villages, showing that plant has high self-protection [12]. Plant tissue is a habitat for endophytic bacteria, providing protection from pathogens and enabling tolerance to various environmental stresses. Therefore, this research aimed to determine potential of endophytic bacteria found in wild passion fruit plant, focusing on the power to control SBR disease in pepper plant and the ability as growth promoters.

#### 2. Materials and methods

### 2.1. Research implementation

This research was carried out at the Agronomy Laboratory of Plantation Crop Cultivation of Politani Samarinda for four months from July to October 2023. Materials used were Potato Dextrose Agar (PDA), NaOCl, alcohol (70%), methylated spirits, tissue, labels, plastic wrap, distilled water, wild passion fruit plant, and Malonan I variety pepper seeds. In addition, the tools used were standard bacteria research tools, a digital camera, and tweezers.

Wild rambusa plant were used in six different sub-district locations in Samarinda City including Samarinda Ulu, Sungai Kunjang, Samarinda Seberang, North Samarinda, Samarinda Ilir, and Palaran sub-district. The roots, stems, and leaves were extracted, resulting in a combination of  $6 \ge 3 = 18$  samples.

#### 2.2. Isolation characterization and test

Bacteria colonies obtained were isolated, characterized, and then tested for the ability to synthesize Indole Acetic Acid (IAA) and produce phosphate enzymes, cellulose, and protease, as well as N fixation [13].

The selected bacteria were tested for antagonism against the pathogen (dual antagonist). Data obtained from the antagonist test were analyzed using a completely randomized design then followed by the least significant difference of 5% when the effect was significant. The best three bacteria obtained were suspended in pepper seedlings from seven internode cuttings, Petaling I varieties, and control. Each level was repeated nine times, resulting in a total of 36 seedlings.

As the initial step, endophytic bacteria isolates were suspended into pepper seedlings that had been grown for four weeks. After the administration, the fungal pathogen *P. capsici* was inoculated on pepper seedlings at 40 mL/polybag. Seedlings were maintained and observed for eight weeks after pathogen treatment. Observations included the level of disease attack and plant growth. Meanwhile, pepper growth data were evaluated from plant tallness and leaves number followed by analysis using a completely randomized design statistical test. Further test was carried

out for the smallest significant difference at 5%.

### 3. Results and Discussion

A total of 18 wild passion fruit plant extract samples produced colony isolates of endophytic bacteria, reaching 54 types. Furthermore, the isolates were characterized and grouped according to color criteria and morphological characteristics [14]. Morphological characterization includes the size of the colony (small, large, and moderate), as well as shape (round, irregular with wavy edges, curved notched, elevation in the form of raised, flat, or convex colony color white, milky white and transparent) [15,16].

According to the differences in colony and cell morphology characterization, 12 different isolates were selected to be tested for microbial potential as biological control agents and phytohormones. The test results on the ability of endophytic bacteria to secrete hormones and function as biological control agents are shown in Table 1.

Isolates Code	IAA synthesis		Dhaanhata	N -	Enzymes	
	Absorbance	IAA (ppm)	Phosphate soluble (cm)	fixation	Cellulase (cm)	Protease (cm)
SIB-02	0.511	28.38	1.6	++	3.1	-
SULB-03	0.433	22.00	-	++	2.6	-
KSA-04	0.431	21.88	-	+	3.4	1.1
KPA-01	0.527	29.68	1.2	+++	2.4	-
KPA-02	0.457	23.99	1.1	++	2.4	-
KPB-05	0.445	23.02	1.2	+	2.5	-
KPA-03	0.463	24.48	1.1	++	3.6	1.3
KPD-05	0.405	19.76	1.1	+	3.0	-
KSA-03	0.469	24.97	1.2	+++	2.9	1.1
SID-02	0.423	21.23	1.4	-	2.9	-
SIA-01	0.446	23.10	1.4	+	2.9	-
SULA-01	0.449	23.34	1.3	++	2.1	-

Note: + (a bit murky), ++ (cloudy), +++ (very cloudy)

### 3.1. IAA Phytohormone Producer

The pink color produced by endophytic bacteria showed the secretion of the IAA hormone. The highest IAA levels in endophytic bacteria cultures of wild passion fruit plant were found in KPA-01 at 29.68 ppm while the lowest was produced by KPD-05 at 19.76 ppm. IAA is a natural auxin that functions to stimulate cell division and development [17]. In general, phytohormones are natural substances capable of stimulating the growth of plant [18] and these hormones are produced by endophytic bacteria in plant [19,20].

### 3.2. Phosphate Solvent

The ability of endophytic bacteria to dissolve phosphate, known as the phosphate

solubilization index, was evidenced in the clear area around the colony. Among the 12 bacteria isolated, only two were unable to dissolve phosphate, namely SULB-03, and KSA-04. The isolate that had the highest ability was SIB-02 with a halo diameter range of 1.6 cm. The difference in the ability of each isolate is attributed to variations in the physiological and biochemical characteristics. The ability of endophytic bacteria to dissolve phosphate varies, depending on the strain [21,22]. In general, these bacteria play a role in dissolving phosphorus in the soil, thereby increasing soil fertility [23].

# 3.3. N fixation

The N fixation ability of bacteria is characterized by the turbidity level of the burk salt media. The higher the level of media turbidity, the greater the ability of bacteria to fix N. As shown in Table 1, endophytic bacteria isolated from wild passion fruit plant showed the ability to fix N with different levels. The highest N fixation ability was obtained in isolates KPA-01 and KSA-03 (very cloudy), while SID-02 was unable to fix N. According to previous research, rhizobacterial isolates have the ability to not only dissolve phosphate but also fix nitrogen [23,24]. Bacteria that act as PGPR play an essential role in biological N fixation from the air [19,20].

## 3.4. Cellulase

Cellulase enzyme-producing bacteria were characterized by the appearance of a clear zone around the microbial colony on carboxymethyl cellulose (CMC) media. The ability to produce cellulase was considered significant when the area appeared larger. As shown in Table 1, all endophytic bacteria isolates from wild passion fruit were able to produce the cellulase enzyme. The highest ability was obtained in isolate KPA-03 at 3.6 cm, showing that endophytic bacteria could produce cellulase enzyme. In general, cellulase enzyme activity is shown by the presence of a yellow halo zone produced by the isolate. The analysis results showed that the isolates produced cellulase enzymes up to 2.1-3.6 U/mL. [14]. Metabolized compounds (metabolites) from endophytic bacteria can induce a process of plant resistance to pathogens, reduce the attack of pathogen infection, and function in disease control [25].

## 3.5. Protease

The presence of proteolytic bacteria was shown by a clear zone around bacteria colonies on Skim Milk Agar (SMA), and the larger the clear zone, the more productive bacteria. The protease enzyme produced can hydrolyze peptide bonds between amino acids that make up the polypeptide chains of proteins [26]. Laboratory results showed the enzyme was only produced by three isolates, namely KSA-04, KPA-03, and KSA-03. Both enzymes (cellulase and protease) produced by the isolate act as a builder inhibition for growth and development of pathogens. The inhibition test against *P. capsici* Leonian disease was further carried out using data from bacteria potential test.

### 3.6. Disease Control

Endophytic bacteria isolates that produced the widest inhibition zone were identified morphologically and subjected to biochemical tests. The inhibition zone was estimated by measuring the clear zone formed around endophytic bacteria isolate capable of producing extracellular compounds that can inhibit growth of fungi (anti-fungal) [14,27,28].

Based on the results, among the 12 isolates, only three showed antibacterial activity. According to previous research, endophytic bacteria found in plant contain chemical compounds/metabolites that inhibit or suppress the development of pathogenic fungi. These bacteria can also function in biocontrol against diseases in plant [29–33].

Hidayat et al. [34] proved that endophytic fungi derived and stems of wild passion fruit plant acted as antagonistic agents of *Fusarium* sp. In this research, the antagonistic test was carried out against *P. capsici* Leonian using a dual test (dual culture) on a petri dish containing PDA media.



Fig. 1. Antibacterial test against the pathogen P. capsici Leonian

**Table 2.** Further Test of Least Significant Difference at 5% level of inhibition of endophytic bacteria against *P. capsici* Leonian

Isolates Code	Inhibition (%)
SIA-01	13.76 cd
SIB-02	16.28 bc
SID-02	0.00 e
SULA-01	0.00 e
SULB-03	11.15 d
KPA-01	14.38 bc
KPA-02	13.64 cd
KPA-03	16.59 b
KPB-05	13.69 cd
KPD-05	0.00 e
KSA-03	19.93 a
KSA-04	11.46 d

The antibacterial test result of endophytes growing wild passion fruit against the fungus *P. capsici* Leonian is shown in Fig. 1. The zone of inhibition formed in endophytic bacteria is greater than that of the *P. capsici* fungus, suggesting endophytic bacteria are anti-fungal pathogens according to Kusumawati et al. [35]. Secondary metabolites produced by endophytic bacteria create a barrier zone that inhibits fungal activity. Based on the completely randomized design, the inhibitory power of endophytic bacteria has a significant effect on *P. capsici* Leonian.

Consequently, the least significant difference test at 5% was carried out and the results are shown in Table 2.

As shown in Table 2, isolates KSA-03, KPA-03, and IB-02 had the highest inhibitory power. Therefore, these three isolates were tested on pepper seedlings and the observations include plant growth and the level of disease attack. The level of treatment was designated as S0 = without treatment, S1 = KSA-03, S2 = KPA-03 and S3 = SIB-02.

#### 3.7. Disease Inhibition in Pepper

The initial symptoms of SBR include root and crown rot, sudden wilting of the leaves, reduced growth, and death without yellowing of the leaves. Symptoms on the leaves are characterized by circular rot with brown margins and necrotic centers [36].

Disease symptoms were observed every week until week eight and the observations include:

- S0 = no damage to five seedlings, heavy attack to one seedling, very heavy attack to three seedlings,
- S1= no damage totaling five seedlings, light attack one seedling, heavy attack one seedling, and very heavy attack two seedlings
- S2 = no damage totaling seven seedlings, light attack two seedlings,
- S3 = no damage six and very heavy attack three seedlings

As shown in Table 3, the lowest disease symptoms were found at the S2 treatment level, which was given endophytic bacteria KPA 03 (Palaran District from the roots). According to Djaya et al., Jana et al., Mohammed et al., and Ouf et al. [37–40], biological agents derived from endophytic bacteria increased resistance to biotic and abiotic diseases in plant.

Isolates Code	Disease severity (%)	<b>Resilience category</b>	Disease incidence (%)
S0 (without bacteria)	40.74	Somewhat vulnerable	44.44
S1 (KSA 01)	37.03	Somewhat resistant	44.44
S2 (KPA 03)	7.41	Very resistant	33.33
S3 (SIB 01)	33.33	Somewhat resistant	33.33

 Table 3. Disease severity and incidence

The lowest disease incidence was obtained at the S2 treatment level which amounted to 7.41% falling into the highly resistant category. Meanwhile, the highest was obtained at the S0 treatment level with a value of 40.74% falling into the somewhat vulnerable category. One way to suppress SBR disease is by increasing plant resistance. This resistance can be obtained by inducing nonpathogenic biotic agents, through the administration of endophytic bacteria [41]. Previous research reported that endophytic bacteria from wild passion fruit plant showed inhibitory effects on microorganisms [42], playing a crucial role in disease control [11].

## 3.8. Pepper plant growth

Pepper plant growth can be enhanced by the availability of nutrients and the help of other microorganisms such as endophytic bacteria. These bacteria increase plant development, growth,

and yield, while also inhibiting growth, contaminating pathogens, dissolving bound phosphate, and binding nitrogen [41,43] Furthermore, bacteria produce important substances such as antioxidants, anti-inflammatory, antibacterial, and others. Pigments such as carotenoids ( $\beta$ carotene) are produced by endophytic bacteria [44]. The carotenoids along with chlorophyll function in photosynthesis and radiation protection [45].

Rhizobacteria in the roots of plant can assist in the absorption of nutrients from the soil. Inoculation of rhizobacteria contributes up to 20-50% of the total Nitrogen needs from the N fixation process [46]. The enzyme nitrogenase, commonly found in nitrogen-fixing bacteria, effectively fixes and converts nitrogen in the air into ammonia [47].

Phytohormones are very important during the formation and vegetative growth of plant, one of which is IAA. This phytohormone plays important roles in the process of cell division and elongation, differentiation, tropism, apical dominance, and abscission [48]. Endophytic bacteria are among the producers of phytohormones, facilitating the absorption of nutrients, thereby contributing to plant enlargement even in less fertile environments [49,50]

Plant growth is also influenced by endophytic bacteria that can fix airborne  $N_2$  [51] as well as phosphate solubilizers [52,53]. Endophytic bacteria that can dissolve phosphate are very helpful in providing elemental phosphorus in the form of solutions to plant [54,55] Based on the 5% completely randomized design test, endophytic bacteria had an insignificant effect on height gain at 30 days and a very significant effect at 60 days as well as increased number of leaves at 30 days and 60 days. Therefore, the LSD test at 5% significance level was carried out and the results are shown in Table 4.

Treatments Level	Plant height (60 days)	Number of leaves (30 days)	Number of leaves (60 days)
S0	8.62 b	0.44 b	2.00 b
S1	16.80 b	0.56 b	2.22 b
S2	39.87 a	1.67 a	5.78 a
S3	26.41 ab	1.11 ab	3.33 ab

 

 Table 4. Least Significant Difference Test at 5% in Endophytic Bacteria and the Effect on Height and Number of Leaves of Pepper Seedlings

Mean numbers followed by the same letter show no significant difference at 5% alpha level

The highest increase in plant height 60 days after treatment was obtained in S2 (KPA 03) at 39.87 cm but was not significantly different from S3 (SIB 02). Furthermore, the highest increase in the number of leaves 30 days after treatment was found in S2 (KPA 03) at 1.67 strands but not significantly different from S3 (SIB 02). The highest increase in the number of leaves 30 days after treatment was obtained in S2 (KPA 03) at 5.78 strands but not significantly different from S3 (SIB 02). The appearance of disease symptoms on the leaves and stems of pepper occurred after substantial damage to the roots [5].

### 3.9. Location Influence

Based on the results, isolates from the Palaran sub-district location had the best number of endophytic bacteria for disease control and pepper plant growth. This is presumably because the Palaran area has not experienced many ecosystem changes, and soil conditions are still considered virgin. In the research by Cho et al. [56], endophytic bacteria isolated from three different areas/regions showed significant diversity based on plant species. Hallmann et al. [57] stated that crop rotation factors and soil conditions influence the population structure of bacteria. Another factor according to Sudewi et al. [58] is the presence of phytopathogens from the location of endophytic bacteria collection.

The results also proved that the production of IAA varied depending on the type of isolate and collection location. The number and presence of endophytic bacteria also depend on the type of plant, soil properties, organic matter, geographical distribution, and sampling time as well as pesticides [14,59].

## 3.10. Bacteria Origin of Plant Tissue

Based on the origin, the roots are the best endophytic bacteria from wild passion fruit plant. Lodewyckx et al. [60] found that roots were the entry point through wounds that occur naturally or via root hairs. Endophytic bacteria from plant roots are better at inducing growth due to the complex interaction with root tissues [61]. Purwanto et al. [62] stated that endophytic bacteria enter plant through the roots, while Zinniel et al. [63] reported a large abundance in the roots but little in the leaves and stems. Therefore, the appearance of these bacteria depends on which part of plant is taken [59].

### 4. Conclusions

In conclusion, isolates collected from the Palaran Sub District had the best number of endophytic bacteria for disease control and pepper plant growth. The application of endophytic bacteria treatment from wild *P. foetida* plant effectively inhibited *P. capsici* as shown by the invitro test. Apart from increasing growth of pepper seedlings, these bacteria also control stem rot disease caused by *P. capsici* Leonian. This research can be applied to pepper plant in the field, as well as other seeds and plantations.

### Abbreviations

- CMC Carboxymethyl Cellulose
- IAA Indole Acetic Acid
- KPA Kecamatan Palaran Akar
- KPB Kecamatan Palaran Batang
- KPD Kecamatan Palaran Daun
- KSA Kota Samarinda Akar

- LSD Least Significant Difference
- PDA Potato Dextrose Agar
- PGPR Plant Growth Promoting Rhizobacteria
- SIA Samarinda Ilir Akar
- SIB Samarinda Ilir Batang
- SID Samarinda Ilir Daun
- SBR Stem Base Rot
- SMA Skim Milk Agar
- SULA Samarinda Ulu Akar
- SULB Samarinda Ulu Batang

## Data availability statement

Data will be shared upon request by the readers.

## **CRediT** authorship contribution statement

NH: Conceptualization, Formal analysis, Methodology, Funding acquisition, Validation, Supervision, Writing – original draft, LM: Data curation, Investigation, Methodology, Writing – reviewing and editing, TRA: Visualization, Writing – review and editing, F: Resources, Supervision, FSDM: Investigation, D: Investigation, Validation, RA: Investigation and SW: Investigation.

# **Declaration of Competing Interest**

The authors declare no competing interest.

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