ENDOPHYTIC BACTERIA: INDUCED SYSTEMIC RESISTANCE ENHANCES BANANA DEFENSE AGAINST BUNCHY TOP DISEASE

Yasmin Suswantini, Ghani Husni Fata, Dina Istiqomah, Dumaris Priskila Purba, Nurtiati, Ruly Eko Kusuma Kurniawan, Ruth Feti Rahayuniati*

Agrotechnology Department, Faculty of Agriculture, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia

*Email: ruth.rahayuniati@unsoed.ac.id

Abstract. Bunchy top virus is a pathogenic agent that drives banana bunchy top disease, causing yield losses that can reach up to 100% within 21.6 days post-infection. The use of endophytic bacteria to induce systemic resistance has the potential to control this virus. This study evaluated the efficacy of four endophytic bacteria i.e. *Bacillus velezensis*, *Serratia* sp., *Enterobacter* sp., and *Stenotrophomonas* sp., for their capacity to elicit systemic resistance in two banana cultivars (*Kepok* and Cavendish) under laboratory and greenhouse conditions. A factorial completely randomized design was employed, with two factors: banana cultivar (*Kepok*, Cavendish) and bacterial treatment (*B. velezensis, Serratia sp., Enterobacter sp., Stenotrophomonas sp*.) plus two non-inoculated controls, each with four replications. Data were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at $\alpha = 0.05$ to identify significant differences. Key findings show that *Enterobacter* sp. and *Stenotrophomonas* sp. significantly reduced disease severity index (DSI) to 10.4% and the area under the disease progression curve (AUDPC) to 21.8 in the Cavendish cultivar. Across cultivars, endophytic bacteria elevated levels of defense-related secondary metabolites, including phenolics, peroxidase activity, saponins, and tannins, indicating induced systemic resistance. Collectively, *B. velezensis, Enterobacter sp.,* and *Stenotrophomonas* sp. effectively promote systemic resistance in banana via enhanced accumulation of these secondary metabolites.

Keywords: Kepok, Cavendish, endophytic bacteria, systemic resistance, banana bunchy top disease

1. Introduction

Banana production in Indonesia increased from 9.3 to 9.69 million tons between 2023 and 2024 [1]. Indonesia exhibits a diverse set of banana cultivars, including Cavendish and *Kepok*, which differ in their characteristics. This diversity presents several challenges, notably the threat of plant pathogenic infections.

Banana bunchy top disease (BBTD) is a major viral disease caused by the bunchy top virus. Transmission occurs via vectors, notably aphids (Pentalonia nigronervosa Coquerel). Symptoms include dwarfing, yellowing of leaves, and reduced fruit quality, with reported yield losses up to 100% within approximately 21.6 days post-infection [2]. Urgent management is required to prevent declines in productivity.

Traditional disease management often relies on chemical insecticides to control vectors. However, chemical controls can have adverse environmental impacts and may contribute to pest resistance. In this context, microbial endophytes offer a potentially environmentally friendly alternative. Prior work indicates that endophytes can colonize both the same host and other hosts [3], with notable endophytic bacteria identified in banana roots and stems of *Ambon, Tanduk*, and *Kepok* cultivars.

Four endophytic isolates i.e., *Bacillus velezensis*, *Serratia* sp., *Enterobacter* sp., and *Stenotrophomonas* sp., were selected for their potential to control these viruses. *Bacillus velezensis* has been reported to promote plant growth and produce secondary metabolites that counteract pathogens [4]. *Serratia* sp. endophytes may enhance plant stress tolerance [5]. *Enterobacter* sp. can degrade herbicidal compounds in vitro [6], and *Stenotrophomonas* sp. endophytes exhibit endosphere colonization and enhancement of plant metabolism [7]. These isolates may induce systemic resistance when applied via root uptake from liquid media prior to viral infection. Therefore, this study evaluates the efficacy of four endophytic bacteria, *B. velezensis, Serratia* sp., *Enterobacter* sp., and *Stenotrophomonas* sp., in inducing systemic resistance in two banana cultivars, *Kepok* and Cavendish.

2. Methods

2.1 Location and Sample Collection

The study was conducted at the Screenhouse and Plant Protection Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman, Purwokerto. Banana seedlings were collected and transplanted from the acclimation medium into the mixture planter medium. Prior to transplantation, the seedlings underwent more than two weeks of acclimation in the screenhouse to adapt to the new environment. The planting medium was prepared by mixing soil, husk, and manure in a 1:1:1 ratio [8], dispensed into 20 × 30 cm polybags, and labeled with treatment codes on name tags.

2.2 Aphids Rearing

Aphids were collected from banana plants in Banyumas and Cilacap regencies, identified as potential viruliferous sources, and transferred to healthy banana seedlings using a wet, soft brush. Rearing followed the aphid life cycle from initial collection through the end of the first generation [9]. Subsequently, nymphs were transferred to additional healthy seedlings to obtain non-viruliferous aphids. Non-viruliferous aphids served as the negative control for evaluating the efficacy of endophytic bacteria against banana bunchy top virus.

2.3. Endophytic Bacteria Efficacy Assay

Endophytic bacteria application began with isolate propagation and quality control through total plant count to obtain the stock for treatment. Four isolates of endophytes were inoculated into NB and shaken at 150rpm for 48 hours. After propagation, the isolates were checked through total plate count, which ranged from 10^8 to 10^{10} CFU/ml. The application was designed by a factorial Completely Randomised Design (CRD), with two banana cultivars (P1: Cavendish and P2: *Kepok*) for the first factor and endophytic bacteria (B1: *B. velezensis*, B2: *Serratia* sp., B3: *Enterobacter* sp., and B4: *Stenotrophomonas* sp.) treatment included two controls (positive: without application and infested with non-viruliferous aphids and negative: without application and infested with viruliferous aphids) for the second factor, repeated four times, and also had 48 treatment units. The endophyte liquid was applied at a rate of 100 mL/plant around the plant roots and incubated for 48 Hours After Inoculation (HAI).

2.4. Viral Transmission

Viral transmission was carried out using viruliferous and non-viruliferous aphids, which were transferred to the banana seedlings test plant with a soft wet brush, applying 10 to 15 aphids (modified) [10,11]. Transmission is placed on the underside of a coiled banana leaf for 48 hours after being transferred, which is based on the feeding period of aphids. After

incubation, aphids were killed off by the sprayed insecticide containing the active ingredient imidacloprid

2.5. Carbohydrate Analysis

A 2 g banana leaves were ground and extracted using 96% ethanol before being filtered onto a paper disk. Iodine added into ethanol leaf extract until showed the colour change indicator into blue for starch, chocolate for glycogen, and red for dextrin [12].

2.6. Sugar Analysis

Banana leaves were collected as much as 1 g for extracted with 5 mL distilled water. Water extract was filtered onto paper disk Whatman No.1 and added 2 mL distilled water. Sugar was detected with DNS reagent that showed the colour change indicator from yellow to reddish-orange in alkali condition after heated at 100^{o}C [12].

2.7. Phenolic Analysis

Phenolic were tested based on modification from Folin-Ciocalteu method which added 0.4 mL Folin-Ciocalteu reagent onto 0.5 mL leaf ethanol extract. After incubated for 5 minutes (min), it mixed with 4 mL $NaCO_3$. The solution was incubated at room temperature for 60 min. Phenolic indicator was showed after changed of the colour solution to blue or dark blue [13].

2.8. Peroxide Enzyme Analysis

According to [14] with slight modification, a 2 g leaf sample was ground and added with 10 mL of pH 7 phosphate buffer, then filtered through Whatman No. 1 paper. A 1.5 mL pyrogallol solution (0.05 M pyrogallol and 0.5 mL of 1% H2O2) was added to the filtrate. Homogenized at 5,000 rpm and 25°C for 10 minutes. Peroxidase activity was assayed by measuring absorbance at 420 nm with a pH 7 phosphate buffer blank. Enzyme activity was expressed as units per milligram per minute (U/mg/min).

2.9. Tannin Analysis

Tannin analysed using leaf ethanol extract from 2 g as much as 2 mL extract. After that, the filtrate was added 2 drops $FeCl_3$ 1%. The colour change indicated tannin content for dark green to dark blue to positive reaction [15].

2.10. Saponin Analysis

Saponins were extracted from 2 g of leaf tissue ground with 96% ethanol and filtered through a paper disk. The leaf ethanol extract was transferred to a tube (up to 1 mL) and mixed with 10 mL of boiled water. The filtrate was shaken for 10 seconds until the colour of the solution changed. After shaking, 1 drop of 2 N HCl was added and the mixture incubated for 10 minutes. Saponins were detected by the formation of a persistent foam 1–3 cm in height [15].

2.11. Research Parameter

Parameters employed in this study include quantitative analyses of incubation period, disease severity index, and the area under the disease progress curve (AUDPC), as well as qualitative analyses of carbohydrate, sugar, phenolic compounds, peroxidase enzyme activity, tannin, and saponin contents.

2.12. Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) at $\alpha = 0.05$ for quantitative parameters, while qualitative data were examined using descriptive analysis. Where significant differences were detected, means were separated using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. All analyses were performed using DSAASTAT version 1.15.

3. Results And Discussion

3.1. The influence of endophytic bacteria on pathosystem components

The incubation period in this study ranged from 14 to 42 days post inoculation (HSI) and was monitored weekly. Disease intensity was evaluated in the Cavendish cultivar (P1). The negative control treatment (K2P1) exhibited the highest disease intensity at 58.3%, a value that differed significantly from other treatments. In contrast, the *Enterobacter* sp. (B3P1) and *Stenotrophomonas* sp. treatments showed markedly lower disease intensities, with a mean of 10.4%, significantly different from the control. These results indicate that *Enterobacter* sp. and *Stenotrophomonas* sp. can suppress disease development and enhance banana resistance to BBTV infection in the Cavendish cultivar. In the *Kepok* cultivar (P2), the pain control treatment (K2P2) yielded a disease intensity of 52.05%. However, four endophytic bacterial treatments reduced disease intensity to 27.05%, a significant difference from the pain control. Collectively, these findings suggest that the endophytic bacteria *Enterobacter* sp. and Stenotrophomonas sp. can suppress BBTV infection and disease progression, likely owing to the production of enzymes and antimicrobial compounds by these strains. It is important to note that disease intensity also varied with cultivar; Cavendish appeared more susceptible to BBTV infection than *Kepok*, indicating cultivar-dependent differences in susceptibility and response to endophytic-bacteria-mediated suppression. This aligns with previous observations that no banana cultivar exhibits complete BBTV resistance, though some cultivars may display greater tolerance than others [16, 17].

In this study, AUDPC values varied across treatments, reflecting differential efficacy of the endophytic bacteria against BBTV in banana plants. The highest AUDPC was observed in the negative control for the K2P2 treatment of the *Kepok* cultivar, at 164.5%, indicating successful infection by banana aphids and subsequent BBTV development. Conversely, the lowest AUDPC was recorded for the B4P1 treatment of the Cavendish cultivar, at 21.83%, suggesting that the endophytic strain Stenotrophomonas sp. effectively suppressed BBTV progression in banana plants. These results illustrate the varying success rates of the endophytic bacteria in mitigating disease progression. A lower AUDPC value denotes greater effectiveness in suppressing pathogen development [18].

Table 1. Influence of endophytic bacteria to the disease severity and AUDPC

Treatment	Disease severity (%)	AUDPC
K1P1	37.4 ab	64.51 bc
K2P1	58.3 c	131.15 a
B1P1	22.8 ab	40.56 bc
B2P1	14.5 ab	36.42 bc
B3P1	10.4 a	26.01 bc
B4P1	10.4 a	21.83 c
K1P2	33.3 ab	72.82 b
K2P2	52.05 c	164.50 a
B1P2	27.05 ab	34.25 bc
B2P2	29.15 ab	29.13 bc
B3P2	29.15 ab	45.77 bc
B4P2	29.15 ab	47.85 bc

Notes: Values within a column with different lowercase letters are significantly (F count>F table). P1 = Cavendish cultivar, P2 = *Kepok* cultivar, K1 = positive control, K2 = negative control, B1 = *B. velezensis*, B2 = *Serratia* sp., B3 = *Enterobacter* sp., and B4 = *Stenotrophomonas* sp.

Table 2. Biochemical and physiological changes after BBTV infection

Treatment	Biochemical		Physiology			
	C	S	Phe	PO	Sp	Tn
K1P1	-	-	++	-	-	+
K2P1	-	-	+	-	-	++
B1P1	-	-	++	-	-	+++
B2P1	-	-	++	-	-	+
B3P1	-	-	++	-	-	+
B4P1	-	-	++++	-	++	-
K1P2	-	-	+++	-	-	+
K2P2	-	-	++++	-	+	++
B1P2	-	-	++	+	-	+
B2P2	-	-	++	-	-	+
B3P2	-	-	++++	-	-	+
B4P1	-	-	+++	-	+	++

Notes: C = carbohydrate, S = sugar, Phe = phenolic, PO = peroxidase, Sp = saponin, Tn = Tannin, P1 = Cavendish cultivar, P2 = *Kepok* cultivar, K1 = positive control, K2 = negative control, B1 = *B. velezensis*, B2 = *Serratia* sp., B3 = *Enterobacter* sp., and B4 = *Stenotrophomonas* sp.; C (- = yellow/not detected, + = dark brown yellowish), S (- = not detected, + = yellow/reddish-orange), Phe (- = green/not detected, + = light blue, ++ = dark blue, +++ = deep blue, ++++ = midnight blue), PO (- = yellow/not detected, + = brownish yellow), Sp (- = not detected/nothing foam, + = foamy, ++ = very foamy), Tn (- = colourless/not detected, + = green, ++ = deep green, +++ = dark green).

Carbohydrate analysis indicated that the tested solutions exhibited discoloration ranging from pale yellow to clear, which suggests an absence of starch (polysaccharides). This observation implies that bananas contained predominantly monosaccharides and disaccharides. In support of this interpretation, previous work has reported that banana leaves generally lack starch, since starch is a complex polysaccharide that serves as the main energy reserve in plant storage organs (e.g., bulbs, stems, and fruits). Bananas are also known to be rich in starch in certain tissues, such as banana pseudostems, underscoring tissue- and organ-specific variation [19].

Sugar content analysis was conducted using the dinitro salicylic acid (DNS) method. Qualitative observations indicated no significant differences in sugar content between plants receiving the control treatment and those inoculated with endophytic bacteria, as evidenced by the absence of a distinct discoloration change in all samples analysed. However, in the disease-control treatment of the Cavendish cultivar (K2P1), a dark yellow coloration was observed, indicating detectable sugar presence, though the colour change did not meet the established criteria for optimal sugar content.

These findings are relevant in the context of Banana Bunchy Top Virus (BBTV) infection, which targets phloem tissue and can manifest as leaf distortion, reduced leaf size, and yield loss, thereby perturbing carbohydrate distribution within the plant. Impaired phloem transport can lead to reduced and sometimes undetectable sugar allocation to sink tissues, including leaves, trunks, and developing fruits [20].

Phenol content analysis was performed using the Folin-Ciocalteu method to assess total phenolics. Qualitative observations indicated that the plant-disease control treatment exhibited a blue-black coloration, reflecting increased phenolic content in BBTV-infected plants relative to healthy controls. An elevated phenolic content was also observed in plants treated with the endophytic bacterium Stenotrophomonas sp. strain (B3P1), which produced a deep dark blue coloration (++++) and, in *Kepok* cultivars, was followed by the disease-control K2P2 treatment with a similar intensity (+++).

The observed increases in phenolics can be attributed to multiple factors: BBTV infection in diseased plants likely activates the plant defence system via the phenylpropanoid pathway, leading to the accumulation of phenolic compounds such as phenolic acids, lignin, and flavonoids. In contrast, endophytic bacteria can induce systemic resistance (ISR), which can elevate phenolic levels, though the response may be less pronounced in plants experiencing severe BBTV infection [21]. Consistent with this, prior work reported that the highest total phenol content can occur in virus-infected leaves, an outcome influenced by the plant's defence mechanisms [22].

Enzymatic and secondary-metabolite indicators of resistance were evaluated across 12 treatment combinations. Peroxidase activity was assessed qualitatively, with positive results observed only for the B1P2 treatment (Bacillus sp. on the *Kepok* cultivar). This suggests that the Bacillus sp. inoculum on *Kepok* can induce peroxidase activity under the conditions tested, whereas other treatment combinations did not exhibit detectable enzymatic activity. Bacillus spp. is reported to possess robust peroxidase-producing capabilities, consistent with the literature [23].

Saponin content, a secondary metabolite associated with plant defence, was also qualitatively assessed. Most treatments yielded negative results, indicated by the absence of foam for the following combinations: K1P1, K2P1, B1P1, B3P1, K1P2, B1P2, B2P2, and B3P2 (Table 2). Saponins were detected in a few treatments: B4P1 showed foaming (++), and both K2P2 and B4P2 displayed faint foaming (+). These observations imply that saponin accumulation is influenced by the specific plant-endophyte combination, with B4P1 notably producing higher saponin levels than most other treatments. Under biotic stress, such as BBTV infection in the K2P2 treatment, plants may upregulate saponin biosynthesis as part of the defence response [24].

Tannin content, another class of phenolic secondary metabolites, was also examined qualitatively. Variation in colour intensity across treatment combinations indicated differential tannin levels. In the Cavendish cultivar (P1), the highest tannin levels were observed in B1P1 (endophytic Bacillus sp.) with a blackish-green intensity (+++), followed by K2P1 (disease control) with an intense green intensity (++). Treatments K1P1, B2P1, and B3P1 showed green (+) and B4P1 showed no detectable tannins (−). In the *Kepok* cultivar (P2), the highest tannin levels were detected in K2P2 (disease control) and B4P2 (endophytic Stenotrophomonas sp.) with intense green intensity (++), while K1P2, B1P2, B2P2, and B3P2 displayed green (+). These results indicate that tannin accumulation is dependent on both cultivar and endophytic treatment, with certain combinations prompting greater tannin responses.

4. Conclusion

Collectively, the four endophytic bacterial isolates effectively suppressed BBTV disease severity and reduced AUDPC, likely by activating multiple defense-associated secondary metabolites, including phenolics, peroxidase, saponins, and tannins, which contribute to systemic resistance.

5. Acknowledgement

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