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Evaluation of *Streptomyces* spp. Isolated from the Rhizosphere of *Avicennia marina* as a Biological Control Agent Against Bacterial Leaf Blight and Their Effects on Rice (*Oryza sativa* L.) Growth

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Abstract: This study aimed to evaluate the potential of three *Streptomyces* spp. isolates obtained from the rhizosphere of *Avicennia marina* at the Mangrove Botanical Garden, Surabaya, for suppressing bacterial leaf blight (BLB) development under in vivo conditions and assessing their effects on plant growth. In a previous study, the three *Streptomyces* spp. isolates (St8, St9, and St19) demonstrated strong inhibitory activity in in vitro assays. The present study was conducted using a factorial Completely Randomized Design (CRD), with the first factor consisting of *Streptomyces* spp. isolates (St8, St9, and St19) and the second factor consisting of biological agent suspension doses (5, 10, and 15 ml plant^{-1}). A diseased control treatment was included for comparison. Each treatment was replicated three times, resulting in a total of 30 experimental units. The collected data were analyzed using analysis of variance (ANOVA) with IBM SPSS Statistics 24 software at a 5% significance level. The in vivo efficacy assay showed that the application of *Streptomyces* spp. isolate St19 at a dose of 5 ml plant^{-1} only delayed the incubation period of *X. oryzae* by 1.1 days and was unable to suppress BLB disease severity. The same treatment significantly affected rice tiller number, reaching 13.6 tillers at 56 days after transplanting (DAT), but did not affect plant height throughout the observation period. The results indicate a discrepancy between the in vitro and in vivo assay outcomes. The potential of the tested *Streptomyces* spp. isolates as biological control agents remains limited; however, they showed a significant effect on rice tiller development.
Keywords: Bacterial leaf blight; *Streptomyces* spp.; biological control; *Oryza sativa* L.; *Avicennia marina*

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops worldwide and serves as the primary staple food for more than half of the global population, including Indonesia. In Indonesia, rice production plays a crucial role in maintaining national food security (Donggulo et al., 2017). According to the Indonesian Central Bureau of Statistics, national rice production reached approximately 54 million tons in 2022, with a harvested area of around 10 million hectares. However, rice productivity in Indonesia remains below its potential yield, which can reach approximately 8 tons ha^{-1} under optimal cultivation conditions (Jauhari et al., 2020). This productivity gap is influenced by several constraints, including agricultural land conversion, limited adoption of advanced cultivation technologies, climate variability, and losses caused by plant pests and diseases (Masganti et al., 2020).

Among the major diseases affecting rice production, bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is considered one of the most destructive bacterial diseases worldwide. The disease can significantly reduce rice yield, particularly under favorable environmental conditions for pathogen development (Nino-Liu et al., 2006). Symptoms of BLB include water-soaked lesions that develop into pale

yellow to grayish necrotic areas along the leaf margins. These lesions progressively expand toward the leaf base, resulting in leaf wilting, drying, and premature senescence (Despita et al., 2018). Therefore, the development of sustainable and environmentally friendly approaches for BLB management is essential to maintain rice productivity.

Currently, BLB management primarily relies on chemical bactericides containing active compounds such as saisentong and copper-based compounds. Although synthetic bactericides can provide rapid disease suppression, their repeated and long-term application may negatively affect environmental quality, microbial diversity, and ecosystem sustainability (Sitompul et al., 2024). Consequently, biological control using beneficial microorganisms has emerged as a promising alternative strategy for reducing dependence on synthetic chemicals. Biological control agents can suppress plant pathogens through various mechanisms, including antibiosis, competition for nutrients and ecological niches, production of hydrolytic enzymes, and induction of plant defense responses (Compant et al., 2005).

Among beneficial microorganisms, *Streptomyces* spp. have attracted considerable attention as potential biological control agents due to their ability to produce diverse secondary metabolites with antimicrobial activities. Members of the genus *Streptomyces* are Gram-positive actinomycetes that produce a wide range of bioactive compounds, including antibiotics, siderophores, and extracellular enzymes, which contribute to pathogen suppression (Hastuti et al., 2012; Olanrewaju and Babalola, 2019). Previous studies have demonstrated the effectiveness of *Streptomyces* spp. against *X. oryzae*. Hastuti et al. (2012) reported that endophytic *Streptomyces* spp. isolate LBR02 inhibited the growth of *X. oryzae* under in vitro conditions. Similarly, Fadil et al. (2023) demonstrated that actinobacteria isolates could suppress BLB development under in planta conditions through the production of secondary metabolites, including protease, cellulase, and amylase enzymes.

However, strong antagonistic activity observed under laboratory conditions does not always correspond to effective disease suppression under plant environments. The effectiveness of microbial biocontrol agents under in vivo conditions is influenced by several factors, including their ability to colonize plant tissues, interaction with indigenous microorganisms, environmental conditions, and stability of antimicrobial compound production (Dow et al., 2023). Therefore, evaluation under plant conditions is necessary to determine whether promising isolates identified through in vitro screening can function effectively as practical biocontrol agents.

In this study, *Streptomyces* spp. isolates St8, St9, and St19 were selected based on their strong antagonistic activity against *Xanthomonas oryzae* in previous in vitro assays. These isolates were obtained from the rhizosphere of *Avicennia marina* at the Mangrove Botanical Garden, Gunung Anyar, Surabaya, East Java. Mangrove ecosystems represent unique environments that harbor diverse actinomycetes with potential biotechnological applications, including the production of antimicrobial compounds and plant growth-promoting substances (Shrivastava et al., 2015). Previous screening showed that isolates St8, St9, and St19 produced inhibition zones of 40 mm, 35.3 mm, and 43.1 mm, respectively, indicating strong antagonistic activity against *X. oryzae*.

The application doses of *Streptomyces* spp. evaluated in this study (5, 10, and 15 ml plant^{-1}) were selected based on previous research demonstrating that different inoculum concentrations may influence the effectiveness of biological control agents. Raihanah et al. (2021) reported that an actinomycete isolate applied at 5 ml plant^{-1} provided the best suppression of *Fusarium oxysporum* infection in shallot plants and

improved plant growth performance. Furthermore, the IR64 rice variety was selected because it is known to be susceptible to BLB caused by *X. oryzae* (Roza et al., 2019), allowing clearer evaluation of the biocontrol potential of the tested isolates.

Despite extensive studies on the antagonistic activity of *Streptomyces* spp., information regarding the effectiveness of mangrove-derived *Streptomyces* isolates against BLB under *in vivo* conditions remains limited. Therefore, this study aimed to evaluate the potential of *Streptomyces* spp. isolates St8, St9, and St19 obtained from the rhizosphere of *Avicennia marina* as biological control agents against bacterial leaf blight and their effects on rice plant growth under *in vivo* conditions.

METHOD

Research Site and Period

The study was conducted at the Plant Health Laboratory and the Screenhouse of the Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Indonesia, from May to December 2025.

Experimental Design

The experiment was arranged using a factorial Completely Randomized Design (CRD). The first factor consisted of *Streptomyces* spp. isolates, namely St8, St9, and St19, while the second factor consisted of biological agent suspension doses of 5, 10, and 15 mL plant^{-1} with a population density of 1.8 × 10^{7} CFU mL^{-1}. One diseased control treatment was included as a comparison. Each treatment was replicated three times, resulting in a total of 30 experimental units.

The treatments were as follows:

- S1D1 = *Streptomyces* spp. isolate St8 at a dose of 5 mL plant^{-1}
- S1D2 = *Streptomyces* spp. isolate St8 at a dose of 10 mL plant^{-1}
- S1D3 = *Streptomyces* spp. isolate St8 at a dose of 15 mL plant^{-1}
- S2D1 = *Streptomyces* spp. isolate St9 at a dose of 5 mL plant^{-1}
- S2D2 = *Streptomyces* spp. isolate St9 at a dose of 10 mL plant^{-1}
- S2D3 = *Streptomyces* spp. isolate St9 at a dose of 15 mL plant^{-1}
- S3D1 = *Streptomyces* spp. isolate St19 at a dose of 5 mL plant^{-1}
- S3D2 = *Streptomyces* spp. isolate St19 at a dose of 10 mL plant^{-1}
- S3D3 = *Streptomyces* spp. isolate St19 at a dose of 15 mL plant^{-1}
- KN = Without *Streptomyces* spp. isolate application (diseased control)

Source of Isolates

The isolates used in this study were cultured on selective media to support their growth. *Streptomyces* spp. isolates were propagated using GNA medium containing 1 g glucose, 1.75 g KH_2PO_4, 0.85 g $NaNO_3$, 2.5 g $MgSO_4 \cdot 7H_2O$, 0.75 g KCl, and 20 g agar in 1000 mL distilled water. The EKG medium was used for both propagation and application of *Streptomyces* spp., consisting of 20 g sugar and 250 g potato in 1000 mL distilled water. Meanwhile, *Xanthomonas oryzae* was propagated using commercial NA medium (Himedia) at a concentration of 20 g per 1000 mL distilled water.

The *Streptomyces* spp. isolates were obtained from the Plant Health Laboratory collection of Universitas Pembangunan Nasional "Veteran" Jawa Timur. These isolates were originally explored by Frida Nur Aisah from the rhizosphere of *Avicennia marina* at the Mangrove Botanical Garden, Gunung Anyar, Surabaya, East Java. Three isolates were evaluated in this study, namely St8, St9, and St19. The isolates were subcultured on GNA medium and incubated for 14 days at 28–30°C.

The *Xanthomonas oryzae* isolate was obtained from the same laboratory collection. The isolate was subcultured on slanted NA (Nutrient Agar) medium and incubated for 48 h at 28–30°C.

Preparation of *Streptomyces* spp. and *Xanthomonas oryzae* Suspensions

The *Streptomyces* spp. suspension was prepared by transferring 20 culture pieces (5 mm diameter) with a density of 1.8×10^7 CFU mL^{-1} into 200 mL of EKG medium in an Erlenmeyer flask. The culture was then shaken at 150 rpm for 7 days. The initial suspension was subsequently filtered, and 15 mL of the filtrate was diluted in 100 mL distilled water before being adjusted according to the required dose for each treatment.

The *Xanthomonas oryzae* suspension was prepared by adding 10 mL distilled water to a slanted NA medium containing *X. oryzae* with a colony density of 4.5×10^8 CFU mL^{-1}. The suspension was then vortexed to obtain a homogeneous bacterial suspension.

In Vivo Antagonistic Test of *Streptomyces* spp.

The antagonistic activity of *Streptomyces* spp. was evaluated in vivo using 14-day-old seedlings of rice variety IR64. The seedlings were transplanted into 30 × 30 cm polybags containing sterilized planting media. Before being transferred into the polybags, the sterilized soil was mixed with compost fertilizer at a 1:1 ratio. The planting medium was then drenched with a 5% *Streptomyces* spp. suspension at 150 mL $polybag^{-1}$ and incubated for 14 days before transplanting.

After transplanting, rice seedlings were treated with *Streptomyces* spp. isolates St8, St9, and St19 at doses of 5, 10, and 15 mL $plant^{-1}$. The biological agent suspension was applied once a week. The control treatment received sterile water application.

Artificial inoculation of *X. oryzae* was performed seven days after the first application of the biological agent using the *spray inoculation* method. The pathogen suspension was prepared from a 48-h pure culture grown on NB medium with a density of 10^8 CFU mL^{-1}. Observations were conducted daily until the first symptoms appeared to determine the incubation period of the pathogen. Subsequent observations after artificial inoculation were performed based on two parameters: incubation period and disease intensity.

Observation Parameters

1. Incubation period

The incubation period was defined as the time required for the pathogenic bacteria to induce visible symptoms on the plant. Observations were conducted daily from the time of artificial inoculation until the first symptoms appeared (Raihanah et al., 2023). The observed symptoms included pale yellowish-gray discoloration on one or both sides of the leaves, which could extend toward the leaf base and cause leaf rolling (Fadil et al., 2023).

2. Disease intensity

Disease intensity was assessed to determine the severity of bacterial leaf blight symptoms by observing disease development. Disease intensity was calculated using the following formula:

$$\text{Disease Intensity} = \frac{\sum(n_i \times v_i)}{Z \times N} \times 100\%$$

Note:

- ni = number of infected leaves in each disease severity category

- vi = severity score value for each category
- Z = maximum severity score
- N = total number of observed leaves

The disease severity scoring system for bacterial leaf blight assessment followed the Standard Evaluation System of IRRI (2014), as follows:

- 0 = no infection
- 1 = infection covering 1–5% of leaf area
- 2 = infection covering 6–12% of leaf area
- 3 = infection covering 13–25% of leaf area
- 4 = infection covering 26–50% of leaf area
- 5 = infection covering 51–100% of leaf area

3. Plant height

Plant height was measured using a ruler by measuring the distance from the base of the stem to the tip of the longest leaf. Measurements were conducted weekly after transplanting until the end of the vegetative phase (56 days after transplanting, DAT).

4. Number of tillers

The number of tillers was determined by counting all stems per plant and subtracting one stem as the main stem. Observations were conducted weekly after transplanting until the end of the vegetative phase (56 DAT).

Data Analysis

The collected data were analyzed using Analysis of Variance (ANOVA), normality tests, and homogeneity tests using IBM SPSS Statistics 24 software. When the analysis indicated significant differences among treatments, further analysis was performed using the Honestly Significant Difference (HSD/BNJ) test at a 5% significance level.

RESULTS AND DISCUSSION

Identification of *Streptomyces* spp. Isolates

The colony characteristics of *Streptomyces* spp. isolates St8, St9, and St19 were characterized by grayish-brown pigmentation with irregular colony margins. The isolates produced spores with a smooth texture (Figure 1). These characteristics are consistent with the description provided by Imani et al. (2024), who reported that *Streptomyces* spp. colonies generally exhibit irregular margins with round to oval shapes. Mature *Streptomyces* spp. colonies containing fully developed spores commonly appear brownish and have a cotton-like texture (Setyawati et al., 2021).

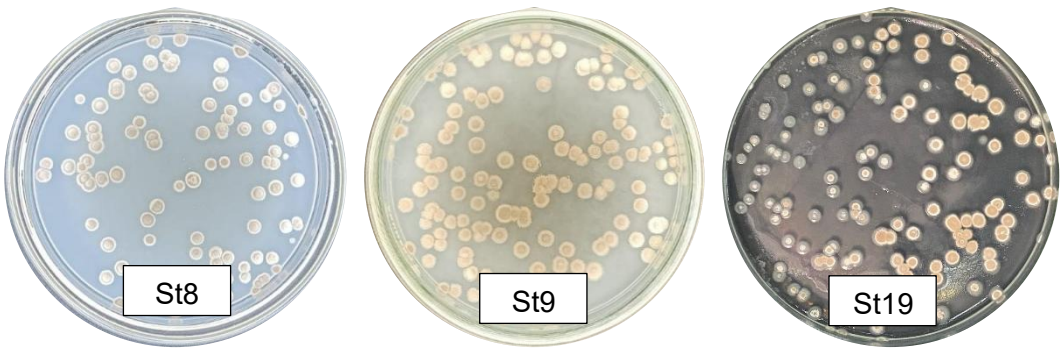

Figure 1. Colony morphology of *Streptomyces* spp. isolates St8, St9, and St19

Microscopic observation of *Streptomyces* spp. isolates St8, St9, and St19 revealed that all isolates consisted of slightly oval-shaped cells forming branched chains resembling spiral-like mycelial structures (Figure 2). This observation agrees

with Goodfellow et al. (2012), who described *Streptomyces* spp. morphology as coccoid cells attached to one another, forming elongated hyphae and developing mycelial structures with various forms, including straight, flexuous, rectiflexible, spiral, and loop configurations.

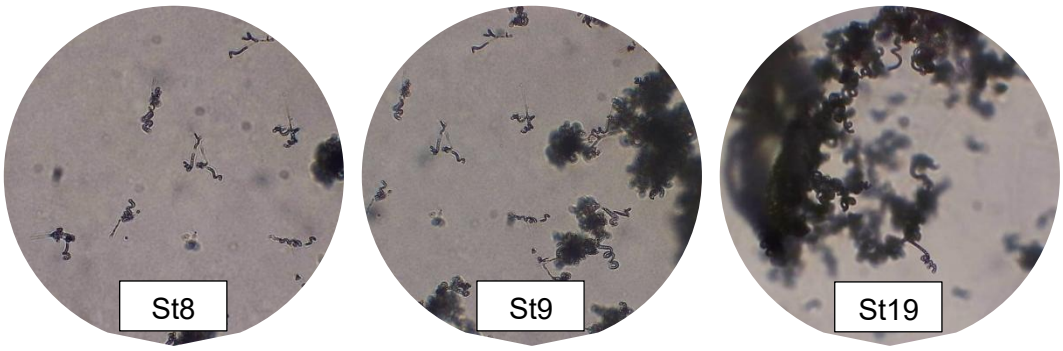

Figure 2. Cells of *Streptomyces* spp. isolates observed at 100× magnification

Identification of *Xanthomonas oryzae*

The pathogenic bacterium *Xanthomonas oryzae* produced yellow-colored and mucoid colonies (Figure 3). These characteristics correspond with the findings of Af'idzatuttama et al. (2025), who reported that *X. oryzae* colonies are typically bright yellow, mucoid, circular, and characterized by entire margins. In addition, individual colonies exhibited a circular shape, smooth surface, elevated appearance, and entire edges (Figure 3). These observations are consistent with Wahyudi (2011), who described *X. oryzae* colonies as mucoid, circular, convex, shiny, and having smooth margins.

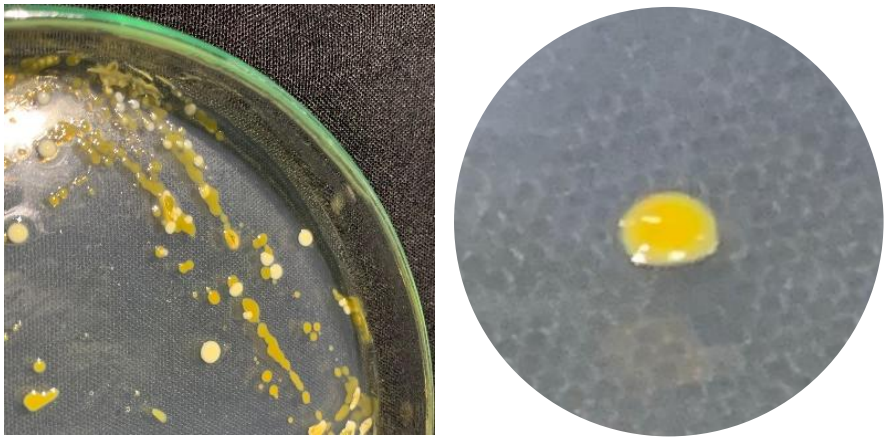

Figure 3. Colony morphology of the pathogenic bacterium *Xanthomonas oryzae*

Pathogenicity Test Results

Symptoms of bacterial leaf blight observed during the pathogenicity test were characterized by yellowish-gray lesions appearing from the leaf tips. The discoloration occurred on both sides of the leaves and gradually expanded toward the leaf base, accompanied by slight curling of the leaf tips (Figure 4). These symptoms correspond with the description by Fadil et al. (2023), who reported that bacterial leaf blight symptoms begin with the appearance of yellowish-gray lesions that subsequently expand toward the leaf base. Leaf curling and drying symptoms may also occur in rice plants older than four weeks (Laraswati et al., 2021).

Figure 4. Symptoms of bacterial leaf blight observed during the pathogenicity test

The average disease intensity of bacterial leaf blight was assessed at 7 days after inoculation (DAI), resulting in a disease intensity of 67%, indicating that the pathogen exhibited high virulence. This finding is consistent with Yunus (2019), who conducted a pathogenicity assay of *Xanthomonas oryzae* and reported disease intensity values ranging from 61–79%. Symptomatic rice leaves were subsequently re-isolated, resulting in colonies with yellow pigmentation, circular colony morphology, and smooth margins.

In Vivo Antagonistic Activity of *Streptomyces* spp.

Incubation Period

The incubation period of *Xanthomonas oryzae* on rice plants showed a significant difference between the *Streptomyces* spp. treatments and the diseased control. Differences in incubation periods were observed from the third day after artificial inoculation. The longest incubation period was recorded in treatment S3D1, which consisted of the application of *Streptomyces* spp. isolate St19 at a dose of 5 ml plant^{-1}, with an average incubation period of 4.5 days (Table 1).

This result indicates that the application of St19 isolate at 5 ml plant^{-1} was able to delay the initial development of the pathogen. This effect may be attributed to the antagonistic mechanisms of *Streptomyces* spp., which potentially suppress pathogen activity before extensive multiplication occurs within plant tissues. These findings are in agreement with Hastuti et al. (2012), who reported that actinobacteria, including *Streptomyces* spp., can inhibit pathogen development at an early stage through antagonistic mechanisms involving the production of bioactive compounds, including lytic enzymes such as glucanase, chitinase, and cellulase.

Table 1. Incubation period of *Xanthomonas oryzae* on rice plants under different treatments

Treatment	Incubation period (days)
S1D1 (*Streptomyces* spp. St8 isolate, 5 ml plant^{-1})	3.7ab
S1D2 (*Streptomyces* spp. St8 isolate, 10 ml plant^{-1})	4.0bc
S1D3 (*Streptomyces* spp. St8 isolate, 15 ml plant^{-1})	3.8ab
S2D1 (*Streptomyces* spp. St9 isolate, 5 ml plant^{-1})	4.0bc
S2D2 (*Streptomyces* spp. St9 isolate, 10 ml plant^{-1})	3.8ab
S2D3 (*Streptomyces* spp. St9 isolate, 15 ml plant^{-1})	3.9bc
S3D1 (*Streptomyces* spp. St19 isolate, 5 ml plant^{-1})	4.5c

Treatment	Incubation period (days)
S3D2 (*Streptomyces* spp. St19 isolate, 10 ml plant^{-1})	4.2bc
S3D3 (*Streptomyces* spp. St19 isolate, 15 ml plant^{-1})	4.2bc
KN (without *Streptomyces* spp. isolate)	3.4a

Note: Values followed by the same letter within the same column indicate no significant difference according to the 5% HSD test.

The application dose of 5 ml plant^{-1} was considered the most effective dose, presumably because it provided a sufficient population of biological control agents to achieve optimal colonization without causing excessive microbial density. In contrast, increasing the application doses to 10 and 15 ml plant^{-1} did not improve disease suppression. This may have occurred because excessive microbial populations could induce competition for available space and nutrients, thereby reducing effective colonization by the biological control agent. This explanation is supported by Khan et al. (2023), who reported that high-density applications of *Streptomyces* spp. may result in uncontrolled inoculum density, leading to intraspecific competition and preventing optimal colonization.

Treatment S3D1, consisting of *Streptomyces* spp. isolate St19 applied at 5 ml plant^{-1}, was the most effective treatment in delaying the incubation period of *X. oryzae* compared with the control treatment. The average incubation period in S3D1 was 4.5 days, whereas the control treatment showed an average incubation period of 3.4 days, representing a delay of 1.1 days.

These results suggest that the application of St19 isolate at 5 ml plant^{-1} delayed the appearance of bacterial leaf blight symptoms due to the antagonistic activity of *Streptomyces* spp. This finding is consistent with El-Shatoury & Diab (2026), who reported that *Streptomyces* spp. can inhibit pathogen metabolism, particularly in Gram-negative bacteria and pathogenic fungi, through mechanisms involving inhibition of cell wall synthesis and protein synthesis, thereby delaying disease symptom development.

Disease Intensity

The symptoms observed during disease intensity assessment were characterized by yellowish-gray lesions appearing from the leaf tips. The discoloration occurred on both sides of the leaves and gradually expanded toward the leaf base, accompanied by slight curling of the leaf tips (Figure 5). The results showed that the application of *Streptomyces* spp. suspension did not significantly affect the average intensity of bacterial leaf blight disease.

Figure 5. Symptoms of bacterial leaf blight on rice plants under all treatments (A. *Streptomyces* spp. isolate St8 applied at 5 ml plant^{-1}; B. *Streptomyces* spp. isolate St8 applied at 10 ml plant^{-1}; C. *Streptomyces* spp. isolate St8 applied at 15 ml plant^{-1}; D. *Streptomyces* spp. isolate St9 applied at 5 ml plant^{-1}; E. *Streptomyces* spp. isolate St9 applied at 10 ml plant^{-1}; F. *Streptomyces* spp. isolate St9 applied at 15 ml plant^{-1}; G. *Streptomyces* spp. isolate St19 applied at 5 ml plant^{-1}; H. *Streptomyces* spp. isolate St19 applied at 10 ml plant^{-1}; I. *Streptomyces* spp. isolate St19 applied at 15 ml plant^{-1}; J. Without *Streptomyces* spp. isolate (diseased control))

At 56 days after transplanting (DAT), the application of *Streptomyces* spp. isolate St19 at 5 ml plant^{-1} resulted in the lowest disease intensity, with an average value of 51%, compared with 55.6% in the control treatment (Table 2). However, statistical analysis showed that these treatments were not significantly different. This result may be associated with differences in pathogen sensitivity to the antagonistic mechanisms produced by the biological control agent. The effectiveness of biological control agents under *in vivo* conditions may vary due to differences in pathogen susceptibility and environmental interactions during plant-based evaluations. This finding is supported by Bardin *et al.* (2015), who stated that the effectiveness of biological control agents is influenced by pathogen sensitivity to the antagonistic mechanisms exhibited by the applied microorganisms.

Table 2. Bacterial leaf blight disease intensity on rice plants under different treatments

Treatment	Disease intensity (%)		
	42 HST	49 HST	56 HST
S1D1	27.1	46.4	55.0
S1D2	25.2	41.6	53.6
S1D3	25.4	43.4	53.5
S2D1	26.3	43.4	51.8
S2D2	27.8	45.8	53.9
S2D3	25.8	42.5	51.8
S3D1	26.4	41.9	51.0
S3D2	26.6	44.2	52.1
S3D3	24.6	39.8	52.2
KN	27.4	47.5	55.6

Note: Values followed by the same letter within the same column indicate no significant difference according to the 5% HSD test.

The *Streptomyces* spp. isolates used in this study were selected based on previous research conducted by Aisah (2025), which reported the highest inhibition zones against *X. oryzae* during antagonistic assays. The inhibition zones were 40 mm for isolate St8, 35.33 mm for isolate St9, and 43.16 mm for isolate St19. These three isolates were categorized as having very strong inhibitory activity because inhibition zones greater than 20 mm are considered highly effective (Maulana et al., 2022).

However, the results of the in vivo antagonistic assay demonstrated that *Streptomyces* spp. isolates St8, St9, and St19 were unable to significantly suppress bacterial leaf blight symptoms in rice plants. This difference may be explained by the contrasting environmental conditions between controlled laboratory assays and plant-based evaluations. In vitro assays provide optimal conditions for microbial interactions, whereas in vivo systems involve more complex factors, including microbial competition, environmental variability, plant responses, and pathogen–host interactions.

This observation is consistent with Dow et al. (2023), who reported that the effectiveness of *Streptomyces* spp. under in vitro and in planta conditions may not always correspond because bioactive compound production, microbial competition, and induction of plant defense responses are strongly influenced by the in vivo environment. The biological control agent suspension used in this study contained *Streptomyces* spp. with a population density of 1.8×10^7 CFU ml^{-1}. This relatively low population density may also have contributed to the limited ability of *Streptomyces* spp. to significantly reduce bacterial leaf blight intensity in rice plants. This explanation is supported by Putri *et al.* (2021), who reported that a high microbial population density is required to improve the effectiveness of biological control agents because higher colony numbers may accelerate colonization and enhance bioactive compound production.

Plant Growth

Plant height

The application of *Streptomyces* spp. did not significantly affect rice plant height from 7 to 56 days after transplanting (DAT) (Table 3). At 28 DAT, the highest plant height was observed in plants treated with *Streptomyces* spp. isolate St8 at a dose of 10 ml $plant^{-1}$, with an average height of 69.7 cm (Figure 6). From 35 to 56 DAT, the highest plant height was observed in plants treated with *Streptomyces* spp. isolate St19 at a dose of 15 ml $plant^{-1}$. However, none of the treatments showed statistically significant differences. Therefore, the application of *Streptomyces* spp. isolates was considered insufficient to promote significant increases in rice plant height.

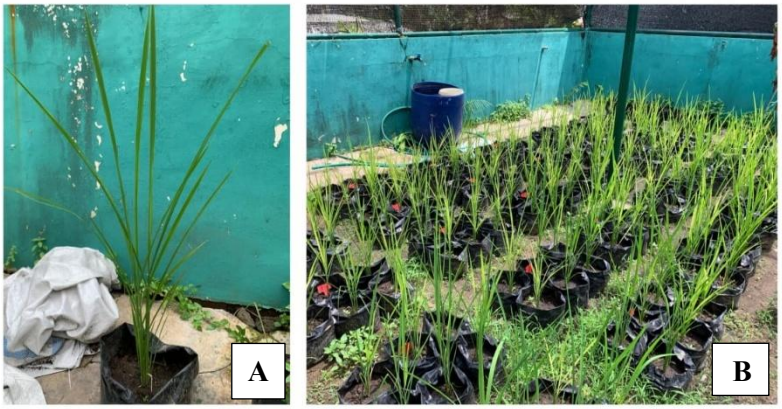

Figure 6. Rice plant height at 28 DAT: (A) the best treatment (*Streptomyces* spp. isolate St8 at 10 ml $plant^{-1}$), and (B) all treatments in the experimental field

Table 3. Rice plant height under different treatments

Treatment	Plant height (cm)				
	28 HST	35 HST	42 HST	49 HST	56 HST
S1D1	60.4	66.6	73.0	78.5	78.5
S1D2	69.7	78.4	85.9	89.7	89.7
S1D3	67.6	73.9	79.3	83.3	83.3
S2D1	59.4	64.7	69.6	73.5	73.5
S2D2	54.5	58.5	63.8	66.3	66.3
S2D3	60.6	67.8	74.5	79.5	79.5
S3D1	61.7	68.7	76.0	80.9	80.9
S3D2	59.5	66.8	73.4	78.5	78.5
S3D3	69.6	78.8	86.8	93.9	93.9
KN	53.8	60.6	65.7	70.5	66.3

Note: Values followed by the same letter within the same column indicate no significant difference according to the 5% HSD test.

The absence of a significant effect on plant height may be associated with differences in plant hormonal regulation and tissue sensitivity to growth-promoting compounds, particularly gibberellic acid (GA). Irvan & Adriana (2017) reported that exogenous application of gibberellin can enhance rice plant height by promoting cell wall loosening and accelerating tissue elongation.

However, not all actinomycetes, including *Streptomyces* spp., are capable of producing gibberellin. This is supported by Anwar et al. (2016), who reported considerable variation among actinomycetes, particularly *Streptomyces* spp., in their ability to synthesize GA. The growth-promoting capacity of these microorganisms may instead depend on other mechanisms, such as indole-3-acetic acid (IAA) production, siderophore production, and phosphate solubilization. Therefore, further screening is required to confirm the ability of the tested *Streptomyces* spp. isolates to biosynthesize gibberellin and other plant growth-promoting compounds.

Number of Tillers

The analysis of rice tiller number showed no significant differences between *Streptomyces* spp.-treated plants and control plants from 7 to 28 DAT (Table 4). This result may be attributed to an adaptation phase occurring in the rice root system, during which colonization by biological control agents had not yet reached its optimal level.

This explanation is supported by Hafni et al. (2019), who reported that early plant growth involves adaptation to environmental conditions and root system development, resulting in limited colonization by biological agents. Consequently, rice tiller formation remains relatively uniform during the early growth stage.

Differences among treatments became apparent at 35 DAT, as indicated by differences in statistical groupings between treated plants and controls. At 42 DAT, more pronounced differences were observed, with the best response obtained from the application of *Streptomyces* spp. isolate St19 at 5 ml plant^{-1}. This treatment produced an average of 13.2 tillers per plant. The same treatment maintained the highest tiller number at 49 and 56 DAT, reaching an average of 13.6 tillers per plant (Table 4).

Table 4. Rice tillers number under different treatments

Treatment	Number of Tillers			
	35 HST	42 HST	49 HST	56 HST
S1D1	9.1ab	12.5cd	12.9bc	13.0bc
S1D2	9.2ab	12.7cd	13.1bc	13.1bc
S1D3	9.2ab	12.7cd	13.0bc	13.0bc
S2D1	9.6ab	12.9cd	13.3bc	13.3bc

Treatment	Number of Tillers			
	35 HST	42 HST	49 HST	56 HST
S2D2	9.0ab	11.9bc	12.2ab	12.2ab
S2D3	9.2ab	12.4cd	12.7bc	12.7bc
S3D1	9.7b	13.2d	13.6c	13.6c
S3D2	8.8ab	11.8ab	12.2ab	12.2ab
S3D3	9.1ab	12.7cd	12.9bc	12.9bc
KN	8.7a	11.5a	12.0a	12.0a

Note: Values followed by the same letter within the same column indicate no significant difference according to the 5% HSD test.

The application of *Streptomyces* spp. isolate St19 at 5 ml plant^{-1} was considered capable of enhancing rice tiller development from 35 to 56 DAT because the applied biological agent was presumed to have reached optimal root colonization, thereby supporting tiller formation. This finding agrees with Gopalakrishnan et al. (2013), who evaluated the potential of *Streptomyces* spp. as plant growth-promoting microorganisms in rice and reported that *Streptomyces* spp. application significantly increased tiller number beginning at 30 DAT.

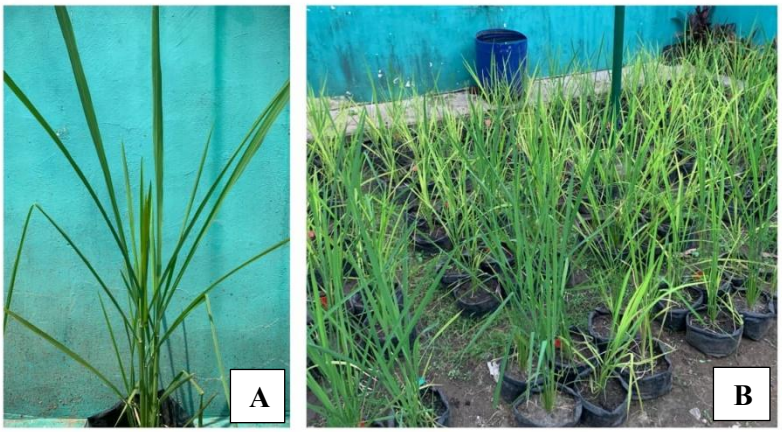

Figure 7. Rice tiller number at 49 DAT: (A) the best treatment (*Streptomyces* spp. isolate St19 at 5 ml plant^{-1}), and (B) all treatments in the experimental field

The application of *Streptomyces* spp. isolate St19 at 5 ml plant^{-1} consistently produced the highest tiller number up to 56 DAT, indicating that this dose provided an optimal condition for biological agent activity. This response may occur because the applied dose was sufficient to support maximum colonization without causing excessive microbial population density. This explanation is consistent with Olanrewaju & Babalola (2019), who reported that optimal application rates of *Streptomyces* spp. promote population balance and maximize microbial colonization.

In contrast, applications of the same isolate at higher doses (10 and 15 ml plant^{-1}) did not produce greater improvements. Excessive microbial populations may increase competition for nutrients and ecological niches, thereby limiting effective colonization. Khan et al. (2023) similarly reported that *Streptomyces* spp. compete for available nutrients, and excessive inoculum density may trigger intraspecific competition, preventing optimal establishment of biological control agents.

The superior performance of *Streptomyces* spp. isolate St19 at 5 ml plant^{-1} may also be related to the origin of the isolate, which was obtained from the rhizosphere of *Avicennia marina*. Such isolates may possess the ability to produce plant growth-promoting compounds that contribute to rice tiller development. This explanation is supported by Shrivastava et al. (2015), who reported that eight *Streptomyces* spp.

isolates obtained from mangrove ecosystems were capable of producing indole-3-acetic acid (IAA) and siderophores, thereby enhancing plant growth.

CONCLUSION

The application of *Streptomyces* spp. isolates demonstrated a significant effect on delaying the incubation period of bacterial leaf blight caused by *Xanthomonas oryzae* in rice plants; however, it did not significantly reduce disease intensity. Among the tested treatments, *Streptomyces* spp. isolate St19 applied at 5 ml plant^{-1} showed the best performance by extending the incubation period to 4.5 days, representing a 1.1-day delay compared with the diseased control treatment. Nevertheless, the tested isolates were unable to significantly suppress bacterial leaf blight severity under screenhouse conditions, indicating that their biocontrol potential remains limited in planta.

The application of *Streptomyces* spp. did not significantly enhance rice plant height but significantly improved tiller development during the late vegetative growth stage. The application of isolate St19 at 5 ml plant^{-1} resulted in the highest tiller number, reaching an average of 13.6 tillers plant^{-1} at 49 and 56 days after transplanting. These findings indicate that although the tested *Streptomyces* spp. isolates have limited effectiveness as direct biocontrol agents against bacterial leaf blight, particularly under screenhouse conditions, isolate St19 has potential as a plant growth-promoting microorganism.

RECOMMENDATION

Further studies are recommended to improve the effectiveness of *Streptomyces* spp. as biological control agents against bacterial leaf blight. Future research should evaluate higher population densities, optimize application timing and frequency, and investigate the mechanisms underlying pathogen suppression and plant growth promotion, including the production of antimicrobial compounds, phytohormones, and other beneficial metabolites. Molecular identification and characterization of promising isolates, particularly St19, are also recommended to support their development as effective biological agents.

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