

## Biocontrol and growth-promoting potential of *Streptomyces hygroscopicus* against bacterial leaf blight in rice

Titik Nur Aeny\*<sup>1</sup>, Radix Suharjo<sup>1</sup>, Sudi Pramono<sup>1</sup>, Suskandini Ratih Dirmawati<sup>1</sup>, Hamim Sudarsono<sup>1</sup>, Selvi Helina<sup>1</sup>, Hening Puji Pangestu<sup>2</sup>

<sup>1</sup>Department of Plant Protection, Faculty of Agriculture, University of Lampung, Indonesia. Jl. Prof. Soemantri Brojonegoro No. 1 Bandar Lampung, Lampung, Indonesia

<sup>2</sup>Department of Agrotechnology, Faculty of Agriculture, University of Lampung, Indonesia. Jl. Prof. Soemantri Brojonegoro No. 1 Bandar Lampung, Lampung, Indonesia

Corresponding author: [titik.nuraeny@fp.unila.ac.id](mailto:titik.nuraeny@fp.unila.ac.id)

ORCID ID: <https://orcid.org/0000-0002-2103-3560>

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**Abstract:** This study evaluated the antagonistic activity and plant growth-promoting potential of *Streptomyces hygroscopicus* against *Xanthomonas oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight (BLB) in rice, under *in vitro* and greenhouse conditions. Experiments were arranged in a completely randomized design. *In vitro* assays compared sterile water, chloramphenicol, and *S. hygroscopicus*, while greenhouse trials assessed three application doses of *S. hygroscopicus* (5, 10, and 15 mL per plant), a bactericide, and a control treatment, each with four replications. The *in vitro* assay demonstrated a strong antagonistic effect of *S. hygroscopicus*, indicated by the formation of distinct inhibition zones against *X. oryzae*. Under greenhouse conditions, foliar application of *S. hygroscopicus* (5-15 mL per plant; 10<sup>8</sup> cfu/mL) delayed disease incubation, significantly reduced disease severity and the area under the disease progress curve (AUDPC). Moreover, higher application doses (10 – 15 mL per plant) enhanced plant height and leaf number. A clear dose-dependent reduction in AUDPC was observed, indicating sustained suppression of disease development over time. Overall, the results highlight the potential of *S. hygroscopicus* as an effective and environmentally friendly biocontrol agent with additional plant growth-promoting benefits. Further studies under field conditions are recommended to confirm its consistency and applicability in integrated management of bacterial leaf blight in rice.

**Keywords:** Actinomycetes; Biological control; Plant growth promotion; Rice bacterial disease; *Xanthomonas oryzae*

### Introduction

*Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the causal agent of bacterial leaf blight (BLB) in rice, that is responsible for considerable yield losses in endemic areas including Indonesia (Chaiharn et al., 2020; Nisha et al., 2012). The impact of BLB is particularly severe in regions, where the disease is established or under serious infestations (Kumar et al., 2013). *Xoo* has been recognized as one of the top ten most significant plant pathogenic bacteria (Mansfield et al., 2012). Prior to the 1970s, BLB was largely confined to Asia, but it has since spread to major rice-growing regions in Central and South America, Australia, and Africa (Eamchit, 1982; Ou Slough, 1985). The extent of yield loss caused by the disease ranges from 15% to 80%, depending on the growth stage of the crop at the time of infection (Reddy and Shang-zhi, 1989; Shanti et al., 2010). In India, BLB-related yield losses have been reported to range between 65% and 95% (Nayak et al., 2008). In Indonesia, up to 35.8% losses have been reported especially when the plants were infected since the seedling stage (Suparyono et al., 2016)

The disease symptoms typically appear on young rice leaves as pale green to grey-green, water-soaked streaks near the tips and edges of the leaves. The pathogen, *X. oryzae* pv. *oryzae* (*Xoo*), is a gram-negative bacterium which infects rice leaves at every stage of plant development, from the nursery phase through to harvest. Infection during the generative phase disrupts the grain filling process, leading to imperfect kernels. The spread of the bacterium is often exacerbated by continuous strong winds and heavy rainfall, which facilitate the dispersal of bacterial droplets from infected plant lesions. In general, the bacterium can be dispersed through various pathways including water, soil, grassy weeds, and contaminated rice seeds.

The distribution and severity of bacterial leaf blight are heavily influenced by environmental conditions such as moisture and temperature, as well as agricultural practices including rice variety selection, and nitrogen fertilization levels. This disease now has near-global distribution, including tropical regions where humid conditions facilitate its proliferation (Niño-Liu et al., 2006); therefore, an integrated management strategy is essential. Several ecological factors contribute to BLB's spread: climate fluctuations, monoculture systems, excessive fertilizer input, and wide movement of contaminated

planting materials (Hernandez et al., 2023; Lahlali et al., 2024). This dynamic complexity has rendered BLB not only as a persistent biological threat but also a serious agronomic challenge requiring integrated and eco-friendly methods.

Chemical bactericides remain the conventional method of BLB control due to their quick and visible effect. Farmers favor them for their practicality and short-term efficacy. However, long-term use has led to the development of resistant strains, accumulation of chemical residues, and detrimental impacts on beneficial soil microorganisms (Ahmad et al., 2024). In light of these limitations, attention has shifted toward biological control strategies that promote ecological sustainability. Among these, actinomycetes particularly those from the genus *Streptomyces* have drawn considerable interest (Bhatti et al., 2017). *Streptomyces* sp. emerging as promising candidates due to their antagonistic properties against phytopathogenic bacteria (Hata et al., 2015; Jacob et al., 2016). These gram-positive, filamentous bacteria are prolific producers of secondary metabolites, including antibiotics and plant growth-promoting substances (Doubou et al., 2005; Viaene et al., 2016). Their dual role as biofertilizers and biopesticides positions them as promising agents in integrated disease management. The effectiveness of *Streptomyces* in suppressing various plant diseases has been well-documented against a broad spectrum of phytopathogens (Ebrahimi-Zarandi et al., 2022; Le et al., 2022). Species such as *Streptomyces hygroscopicus* has exhibited strong *in vitro* inhibitory activity against *Dickeya* spp., the causal agent of soft rot in pineapple (Aeny et al., 2018), *Alternaria alternata* of tobacco brown spot (Cai et al., 2023), *Colletotrichum gloeosporioides* in orchid (Prapagdee et al., 2008). In fact, the antagonistic activity of *S. hygroscopicus* has been documented as early as 1991 against turfgrass fungi (Chamberlain and Crawford, 1999).

The biocontrol efficacy of *Streptomyces* is attributed to multiple integrated modes of action including: (i) synthesis of antimicrobial metabolites such as streptomycin, tetracycline, and other bioactive compounds; (ii) competitive exclusion of pathogens via rhizosphere colonization; (iii) induction of systemic resistance within the plant host, which enhances defense responses through signaling molecules like salicylic acid and jasmonic acid; and (iv) degradation of organic matter, indirectly improving soil structure and plant resilience. An effective alternative to chemical antimicrobials in soil is the use of antibiotic-producing bacteria as biocontrol agents. Among these, *Streptomyces* species are well-known for synthesizing a wide variety of bioactive organic compounds, which can influence plant growth and development either directly or indirectly, in addition to providing antimicrobial protection against numerous plant pathogens (Wati et al., 2024; Khan et al., 2023; Pacios-Michelena et al., 2021; Viaene et al., 2016). Previous studies, including Promnuan et al. (2020), reported certain *Streptomyces* strains suppress *X. oryzae* pv. *oryzae* through multiple biochemical mechanisms, such as the production of antimicrobial secondary metabolites and siderophores. These findings support the potential use of *S. hygroscopicus* as an eco-friendly component of integrated management strategies for bacterial leaf blight in rice. They have demonstrated that *Streptomyces* can inhibit the growth of *Xoo* through several mechanisms, including the production of secondary metabolites, siderophores, and other growth-promoting substances. The antagonistic effect of *Streptomyces* against *Xoo* could provide an environmentally friendly approach to manage BLB in rice, thus contributing to sustainable agricultural practices in Indonesia.

Although *Streptomyces* species are recognized for their potential as biocontrol agents, the role of *S. hygroscopicus* in controlling BLB has not been well studied. More research is needed to evaluate its antagonistic effects on *Xoo* and its possible contribution to reducing BLB and supporting plant growth. Exploring this gap could provide important insights into how *S. hygroscopicus* suppresses pathogenicity and interacts with plants, which in turn, may support the development of sustainable strategies for integrated pest management (Elshafie and Camele, 2022; Ebrahimi-Zarandi et al., 2009). The novelty of this study lies not only in the identification and characterization of *S. hygroscopicus* as a biocontrol agent against *Xoo* but also in the exploration of its underlying mechanisms of action. This research will contribute to the broader understanding of actinomycetes in biocontrol as well as in plant growth and development.

## Results and Discussion

### **Morphological characteristics of the isolates**

The culture of *Xoo* displayed vigorous growth on PPGA medium, forming yellow, mucoid colonies with glistening surfaces, smooth, shiny, light yellow, raised and a slightly convex elevation with circular to irregular margin within 48 hours of incubation. These morphological traits are characteristic of *Xoo*, as previously described by Jonit et al. (2016) and Sheik et al. (2017). The production of extracellular polysaccharides gives the glossy and mucoid appearance.

In contrast, *S. hygroscopicus* exhibited slow growth on MEA medium, forming white, powdery colonies with irregular margins and a coarse surface texture. These features are typical of *Streptomyces* species, which are known for their filamentous morphology, aerial mycelium development, and sporulation capability. This observation aligns with those reported by Kováčsová et al. (2015) who emphasized that the distinct colony morphology of actinomycetes on nutrient-rich media is indicative of their metabolic diversity and potential for antimicrobial activity.

### **In vitro antagonistic activity**

The antagonistic potential of *S. hygroscopicus* against *X. oryzae* was clearly demonstrated by the formation of inhibition zones surrounding the actinomycete colonies (Figure 1). In the agar diffusion assay, these actinomycetes exhibited strong inhibitory activity, producing a distinct clear zone with an average diameter of 14.41 mm after five days of incubation (Table 1). This zone of inhibition was substantially larger than that of the positive control (chloramphenicol), which averaged only 4.88 mm, while no inhibitory effect was observed in the sterile water control. Mean separation with Tukey's Test indicated

**Table 1.** Mean diameter of inhibition zones (mm) produced by different treatments (sterile water, chloramphenicol, and actinomycetes) at 1 to 5 days after inoculation (dai).

Treatment	Diameter of inhibition zones (mm),		
	1 dai	3 dai	5 dai
Sterile water	0.00 c	0.00 c	0.00 c
Chloramphenicol	2.94 ± 0.34 b	3.79 ± 0.54 b	4.88 ± 0.49 b
<i>Streptomyces</i>	10.25 ± 1.19 a	12.44 ± 1.85 a	14.41 ± 1.19 a

Notes: Values are means of 4 replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Test at a significance level of  $p < 0.05$ . dai = days after inoculation.

**Table 2.** Incubation period of BLB pathogen after the treatments.

Treatment	Incubation period (days)
Sterile water	4.63 ± 0.50 c
Bactericide 2.5 g/L	4.69 ± 0.48 bc
<i>Streptomyces</i> 5 ml/plant	4.88 ± 0.50 abc
<i>Streptomyces</i> 10 ml/plant	5.19 ± 0.54 ab
<i>Streptomyces</i> 15 ml/plant	5.44 ± 0.51 a

Notes: Values are means of 4 replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Test at a significance level of  $p < 0.05$ .

**Table 3.** Disease severity of bacterial leaf blight at three and five weeks after the treatment.

Treatment	Disease severity (%)			
	1 wat	3 wat	5 wat	6 wat
Sterile water	33.75 ± 2.3 a	53.75 ± 3.1 a	71.25 ± 4.2 a	78.75 ± 3.1 a
Bactericide	28.75 ± 1.2 b	48.75 ± 2.3 a	68.75 ± 3.1 a	73.75 ± 1.2 a
<i>Streptomyces</i> 5 ml/plant	26.25 ± 1.2 b	37.50 ± 1.4 b	51.25 ± 1.2 b	55.00 ± 2.0 b
<i>Streptomyces</i> 10 ml/plant	25.00 ± 0.0 b	36.25 ± 2.3 b	48.75 ± 2.3 b	53.75 ± 4.2 b
<i>Streptomyces</i> 15 ml/plant	26.25 ± 1.2b	36.25 ± 2.3 b	47.50 ± 2.5 b	47.50 ± 2.5 b

Notes: Values are means of 4 replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Test at a significance level of  $p < 0.05$ . wat = weeks after treatment.

that actinomycetes had a significantly larger diameter of clear zones than those of chloramphenicol and sterile water, indicating its inhibition effect on the growth of Xoo (Table 1).

This inhibitory effect is likely attributed to the production of bioactive secondary metabolites by *S. hygroscopicus* as reported by Bhattacharyya et al., (1998); Chaiharn et al., (2020); Pacios-Michelena et al., (2021); Rajan and Kannabiran, (2014). The antagonistic effect observed in this study suggests that the *Streptomyces* produce bioactive secondary metabolites. A previous study reported the presence of saponins, triterpenoids, and anthraquinone glycosides in the extract of *S. hygroscopicus* (Setyaningrum et al., 2021). Further chemical profiling will be required to identify more specific compounds. These findings align with the work of Alekhya and Gopalakrishnan, (2011); Baltz, (2016); Chaiharn et al., (2020), who reported that actinomycetes synthesize a wide range of secondary metabolites, many of which interfere with bacterial protein synthesis or compromise cell wall integrity.

#### **Incubation period of BLB pathogen**

Application of *S. hygroscopicus* suspensions at 10 and 15 mL per plant significantly delayed the onset of bacterial leaf blight (BLB) symptoms, extending the incubation period to 5.25 and 5.40 days, respectively, compared to 4.55 days in the untreated control (Table 2). These results suggest that *S. hygroscopicus* may function by delaying the onset of symptoms, possibly by interfering with early pathogen colonization. An extended incubation period is advantageous for disease management, as it allows the host plant more time to activate basal defense mechanisms. These findings are consistent with previous reports demonstrating that actinomycetes can elicit induced systemic resistance (ISR) in rice (Chaiharn et al., 2020; Ebrahimi-Zarandi et al., 2022).

#### **Disease severity and area under the disease progress curve (AUDPC)**

Statistical analysis revealed that *S. hygroscopicus* treatments significantly lowered disease severity over the observation period (Table 3). Statistical differences among treatments were further confirmed using AUDPC values (Table 4). The substantial decline in disease severity and AUDPC observed in this study highlights the effectiveness of *Streptomyces hygroscopicus* in suppressing bacterial leaf blight. The dose-responsive reduction in AUDPC suggests a sustained antagonistic effect during disease development. Similar suppressive activity of actinomycetes against *Xanthomonas campestris* and *Ralstonia solanacearum* has also been reported, supporting the broad-spectrum disease-suppressive potential of *Streptomyces* spp. (Pacios-Michelena et al., 2021; Le et al., 2022).

The reduction in disease severity highlights the potential of *S. hygroscopicus* as biocontrol agents, even following the initial infection. As demonstrated by Le et al. (2022) *Streptomyces* spp. isolated from suppressive soils can inhibit a broad spectrum

**Table 4.** The Area Under the Disease Progress Curve (AUDPC).

Treatment	AUDPC
<i>Streptomyces</i> 15 ml/plant	73.00
<i>Streptomyces</i> 10 ml/plant	74.00
<i>Streptomyces</i> 5 ml/plant	77.00
Bactericide	99.00
Sterile water	107.00

**Table 5.** Plant height at three and five weeks after the treatment (wat).

Treatment	Plant height (cm)	
	3 wat	5 wat
Sterile water	25.18 ± 4.59 a	30.30 ± 12.98 b
Bactericide	25.10 ± 5.38 a	30.40 ± 11.11 b
<i>Streptomyces</i> 5 ml/plant	27.20 ± 3.34 a	39.00 ± 4.77 a
<i>Streptomyces</i> 10 ml/plant	26.73 ± 3.34 a	37.10 ± 6.57 ab
<i>Streptomyces</i> 15 ml/plant	27.20 ± 2.00 a	36.80 ± 5.36 ab

Notes: Values are means of 4 replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Test at a significance level of  $p < 0.05$ .

**Table 6.** Number of leaves at three and five weeks after the treatments.

Treatment	Numbers of leaves	
	3 wat	5 wat
Sterile water	5.20 ± 0.62 b	6.50 ± 1.73 b
Bactericide	5.30 ± 0.44 b	6.90 ± 1.33 b
<i>Streptomyces</i> 5 ml/plant	5.60 ± 0.50 ab	7.90 ± 1.37 a
<i>Streptomyces</i> 10 ml/plant	5.90 ± 0.59 a	8.20 ± 1.49 a
<i>Streptomyces</i> 15 ml/plant	5.90 ± 0.49 a	8.02 ± 1.44 a

Notes: Values are means of 4 replicates. Means within a column followed by the same letter are not significantly different according to Tukey's Test at a significance level of  $p < 0.05$ .

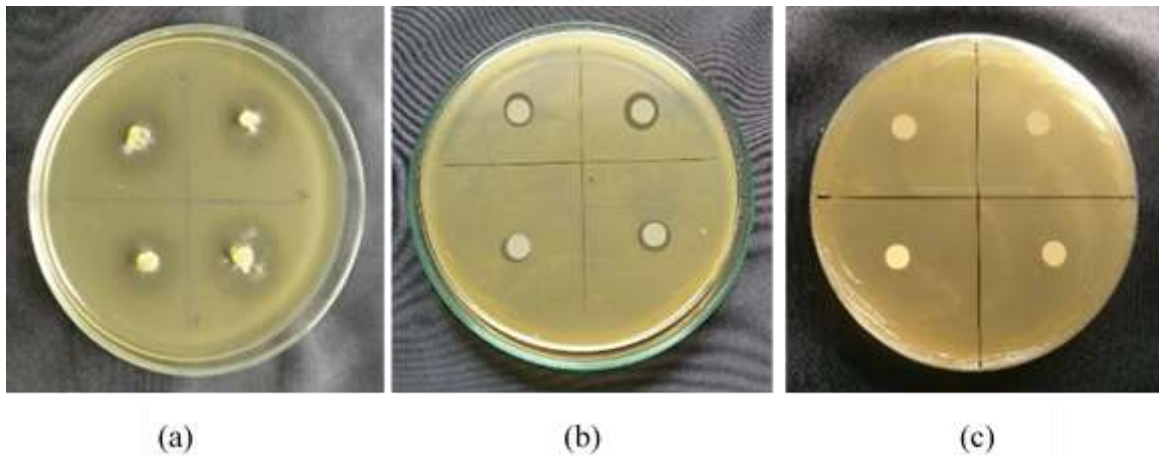


Figure 1. Clear zone formation in the in vitro antagonism assay (5 days old): (a) *S. hygroscopicus* subsp. *hygroscopicus* isolate, (b) chloramphenicol, and (c) sterile water.

of phytopathogens. Their effectiveness is largely attributed to the production of diverse secondary metabolites and is influenced by environmental conditions. Actinomycetes are prolific producers of antimicrobial compounds, reinforcing their potential as sustainable disease management tools (De Simeis and Serra, 2021) and field performance of microbial antagonists depends heavily on both biotic and abiotic factors (Bonaterra et al., 2022). Actinomycetes can produce several bioactive secondary metabolites with antibacterial, antifungal as well as antiviral (Elshafie and Camele, 2022; Rahma et al., 2023; Tistechok et al., 2019).

Based on the AUDPC data (Table 4), a clear dose-dependent effect of the *Streptomyces* treatment was observed, which serves as a strong indicator of its efficacy. Over the 6-week period, plants treated with sterile water showed the most severe disease progression, with the highest AUDPC value of 107, indicating rapid symptom appearance and disease spread which was significantly higher than all *Streptomyces* treatments ( $p \leq 0.05$ ). In contrast, the bactericide treatment provided only a slight reduction in disease progression, with an AUDPC of 99. The most promising results came from the actinomycete treatments, which showed a progressive decrease in AUDPC values with increasing dosage. The 5 ml dose resulted in an AUDPC of 77, the 10 ml dose in 74, and the highest concentration of 15 ml/plant achieved the lowest AUDPC of 73. This consistent, gradual

reduction in AUDPC values as the dose increases strongly suggests that the biocontrol agent's suppressive activity is directly proportional to its concentration. These lower AUDPC values not only indicate a delay in symptom appearance but also fewer and slower-spreading lesions, highlighting the potential for improved plant resistance (Hastuti et al., 2012). This dose-dependent response adds significant weight to the conclusion that *S. hygroscopicus* is a highly effective biocontrol agent for managing bacterial leaf blight.

### ***Effect on plant growth parameters***

Beyond its disease-suppressive activity, application of *Streptomyces* also exerted a positive effect on rice plant growth. Significant increases were observed in plant height (Table 5) and leaf development (Table 6) over the five-week observation period. At three weeks after application, actinomycete treatments at 5, 10, and 15 mL per plant showed a general tendency toward increased plant height compared with the sterile water and bactericide controls, although these differences were not statistically significant. By five weeks; however, all *Streptomyces* treatments resulted in significantly greater plant height than the controls.

Similarly, a positive pattern was evident in leaf development (Table 6). At three weeks, the higher doses (10 and 15 mL/plant) already showed a statistically significant increase in leaf count compared to the controls. By five weeks, all *Streptomyces* treatments, including the 5 mL dose, had produced a significantly higher number of leaves than the control plants, which is a strong indicator of overall vegetative growth. This suggests that the bacteria's beneficial effects are cumulative, requiring time for them to establish a relationship with the plant roots before the growth-promoting benefits become fully apparent. These growth-promoting effects are likely attributed to the production of phytohormones like indole-3-acetic acid (IAA) and gibberellins, as well as siderophores and enzymes that enhance nutrient availability, as reported in other studies (Alekhya and Gopalakrishnan, 2011; Gopalakrishnan et al., 2013; Jog et al., 2014) where *Streptomyces* strains improved growth in maize and other crops under field conditions. Our findings align with other work that has shown *Streptomyces* strains can improve plant growth parameters like shoot length and leaf production in other crops (Devi et al., 2022). This dual benefit on both disease suppression and plant growth underscores the potential of *S. hygroscopicus* as a powerful and sustainable biostimulant in agriculture.

## **Materials and Methods**

### ***Plant material and cultivar***

Rice plant material used in this study consisted of the Indonesian commercial cultivar Ciherang, a widely cultivated variety known for its high susceptibility to bacterial leaf blight. Seeds were obtained from the Indonesian Center for Rice Research (ICRR), Subang, West Java. Prior to sowing, seeds were surface-sterilized with 70% ethanol, rinsed with sterile distilled water, and soaked for 24 hours to ensure uniform germination. The use of cv. Ciherang was chosen to allow consistent disease development and to evaluate the effectiveness of *S. hygroscopicus* as a biocontrol agent.

### ***Experimental site description***

The experiment was conducted at the Biotechnology Laboratory and the Integrated Field Laboratory, Faculty of Agriculture, University of Lampung, Indonesia. Laboratory activities, including microbial culture preparation and *in vitro* antagonism assays were carried out in the Biotechnology Laboratory using standard microbiological equipment. The *in planta* experiment was performed in the greenhouse of the Integrated Field Laboratory, where rice plants (var. Ciherang) were grown in buckets filled with a sterilized soil:manure mixture (1:1). Greenhouse conditions reflected typical lowland tropical environments, allowing consistent monitoring of disease development and plant responses throughout the study period.

### ***Source of bacterial isolates and study location***

The *Streptomyces* and *Xanthomonas* isolate used in this study were obtained from the collections of Biotechnology Laboratory Faculty of Agriculture University of Lampung, and has been identified as *Streptomyces hygroscopicus* subsp. *hygroscopicus* (Aeny et al., 2018) and *Xanthomonas oryzae*. The *Xanthomonas oryzae* was isolated from a rice field in Central Lampung, Indonesia. The research was conducted at the Biotechnology Laboratory and the greenhouse facilities of the Integrated Field Laboratory, Faculty of Agriculture, University of Lampung, Indonesia, from January to October 2023.

### ***In vitro antagonism assay***

A completely randomized design was employed for both the *in vitro* and *in planta* experiments. The *in vitro* assay following agar diffusion method (Barakate et al., 2002) was set to observe the growth of *Xanthomonas* on media containing sterile distilled water (negative control), chloramphenicol at 1 mg/mL (positive control), and *S. hygroscopicus* at  $10^8$  cfu/mL. Each treatment was replicated four times. The variable observed in this experiment was the diameter of inhibition zones or the clear zone (mm).

Four agar plugs (5 mm in diameter) containing 14-day-old *S. hygroscopicus* colonies were aseptically transferred onto PPGA plates previously inoculated with a suspension of *X. oryzae* (approximately  $10^6$  cfu/mL), which had been evenly spread to form a uniform bacterial lawn. For comparison, sterile 5-mm paper discs soaked in chloramphenicol solution and sterile distilled water were placed on separate plates. All plates were incubated at room temperature, and the diameters of the inhibition zone were measured daily over a five-day period.

### ***In planta* experiment and disease assessment**

The *in planta* experiment comprised of five treatments: (1) sterile distilled water (negative control), (2) Nordox 56 WP (bactericide, active ingredient copper oxide) at 2.5 g/L (positive control), and (3–5) three dose levels of the *S. hygroscopicus* suspension: 5 mL, 10 mL, and 15 mL per plant, respectively. All treatments were applied to four replicates. The measured variables are incubation periods, disease severity, area under disease progress curve, plant height, and leaves number.

Rice seeds (cv. Ciherang) were surface-sterilized using 70% ethanol, rinsed thoroughly with sterile distilled water, and soaked for 24 hours (Hussain et al., 2015). Prior to sowing, the seeds were immersed in a *S. hygroscopicus* suspension for 5 minutes. The treated seeds were then sown in seedling trays with rock wool. After 15 days, the seedlings were transplanted into buckets filled with a sterilized 1:1 (v/v) mixture of topsoil and organic manure, five seedlings per bucket. All rice plants were carefully maintained, with care including watering, weeding, fertilization, and mechanical pest control. Fertilization was applied according to the recommendations of Husnain et al. (2020).

Twenty-five days post-transplantation, rice plants were inoculated with *X. oryzae* using the standard leaf-clipping method (Kauffman et al., 1973; Khaeruni et al., 2014). In this method, the bacterial suspension was prepared at a concentration of approximately  $1 \times 10^8$  cfu/mL ( $OD_{600} \approx 0.5$ ). Plants were inoculated by leaf-clipping method using scissors dipped in *X. oryzae* suspension before clipping 2–3 cm from the leaf tip. Plants were then maintained under greenhouse conditions until symptom development. Fourteen days after inoculation, lesion lengths were measured, and disease severity was calculated based on the percentage of diseased leaf area. *Streptomyces* suspensions ( $10^8$  cfu/mL, with the dose of 5, 10, or 15 mL per plant) were applied as foliar spray at two-week intervals, for a total of three times applications. Bactericide and sterile water were applied similarly to ensure uniform treatment procedures.

Following inoculation, plants were observed daily to determine the incubation period, defined as the time between inoculation and the appearance of initial symptoms. BLB severity was measured using the IRRI Standard Evaluation System (0–9 scale) where 0 = no symptoms and 9 = more than 75% leaf area infected (IRRI, 2013). The development of the disease was observed weekly for two months and the disease severity was calculated using the following formula:

$$\text{Disease severity (\%)} = \left[ \frac{\sum (n_i \times v_i)}{N \times V} \right] \times 100,$$

where  $n_i$  is the number of leaves in the  $i$ -th disease category,  $v_i$  is the corresponding disease rating score,  $N$  is the total number of observed leaves, and  $V$  is the maximum disease rating based on the IRRI Standard Evaluation System (IRRI, 2013).

Weekly disease severity data were used to construct disease progress curves, while inferential statistical comparisons among treatments were performed using AUDPC values following formula by Rahma et al. (2023). The area under the disease progress curve was calculated based on severity scores over time. After obtaining the AUDPC value, the level of control effectiveness was determined by calculating the disease suppression index value.

The AUDPC was calculated using this formula:

$$AUDPC = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

where:  $y_i$  = the percentage of disease severity at the  $i$ -th observation

$t_i$  = the time of the  $i$ -th observation (in days)

$n$  = the number of observations

### **Statistical analysis**

Data were analysed using analysis of variance (ANOVA). Prior to analysis, data were checked for normality and homogeneity of variances. When significant treatment effects were detected, mean separation was performed using Tukey's honestly significant difference (HSD) test at a significance level of  $p \leq 0.05$ . All statistical analyses were conducted using R software (R Core Team, 2024).

### **Conclusion**

*Streptomyces hygroscopicus* demonstrates strong antagonistic activity against *X. oryzae* both *in vitro* and *in planta*, significantly reducing bacterial leaf blight severity and enhancing plant growth. It has capacity to extend disease incubation periods and reduce AUDPC indicates its dual role as a biocontrol and plant growth-promoting agent. These findings highlight its promise for sustainable rice cultivation

Future studies should aim to investigate the molecular and physiological mechanisms underlying *S. hygroscopicus*-mediated disease suppression and plant growth promotion. In addition, its consistency and effectiveness under field conditions across diverse agroecological settings warrant further evaluation.

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### **Competing of interest**

The authors declare that they have no competing interests related to the content of this manuscript.

### Statement of author contributions

TNA was responsible for conception of the experiment, methodology design, scientific writing, manuscript preparation, review and submission; RS and SRD contributed to orientation, revision, and writing review; SP and SH were responsible for data collection and manuscript review; HS performed the statistical analysis, translations, and manuscript review prior to submission; HPP contributed to data collection and data entry.

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