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Forest-Derived Actinomycetes from Indonesian National Parks: A Novel Approach for Bacterial Leaf Blight Control in Rice

Giyanto[1*], Ruly Anwar[1], Muhamad Hidayanto[2] and Syahri[2]

[1]Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, Indonesia; [2]Research Center for Food Crops, National Research and Innovation Agency (BRIN), Bogor, Indonesia

[*]*Corresponding author:* giyanto@apps.ipb.ac.id

Abstract

The potential of forest-derived actinomycetes from Indonesian national parks for managing bacterial leaf blight (BLB) remains largely unexplored. This study aimed to evaluate the biocontrol potential of forest-derived actinomycetes from Kutai National Park against *Xanthomonas oryzae* pv. *oryzae* and to assess their ability to promote rice seed germination, seedling vigor, and root colonization. A total of 12 actinomycete isolates were screened for *in vitro* antagonism against *Xoo* race 8. Further investigation *in planta* revealed that 3 isolates (KrK_1K_1, DrK_1T_{20}, and DrK_1T_{21}) significantly reduced disease severity by 77.77%, 86.74%, and 82.85%, respectively. Molecular identification of the 3 potential isolates revealed that KrK_1K_1, DrK_1T_{20}, and DrK_1T_{21} are identified as *Streptomyces parvulus*, *Tsukamurella tyrosinosolvens*, and *Salinispora tropica*, respectively. Three selected isolates also significantly ($p < 0.05$) enhanced the seed germination rate (25.05%) and the vigor index (51.11%). Filtrate bioassays at 5%, 10%, and 15% concentrations demonstrated that only *T. tyrosinosolvens* DrK_1T_{20} effectively inhibited *Xoo* growth. All 3 isolates produced siderophores and chitinase, whereas phosphate-solubilizing activity was detected only in *S. parvulus*. Scanning electron microscopy confirmed effective colonization of rice roots by actinomycetes, indicating a successful interaction between the roots and the actinomycete isolates. These abilities strongly support the potential of forest-derived actinomycetes to control BLB and improve plant growth in the field.

Keywords: biological control agent; root colonization; siderophores; *Streptomyces*; *Xanthomonas oryzae*

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INTRODUCTION

Bacterial leaf blight (BLB) is a deadly disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Sanya et al., 2022), which typically infects rice during the seedling and early tillering stages (Sawatphanit et al., 2022). India and Indonesia's rice yields have been reduced by 20 to 80%, Malaysia by 50%, the Philippines and China by 30 to 50%, Bangladesh by 20 to 40%, and Mali by 50 to 80% due to BLB (Amin et al., 2023; Syahri and Somantri, 2024). Generally, it may lead to a 20 to 30% loss in rice yield (Song et al., 2023). It management challenges due to multiple races or pathotypes capable of causing severe damage (Irpawa et al., 2024). As a notorious pathogenic agent, *Xoo* rapidly generates new races with diverse capacities for causing disease.

Various management practices, including seed treatment, pesticides, and planting resistant varieties, are rendered less successful (Irpawa et al., 2024) due to the rapid mutability of *Xoo* (Sudir and Yuliani, 2016). *Xoo* can break down the resistance of rice varieties (Suparyono et al.,

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2004; Irpawa et al., 2024). It is caused by resistant rice containing *BB R* genes, which have been grown in Asia for decades, affecting *Xoo* evolution through their interaction with rice hosts (Song et al., 2023). Furthermore, *Xoo* races with diverse resistance to various rice cultivars have been reported in Indonesia (Suparyono et al., 2004; Khaeruni and Wijayanto, 2013; Sudir and Yuliani, 2016).

In addition, the widespread use of chemical pesticides in agriculture, although effective for pest control, has led to persistent environmental and food system contamination, posing serious acute and chronic risks to human health (Ahmad et al., 2024; Muñoz-bautista et al., 2025). Consequently, biological control strategies are needed to enhance varietal resistance, particularly through the application of actinomycetes. Although microbial pesticides do not directly eliminate germs, they are thought to regulate disease outbreaks by inhibiting the proliferation of harmful bacteria through nutritional competition (Adachi et al., 2012). Actinomycetes constitute a significant portion of rhizosphere microbial communities, contributing critically to soil nutrient cycling and improving plant development and productivity (Sreevidya et al., 2016).

Forests are one of the richest sources and reservoirs of actinobacteria due to their complexity and heterogeneity (Hamdi et al., 2023). The diversity and distribution of forest actinobacteria are strongly influenced by microenvironmental heterogeneity (Hamdi et al., 2023). The detected forest microbe predominantly belongs to actinobacteria, constituting 59% and 63% of vegetated and revegetated forests, respectively (Emmyrafedziawati and Stella, 2015). Actinobacteria are indispensable for the well-being of forest ecosystems. Its capacity to supply nutrients is crucial for stimulating plant growth. Due to their role in nutrient cycling and soil health (Jain et al., 2016), environmental and water availability changes responsibility (Zhou et al., 2016; Tian et al., 2018), ability to produce bioactive compounds (Sharma and Thakur, 2020), and maintain ecological balance (Rahmansyah and Sudiana, 2010), actinobacteria can serve as essential sustainability indicators. Forest soil–derived actinobacteria are known to exhibit broad-spectrum antimicrobial activity due to the production of biologically active compounds (Sharma et al., 2016). Moreover, some actinobacteria can adapt to low-temperature environments (psychrotolerant) (Prokopenko et al., 2019).

The use of actinomycetes to control *Xoo* has been widely reported (Van Hop et al., 2014; Ilsan et al., 2016; Ashwini et al., 2023; Rahma et al., 2023b). However, no reports have been published regarding using forest actinomycetes to manage BLB in rice. Forest-derived actinomycetes have rarely been used to control it due to differences in ecological niches. Previously, *Streptomyces corchorusii*, *S. levoris*, *S. pluricolorescens*, *S. aburaviensis*, *S. monomycini*, and *S. filamentosus* were known as actinomycetes derived from the forest to control non-forest-plant pathogens, i.e., *Ulocladium*, *Aspergillus flavus*, and *Alternaria alternata* (Shirokikh and Shirokikh, 2017b); *Catenulispora pinisilvae* for controlling *Fusarium culmorum*, *F. graminearum*, and *F. oxysporum* (Świecimska et al., 2021). Even *Streptomyces* sp. strain ZX01T from the forest is a biocontrol agent of tobacco mosaic virus (TMV) (Han et al., 2015).

Moreover, forest actinomycetes widely control human pathogenic bacteria (Chanthasena and Nantapong, 2016; Sharma et al., 2016; Retnowati et al., 2018; Sharma and Thakur, 2020). Furthermore, this study evaluated the efficacy of forest actinomycetes in controlling *Xoo* and preserving seeds from pathogenic attacks. This research demonstrates the capacity of forest-derived actinomycetes to act as biological control agents against BLB while simultaneously enhancing rice growth at the seedling stage.

MATERIALS AND METHOD

Pathogen preparation

Pathogen *Xoo* race 8 was obtained from the Bacteriology Laboratory of the Plant Protection Department at IPB University. A 24-hour-old bacterial culture, cultivated on nutrient agar (Himedia, India), was subsequently transferred to nutrient broth (Himedia, India) as the inoculum source, with an optical density (OD) at 600 nm set at 1.0 for inoculation.

Isolation of actinomycetes from soil, leaves, bark, and roots of Kutai National Park

The forest actinomycete used in this study was isolated from several sample types (roots, soil, stems, barks, and leaves) of Kutai National Park, East Kalimantan, Indonesia (GPS coordinates: 117°28′31.80″ E, 0°22′07.80″ N; 117°29′54.18″ E, 0°08′47.22″ N; 117°30′55.98″ E, 0°19′15.12″ N; and 117°31′10.44″ E, 0°18′58.20″ N). The serial dilution spread plate technique was used to isolate actinomycetes from the rhizosphere and soil (Balakrishna, 2012). A 300 ml erlenmeyer

flask was used to mix 10 g of soil sample with sterile aquadest until a total volume of 100 ml was achieved. The mixture was homogenized by stirring. Actinomycetes were isolated by the quadrant streak method and serial dilution. Each dilution was cultured on starch casein agar (SCA) medium (Himmedia, India), which contained 0.3 g vitamin-free casein, 10 g soluble starch, 2 g KNO_3, 0.05 g $MgSO_4 \cdot 7H_2O$, 2 g NaCl, 2 g K_2HPO_4, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.02 g $CaCO_3$, and 18 g agar per liter and incubated for 4 days. The ISP2 medium (4 g dextrose, 10 g malt extract powder, 4 g yeast extract powder, and 20 g agar per liter) was used to purify colonies, which were incubated for 4 days for additional testing.

The phyllosphere (leaves/bark) actinomycetes were isolated using the method described by Ilsan et al. (2016). Leaves or barks (10 g) were chopped into small fragments and dissolved in 100 ml of sterile water. The mixture was agitated using a shaker at 150 rpm for 15 minutes. The isolation process was performed using the serial dilution and quadrant streak method on SCA medium (Himmedia, India). The cultures were incubated for 4 days. Actinomycetes-like bacterial colonies were purified on ISP2 medium after they appeared and incubated for 4 days.

Screening of actinomycete isolates

Eighty-nine forest actinomycete isolates were tested for biosafety, including hypersensitivity reactions (Ezrari et al., 2021) and hemolysis assay (Vallet-Gely et al., 2010). Further selection was carried out on the ability of actinomycetes to inhibit the growth of *Xoo* through an antibiosis mechanism using the plate diffusion assay and cross-streak methods (Velho-Pereira and Kamat, 2011), resulting in 12 actinomycete isolates (KrK_1T_{18}, KrK_1K_1, DrK_1T_{20}, DcK_1D_8, DrK_1T_{21}, DrK_1T_{22}, RwK_1R_4, RwK_1R_2, KrK_1T_6, DcK_1D_6, DcK_1D_4, and KrK_1T_{13}). Three isolates with the most excellent pathogen-inhibiting capacity were selected for further analysis, including molecular characterization, assessment of filtrate activity, and evaluation of their role as plant growth promoters.

Inhibition of Xoo in vitro

The cross-streak method (Velho-Pereira and Kamat, 2011) and the paper disc diffusion test were utilized to evaluate the inhibitory capacity of actinomycetes. This procedure involved meticulously streaking the actinomycete isolates onto one-third of ISP2 medium (Himmedia, India) and incubating them for 3 days. Following incubation, a single line streak of the pathogen (5 cm in length and 0.2 cm in width) was applied around 0.5 cm from the opposing side of the actinomycete on the medium. The only pathogen was streaked on a petri dish as a control. Equation 1 was used to calculate the relative inhibition rate (RIR).

$$\text{RIR} = \frac{\text{LA - LC}}{\text{LC}} \times 100\% \quad (1)$$

Where LC denotes the length of the *Xoo* streak in control (cm), while LA indicates the streak's length under treatment (cm).

In the paper disc diffusion method, 0.1 ml of *Xoo* suspension (10^8 cfu ml^{-1}) was uniformly plated on the nutrient agar. Twenty microliters of a 4-day-incubated actinomycete suspension were dropped onto each filter paper disc (5 mm), with 12 discs applied to each petri dish. The culture was incubated for 48 hours. The clear zone surrounding the disc indicated the pathogen reduction. For this assay, each treatment was evaluated on a single petri dish.

In vivo assay for disease progression.

There are 13 treatments in this study, and each is repeated 3 times. The actinomycete isolates were used as the treatment, and sterile water was used as the control. The 50 g of rice seed IPB 3S cultivar (IPB University) in each replication was surface-sterilized with 1% NaOCl for 3 minutes and rinsed with sterile water 5 times. The sterilized seeds were soaked in an actinomycetes suspension (OD_{600} = 1.0) while agitated on a shaker at 150 rpm for 12 hours (the control treatment was only soaked in sterile water). After 12 hours, the bacterial suspension was discarded. The treated rice seeds were spread on a 30 cm × 15 cm tray containing a sterile soil medium. Suspension of *Xoo* in LB broth (2 days incubation, OD_{600} = 3.0) was sprayed on 13-day-old rice seedlings, and the plants were covered immediately with transparent plastic for 24 hours. Twenty-four hours after inoculation, a second application of the actinomycetes was performed by evenly spraying a 5-day-old actinomycetes suspension, while sterile water was sprayed in the control. The trays were covered again with plastic for 24 hours.

Disease severity was assessed on days 4, 6, 8, 10, 12, and 15, and calculated using the Standard Evaluation System for Rice (SESR) formula (IRRI, 2014) as shown in Equation 2.

$$Is = \left[\frac{\sum(n_i \times i)}{(N \times V)}\right] \times 100\% \quad (2)$$

Where Is = Disease intensity, ni = Number of samples with score i, i = Disease score (0 to 9), V = Highest disease score, and N = Total number of observed samples. Meanwhile, the disease severity score (V) is based on the percentage of leaf area infected as follows: 0 (no infection); 1 (< 1%); 3 (1 to 5%); 5 (6 to 25%); 7 (26 to 50%); 9 (51 to 100%).

The effect of treatment was shown on the disease progress curve (AUDPC) using Equation 3 as described by Simko and Piepho (2012).

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right) x(t_{i+1} - t_i) \quad (3)$$

Where n = Number of observations, y_i = Disease severity at observation i; t_i = Time at observation i.

The control efficacy was assessed using Equation 4 (Cooke, 2006).

$$E = \frac{S_C - S_T}{S_c} \times 100\% \quad (4)$$

Where E = Control efficacy; S_C = AUDPC in the positive control; S_T = AUDPC in the treated plant.

Molecular identification of selected actinomycetes

The 3 most effective isolates for suppressing the pathogen under both *in vitro* and *in vivo* conditions were molecularly identified using polymerase chain reaction (PCR). DNA was extracted according to the protocol described by Abd-Elsalam et al. (2003). The PCR process used the specific primer pair 27F and 16Sact1114R. The 1% agarose gel PCR product was visualized under UV transillumination. DNA amplicon from each isolate was purified and subjected to Sanger sequencing at First Base Malaysia via PT Genetika Science. The nucleotide was analyzed using BioEdit (Hall, 1999), and alignment analysis was performed using Basic Local Alignment Search Tool (BLAST) Nucleotide on the NCBI website (https://blast.ncbi.nlm.nih.gov/).

Closely related sequences were then aligned using CLUSTAL W in BioEdit. Phylogenetic analysis was performed using the neighbor-joining method, as implemented in Molecular Evolutionary Genetics Analysis (MEGA) version 10.2.6. The robustness of the phylogenetic tree was evaluated by bootstrap analysis with 1,000 replications.

Inhibition assay of selected actinomycete filtrates

Their filtrates evaluated 3 selected isolates (KrK_1K_1, DrK_1T_{20}, and DrK_1T_{21}). The filtrate was prepared by growing the actinomycete in an ISP medium and incubating it for 7 days on a shaker. The supernatant was collected by centrifugation at 4,000 rpm for 20 minutes and subsequently filtered through sterile filter paper (Syahri et al., 2025). The activity of the metabolites was tested using the poisoned-food technique (Nurbailis et al., 2023), with minor modifications. The *Xoo* was cultivated in media containing actinomycete metabolites at concentrations of 0%, 5%, 10%, and 15% (v/v). Optical density (OD) of culture was used to predict *Xoo* growth at 2, 4, 6, 10, 12, and 14 hours. Pathogen inhibition was visualized through differences in absorbance between treatment and control.

***In vitro* characterization of actinomycetes**

The ability of actinomycetes to synthesize IAA was measured using the modification protocol of Nimnoi et al. (2010). The phosphate solubilization capability test was identified by growing the actinomycete on Pikovskaya agar in 3 replications (Teymouri et al., 2016). The activity of siderophores was visualized using chrome azurol sulfonate (CAS) agar, based on Abedinzadeh et al. (2019). Actinomycete isolates were streaked on ISP and cultivated for 5 to 7 days. The color change from blue to orange indicates the presence of siderophore activity. The protease production was conducted by culturing the isolates on skim milk agar (Fernandez et al., 2018).

Plant growth promotion evaluation

The plant growth–promoting effects of actinomycetes on rice were evaluated using seed germination and seedling growth bioassays. Rice seeds of the IPB 3S variety were surface-sterilized with 1% NaOCl for 3 minutes and rinsed 5 times with sterile water before use in both assays. The effects of actinomycetes on seed germination were assessed using the "Ragdoll" method (Whitten, 2003) with modifications. A hundred surface-sterilized seeds were subsequently soaked in a suspension of actinomycetes (KrK_1K_1, DrK_1T_{20}, and DrK_1T_{21}) (OD 1.0) and shaken at 150 rpm for 12 hours, while the control was soaked in sterile water. After 12 hours of incubation, the seeds were air-dried and subsequently arranged on a sheet of straw paper.

The number of germinated seeds was recorded at 7 and 14 days, and germination rate (GR), vigor index (VI), root length, shoot length, and seedling fresh weight were quantified. The GR was calculated as GR = Nt/N0 × 100%, where N0 represents the total number of seeds and Nt represents the total number of germinated seeds at 7 days (Niu et al., 2022). The VI was measured using VI = germination (%) × (shoot length + root length) (Song et al., 2015). The treatment was repeated in 3 replicates.

The effect of actinomycetes on rice growth was evaluated by immersing surface-sterilized seeds in actinomycete suspensions before planting (Niu et al., 2022). Three isolates (KrK_1K_1, DrK_1T_{20}, and DrK_1T_{21}) were used for this experiment, whilst control seeds were immersed in sterile water. The treatment was repeated in 10 pots. Approximately 2 seeds were sown in a pot filled with sterile zeolite. The pots were cultivated in a greenhouse for up to 14 days. Ten random seedlings in each treatment were collected to measure growth parameters.

Visualization of root-actinomycete interaction by scanning electron microscopy

The root-microbe interaction was visualized using a scanning electron microscope (SEM, Thermo Scientific) at the i-Lab National Research and Innovation Agency of Indonesia. The analysis was modified from the protocols of Sreevidya et al. (2016). Fourteen days after the previous step, seedlings were pulled out for colonization analysis (Khomampai et al., 2024). Around 5 mm of root was cut and rinsed with sterile water to remove growing media. The sample was immersed in 2.5% glutaraldehyde for several hours. It was fixed in 2% tannic acid for 6 hours, then rinsed 4 times in cacodylate buffer for 5 minutes each. Every procedure was conducted at 4 °C. The ethanol dehydration series was performed at room temperature, consisting of 4 immersions: 5 minutes in 50% ethanol, 20 minutes in 70% ethanol, 20 minutes in 85% ethanol, and 20 minutes in 95% ethanol, followed by 2 final 10-minute immersions in absolute alcohol. The final step is dehydrating, preceded by 2 10-minute immersions in a tert-butanol solution. Before SEM observation, desiccated specimens were fixed to stubs and coated with Au.

Statistical analysis

All statistical analyses were performed using Minitab 20. Statistical differences were identified by one-way ANOVA followed by Fisher's least significant difference (LSD) post-hoc tests at $p < 0.05$. The relationships among growth parameters, actinomycetes enzymatic activities, and their effects on disease development were analyzed using principal component analysis (PCA) and a complete-linkage dendrogram based on Euclidean distance, performed with Minitab 20.

RESULTS AND DISCUSSION

The pathogen inhibition by actinomycetes

Eleven isolates inhibited the growth of *Xoo in vitro* (Table 1). Inhibition levels varied considerably among all isolates. Based on the paper disc diffusion test, the RwK_1R_4 isolate was the only one to fail to inhibit *Xoo*. The most significant pathogen inhibition was observed with the DcK_1D_6 isolate, which reduced *Xoo* by 51.56%. Simultaneously, a secondary assessment using the paper disc diffusion technique revealed the extensive inhibitory zones, ranging from 6 to 18 mm in diameter.

Table 1. The inhibition of *Xanthomonas oryzae* pv. *oryzae* by actinomycetes isolates

Forest actinomycetes isolate	Pathogen reduction	
	Percentage of *Xoo* inhibition by the cross-streak method (%)	Diameter of clear zone based on paper disc diffusion test (mm)
KrK_1T_{18}	6.25	10
KrK_1K_1	6.25	11
DrK_1T_{20}	21.88	18
DcK_1D_8	14.06	10
DrK_1T_{21}	43.75	11
DrK_1T_{22}	18.75	7
RwK_1R_4	9.38	0
RwK_1R_2	25.00	8
KrK_1T_6	20.31	11
DcK_1D_6	51.56	8
DcK_1D_4	45.31	10
KrK_1T_{13}	29.69	6

Broad-spectrum pathogen suppression is advantageous when actinomycetes are applied under field conditions, where pathogen populations and strain composition may vary. The finding corresponds with studies demonstrating that the composition and distribution of *Xoo* pathotypes generally vary by location (Suparyono et al., 2004; Khaeruni and Wijayanto, 2013). For example, 3 dominant *Xoo* pathotypes in Indonesia–III, IV, and VIII–have distinct characteristics (Sudir and Yuliani, 2016). Pathotype variation may result from differences in rice cultivars and irrigation techniques across locations (Khaeruni et al., 2014). Consequently, biocontrol agents with inhibitory activity against various pathogen strains are actively sought for practical field applications.

Efficacy of actinomycetes isolates in suppressing disease severity in rice seedlings

Seedlings represent a vulnerable stage of plant development, encountering numerous biotic and abiotic stressors, including susceptibility to plant disease infections. The presence of pathogens in seeds or in the field during the seedling stage heightens rice's susceptibility to diseases, even with the cultivation of resistant varieties. Several pathogens have been reported to attack rice seedlings, including bacterial seedling rot and seedling blight (Adachi et al., 2012), blast disease (Palupi and Riyanto, 2020), and several fungal species that cause seed rots (Verma et al., 2018b). BLB is one of the most critical diseases during the seedling phase. As a polycyclic disease, it predominantly persists between seasons on rice stubble and weeds, the primary sources of inoculum. In contrast, *Xoo* has a limited lifespan outside its host, rendering it less viable in soil, with inconsistent data on its survival on seeds (Niones et al., 2022). The study findings indicated a substantial reduction in severity in treated rice (Figure 1).

Figure 1 depicts the swift advancement of BLB disease throughout the growth of rice seedlings. The severity of the disease consistently escalated from the initial inoculation to the final observation. Without actinomycete, severity increased from 13.3% on day 4 to 35.0% by day 15. A notably elevated severity was also recorded in the KrK_1T_6 treatment ($p > 0.05$). KrK_1K_1 demonstrated *in vitro* efficacy in inhibiting *Xoo* growth but failed to show significant disease suppression in seedlings. Conversely, RwK_1R_4, which showed little *in vitro* pathogen suppression, significantly reduced disease progression. Actinomycetes are microorganisms whose activity is highly influenced by both biotic and abiotic factors. Their survival, growth, and antagonistic potential are affected by variables

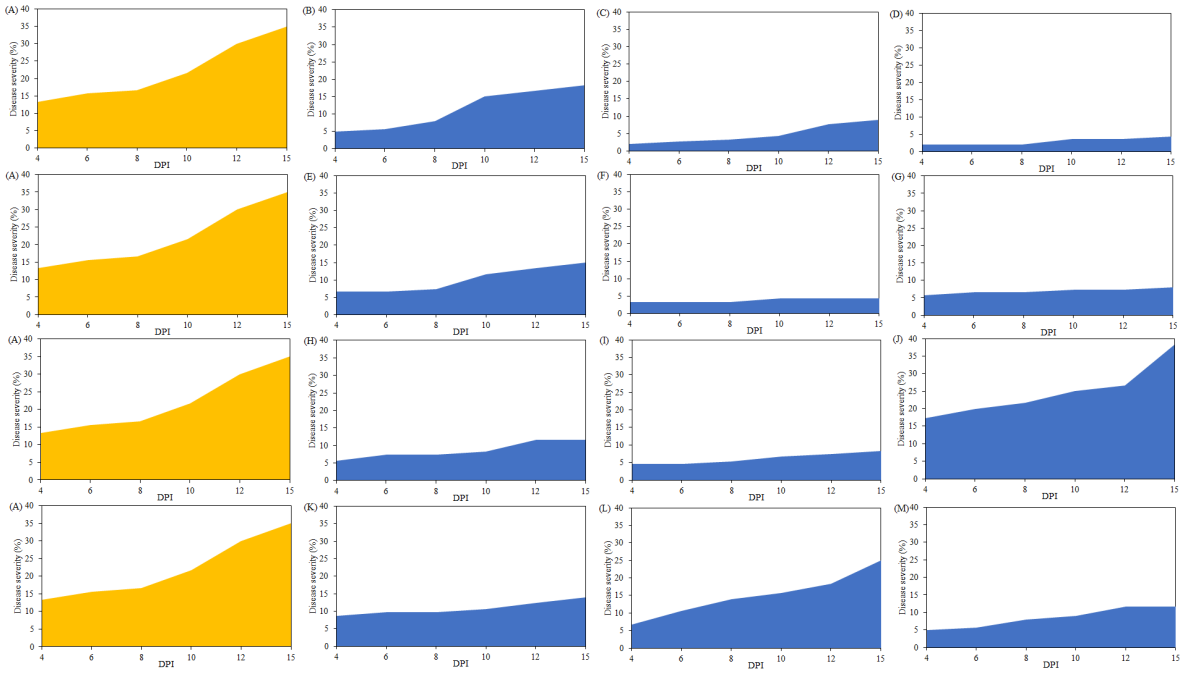


Figure 1. Progression of disease severity in rice seedlings after actinomycete treatment
Note: A = Untreated, B = KrK_1T_{18}, C = KrK_1K_1, D = DrK_1T_{20}, E = DcK_1D_8, F = DrK_1T_{21}, G = DrK_1T_{22}, H = RwK_1R_4, I = RwK_1R_2, J = KrK_1T6, K = DcK1D6, L = DcK1D4, M = KrK1T13 (experiment conducted in triplicate). DPI = Days post-inoculation

such as soil pH, moisture, temperature, nutrient availability, and their association with the plant. As a result, their ability to suppress pathogens under field conditions depends heavily on these complex interactions. It explains why actinomycetes may exhibit strong antagonistic effects against pathogens *in vitro*, yet fail to demonstrate similar efficacy in the field, vice versa.

KrK_1K_1 and DrK_1T_{20} isolates demonstrated a considerable effect, effectively reducing infection and limiting disease severity to 2.0% by the 5 days post-inoculation (DPI). Disease progression was notably slowed across all actinomycete treatments, with severity reaching only 4.3% and 9.0% by the 15th-DPI. DrK_1T_{21} proved to be another effective bioagent, suppressing disease and resulting in a final severity of 4.3%. The success of various actinomycete isolates in controlling BLB aligns with findings from previous studies (Vap Hop et al., 2014; Ilsan et al., 2016; Rahma et al., 2023a). Moreover, forest actinomycetes have been reported to reduce various fungal species, i.e., *Fusarium culmorum*, *F. oxysporum*, and *F. graminearum* (Sacramento et al., 2004; Shirokikh and Shirokikh, 2017a; Świecimska et al., 2021).

The AUDPC (Figure 2) showed that actinomycete treatment had a highly marked effect on suppressing BLB during the seedling stage ($p < 0.01$). Disease development was significantly lower than the control, except for the KrK_1T_6 and DcK_1D_4 treatments. The lowest AUDPC value was recorded in the DrK_1T_{20} isolate treatment at 33.0, followed by KrK_1K_1 and DrK_1T_{21}, with values of 55.3 and 42.67, respectively. The low AUDPC readings indicate that the severity of *Xoo*-induced disease can be effectively mitigated during its progression. The capacity of actinomycetes to reduce disease severity during the seedling phase must be beneficial when rice is cultivated in endemic regions. Actinomycetes' colonization of plant roots enables these biocontrol agents to reduce *Xoo* or enhance plant growth directly through enzymatic activities. The development and symptoms of BLB disease in rice treated with actinomycetes are presented in Figure 3. In the actinomycete treatment, disease symptoms were limited only to the leaf tips. In contrast, in the control, the symptoms were observed on nearly half of the leaf area.

The control efficacy of actinomycetes presented a significant impact. The effectiveness of disease control by actinomycete varied between 31.39% and 86.74%, except for the KrK_1T_6 treatment, which failed to impede disease progression. The elevated control percentage indicates that actinomycetes have significant potential to mitigate yield loss and disease prevalence effectively. Based on these results, 3 isolates–KrK_1K_1, DrK_1T_{20}, and DrK_1T_{21}–were highly effective in suppressing disease development in rice seedlings.

The ability of actinomycetes to colonize rice roots is an important factor in inhibiting disease development. It was confirmed that the DrK_1T_{20}

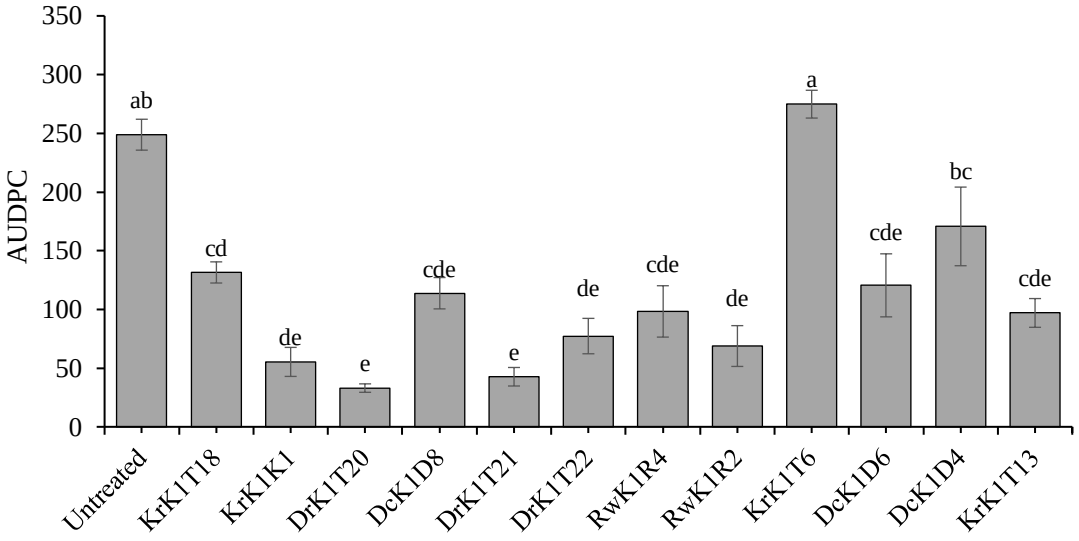


Figure 2. AUDPC of BLB in rice seedlings after actinomycete treatment
Note: The bars indicate the mean±SE; the same letters above the bars show no significant difference based on Fisher's LSD test at a 95% confidence level

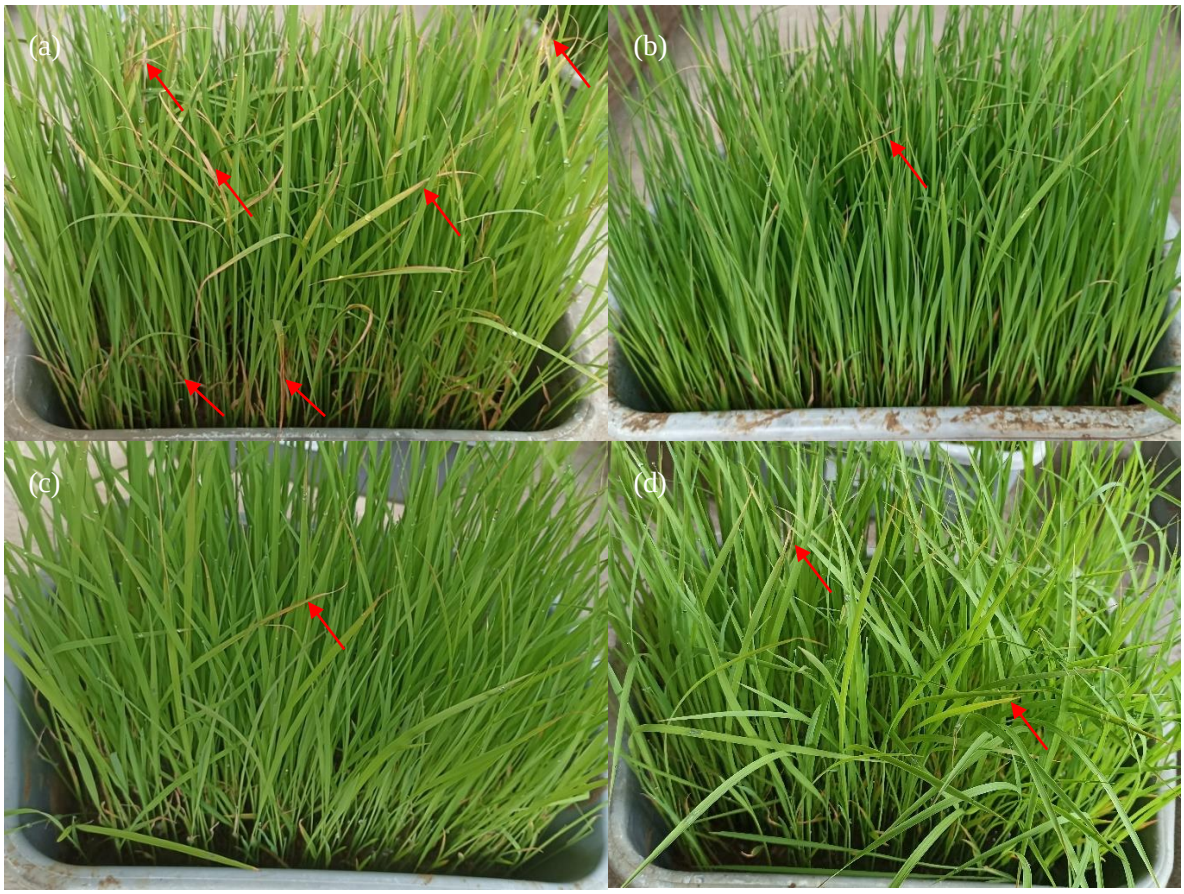


Figure 3. The effect of actinomycete application on the disease progression in the rice seedlings (a = Positive control, b = KrK_1K_1, c = DrK_1T_{20}, d = DrK_1T_{21})
Note: The red arrow indicates disease symptoms

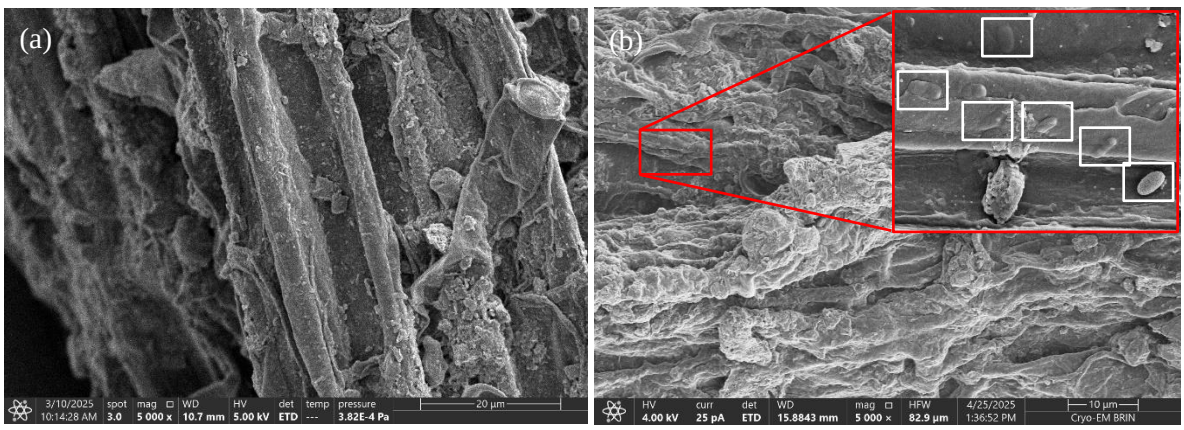


Figure 4. SEM image of IPB 3S rice root colonized by the DrK_1T_{20} isolate. (a) A non-inoculated and (b) Inoculated seed by DrK_1T_{20} isolate (5000X magnification and inzet visualize actinomycetes)

isolate successfully colonized the rice root surface, as shown in Figure 4. Effective root and rhizosphere colonization is a key requirement for successful actinomycete-based biofertilizers and biopesticides, enabling sustained establishment and pathogen suppression (Silva et al., 2022). It is a fundamental trait of plant growth–promoting actinomycetes, driven by attraction to root exudates and their ability to adhere to roots while producing protective compounds that enhance plant resistance to biotic and abiotic stresses (Djebaili et al., 2021).

Molecular identification

Molecular analysis was performed only on 3 actinomycete isolates–KrK_1K_1, DrK_1T_{20}, and DrK_1T_{21}–that exhibited optimal disease suppression. As shown in Table 2, the BLAST analysis confirmed that KrK_1K_1 belongs to *Streptomyces parvulus* (Accession number: PX806337). The strain was closely related to *S. parvulus* strain DS261, showing a 100% query cover and 99.18% similarity, indicating a nearly identical match to the reference strain from the Philippines (Accession number: MW217111.1). DrK_1T_{20} isolate was identified as *Tsukamurella tyrosinosolvens* (Accession number: PX806340) and closely related to strain PD-X-1, with a 98% query cover and 96.81% similarity, suggesting a slightly lower but still significant match to the reference strain from China (Accession number: MG763891.1). The actinomycetes listed in GenBank have the highest similarity to the KrK_1K_1 and DrK_1T_{20} strains and originate from various countries. KrK_1K_1 isolate closely resembles the *S. parvulus* strain from an Asian country, while DrK_1T_{20} shows similarity to strains from Asia and America. Meanwhile, the DrK_1T_{21} was closely related to the *Salinispora tropica* strain from the USA, with a 100% query cover and 96.26% similarity. Phylogenetic analysis revealed that the Indonesian *S. parvulus* clustered within the same clade as *S. parvulus* from the Philippines, while *T. tyrosinosolvens* grouped with a strain from South Korea, and *S. tropica* clustered with a strain from the United States (Figure 5).

The effect of the crude filtrate of the actinomycete on pathogen growth

The results of the actinomycetes filtrate in suppressing pathogen growth *in vitro* are shown in Figure 6. Changes in the pathogen population were determined based on observed absorbance values. The most widely used technique for estimating cell count in a liquid suspension is measuring OD at 600 nm (Beal et al., 2020).

Table 2. Identification of the actinobacterial isolates based on 16S rDNA

Strain	BLAST analysis	Query cover (%)	Similarity (%)	Country	Accession number
KrK_1K_1	*Streptomyces parvulus* strain DS261	100	99.18	Philippines	MW217111.1
	Streptomyces parvulus	100	99.18	India	KT906299.1
	Streptomyces parvulus strain DSD1692	100	99.18	Philippines	MW217106.1
	Streptomyces parvulus strain MX9	100	99.18	Vietnam	ON026158.1
	Streptomyces parvulus strain DSD982	100	99.18	Philippines	MW217122.1
DrK_1T_{20}	*Tsukamurella tyrosinosolvens* strain PD-X-1	98	96.91	China	MG763891.1
	Tsukamurella tyrosinosolvens strain DSM 44234	98	96.91	USA	NR_042801.1
	Tsukamurella tyrosinosolvens strain IFM-10571	98	96.91	Japan	AB478953.1
	Tsukamurella tyrosinosolvens strain Y2	98	96.91	South Korea	NR_044516.1
	Tsukamurella sp. strain H404	98	96.91	Indian Ocean	HQ622532.2
DrK_1T_{21}	*Salinispora tropica* isolate U3T_8104	100	96.26	USA	LT682689.1
	Salinispora tropica isolate 517N_15980	100	96.26	USA	LT690564.1
	Salinispora tropica isolate D3T_15479	100	96.26	USA	LT690063.1
	Salinispora tropica isolate D3T_15468	100	96.26	USA	LT690052.1
	Salinispora tropica isolate 517N_16009	100	96.26	USA	LT690593.1

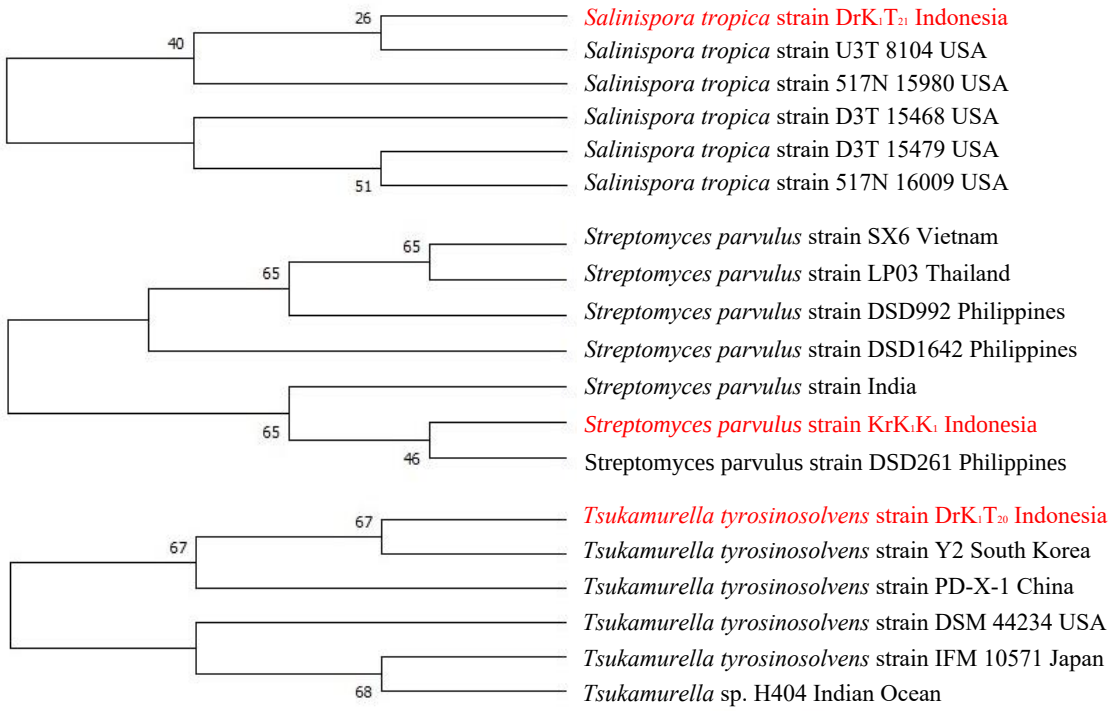


Figure 5. Phylogenetic tree representing the relationships between Indonesian actinomycetes and other strains worldwide

The same method has been used to determine the effect of *Streptomyces rameus* on the growth of ESKAPE pathogens (Dar and Ahmad, 2025). However, the correlation between cell concentration and OD is complex, as it is influenced by light path length, particle size, and particle concentration. Figure 6 shows the proliferation of pathogens, as indicated by absorbance values, which exhibited variations among treatments. The antimicrobial activity of actinomycete filtrates varied considerably among isolates. The filtrate of *S. parvulus* exhibited the most potent inhibitory effect, as indicated by a flattened *Xoo* growth curve over 14 hours of incubation. *T. tyrosinosolvens* showed growth inhibition after 10 hours of incubation, whereas *S. tropica* displayed no inhibitory activity, as evidenced by a continuous increase in absorbance throughout the incubation period.

In the control (red line), pathogen growth rapidly increases after 4 hours of incubation. Between 6 and 10 hours post-application, the *Xoo* population proliferated significantly in the filtrate of *T. tyrosinosolvens* and *S. tropica* treatments. A significant decline in OD values occurred after 10 hours and continued until 14 hours post-application. The filtrate of *S. parvulus* exhibited a superior capacity to suppress pathogens, as indicated by its OD_{600} value remaining below 0.3 in all concentrations. However, increasing the filtrate concentration did not consistently lead to a proportional inhibition of pathogen growth *in vitro*. The activity of actinomycete filtrates is closely associated with their ability to produce a range of metabolites. Actinomycetes are major soil microbes known for creating a diverse range of bioactive secondary metabolites, including antibiotics and extracellular enzymes (Hata et al., 2015).

Characteristics of selected actinomycete isolates

The research findings demonstrate that the biochemical synthesis capacities of actinomycetes, which are intimately linked to their ability to boost plant growth, exhibit considerable variability (Table 3). Most isolates can synthesize siderophores and produce lytic enzymes such as chitinase and protease. However, they did not show the capacity to synthesize cellulase or IAA. The pathogen-suppressing activities of actinomycetes are attributed to their capacity to synthesize a variety of lytic enzymes, including protease, amylase, chitinase, cellulase, esterases, and lecithinase, which play crucial roles in breaking down pathogen cell walls and disrupting their metabolism (Loliama et al., 2013; Lahmyed et al., 2021; Díaz-Díaz et al., 2022). These enzymes also play a crucial role in catalyzing the formation of various compounds

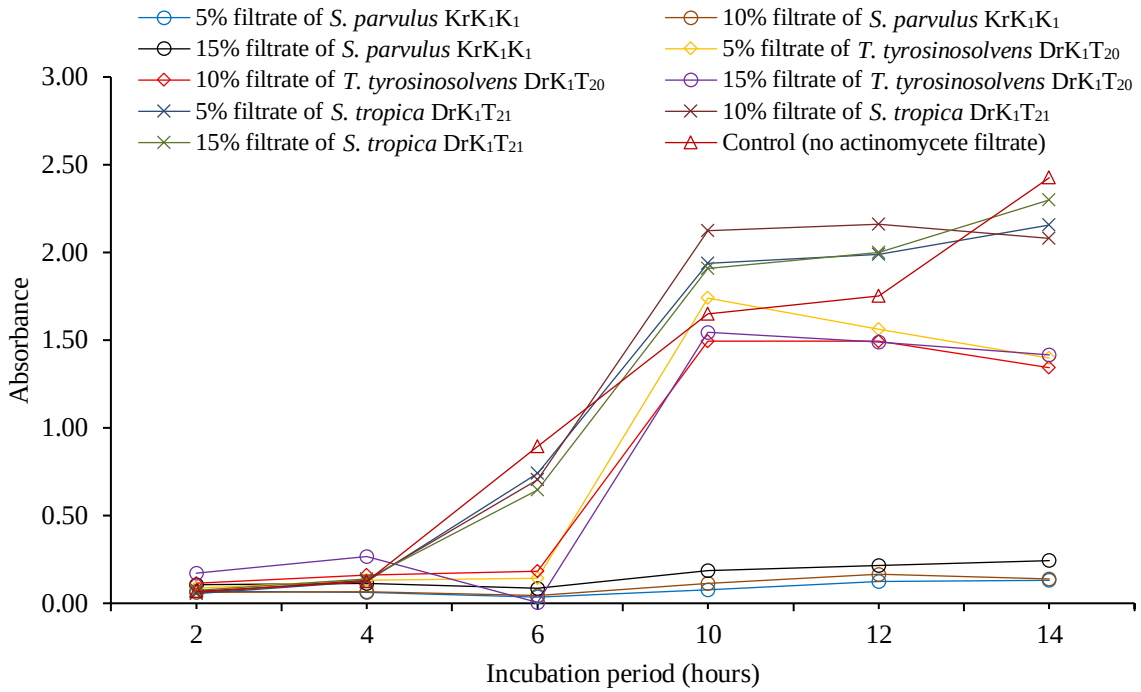


Figure 6. The impact of different concentrations of actinomycete filtrate on decreasing pathogen growth in a liquid medium

Note: Absorbance was measured at $\lambda_{600\ nm}$, and each treatment was replicated twice

Table 3. Characteristics of actinomycetes isolated from the forest of Kutai National Park

Parameters	Actinomycete species		
	S. parvulus KrK_1K_1	*T. tyrosinosolvens* DrK_1T_{20}	*S. tropica* DrK_1T_{21}
Production of siderophore	0.79	0.32	1.03
Production of phosphatase solubilization	0.54	-	-
Production of IAA	-	-	-
Production of cellulase	-	-	-
Production of protease	-	0.67	1.79
Production of chitinase	0.49	0.19	1.75

Note: - represents that there is no activity

during seed germination, making them available for seed growth (Din et al., 2014; Joshi, 2018). The ability to produce phosphatase was found only in *S. parvulus*. The *S. parvulus* produces various enzymes, including protease, amylase, lipase, and cellulase, and exhibits phosphate solubilization ability (Kadaikunnan et al., 2023).

Meanwhile, *T. tyrosinosolvens* DrK_1T_{20} and *S. tropica* DrK_1T_{21} isolates share distinct characteristics that set them apart from *S. parvulus*: their ability to produce protease. These microbial proteases play a crucial role in the recycling of organic matter in soil and in the breakdown of cell walls. Proteases are key components of plant defense, functioning in pathogen recognition, programmed cell death, and degradation of microbial proteins (Paiva-silva et al., 2025), while in phytopathogenic bacteria, they act as critical virulence determinants by regulating infection-related processes, modulating virulence factor expression, and secreting enzymes that suppress host immune recognition and defense responses (Figaj et al., 2019). The ability of actinomycetes to synthesize proteases is crucial for mediating interactions with host cells and minimizing host defense responses.

Actinomycetes produce siderophores that limit pathogen access to iron, alongside secondary metabolites such as kaempferol, iso-scutellarin, umbelliferone, and cichoriin, which contribute to plant defense mechanisms and overall plant health (Jaber and Fayyadh, 2019). Iron is a vital element for almost all living organisms, functioning as a key cofactor in numerous enzymatic processes,

including photosynthesis, respiration, and DNA synthesis (Pandey, 2023). Iron availability and its regulation through siderophore-mediated uptake are crucial for *Xoo* virulence, as iron metabolism directly controls the bacterium's ability to grow, respond to environmental signals, and cause disease (Subramoni and Sonti, 2005). More than 50% of the tested actinomycetes in this study exhibit siderophore synthesis activity, with the *S. tropica* DrK_1T_{21} demonstrating the highest capability. The siderophore-producing ability of these actinomycetes is highly beneficial for reducing iron availability, which is also required by *Xoo*. Iron metabolism and regulation plat critical determinant of its virulence (Verma et al., 2018a).

Actinomycetes also secrete several antibiotics and antifungal compounds, which further inhibit pathogen growth and produce plant growth-promoting factors that enhance crop resilience (Zulfa et al., 2021). The production of these bioactive compounds, which are closely linked to their plant growth-promoting potential, is vital when these actinomycete species are applied to plants. Plants need nutrients to grow, and nutritional imbalances can significantly affect a plant's susceptibility to pathogen attacks (Val-Torregrosa et al., 2022). This study is consistent with the findings that different actinomycete strains produce different biochemical compounds (Chaudhary et al., 2013). Furthermore, variations in the growth medium type can lead to differences in the metabolites produced by actinomycetes (Charousová et al., 2017). The disparities in compound production influence their efficacy in enhancing plant development and inhibiting infections. The various capabilities of actinomycete strains to enhance plant growth, including nutrient solubilization, nitrogen fixation, and phytohormone synthesis, indirectly contribute to the management of plant diseases (Djebaili et al., 2021).

In this study, *S. parvulus* KrK_1K_1 and *T. tyrosinosolvens* DrK_1T_{20} were the key species exhibiting these beneficial properties. *S. parvulus* has been shown to increase growth and decrease the incidence of Fusarium wilt in *Vigna radiata* (Kadaikunnan et al., 2023) and damping-off disease in *Phaseolus vulgaris* (Korayem et al., 2020). Additionally, *S. parvulus* VNUA74, isolated from the banana rhizosphere, exhibited vigorous antagonistic activity against multiple pathogens (Nguyen et al., 2025). This species has been reported to produce Actinomycin D, inhibiting various pathogenic bacteria, including streptomycin-resistant strains. Meanwhile, *Tsukamurella tyrosinosolvens* have been reported to promote drought resistance and stimulate peanut growth by enhancing leaf water retention, optimizing photosynthesis, controlling stomatal closure, and increasing antioxidant enzyme

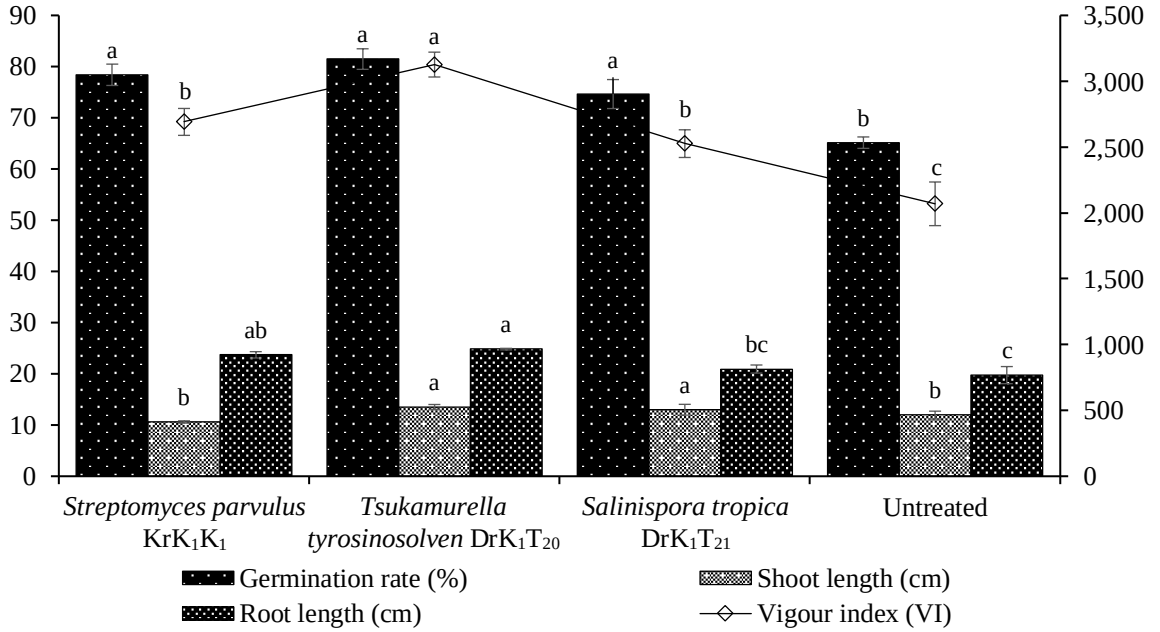


Figure 7. Performance of seedlings treated with different actinomycete species
Note: Different letters above the mean±SE bars show significant differences ($p < 0.05$) based on Fisher's LSD

activity (Long et al., 2023). This ability is strongly believed to be influenced by root exudates (Jiang et al., 2023).

The ability of actinomycetes as a growth promoter

Preserve seed germination

The germination potential and vigor index of seeds were increased after treatment with actinomycetes compared with the control (Figure 7). The actinomycetes treatment showed a substantial impact on the germination of rice seeds. Germination rate, vigor index, and seedlings' primary root lengths increased significantly compared to the control group ($p < 0.05$). Meanwhile, the shoot length was not significantly different from that of the control ($F = 3.39$, $p = 0.074$).

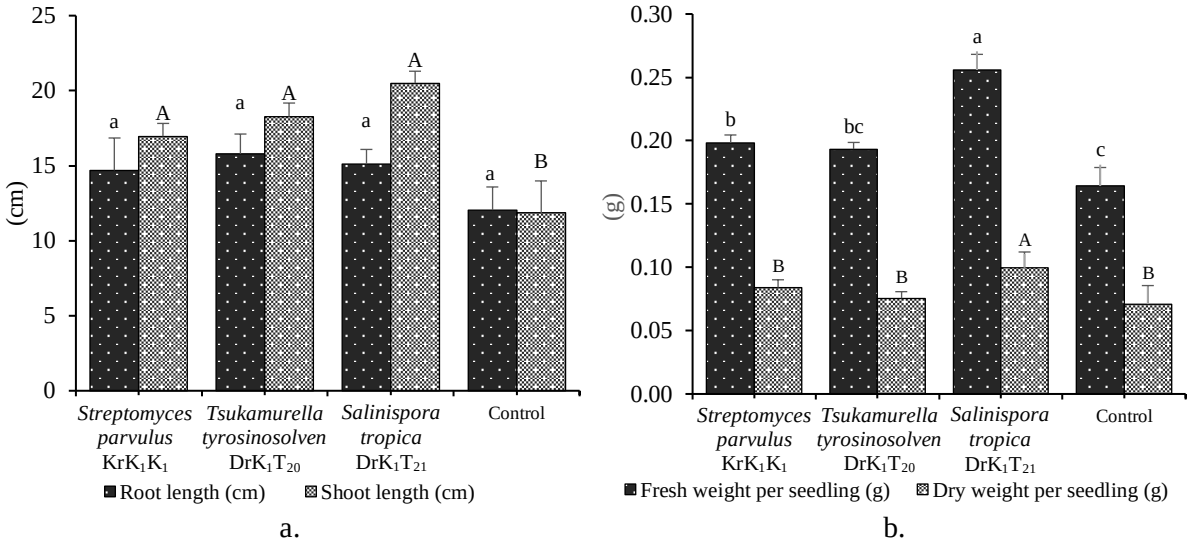
The application of *T. tyrosinosolvens* DrK_1T_{20} to seeds resulted in the highest germination rate and vigor index among all treatments, with values of 81.47% and 3,126.5, respectively. Seed germination activity is closely linked to the activity of various hydrolytic enzymes, including proteases. Protease enzymes catalyze the breakdown of seed proteins into soluble proteins, free amino acids, and peptides, which are then transported to the developing embryo (Din et al., 2014; Joshi, 2018). Protease activity in rice seeds treated with PGPR increased during the first 1 to 2 days of germination and subsequently declined due to the degradation of soluble proteins into amino acids (Din et al., 2014). Therefore, it is reasonable that the germination and vigor index in actinomycete-treated seeds is higher than in the control.

The capability of this actinomycete is essential for minimizing farmers' excessive seed consumption, which often results from poor seed quality or seed-borne diseases. Plants with good germination and vigor are better able to tolerate pathogen infection during the seedling stage (Hassani et al., 2019). Actinomycetes have been demonstrated to enhance seedling performance. On the 14th day, root and shoot lengths showed considerable growth compared with the control. Two weeks post-testing, *T. tyrosinosolvens* DrK_1T_{20} exhibited superior seedling growth, as evidenced by the longest shoot and root lengths among all treatments.

Plant growth enhancement

Actinomycetes enhance germination and promote rice seedlings' growth after pathogen inoculation (Figure 8). Applying actinomycetes to zeolite-enriched media enhanced seedling performance. ANOVA indicated that actinomycete treatment significantly influenced fresh weight, plant height, and dry weight of rice seedlings ($p < 0.05$), while root length did not increase substantially ($p > 0.05$).

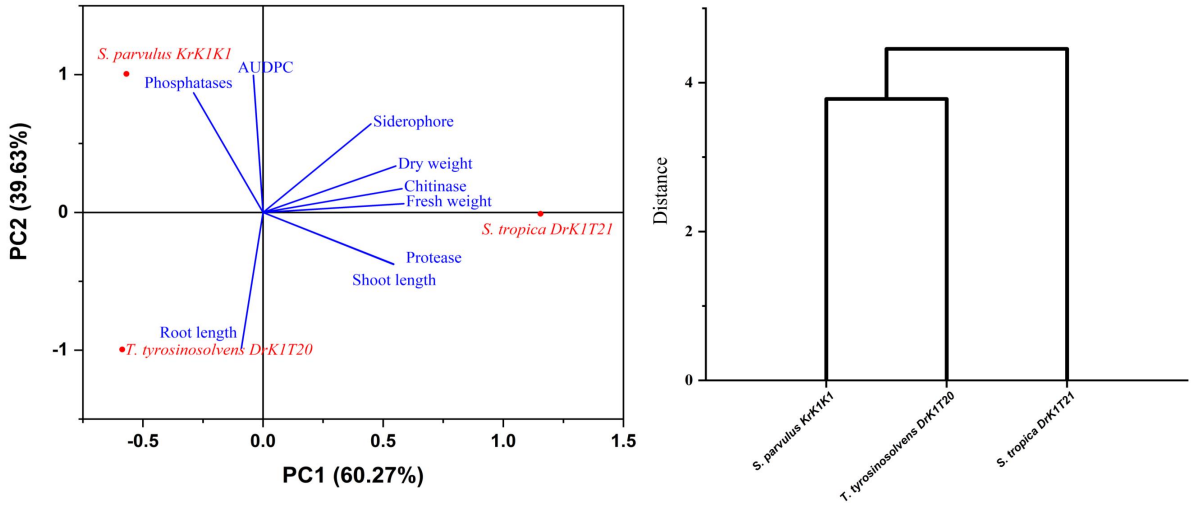
The shoot length of seeds treated with actinomycete species was significantly greater than that of untreated seeds, averaging 16.95 to 20.50 cm. Although actinomycetes did not cause a significant increase in root length, their application produced root length greater than that of the control. Concurrently, root length in



a. b.

Figure 8. Effects of actinomycete on the seedlings' growth at 14 days (a = Root and shoot length, b = Fresh and dry weight)

Note: Different letters show that the mean±SE is a significant difference at the 0.05 level (Fisher's LSD)



a. b.

Figure 9. PCA illustrating the interactions among growth parameters, AUDPC, and enzymatic activities of actinomycetes (a), and a complete-linkage dendrogram based on Euclidean distance showing the clustering of 3 actinomycete isolates (b)

the actinomycete treatments varied from 14.69 to 15.79 cm. Actinomycetes consistently enhanced seedling growth, evidenced by superior plant height, root length, fresh weight, and dry weight relative to the control. The best seedling growth was observed with the application of *S. tropica*, which produced the best seedling stem length, fresh weight, and dry weight. These 4 seedling growth parameters can be important factors in explaining the effectiveness of actinomycetes in promoting seedling growth.

To explore the relationships between plant growth parameters and disease suppression, multivariate analyses were conducted using PCA and hierarchical clustering with a complete-linkage dendrogram, as presented in Figure 9. Multivariate analyses revealed clear functional differentiation among forest-derived actinomycete strains, based on their plant growth–promoting and antagonistic traits. PCA showed that PC1 and PC2 accounted for 60.27% and 39.63% of the total variance, respectively, indicating strong discrimination among isolates. PC1 was mainly associated with siderophore production, hydrolytic enzyme activities, and biomass-related traits, indicating pathogen reduction and growth promotion, whereas PC2 was influenced by root length and phosphatase activity, indicating root-associated functions and nutrient mobilization. The PCA successfully separated the 3 species into distinct quadrants. The PCA biplot highlighted strain-specific functional characteristics: *S. tropica* DrK_1T_{21} associated with siderophore production and shoot biomass; *S. parvulus* KrK_1K_1 correlated with phosphatase activity; and *T. tyrosinosolvens* DrK_1T_{20} was closely related to root development, suggesting superior root colonization and induced systemic resistance.

Hierarchical clustering supported these patterns, confirming functional heterogeneity among isolates. Overall, the integration of PCA and clustering analyses demonstrates that the superior performance of strain DrK_1T_{20} is likely attributable to its multi-functional traits, particularly those related to root interaction and belowground growth promotion. This isolate promoted relatively good seed germination and a high vigor index, as well as root growth; however, it did not enhance stem growth or plant biomass as effectively as isolate DrK_1K_{21}. These findings support the hypothesis that forest actinomycetes possess unique and complementary mechanisms, shaped by evolutionary pressures in complex ecosystems. Such functional diversity provides a strong foundation for selecting effective biological control agents against *Xoo*, highlighting the potential of niche-adapted actinomycetes in sustainable rice disease management.

CONCLUSIONS

Three species of forest-derived actinomycetes were identified as *S. parvulus* KrK_1K_1, *T. tyrosinosolvens* DrK_1T_{20}, and *S. tropica* DrK_1T_{21}, which effectively suppressed the BLB in rice

seedlings by 77.77%, 86.74%, and 82.85%, respectively. Moreover, *T. tyrosinosolvens* DrK_1T_{20} enhanced the seed germination rate and vigor index by 25.05% and 51.11%, respectively. After pathogen inoculation, however, *S. tropica* DrK_1T_{21} showed the best growth-promoting activity. These findings suggest a trade-off between the induction of plant defense responses and the promotion of growth. The successful suppression of disease is primarily supported by actinomycete root colonization and their ability to produce various bioactive compounds, including lytic enzymes and siderophores.

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AI Usage Statement

During the production of this publication, the authors used QuillBot and Grammarly Premium to paraphrase the text and verify its grammatical accuracy, respectively. The authors accept complete responsibility for the content of this publication and have reviewed and edited it as necessary after utilizing this tool or service.

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