BIODIVERSITAS
Volume 25, Number 6, June 2024
Pages: 2661-2669

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250636

Exploring the biocontrol and plant growth-promoting potential of *Streptomyces kronopolitis* isolate SA18 against durian foliar blight and dieback disease

PARINYA KRAIVUTTINUN[1], KUNCHIT PIRAPAK[2], PATTACHAI PINNAK[3], WANWISA PIRAPAK[3,♥]

[1]Program in Environment, Faculty of Science and Technology, Uttaradit Rajabhat University. Tha-it, Meaung, Uttaradit, 53000, Thailand
[2]Program in Survey Technology and Geo-Informatics, Faculty of Industrial Technology, Uttaradit Rajabhat University. Tha-it, Meaung, Uttaradit, 53000, Thailand
[3]Program in Biology, Faculty of Science and Technology, Uttaradit Rajabhat University. Tha-it, Meaung, Uttaradit, 53000, Thailand.
Tel.: +66-89-6400191, ♥email: wanwisa.pir@uru.ac.th

Manuscript received: 9 May 2024. Revision accepted: 22 June 2024.

Abstract. *Kraivuttinun P, Pirapak K, Pinnak P, Pirapak W. 2024. Exploring the biocontrol and plant growth-promoting potential of* Streptomyces kronopolitis *isolate SA18 against durian foliar blight and dieback disease. Biodiversitas 25: 2661-2669.* Actinomycetes have been extensively studied for their potential as plant growth promoters and biocontrol agents against fungal plant pathogens. In Thailand, durian is a highly valuable fruit crop, celebrated for its delectable fleshy arils. *Rhizoctonia solani* destruction causes foliar blight and dieback, a severe disease on durian plantations. Our investigation objective was to isolate, select, and identify actinomycetes that exhibit antagonistic properties and could improve the growth of durian. This study revealed that 37 actinomycete isolates were obtained, comprising 14 endophytic isolates and 23 soil-derived actinomycete isolates from durian plantations in Thailand's Uttaradit and Sukhothai Provinces. All actinomycete isolates demonstrated the ability to inhibit pathogen growth through in vitro antagonistic bioassay. Isolate SA18 exhibited the highest percentage inhibition of radial growth against *R. solani* isolate 01, achieving an impressive 92% inhibition. Furthermore, isolate SA18 demonstrated the capacity to produce 5.91 µg/mL of indole acetic acid after seven days of culture, increasing to 45.72 µg/mL after 14 days. This isolate also exhibited phosphate solubilization ability. In the detached leaf bioassay, isolate SA18 effectively controlled the spread of fungal pathogens, reducing disease severity significantly from 93.75% in the control treatment to 53.75% with a concentration of 10^8 spores/mL of isolate SA18. Morphological examination and 16S rRNA gene sequencing revealed that isolate SA18 formed smooth-surfaced spores in chains and showed a close relationship with *Streptomyces kronopolitis* based on the 16S rDNA sequence. This study is the first to report the presence of *S. kronopolitis* in soil samples collected from durian orchards in Thailand. This isolate can improve plant growth and control *R. solani* in bioassays. *S. kronopolitis* isolate SA18 can potentially be a biocontrol agent for durian plantations.

Keywords: Biocontrol, dieback, durian, foliar blight, *Streptomyces*

INTRODUCTION

Durian (*Durio zibethinus* Murr.) is Thailand's most profitable fruit crop, renowned for its delicious fleshy arils (Surat Thani Provincial Agriculture and Cooperatives Office 2021). Pests and diseases pose significant constraints on crop production. In addition to the devastating diseases caused by *Phytophthora palmivora*, *Rhizoctonia* foliar blight and dieback, caused by the pathogen *Rhizoctonia solani* Kuhn, present equally damaging threats. This disease can destroy seedlings and mature trees, resulting in up to 40% losses. On mature trees, it can lead to twig dieback and defoliation. Rhizoctonia foliar blight and dieback have become prevalent in Thailand's durian-growing regions, particularly during warm and wet seasons (Office of Agricultural Research and Development Region 6 2022). Chemical-based disease management is unsuitable for controlling diseases in large plantation areas due to concerns about their environmental and human health impacts. Consequently, investigating the use of biological control agents to prevent dieback disease in durian is worthwhile.

Numerous microbial taxa, including viruses, bacteria, and fungi, have been identified as biocontrol agents. Gram-positive bacteria, known as actinomycetes, can be found in various pHs, temperatures, humidities, and environments (Jagannathan et al. 2021). Various habitats, including terrestrial, marine, hypersaline, wetlands, and plant endophytes, have been reported to harbor actinomycetes (Tatar 2021). The phylum Actinobacteria consists of over 800 species with validly published names, with the genus *Streptomyces* was first named by Waskman and Henrici (1943), being the largest taxon (Parte 2018). Actinomycetes have been used as biocontrol agents against phytopathogenic fungi in recent years, providing an alternative to synthetic fungicides (Boukaew et al. 2022). Actinomycetes exhibit various antagonistic mechanisms, including competition for resources, production of antibiotics, siderophores, lytic enzymes, and stimulation of host resistance (Torres-Rodriguez et al. 2022). In addition, they can enhance plant growth by producing plant growth regulators and serving as biocontrol agents (Sreevidya et al. 2016; Nazari et al. 2023).

The rhizosphere is a fascinating zone where plants and microbes, including actinomycetes, interact. The bacterial community's diversity, structure, and diversity in the rhizosphere vary depending on the plant species and root zones (Iqbal et al. 2023). A habitat known as an endophytic environment is home to numerous bioactive actinomycetes. Actinomycetes found in the rhizosphere and inside plants have been shown to have important functions in promoting plant growth, inducing disease resistance, stimulating drought tolerance, and acting as biocontrol agents (Oberhofer et al. 2019). Plant Growth Regulators (PGRs) are organic compounds produced by plants in response to specific stimuli and are present in very low concentrations (Soumare et al. 2021). Plant growth regulators are categorized into three groups based on their origin: those synthesized by plants, those of microbial origin, and synthetic compounds (Blázquez et al. 2020). Therefore, five primary categories of plant growth regulators can be differentiated based on their chemical compositions and impacts: auxins, cytokinins, gibberellins, abscisic acid, and ethylene (Pour et al. 2019). *Streptomyces* can form symbiotic relationships with crop plants, producing hormones like gibberellic acid and indole-3-acetic acid (IAA) (Himaman et al. 2016; Devi et al. 2022). IAA, a crucial phytohormone, is vital in promoting root growth. The synthesis of IAA has been documented in different *Streptomyces* species (Myo et al. 2019; Suárez-Moreno et al. 2019).

This study aimed to select actinomycetes from durian roots and rhizosphere soils for use as biocontrol agents against the fungal pathogen responsible for durian foliar blight and dieback disease and investigate their potential to promote plant growth. The selected isolates were evaluated for their capacity to mitigate *R. solani* infection, a prevalent cause of durian foliar blight and dieback disease in nurseries and plantation environments.

MATERIALS AND METHODS

Study area

A total of six samples of durian roots and five samples of rhizospheric soil were collected from durian plantations in Uttaradit and Sukhothai Provinces of Thailand. The geographic scope of this study includes the area of approximately location 1: 17°40'9 N latitude and 99°56'22 E longitude; location 2: 17°43'2 N latitude and 100°0'46 E longitude; and location 3: 17°49'42 N latitude and 100°6'34 E longitude. The collection period was extended from April 2022 to January 2023. Specimens of durian leaves displaying symptoms of dieback disease were collected from a durian plantation area at location 2. The infected leaves were carefully placed in plastic bags and transported to the laboratory for fungal pathogen isolation.

Procedures

Sample preparation

The durian root samples were carefully excavated to ensure optimal acquisition and transportation of the maximum amount of root material to the laboratory. Before processing, the soil surrounding the roots, known as rhizosphere soil, was separated and allowed to dry naturally at room temperature.

Isolation of actinomycetes from roots and rhizosphere soils

The durian root samples underwent surface sterilization using a method described by Mingma et al. (2014) with some modifications. Initially, the roots were rinsed in flowing water to remove soil particles. Subsequently, they were subjected to surface sterilization by shaking in a 0.1% solution of Tween 20 for five minutes, followed by immersion in a 1.0% solution of NaOCl for 10 minutes, then in 2.5% $Na_2S_2O_3$ for another 10 minutes, and finally in a 75% ethanol solution for five minutes. The samples were then rinsed three times in sterile water to remove surface sterilization agents. The efficacy of the surface sterilization technique was assessed by applying 100 µL of the final wash sample onto ISP2 agar plates (Himaman et al. 2016) and incubating them at 28°C for 1-2 days to observe microbial colony growth. After surface sterilization, the samples were immersed in a 10% $NaHCO_3$ solution for 10 minutes. Subsequently, the samples were crushed using a sterile glass rod and mixed with 0.85% normal saline. The prepared root suspensions were then applied to two specific media: Starch Casein (SC) agar (Ser et al. 2015) and humic acid-vitamin (HV) agar (Krishanti et al. 2018) using the spread plate method. These media were supplemented with nalidixic acid (25 µg/mL) and ketoconazole (100 µg/mL). The plates were then incubated at 28°C for 14 to 21 days.

The dilution plate method was employed to isolate rhizospheric actinomycetes from air-dried soil samples collected from the rhizosphere (Himaman et al. 2016). Each soil sample was suspended by mixing one Gram of soil with 9 mL of sterile distilled water and then diluted in a series of up to 10^{-5}. Soil suspensions of 0.1 mL, ranging from 10^{-3} to 10^{-5}, were then applied onto SC and HV agar plates supplemented with antibiotics, following the previously described method. After a 14-day incubation period at 28°C, the growth of actinomycete colonies was assessed. Colonies exhibiting distinct morphological features were isolated on ISP2 agar and preserved for storage in a 20% (v/v) glycerol solution at -20°C. The isolates obtained from the roots of durian trees and the soil surrounding the roots were designated as "RDR" and "SA" abbreviations, respectively.

Isolation and pathogenicity test of a fungal pathogen in durian foliar blight and dieback disease

The fungal pathogen was obtained from the durian leaves using the tissue transplanting technique. Branches and segments of diseased durian plants measuring approximately 1x1 cm were excised and immersed in a 1.2% sodium hypochlorite solution for three minutes. Subsequently, the samples were rinsed with sterilized distilled water 2-3 times and gently dried at room temperature. The samples were cultured on Potato Dextrose Agar (PDA) medium and incubated at 25°C for 2-4 days. The hyphal tip was isolated on PDA under a stereo microscope and then cultured on PDA plates. The pathogenic isolate on PDA slants was preserved for long-term storage by covering it with sterile liquid paraffin.

For the pathogenicity test, the fungi were cultivated on PDA medium at 30°C for seven days, after which the fungus was transferred onto the leaves of 6-month-old Mon Thong variety durian plants. The inoculation process involved two methods: wound and non-wound. Subsequently, the specimens were placed in a moist chamber for 24 hours. Disease occurrence and symptoms were recorded after seven days of inoculation.

Identification of a fungal pathogen

Fungal identification based on morphological characteristics was performed on a PDA medium at an ambient temperature of 28-30°C. The study examined the colony's characteristics, dimensions, and pigmentation of hyphae. The quantity of nuclei present in the mycelium cells was also assessed. Mycelium was stained with safranin O and a 3% KOH solution and then observed under a compound microscope at 100X, 400X, and 1,000X magnifications. Additionally, the color of the sclerotia granules was examined using a stereo microscope under a magnification range of 20X-40X (Lim et al. 1987).

According to Ramos-Molina et al. (2016), fungal mycelium was cultured in potato dextrose broth and incubated for 5 days for molecular identification based on ribosomal DNA region sequencing. The cultivation was conducted on a shaker at a speed of 75 rpm. Subsequently, the mycelium was harvested through filtration and freeze-drying for approximately 48 hours. DNA extraction was performed using a GenElute kit (Sigma-Aldrich), following the manufacturer's instructions. The isolate was identified using Polymerase Chain Reaction (PCR) with primers specific for the ribosomal DNA region ITS-5.8S (Ramos-Molina et al. 2016). A universal primer pair, ITS4 and ITS5, was utilized for amplification. The PCR conditions utilized were the same as Ramos-Molina et al. (2016). The ribosomal DNA ITS-5.8S was sequenced using the primers ITS4 and ITS5. The PCR product sequence was performed by Macrogen (South Korea). Phylogenetic analyses were conducted using the neighbor-joining method and the Kimura model for genetic distance in MEGA v.11.0 (Tamura et al. 2021). The statistical significance of each clade in the obtained tree was evaluated by performing bootstrap resampling with 1,000 permutations.

In vitro antagonistic bioassay

The actinomycete isolates collected from both roots and rhizospheric soils were evaluated for their ability to inhibit the growth of *R. solani* using a dual culture technique. A mature actinomycete colony on ISP2 agar was transferred onto PDA plates in a straight line, positioned 3 cm from the center of the plate. After a 7-day incubation period at 30°C, a fungal disc (5 mm in diameter) was placed directly across from the actinomycete colony, also positioned 3 cm away from the center of the petri dish. A fungal mycelial disc was positioned on PDA plates in the same region as the experimental set as a control. The plates were then incubated at 28°C until the fungal mycelium on the control plate grew to a radius of 6 cm. The percentage of inhibition of radial growth (PIRG%) was calculated using the formula $[(r1-r2)/r1]\times100$, where r1 represents the radius of fungal mycelial growth in the control group and r2 represents the radius of fungal mycelial growth in the presence of actinomycetes. The experiment was replicated.

Investigation of the plant growth-promoting abilities and biochemical properties of selected actinomycetes

The study assessed plant growth-promoting traits, specifically indole acetic acid and phosphate solubilization activity. At the same time, the study of biochemical properties was concentrated on determining cellulase and chitinase enzymes.

The quantification of IAA was conducted using the methodology established by Anwar et al. (2016), with certain adjustments. Discs of Actinomycete culture, grown for seven days on ISP2 medium, were added to 5 mL of GYE broth (containing 10 g/L glucose and 10 g/L yeast extract), supplemented with 0.2% (w/v) L-tryptophan. The mixture was placed on a rotary shaker at 170 rpm and incubated in the dark at 28°C for seven days. The cultures were subjected to centrifugation at 5,800 rpm for 10 minutes. After that, 1 mL of the liquid remaining after centrifugation was combined with 1 mL of Salkowski's reagent and left to react in the dark for 30 minutes at room temperature. The presence of a pink color indicated the production of IAA. The reaction mixture was measured using a spectrophotometer to determine its absorbance at a wavelength of 530 nm. The concentration of IAA produced per milliliter of culture (μg/mL) was determined using a standard curve of indole acetic acid (0.5-100 μg/mL).

The phosphate solubility was investigated using Pikovskaya's (PVK) medium according to the method outlined by Selvi et al. (2017). Discs of Actinomycete culture, aged seven days and grown on ISP2 medium, were placed on a PVK agar plate containing 0.5% tricalcium phosphate as the sole source of phosphorus. The plates were placed in an incubator and maintained at 28°C for two weeks. The colony producing the halo zone was regarded as a phosphate-solubilizing isolate. The experiments were conducted in duplicate.

The cellulase enzyme-producing isolates were screened by cultivating selected actinomycetes on carboxymethyl-cellulose medium plates. The plates were cultured for 14 days at 30°C. The clearing zones around the isolates were observed following the flooding of the plates with a 0.1% solution of Congo red. The plates were incubated for 30 minutes and subsequently rinsed with 1 M NaCl. Clearing zones indicate a positive result regarding the isolate's ability to produce cellulase.

Actinomycetes were surveyed for chitinolytic activity. The specimen was cultured in an ISP2 medium containing 1.0% colloidal chitin to induce chitinase production. The inoculated plates were incubated at 30°C for 10 days. A degradation halo surrounding the colonies indicated the hydrolysis of chitin.

Detached leaves bioassay

The durian leaves were taken from healthy plants, cleaned using running tap water, and sterilized on the surface following the method described by Himaman et al. (2016). The detached leaf assay was conducted with four

treatments, as outlined: (1) Durian leaves were immersed in actinomycete spores at a 10^8 spores/mL concentration for 60 minutes. (2) Durian leaves were immersed in actinomycete spores, following the same procedure described previously, and then treated with a suspension of *R. solani* mycelium (10^8 cells/mL) to ensure complete leaf coverage. (3) Durian leaves were treated with a suspension of *R. solani* mycelium (10^8 cells/mL) to ensure complete leaf coverage. (4) Durian leaves were treated only with sterile distilled water as a control. The treatments were stored in a moist, sterile chamber with wet filter paper. The chamber was sealed with paraffin film to retain moisture in the leaves. The storage temperature was maintained at 28°C for five days. Each treatment used 15 leaves. The impact of actinomycetes on disease suppression in detached leaves was evaluated by measuring the proportion of infected leaf area. The degree of disease severity, indicated as a percentage of the lesion area on the leaf surface, was assessed using a scale ranging from 0 to 4. The scores were assigned as follows: Score 0 indicates no symptoms; Score 1 indicates that 1-25% of the leaf area is infected; Score 2 indicates that 26-50% of the leaf area is infected; Score 3 indicates that 51-75% of the leaf area is infected; and Score 4 indicates that more than 75% of the leaf area is infected. The formula used by Himaman et al. (2016) was employed to calculate disease severity and biocontrol efficacy. The formula for disease severity is calculated by:

Disease severity = [∑ (the disease score × the number of leaves displaying symptoms)/(total number of leaves investigated × 4)] × 100, where the number 4 represents the highest disease score.

Cultural characteristics and 16S rRNA gene sequencing

The selected actinomycetes were cultured on nutrient agar (NA), PDA, and International Streptomyces Project (ISP) solid medium, including yeast malt extract agar (ISP 2), oatmeal agar (ISP 3) and glycerol asparagine agar (ISP 5), using the streak plate method, and incubated at 30°C for 14 days. Gram staining was examined in a 1-day-old colony on NA medium. The characteristics of colonies were described, and the color of the colonies was analyzed using the ISCC-NBS Color Chart (Kelly 1958). Spores and hyphae were examined under a light microscope.

The actinomycete was identified through the process of 16S rRNA gene sequencing. The genomic DNA of the isolate was obtained using the modified extraction method described by Daigham and Mahfouz (2020). The PCR amplification of 16S rRNA gene fragments was performed using a TopTaq Master Mix kit (Qiagen) and two sets of primers. The first set of primers, 1F (5'-TCACGGAGAGTTTGATCCTG-3') and 1530R (5'-AAGGAGGTGWTCCARCC-3'), was used as described by Kataoka et al. (1997). Additionally, the Mg4F (5'-ATTCCTGGTGTAGCGGT-3') and 782R (5'-ACCAGGGTATCTAATCCTGT-3') primers, as described by Chun (1995), were occasionally applied. The PCR protocol involved an initial hot start incubation at 94°C for 10 minutes, followed by 30 cycles of denaturation at 94°C for one minute, annealing at 53°C for one minute, and extension at 72°C for one minute. This was followed by a final extension at 72°C for 10 minutes. The PCR products were purified using a Qiagen QIAquick PCR clean-up kit (Qiagen, Chatsworth, CA). The purification process followed the instructions of the manufacturer's protocols. After purification, the PCR products were sent to Macrogen, South Korea, for sequencing. The DNA sequences were edited and aligned using MEGA v.11.0. A phylogenetic tree was generated using the neighbor-joining method with 1000× bootstrap analysis in MEGA v.11.0.

Data analysis

The observation data of PIRG%, disease scores, and disease severity went through statistical analysis of variance (ANOVA), and the treatment means were compared using least significance tests at a significance level of $P<0.05$. The data were analyzed using the SPSS program (version 22.0).

The process of aligning and visualizing sequences was performed using ClustalW on MEGA v.11.0 (Tamura et al. 2021). The final alignment was confirmed using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure the samples were real and see how similar their sequences were to those in GenBank. Therefore, to do a phylogenetic analysis, a phylogenetic tree was made using the neighbor-joining method and Kimura-2-parameter substitution models (Kimura 1980). The tree was reconstructed with 1,000 replicates of the bootstrap value, and the genetic distance between species was examined using MEGA v.11.0 software (Kumar et al. 2018).

RESULTS AND DISCUSSION

Isolation of actinomycetes from roots and rhizosphere soils

This study aimed to collect and examine actinomycete strains from rhizosphere soils and the roots of durian trees to identify those with the ability to inhibit the growth of other organisms and promote the growth of plants. Soils in the rhizosphere and roots of durian trees were obtained from the Uttaradit and Sukhothai Provinces in Thailand. This study used fine root samples of durian with a 3-5 mm diameter, obtained from a depth of 10-25 cm below ground, to isolate endophytic actinomycetes. Therefore, 37 actinomycete isolates were collected, consisting of 14 endophytic isolates and 23 soil-derived actinomycete isolates.

Isolation and pathogenicity test of a fungal pathogen in durian foliar blight and dieback disease

Collected samples of the pathogen causing foliar blight and dieback disease were obtained from durian orchards in Laplae District, Uttaradit Province. The samples exhibited symptoms of the disease in the following manner: Early signs include burns on the leaves (Figure 1.A). Eventually, the wound grows larger and changes color to a deep brown shade. The fungus responsible for the disease forms hyphae that resemble spider webs, which bind the leaves together (Figures 1.A and 1.B). The leaves become infected and

eventually wither, clinging to the branch (Figure 1C). Before gently descending and encountering the foliage below, the disease will spread, resulting in the appearance of patches of burned leaves. There may be a sensation of burning on the leaf margins and at the tips of the leaves. The dry leaves adhere to other leaves, and clusters dangle from the branches. In due time, the leaves will drop off, leaving behind only the sturdy branches, and the branch eventually dries up. After microscopic examination, it was discovered that the fibers growing on the branch possess a wall, indicating the presence of the disease-causing fungus. No reproductive cells were discovered. The fibers exhibit perpendicular side branches (T cells) (Figure 1.E) and show more than two nuclei (multiple nuclei) (Figure 1.F).

After experimenting on the PDA medium for eight days, the pathogens displayed distinct colony characteristics. They produce fibers without color. Eventually, it will transition from a shade of brown to black-brown after seven days (Figure 1.G).

The pathogenicity test on durian leaves was investigated by observing brown wounds on the backs of the leaves after inoculating and incubating for three days. The injury appeared discolored. After seven days of incubation, it was observed that the tissue in the inoculated area underwent a significant color change, appearing dark brown, almost black. The tissue became dehydrated and ultimately died (Figure 1.D).

Identification of a fungal pathogen

Therefore, using morphological features to identify fungi, it was found that the mycelium is between 4.5 and 8.5 µm wide and contains multiple nuclei, forming brown to black-brown sclerotia granules. The shape is not clearly defined.

DNA sequence analysis of the ITS-nrDNA region determined that the fungus isolate 01 causing durian foliar blight and leaf blight disease is *Rhizoctonia solani*. In the tree of evolutionary relationships, the pathogen (PP770709) shows a striking resemblance of 100% to *R. solani* KJ577141 and a significant similarity of 78% to *R. solani* isolates KP202862, JQ292803, and MH122653 (Figure 2).

In vitro antagonistic bioassay

Using the dual-culture plate technique on a PDA medium, the growth of *R. solani* was investigated by examining 37 actinomycete isolates (isolates SA01-23 and RDR1-14). After 10 days of infection, it was observed that most actinomycete isolates demonstrated the capacity to inhibit the growth of the pathogen (Figure 3). Isolate SA18 exhibited the highest level of PIRG% against *R. solani*, with an impressive percentage of 92%. Actinomycetes play a crucial role in preventing the growth of fungal hyphae that lead to leaf blight. This is evident from the clearly visible clear zone of growth inhibition (Figure 4).

Investigation of the plant-growth-promoting abilities and biochemical properties of selected actinomycetes

An actinomycete isolate, SA18, described for its exceptional effectiveness in controlling *R. solani*, was investigated in this experiment. The investigation focused on producing IAA, phosphate degradation, cellulase, and chitinase enzymes. Isolate SA18 demonstrated the capacity to generate 5.91 µg/mL of IAA after seven days of culture, which increased to 45.72 µg/mL after 14 days. Through phosphate degradation, it was discovered that isolate SA18 could break down phosphate when grown on PVK's medium containing 0.5% tricalcium phosphate. This formed a distinct, clear zone surrounding the colony after seven days of incubation. Testing the ability to produce cellulase and chitin-degrading enzymes on a test medium for 10 days revealed that isolate SA18 could not produce either type of enzyme.

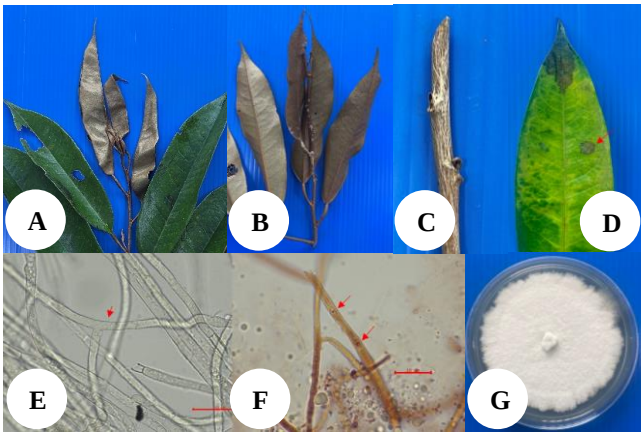

Figure 1. Foliar blight and dieback disease symptoms on leaves (A, B) and branches (C) on a durian tree; pathogenicity test on durian leaves (D); T-cell mycelium (E); and mycelium with 2 nuclei (F); and a colony of dieback disease pathogen isolate 01 on PDA after incubation for 7 days (G)

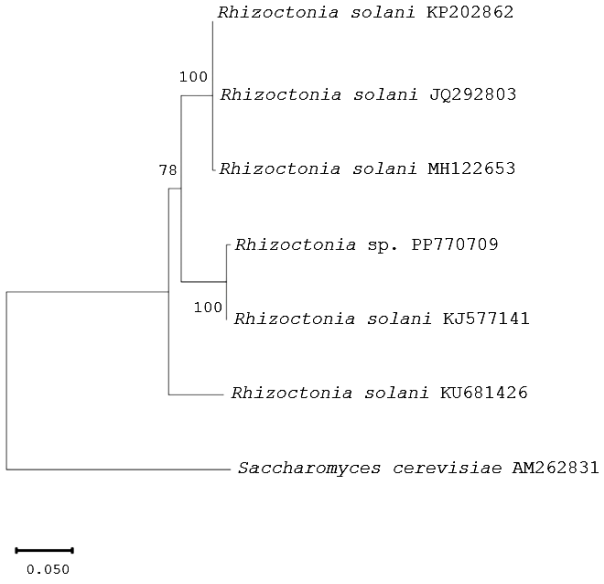

Figure 2. Neighbor-joining tree based on ITS-nrDNA region sequences showing the position of the foliar blight and dieback disease pathogen (PP770709) versus related species of *Rhizoctonia*. The nodes' numbers indicate the bootstrap support levels (%) based on 1,000 reassembled datasets, in which only the branches with >50% bootstrap support are labeled. The scale bar at the bottom indicates genetic distance units based on Nei's genetic distance

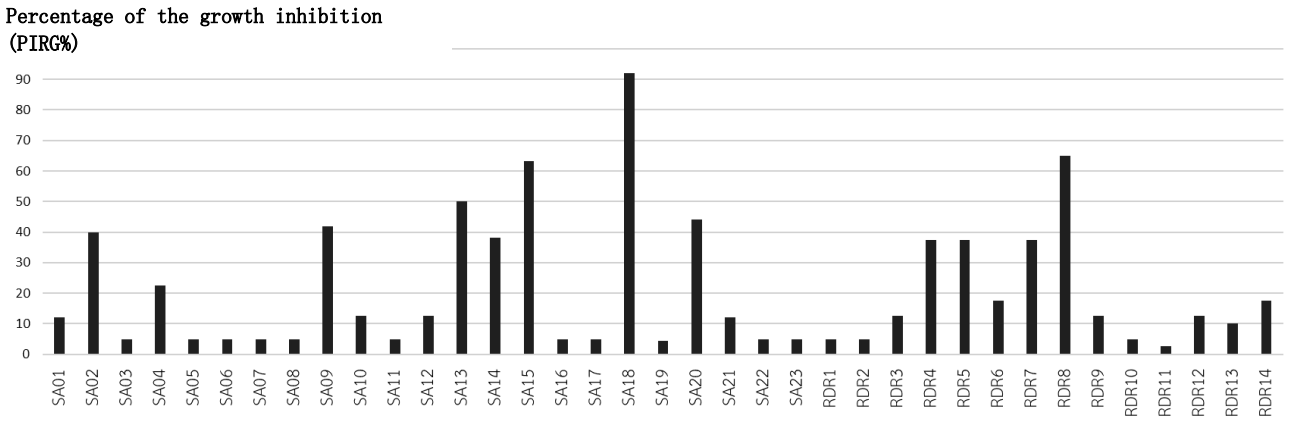

Figure 3. Percentage of the growth inhibition of different actinomyces isolates against *R. solani* isolate 01 using the Dual Culture method after a 10-day incubation period

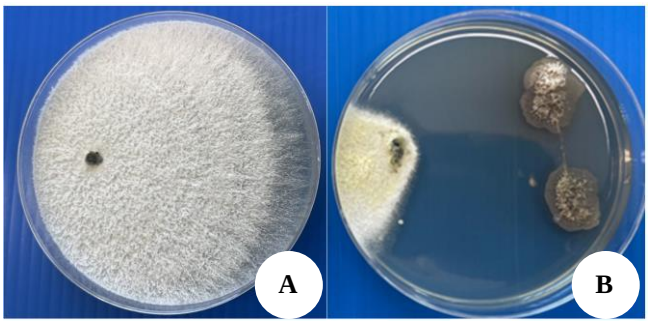

Figure 4. *In vitro* antifungal activity of the isolate SA18, control (A), anti-R. *solani* isolate 01 (B)

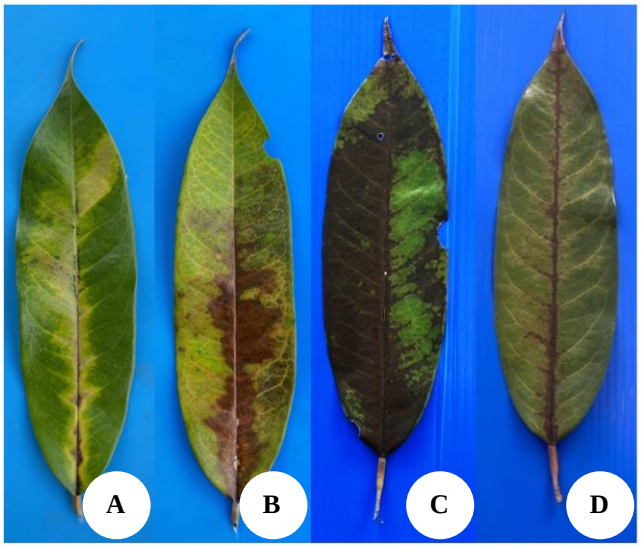

Figure 5. Detached leaves assay of durian with *R. solani*: (A) leaf inoculated with isolate SA18; (B) leaves pre-treated with isolate SA18 before inoculated with *R. solani*; (C) leaf inoculated with *R. solani* (showing the serious symptom of leaf blight disease); and (D) leaf from the control treatment. The leaves were observed after incubation at 28°C for 7 days

Table 1. Comparison of disease score levels, number of leaves displaying symptoms, and percentage of disease severity in all treatments

Treatment	Disease score	No. of leaves displaying symptoms	Disease severity
1 (SA18)	1.00 c	15	25.00 c
2 (SA18 and *R. solani*)	2.15 b	15	53.75 b
3 (*R. solani*)	3.75 a	15	93.75 a
4 Control (sterile distilled water)	1.07 c	15	26.75 c

Detached leaves bioassay

In the investigation of all treatments, it was evident that the control leaves (treatment 4) displayed only a few symptoms (Figure 5.D). However, the durian leaves treated with *R. solani* exhibited severe leaf blight symptoms (Figure 5.C). A few symptomatic developments were observed when leaves were inoculated with the isolate SA18 (Figure 5.A). When detached leaves treated with isolate SA18 spores were inoculated with *R. solani*, the growth of the fungus was inhibited (Figure 5.B). It was found that the isolate SA18 could control the spread of fungal pathogens. The disease severity decreased significantly from 93.75% in the control treatment to 53.75% when using a concentration of 10^8 spores/mL of isolate SA18 (Table 1).

Cultural characteristics and 16S rRNA gene sequencing

Based on the morphological study of the actinomycete isolate SA18 (Figure 6), it was found that it was able to grow on various solid media, including ISP-2, ISP-3, ISP-5, NA, and PDA (Figures 6.A-6.E). Colonies exhibit distinct characteristics based on the type of medium. Pigments and spores were produced on all media types (Table 2). When examining cell morphology and sporulation under a light microscope, it was determined that isolate SA18 was gram-positive based on Gram staining results (Figure 6.F). It was observed that isolate SA18 formed spores in a chain with a smooth surface (Figure 6.F).

Based on the 16S rDNA sequence, isolate SA18 was analyzed for its relatedness to known species. It was evident that isolate SA18 (PP770720) was closely related to *Streptomyces kronopolitis* (KN050495) (99%) (Figure 7).

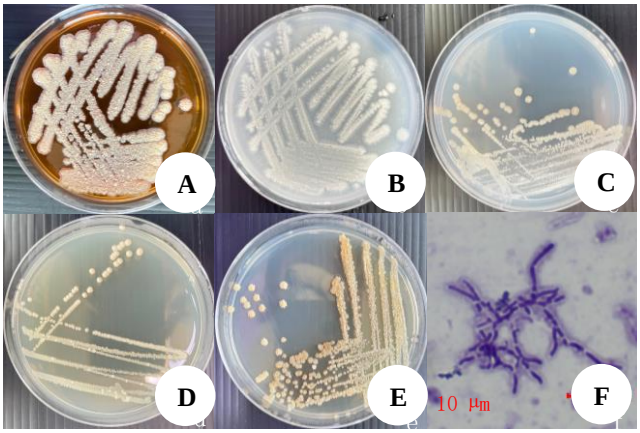

Figure 6. Cultural characteristics of isolate SA18, on ISP 2 (A), ISP 3 (B), ISP 5 (C), NA (D), and PDA (E), respectively, and Gram stain of cells under a microscope (x100) (F)

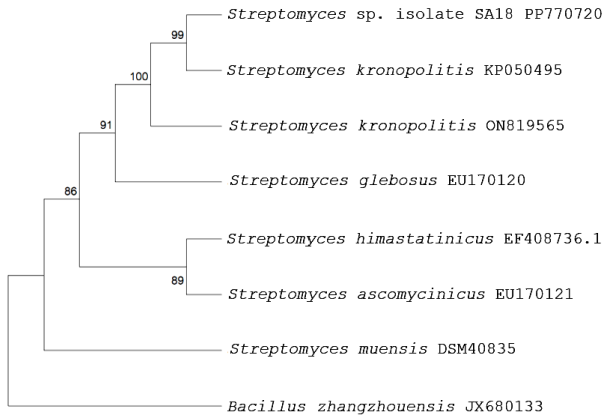

Figure 7. A neighbor-joining tree based on 16S rRNA gene sequences shows the position of isolate SA18 (PP770720) versus related *Streptomyces* species. The nodes' numbers indicate the bootstrap support levels (%) based on 1,000 reassembled datasets

Table 2. Morphology characterization of antagonistic actinomycetes isolates SA18 culture on various media

Medium	Color of aerial mycelium	Color of substrate mycelium
ISP-2	Yellowish white	Pale yellow
ISP-3	Yellowish white	Greenish white
ISP-5	Yellowish white	Pale yellow
NA	Yellowish white	Pale yellow
PDA	Pale yellow	Dark grayish-yellow

Discussion

Currently, extensive research is being conducted on using actinomycetes as biological control agents and biofertilizers in various agricultural crops (Myo et al. 2019; Torres-Rodriguez et al. 2022), aiming to reduce reliance on chemicals and fertilizers. There is also interest in employing microorganisms that aid in plant disease biocontrol to enhance plant growth and mitigate disease infections. However, there is currently a lack of information regarding the isolation of actinomycetes from soil and durian, as well as their ability to combat foliar blight and dieback disease in durian trees.

In this study, a disease pathogen was isolated and identified as *R. solani* based on morphology and DNA sequence analysis of the ITS-nrDNA region. Lim et al. (1987) reported that foliar blight and dieback in durian seedlings and trees in Peninsular Malaysia were attributed to *R. solani* (teleomorph, *Thanatephorus cucumeris*). These organisms were harmful to various plants, including durian, papaya, cucumber, long bean, Mikania weed, padi, musk melon, mung bean, Zoysia grass, Bermuda grass, and St. Augustine grass. However, various pathogens are responsible for causing symptoms of foliar blight and dieback in durian. Pongpisutta et al. (2020) investigated the occurrence of durian branches with dieback symptoms and brown lesions on the stems in orchards in Chumphon, Chanthaburi, and Trat provinces in Thailand. The fungal pathogen was analyzed and identified as *Fusarium solani*.

Moreover, 37 actinomycete isolates have been obtained from the rhizosphere soils and roots of durian samples (Figure 3). All the isolates were examined for their antagonistic effects in vitro. It was discovered that most actinomycete isolates could inhibit the growth of the pathogen. The *Streptomyces* genus is commonly found in plant roots and rhizospheric soils. They are most renowned for their remarkable capacity to produce secondary metabolites, particularly antibiotics. However, actinomycetes that are not streptomycetes are also highly regarded as potential sources for discovering new bioactive compounds and antibiotics because of their impressive metabolic diversity (Himaman et al. 2016; Nazari et al. 2023). Fatmawati et al. (2020) indicate that using certain actinomycetes found in the soybean rhizosphere shows potential as a biocontrol agent for combating damping-off disease caused by *R. solani*; of 26 isolates, 18 demonstrated diverse antifungal activities against *R. solani*, with PIRG% ranging from 18.9 to 64.8%. Whereas, in this study, isolate SA18 had the highest level of PIRG% against *R. solani*, with an impressive 92% (Figure 3). According to a recent study by Meena et al. (2022), certain strains of *Streptomyces* found in soil have demonstrated a remarkable ability to reduce the growth of *R. bataticola* mycelium by an impressive 65.3%. A study on Streptomyces sp. found in a terrestrial environment that *Botrytis cinerea*'s mycelial growth was significantly reduced by 77% (Vijayabharathi et al. 2018). Torres-Rodriguez et al. (2022) highlighted how actinomycetes exhibit antagonistic behavior. These include their ability to compete for resources and space, produce antibiotics, siderophores, and lytic enzymes, and induce host resistance, among other mechanisms.

Observing the formation of an inhibition zone in an antagonistic bioassay, isolating SA18's ability to produce antibiotics is the primary factor contributing to its antagonistic behavior. Compared to the control treatment, this isolate effectively suppressed the growth of the pathogenic fungi and caused an apparent change in the color of the mycelium morphologically (Figure 2).

Regarding plant growth enhancement, there have been reports on the effectiveness of different microorganisms, such as actinomycetes, in promoting plant growth (Himaman et al. 2016; Suksaard et al. 2017; Nonthakaew et al. 2021). IAA is a significant phytohormone that promotes root elongation and cellular division (Myo et al. 2019). It is commonly used in agriculture to enhance plant growth and development. For this study, the initial test for IAA production involved observing the formation of a pink-red color using Salkowski's reaction. The production of IAA was observed at a concentration of 45.72 µg/mL within 14 days. Microorganisms produce IAA through either tryptophan-dependent or tryptophan-independent pathways. Myo et al. (2019) reported that it was found that the kaolin-based formulation has the potential to enhance plant growth through the use of *Streptomyces fradiae*. Additionally, isolate SA18 exhibited the capability to solubilize phosphate. Microbial mechanisms can enhance phosphorus availability for plants, leading to improved growth and yield. In recent years, the ability of phosphate-solubilizing actinomycetes has become a topic of interest among researchers (Salcedo et al. 2014).

Isolate SA18, one of the antagonists, exhibited the most potent activity against *R. solani* in the in vitro assay. The fungus control ability was also demonstrated in a detached leaf assay. The disease severity decreased significantly from 93.75% in the control treatment to 53.75% when using a concentration of 10^8 spores/mL of isolate SA18. This isolate indicates great potential for future use as a biocontrol and plant growth promoter in durian field conditions. Himaman et al. (2016) showed that the control of *Eucalyptus* leaf and shoot blight resulted in disease severity of only 30% in *Streptomyces* isolate EUSKR2S82-inoculated *Eucalyptus* leaves compared to 95% in the control treatment. Goudjal et al. (2014) reported that *Streptomyces* sp. CA-2 and AA-2 have shown great potential in controlling *R. solani* damping-off and promoting the growth of tomato seedlings.

The morphological study and 16S rRNA gene sequencing revealed that isolate SA18 formed spores in a smooth-surfaced chain, and the 16S rDNA sequence clearly indicated a close relationship with *Streptomyces kronopolitis*. In a study by Liu et al. (2016), *S. kronopolitis* was initially observed in millipedes (*Kronopolites svenhedind* Verhoeff). The DNA sequence of *S. kronopolitis* was similar to that of *S. lydicus* and *S. chattanoogensis*. The ability to produce antibiotics, phoslactomycins, was present. *Streptomyces kronopolitis* may isolate SA18 and has the potential to produce phoslactomycins, which are effective in controlling plant pathogenic pathogens. De Azevedo et al. (2024) reported that *Streptomyces kronopolitis* ARH (A3) was isolated from soil and showed the ability to act as an antagonist to *Staphylococcus aureus*, *Corynebacterium diphtheriae*, and *Mycobacterium abscessus*.

This study is the first to report the presence of *S. kronopolitis* in soil samples collected from durian orchards in Thailand. This finding is attributed to its remarkable capacity to produce substances that enhance plant growth. The selected actinomycete, *Streptomyces kronopolitis*, was shown for the first time in this study to have a remarkable ability to control *R. solani* in both in vitro bioassays and detached leaf assays. According to this experiment, it appears that *S. kronopolitis* isolate SA18 has the potential to produce the antibiotic phoslactomycin. Based on its characteristics, *S. kronopolitis* isolate SA18 shows potential for use as a biocontrol agent for durian plantations. Future research must analyze the type and quantity of organic compounds or antibiotics in *S. kronopolitis* isolate SA18 using GC-MS. Furthermore, considering the wide range of plant-microbe interactions across various ecosystems and plant types, it is crucial to acknowledge the potential limitations of generalizing the findings of this study to all plant species. In addition, various environmental factors, such as soil composition or climate, can influence plant growth-promoting traits more than Rhizospheric Actinomycetes. Considering the limitations of studying in vitro and in vivo traits is crucial when examining plant-microbe interactions in real-world environments.

ACKNOWLEDGEMENTS

The authors express gratitude to the National Research Council of Thailand (NRCT) for providing funding and to the Program of Biology and Environment, Faculty of Science and Technology, Uttaradit Rajabhat University, Uttaradit, Thailand, for allowing us to use their research equipment.

REFERENCES

Anwar S, Ali B, Sajid I. 2016. Screening of rhizospheric Actinomycetes for various *in-vitro* and *in-vivo* Plant Growth Promoting (PGP) traits and for agroactive compounds. Front Microbiol 7: 1334. DOI: 10.3389/fmicb.2016.01334.

Blázquez MA, Nelson DC, Weijers D. 2020. Evolution of plant hormoneresponse pathways. Annu Rev Plant Biol 71: 327-353. DOI: 10.1146/annurev-arplant-050718-100309.

Boukaew S, Yossan S, Cheirsilp B, Prasertsan P. 2022. Impact of environmental factors on *Streptomyces* spp. metabolites against *Botrytis cinerea*. J Basic Microbiol 62 (5): 611-622. DOI: 10.1002/jobm.202100423.

Chun J. 1995. Computer-assisted classification and identification of actinomycetes. [PhD thesis]. University of Newcastle, Australia.

de Azevedo VLS, Rosa FC, Dias LRL, Batista LA, Melo MC, Sales LA, Branco AJM, Araújo TRR, de Miranda RCM, Aliança ASDS. 2024. An evaluation of the antibacterial, antileishmanial, and cytotoxic potential of the secondary metabolites of *Streptomyces* sp. ARH (A3). Microorganisms 12 (3): 476. DOI: 10.3390/ microorganisms12030476.

Devi S, Sharma M, Manhas RK. 2022. Investigating the plant growth promoting and biocontrol potentiality of endophytic *Streptomyces* SP. SP5 against early blight in *Solanum lycopersicum* seedlings. BMC Microbiol 22: 285. DOI: 10.1186/s12866-022-02695-8.

Daigham GE, Mahfouz AY. 2020. Isolation, characterization, and screening of actinomycetes producing bioactive compounds from

Egyptian soil. Egypt Pharm J 19 (4): 381-390. DOI: 10.4103/epj.epj_44_20.

Fatmawati U, Meryandini A, Nawangsih AA, Wahyudi AT. 2020. Damping-off disease reduction using Actinomycetes that produce antifungal compounds with beneficial traits. J Plant Prot Res 60 (3): 233-243. DOI: 10.24425/jppr.2020.133318.

Goudjal Y, Toumatia O, Yekkour A, Sabaou N, Mathieu F, Zitouni A. 2014. Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic Actinomycetes isolated from native plants of Algerian Sahara. Microbiol Res 169 (1): 59-65. DOI: 10.1016/j.micres.2013.06.014.

Himaman W, Thamchaipenet A, Pathom-aree W, Duangmal K. 2016. Actinomycetes from *Eucalyptus* and their biological activities for controlling *Eucalyptus* leaf and shoot blight. Microbiol Res 188-189: 42-52. DOI: 10.1016/j.micres.2016.04. 011.

Iqbal MM, Nishimura M, Haider MN, Yoshizawa S. 2023. Microbial communities on eelgrass (*Zostera marina*) thriving in Tokyo Bay and the possible source of leaf-attached microbes. Front Microbiol 13: 1102013. DOI: 10.3389/fmicb.2022.1102013.

Jagannathan SV, Manemann EM, Rowe SE, Callender MC, Soto W. 2021. Marine Actinomycetes, new sources of biotechnological products. Mar Drugs 19 (7): 365. DOI: 10.3390/md19070365.

Kelly KL. 1958. Central notations for the revised ISCC-NBS color name blocks. J Res Natl Bur Stand 61 (5): 427-431. DOI: 10.6028/jres.061.035.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120. DOI: 10.1007/BF01731581.

Krishanti NPRA, Zulfiana D, Wikantyoso B, Zulfitri A, Yusuf S. 2018. Antimicrobial production by an Actinomycetes isolated from thetermite nest. J Trop Life Sci 8 (3): 279-288. DOI: 10.11594/jtls.08.03.10.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35 (6): 1547-1549. DOI: 10.1093/molbev/msy096.

Kwee LT, Chong NC, Lan CC. 1987. Etiology and control of durian foliar blight and dieback caused by *Rhizoctonia solani*. Ann Appl Biol 111 (2): 301-307. DOI: 10.1111/j.1744-7348.1987.tb01456.x.

Liu C, Ye L, Li Y, Jiang S, Liu H, Yan K, Xiang W, Wang X. 2016. *Streptomyces kronopolitis* sp. nov., an Actinomycete that produces phoslactomycins isolated from a millipede (*Kronopolites svenhedind* Verhoeff). Intl J Syst Evol Microbiol 66 (12): 5352-5357. DOI: 10.1099/ijsem.0.001520.

Meena LI, Rajeswari E, Ahiladevi P, Kamalakannan A, Kalaiselvi T. 2022. Antifungal potential of *Streptomyces rameus* GgS 48 against mungbean root rot (*Rhizoctonia bataticola* (Taub.) Butler). J Biosci 47 (1): 10. DOI: 10.1007/s12038-021-00244-5.

Mingma R, Pathom-aree W, Trakulnaleamsai S, Thamchaipenet A, Duangmal K. 2014. Isolation of rhizospheric and roots endophytic Actinomycetes from Leguminosae plant and their activities to inhibit soybean pathogen *Xanthomonas campestris* pv. *glycine*. World J Microbiol Biotechnol 30: 271-280. DOI: 10.1007/s11274-013-1451-9.

Myo EM, Ge B, Ma J, Cui H, Liu B, Shi L, Jiang M, Zhang K. 2019. Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. BMC Microbiol 19: 1-14. DOI: 10.1186/s12866-019-1528-1.

Nazari MT, Schommer VA, Braun JC, dos Santos LF, Lopes ST, Simon V, Machado BS, Ferrari V, Colla LM, Piccin JS. 2023. Using *Streptomyces* spp. as plant growth promoters and biocontrol agents. Rhizosphere 27: 100741. DOI: 10.1016/j.rhisph.2023.100741.

Nonthakaew N, Panbangred W, Songnuan W, Intra B. 2021. In-vitro evaluationof actinomycetes with plant growth-promoting properties. Asia-Pac J Sci Technol 27: 1-8. DOI: 10.14456/apst.2022.33.

Oberhofer M, Hess J, Leutgeb M, Gössnitzer F, Rattei T, Wawrosch C, Zotchev SB. 2019. Exploring Actinobacteria associated with rhizosphere and endosphere of the native alpine medicinal plant *Leontopodium nivale* subspecies *alpinum*. Front Microbiol 10: 2531. DOI: 10.3389/fmicb.2019.02531.

Office of Agricultural Research and Development Region 6. 2022. Integrated technology for prevention and elimination Durian root rot and stem rot disease, Eastern Region. Department of Agriculture. 63 p. [Thailand]

Parte AC. 2018. List of prokaryotic names with standing in nomenclature (bacterio. net), 20 years on. Intl J Syst Evol Microbiol 68 (6): 1825-1829. DOI: 10.1099/ijsem.0.002786.

Pongpisutta R, Rattanakreetakul C, Bincader S, Chatchaisiri K, Boonruangrod P. 2020. Detection of fungal pathogen causing durian dieback disease. Khon kaen Agric J 48 (4): 703-714. DOI: 10.14456/kaj.2020.65.

Moradipour M, Saberi-Riseh R, Mohammadinejad R, Hosseini A. 2019. Nano-encapsulation of plant growth-promoting rhizobacteria and theirmetabolites using alginate-silica nanoparticles and carbon nanotube improves UCB1 pistachio micropropagation. J Microbiol Biotechnol 29: 1096-1103. DOI: 10.4014/jmb.1903.03022.

Ramos-Molina LM, Chavarro-Mesa E, Pereira DA, Silva-Herrera MD, Ceresini PC. 2016. *Rhizoctonia solani* AG-1 IA infects both rice and signalgrass in the Colombian Llanos. Pesq Agropec Trop 46 (1): 65-71. DOI: 10.1590/1983-40632016v4638696.

Salcedo LDP, Prieto C, Correa MF. 2014. Screening phosphate solubilizing actinobacteria isolated from the rhizosphere of wild plants from the Eastern Cordillera of the Colombian Andes. Afr J Microbiol Res 8 (8): 734-742. DOI: 10.5897/AJMR2013.5940.

Selvi KB, Paul JJA, Vijaya V, Saraswathi K. 2017. Analyzing the efficacy of phosphate solubilizing microorganisms by enrichment culture techniques. Biochem Mol Biol J 3 (1): 1-7.

Ser H-L, Ab Mutalib N-S, Yin W-F, Chan K-G, Goh B-H, Lee L-H. 2015. Evaluation of antioxidative and cytotoxic activities of *Streptomyces pluripotens* MUSC 137 isolated from mangrove soil in Malaysia. Front Microbiol 6: 1398. DOI: 10.3389/fmicb.2015.01398.

Soumare A, Diédhiou AG, Arora NK, Tawfeeq Al-Ani LK, Ngom M, Fall S, Hafidi M, Ouhdouch Y, Kouisni L, Sy MO. 2021. Potential role and utilization of plant growth promoting microbes in plant tissue culture. Front Microbiol 12: 649878. DOI: 10.3389/fmicb.2021.649878.

Sreevidya M, Gopalakrishnan S, Kudapa H, Varshney RK. 2016. Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. Braz J Microbiol 47 (1): 85-95. DOI: 10.1016/j.bjm.2015.11.030.

Suárez-Moreno ZR, Vinchira-Villarraga DM, Vergara-Morales DI, Castellanos L, Ramos FA, Guarnaccia C, Degrassi G, Venturi V, Moreno-Sarmiento N. 2019. Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. Front Microbiol 10: 290. DOI: 10.3389/ fmicb.2019.00290.

Suksaard P, Pathom-aree W, Duangmal K. 2017. Diversity and plant growth promoting activities of actinomycetes from mangroves. Chiang Mai J Sci 44: 1210-1223.

Surat Thani Provincial Agriculture and Cooperatives Office. 2021. Product list (Durian): Information for development planning Agriculture and product cooperatives in Surat Thani Province (durian). Office of the Permanent Secretary, Ministry of Agriculture and Cooperatives. 100 p. [Thailand]

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 38 (7): 3022-3027. DOI: 10.1093/molbev/msab120.

Tatar D. 2021. Isolation, phylogenetic analysis and antimicrobial activity of halophilic actinomycetes from different saline environments located near Çorum province. Biologia 76: 773-780. DOI: 10.2478/s11756-020-00612-w.

Torres-Rodriguez JA, Reyes-Pérez JJ, Quiñones-Aguilar EE, Hernandez-Montiel LG. 2022. Actinomycete potential as biocontrol agent of phytopathogenic fungi: Mechanisms, source, and applications. Plants 11 (23): 3201. DOI: 10.3390/plants11233201.

Vijayabharathi R, Gopalakrishnan S, Sathya A, Vasanth Kumar M, Srinivas V, Mamta S. 2018. *Streptomyces* sp. as plant growthpromoters and host-plant resistance inducers against *Botrytis cinerea* in chickpea. Biocontrol Sci Technol 28 (12): 1140-1163. DOI: 10.1080/09583157.2018.1515890.

Waskman SA, Henrici AT. 1943. The nomenclature and classification of the actinomycetes. J Bacteriol 46 (4): 337-341. DOI: 10.1128/jb.46.4.337-341.1943.