Indonesian Journal of Applied Research
IJAR

Isolation and Antagonistic Activity of Actinomycetes Associated with Strawberry Plants (*Fragaria x ananassa* Duch.) Against *Colletotrichum acutatum* Simmonds

Oktira Roka Aji[1], Iis Nisya Nur Aisyah[1], Karomah Indah Fitria[2]

[1]Department of Biology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Indonesia
[2]Laboratory of Microbiology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Indonesia

Volume 5 Issue 3
(December 2024)
e-ISSN 2722-6395
doi: 10.30997/ijar.v5i3.519

ARTICLE INFO

Article history:
Received: 07-31-2024
Revised version received: 10-3-2024
Accepted: 10-8-2024
Available online: 12-27-2024

Keywords:
actinomycetes; antagonistic; biocontrol; dual-culture assay; strawberry.

How to Cite:
Aji, O. R., Aisyah, I. N. N., & Fitria, K. I. (2024). Isolation and Antagonistic Activity of Actinomycetes Associated with Strawberry Plants (Fragaria x ananassa Duch.) Against Colletotrichum acutatum Simmonds. *Indonesian Journal of Applied Research (IJAR)*, *5*(3), 196-207. https://doi.org/10.30997/ijar.v5i3.519

Corresponding Author:
Oktira Roka Aji
oktira.aji@bio.uad.ac.id

ABSTRACT

Strawberries (*Fragaria x ananassa* Duch.) are a valuable horticultural crop in Indonesia, but their productivity is threatened by anthracnose disease caused by *Colletotrichum acutatum* Simmonds. Traditional control methods often involved synthetic fungicides, which posed risks of pesticide residues and environmental harm. This study investigated Actinomycetes isolated from various strawberry plant organs and their rhizosphere as potential biological control agents. The isolation processes included the collection of Actinomycetes from strawberry plant organs and the rhizosphere, along with the regeneration of *C. acutatum*. The isolates were identified through observations of their morphological characteristics. Screening of antagonistic activity was performed using dual-culture assays. Over 10 Gram-positive Actinomycetes strains were successfully isolated and characterized. Two isolates exhibited the highest antagonistic activity against *C. acutatum*, with inhibition percentages of 67.17% and 55.80%, respectively. Data analysis revealed that these isolates demonstrated significant biocontrol potential through mechanisms such as competition and antibiosis. The results suggested that Actinomycetes could serve as effective, eco-friendly alternatives to chemical pesticides in strawberry cultivation. Future research should focus on optimizing these strains for broader agricultural applications.

1. Introduction

Strawberries (*Fragaria* × *ananassa* (Duchesne ex Weston) Duchesne ex Rozie) are high-value horticultural plants often grown for agrotourism in Indonesia (Aristya et al., 2019). They are popular due to their attractive appearance, sweet taste, and high content of antioxidants, vitamins, and nutrients (Karina et al., 2012). The demand for strawberries increases by up to 68% annually. However, strawberries are vulnerable to pathogen attacks, which can affect fruit quality and productivity (Istifadah et al., 2018; Sari et al., 2021). Anthracnose disease, caused by the fungus *Colletotrichum acutatum* Simmonds, poses a major threat to strawberry productivity, affecting fruits, stems, stolons, petioles, and leaves, and leading to significant yield losses (Lelana et al., 2015). Control of anthracnose in strawberries primarily relies on synthetic fungicides, particularly quinone-outside inhibitors (QoIs). However, overuse has led to resistance in *C. acutatum* isolates, especially to azoxystrobin and pyraclostrobin in Florida (Forcelini et al., 2016). Other fungicides, like propiconazole and bitertanole, have been evaluated but can cause phytotoxic effects (de los Santos García de Paredes & Romero Muñoz, 2002). Although alternative fungicides such as benzovindiflupyr and penthiopyrad have shown promise, concerns about resistance remain (Rebello et al., 2022). Furthermore, resistance to thiophanate-methyl is widespread in strawberry nurseries (Wu et al., 2022). The continuous use of synthetic fungicides can result in pesticide residues in fruit, environmental pollution, pathogen resistance, and harm to other microorganism (Lelana et al., 2015; Nurjasmi & Suryani, 2020). Therefore, there is a critical need for eco-friendly control methods to manage *C. acutatum*. Biological control, which involves using microorganisms with antagonistic properties against pathogens, presents a more environmentally friendly long-term solution (Syafriani et al., 2019). Microorganisms are advantageous as biocontrol agents due to their rapid growth on inexpensive substrates and their ability to produce potent enzymes (El-Saadony et al., 2022).

Actinomycetes, which are a group of Gram-positive bacteria found in plant tissues and soil, are known for producing bioactive compounds with antibiotic, anticancer, and antioxidant (De Simeis & Serra, 2021; Janardhan et al., 2014; Olano et al., 2009). They also produce antifungal compounds that inhibit pathogen growth through mechanisms such as competition for space and nutrients, antibiosis, and mycoparasitism (Malek et al., 2015; Marsaoli et al., 2020). Actinomycetes, particularly from the genus *Streptomyces*, have been widely studied for their antagonistic activity against plant pathogens, including species of *Colletotrichum*, which are responsible for anthracnose diseases in various crops. Numerous studies have demonstrated the potential of Actinomycetes as biological control agents. For example, *Streptomyces* isolated from avocado rhizosphere showed significant inhibition against *Colletotrichum gloeosporioides*, a common pathogen of avocado and other crops, with 15% of the isolates demonstrating mycelial growth inhibition (Trinidad-Cruz et al., 2021). Similarly, isolates from chili pepper rhizosphere exhibited antifungal activity against *C. gloeosporioides*, achieving a substantial reduction in anthracnose lesions (Suwan et al., 2012). In Vietnam, *Streptomyces* strains isolated from national parks showed broad-spectrum antifungal activity, including inhibition of *C. gloeosporio*ides (Van et al., 2020). Further, in the case of mango, *Streptomyces* isolates from soil significantly inhibited the growth of *Colletotrichum* spp., the causative agent of anthracnose, with inhibition rates reaching up to 87.79% (Urtgam et al., 2024). Post-harvest studies on bananas revealed that Actinomycetes isolates were able to reduce anthracnose caused by *Colletotrichum musae*, demonstrating disease inhibition up to 85.87% (Ara et al., 2012). Despite the growing research on the use of Actinomycetes for biocontrol of *Colletotrichum* species, studies specifically targeting *C. acutatum* in strawberry plants are still limited. This study aims to explore the potential of Actinomycetes isolated from strawberry plant organs and rhizosphere as natural antifungal

agents against anthracnose disease in strawberries caused by *C. acutatum.* Understanding their efficacy in inhibiting *C. acutatum* could lead to the development of environmentally friendly strategies for managing anthracnose in strawberries, contributing to sustainable agricultural practices.

2. Methods

2.1. Equipment and Materials

The equipment used includes Erlenmeyer flasks, Petri dishes, measuring cylinders, pipettes, autoclaves (SX500, TOMY), Bunsen burners, scalpels, slides, microscopes (CX22, Olympus), mortars, pestles, analytical balance, incubators (IN30, Memmert), ovens (UN55, Memmert), microwaves, vortexes, rulers, test tubes, and clamps. Materials used include strawberry plants (*Fragaria x ananassa* Duch.), *Colletotrichum acutatum* Simmonds fungus, soil samples, Starch Casein Agar (SCA) medium (HiMedia), Potato Dextrose Agar (PDA) medium (Oxoid), and various chemicals such as sterile distilled water, 70% alcohol, 0.5% NaClO solution, nystatin (Sigma-Aldrich), chloramphenicol (Sigma-Aldrich), and gram stain kit (K001, HiMedia).

2.2. Research Procedure

2.2.1. *Isolation of Actinomycetes from Strawberry Plant Organs (Fragaria x ananassa)*

Healthy strawberry plants (*Fragaria x ananassa*) were collected from Magelang, Central Java. Roots, stems, and leaves were washed with running water. The organ samples were surface-sterilized with 70% (v/v) alcohol for 1 minute, 0.5% (v/v) NaClO solution for 3 minutes, 70% (v/v) alcohol for 30 seconds, then rinsed twice with sterile distilled water and dried in sterile Petri dishes. The organ samples were then cut and ground. SCA medium was prepared and supplemented with 50 g/mL nystatin and 25 mg/mL rifampicin. The organ samples were placed on the medium and incubated at room temperature (25-28°C) for 2-3 weeks. Each Actinomycetes isolate that grew was subcultured using the 4-quadrant streak method on SCA medium (Passari et al., 2015).

2.2.2. *Isolation of Actinomycetes from Strawberry Rhizosphere (Fragaria x ananassa)*

Soil samples from strawberry plants (*Fragaria x ananassa*) were randomly taken from four points at a distance of 0-10 cm from the plant stand and at a depth of 3-20 cm. The surface soil layer of 0-3 cm was discarded. The soil samples were placed in clean zip lock bags and brought to the laboratory. The samples were mixed and placed in Petri dishes, then left in the open air for 5 days (Nurrohman et al., 2016; Susilowati et al., 2007). One gram of soil sample was placed in a test tube containing 9 mL of sterile 0.85% NaCl solution and shaken for 5 minutes for a 10^{-1} dilution. The sample suspension was placed in a water bath at 50°C for 10 minutes, then serially diluted. One milliliter was transferred to another test tube containing sterile 0.85% NaCl solution for a 10^{-2} dilution. This step was repeated until a 10^{-4} dilution was obtained. Suspensions at 10^{-3} and 10^{-4} dilutions were inoculated using the pour plate method. SCA medium and 1% nystatin were added to Petri dishes containing the 10^{-3} and 10^{-4} dilution suspensions. The plates were gently shaken to homogenize and incubated at 25°C for 4 days to 2 weeks (Sembiring et al., 2000). Isolated actinomycete strains were coded as 'A' for roots, 'B' for stems, 'D' for leaves, 'T' for soil, with numbers indicating the sequential order of isolation from each source.

2.2.3. *Regeneration of Colletotrichum acutatum*

The strain of *C. acutatum* was obtained from the Laboratory of Biology at Universitas Ahmad Dahlan in Yogyakarta. The isolate was cultured on PDA medium supplemented with

chloramphenicol. The mycelia were then transferred to Petri dishes containing PDA medium and incubated for 7 days at a room temperature of 25-28°C (Nurjasmi & Suryani, 2020).

2.2.4. *Observation of Morphological Characteristics of Actinomycetes*

Actinomycetes isolates were grown on SCA media for 10 days at room temperature (25-28°C), then observed the morphological characteristics of colonies including macroscopic and microscopic observations. Macroscopic observations consisted of edges, elevations, colony shape, presence or absence of aerial mycelium, aerial mycelium color, and substrate mycelium color. Microscopic observations are carried out using the gram staining stage first, then the cell shape of the Actinomycetes is observed (Li et al., 2016).

2.2.5. *Screening of Antagonism Activity of Actinomycetes against Fungus Colletotrichum acutatum*

At this stage, the treatment involved testing the antagonistic potential of Actinomycetes from four different sources: roots, stems, leaves, and soil of strawberry plants against *C. acutatum* using the dual-culture method. These four treatments were based on the origin of the Actinomycetes isolates, and the inhibition percentage was calculated to measure the antifungal efficacy of each treatment group. *C. acutatum* fungus was revived, and Actinomycetes isolates were cultivated on SCA medium as stock for 3 days. Pieces of pure culture of *C. acutatum* fungus (± 5 mm) were inoculated in the center of petri dishes containing PDA media. Actinomycetes isolates were streaked (± 2 cm) on all four sides of the petri dish at a distance of 2.5 cm from the center. In the negative control, pieces of *C. acutatum* fungus (± 5 mm) were placed in the center of the Petri dish containing PDA media without adding Actinomycetes isolates. Both treatment dishes were then incubated at room temperature (25-28°C) for 5-7 days until the fungal colonies of *C. acutatum* in the negative control treatment grew to fill the petri dish. The radius of the fungal colonies formed was measured every day for 5 days (Khucharoenphaisan et al., 2016; Audinah and Ilmi, 2019). The percentage of inhibition of Actinomycetes against *C. acutatum* was calculated using the formula from Alimuddin et al. (2011):

$$\text{PH} = \left(\frac{R1 - R2}{R1}\right) x100\%$$

In this formula, PH represents the percentage of inhibition, R1 is the growth radius of *C. acutatum* in the control, and R2 is the growth radius in the treatment. This calculation quantifies the effectiveness of Actinomycetes in inhibiting fungal growth.

2.2. Data Analysis

The experiment was repeated three times to ensure reliability. Data obtained from the percentage of inhibition results were analyzed to determine which Actinomycetes isolates had the highest efficacy in inhibiting the fungus *C. acutatum* during the antagonism activity screening. A One-Way ANOVA test was used to analyze the data. If the One-Way ANOVA test indicated significant differences among the mean values, the Duncan test was subsequently performed to identify which isolates significantly differed from others.

3. Results and Discussion

3.1. Results

Actinomycetes isolates were successfully obtained from various plant organs and the rhizosphere of strawberry plants (*Fragaria x ananassa*). While some isolates exhibited similar characteristics, 10 distinct isolates were selected based on their unique traits. The isolation process yielded 10 Actinomycetes isolates: 3 from the roots, 2 from the stems, 2 from the leaves, and 3 from the rhizosphere (soil). The highest number of isolates were obtained from

the roots and rhizosphere of the strawberry plants. The appearance of these isolates is displayed in Figure 1. Macroscopically and microscopically, all isolates have distinct colony forms. These observations suggest that the isolates in groups A, B, D, and T represent different species or strains of Actinomycetes, indicating varied potential functions or activities.

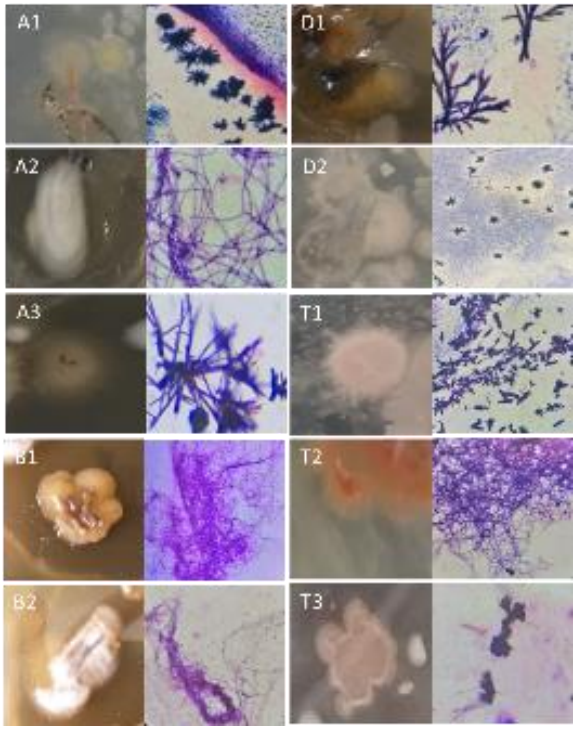

Figure 1 Actinomycetes isolates from strawberry plant organs and rhizosphere (*Fragaria x ananassa*) on SCA medium. The colony morphology of Actinomycetes on the left and the microscopic observation of cell morphology on the right

Table 1 Macroscopic and microscopic characterization of microbial isolates obtained from strawberry plant organs and rhizosphere (*Fragaria x ananassa*)

Source	No	Isolate Code	Colony Shape	Edge	Elevation	Cell Shape	Aerial Mycelium Color	Substrate Mycelium Color
Plant organs	1.	A1	Circular	Undulate	Umbunate	Monoverticillate no spirals	Yellowish white	Yellowish brown
	2.	A2	Circular	Entire	Convex	Primitive spirals	Yellowish white	Yellowish white
	3.	A3	Circular	Undulate	Raised	Straight	Yellowish white	Yellowish white
	4.	B1	Circular	Entire	Convex	Primitive spirals	Brownish white	Brownish white
	5.	B2	Circular	Entire	Convex	Primitive spirals	Yellowish white	Kuning kecoklatan
	6.	D1	Circular	Undulate	Umbunate	Flexous	Oranye	Oranye
	7.	D2	Circular	Undulate	Convex	Fascicled	Yellowish white	Yellowish white
Soil	8.	T1	Circular	Felamen-tous	Flat	Bivarticillate no spirals	Pink-white	White
	9.	T2	Circular	Filamen-tous	Flat	Monoverticillate no spirals	Pink	Pink
	10.	T3	Circular	Filamen-tous	Flat	Bivarticillate no spirals	Pink-white	Pink-white

Note: Isolated actinomycete strains were coded as 'A' for roots, 'B' for stems, 'D' for leaves, 'T' for soil, with numbers indicating the sequential order of isolation from each source.

Based on Figure 1. and Table 1., the 10 isolates observed are suspected to belong to the Actinomycetes group due to their colony shapes (circular) and various other characteristics such as colony edge, elevation, cell shape, aerial mycelium color, and substrate mycelium color. The Gram staining results indicated that all isolates produced a purple color, indicating that they are gram-positive bacteria, like Actinomycetes.

The results of the antagonistic activity screening test of Actinomycetes isolates from the plant organs and rhizosphere of strawberry plants (*Fragaria x ananassa*) against the fungus *C. acutatum* after 5 days of incubation are presented in Figure 2. All isolates show varying degrees of inhibition. Isolate A3, exhibit significant inhibition zones, indicating strong antagonistic activity. Isolate T2 also shows considerable inhibition. The percentage of inhibition for each isolate was calculated and analyzed, as shown in Figure 3.

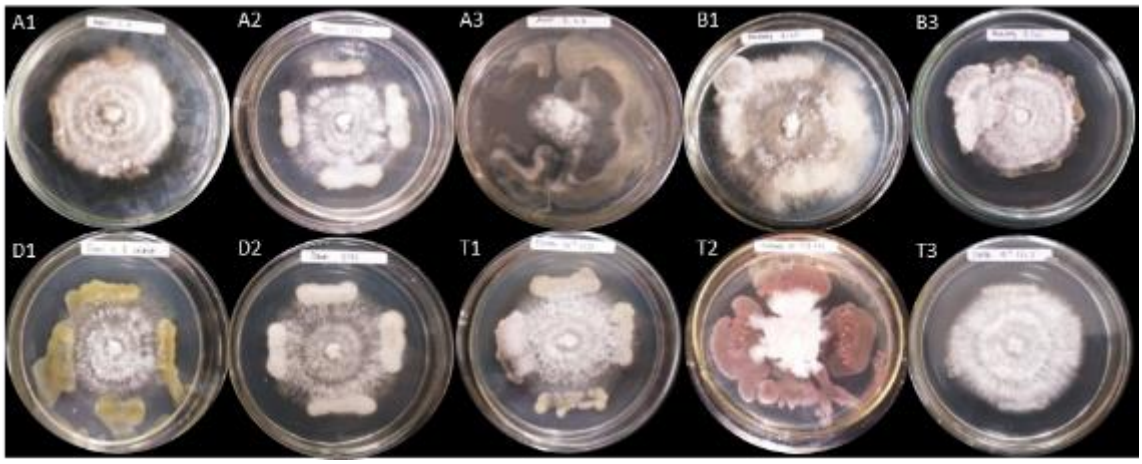

Figure 2 Antagonistic activity screening results of Actinomycetes isolates from strawberry plant organs and rhizosphere (*Fragaria x ananassa*) against *C. acutatum*.

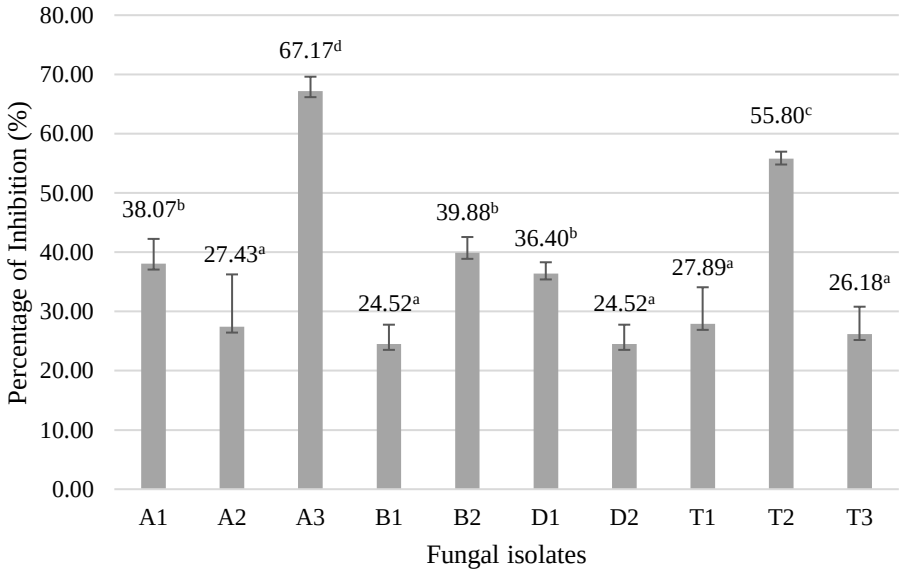

Figure 3 Percentage of inhibition of fungal isolates against *C. acutatum*. Different letters denote statistically significant differences based on Duncan's test at $p < 0.05$. A for roots, B for stems, D for leaves, and T for soil

Based on Figure 3, the screening results of the antagonistic activity of Actinomycetes isolates, which were isolated from strawberry plant organs and rhizosphere (*Fragaria x ananassa*), against the fungus *C. acutatum*, showed that all 10 Actinomycetes isolates had the ability to inhibit the fungus. The isolate A3 stands out with the highest inhibition percentage of 67.17% and is significantly different from all other isolates. The isolate T2 also shows a high inhibition percentage of 55.80%, significantly different from many other isolates except

A3. Isolate A1, B1, and B2 have moderate inhibition percentages ranging from 36.40% to 39.88%, and they are not significantly different from each other. Isolates A2, D1, D2, T1, and T3 show lower inhibition percentages, ranging from 24.52% to 27.89%, and are not significantly different from each other.

3.2. Discussion

The samples used as sources of Actinomycetes isolates were taken from the organs (roots, stems, and leaves) and the rhizosphere of strawberry plants (*Fragaria x ananassa*), isolated using the planting method. The choice of vegetative organs and rhizosphere as sources of Actinomycetes is based on their presence in the healthy vegetative parts of plants (Elshafie & Camele, 2022). Additionally, Sastrahidayat (2014) stated that at soil depths of 3-20 cm, there is an abundant and diverse population of Actinomycetes. The isolation process resulted in more than 10 bacterial isolates from plant organs and the rhizosphere. The root organs and rhizosphere were the most abundant sources of isolates due to their ability to produce and release root exudates, which serve as energy sources for Actinomycetes, influencing microbial diversity and population in the roots and rhizosphere. Soil properties, such as pH conditions, also affect microbial populations (Zunairoh et al., 2019).

The successful isolation of 10 distinct Actinomycetes isolates from different organs and the rhizosphere of strawberry plants, as illustrated in Figure 1 and detailed in Table 1, highlights the diversity of microbial life in these environments. The high number of isolates from the roots and rhizosphere aligns with previous studies showing these areas' rich microbial population. The microscopic and macroscopic characterizations (Table 1) further confirm the isolates as Actinomycetes, consistent with Gram-positive staining results. These findings align with Fatmawati et al. (2014), indicating Actinomycetes are Gram-positive bacteria. Sulistyani & Akbar (2014) also noted Actinomycetes colonies are round or circular with varied edges and surfaces. The observed morphological characteristics align with those reported by Fatmawati et al. (2014) and Sulistyani & Akbar (2014), suggesting the 10 isolates from strawberry plants and the rhizosphere belong to the Actinomycetes group and can be used for further testing. Morphological characterization of Actinomycetes serves as a reference for classification but cannot determine specific types of Actinomycetes (Barka et al., 2016).

The antagonist test assesses the bacteria's ability to inhibit fungal growth (Lestari, 2017). The 5-day incubation was chosen because the petri dish containing only *C. acutatum* (negative control) was fully occupied. According to Khucharoenphaisan et al. (2016), inhibition percentages of 61%-100% are categorized as highly efficient in inhibiting growth, while percentages of 41%-60% indicate the ability to inhibit fungal mycelium growth. Referring to Khucharoenphaisan et al. (2016), only two isolates had average inhibition percentages above 61% with A3 (highly efficient) and T2 above 41% (able to inhibit growth). The antagonistic activity screening, as depicted in Figure 2, reveals varying degrees of inhibition among the isolates. Isolates A3 and T2, in particular, show the strongest inhibition against *Colletotrichum acutatum* (Figure 3), with A3 achieving a significant inhibition percentage of 67.17%, as confirmed by statistical analysis. These findings suggest the potential of these isolates as biocontrol agents. The mechanism of inhibition by Actinomycetes against *C. acutatum*, known as antagonistic activity in biological control, includes competition for space and nutrients, antibiosis, and microparasitism (Marsaoli et al., 2020). Actinomycetes also produce antifungal compounds that inhibit pathogenic fungal growth and are antagonistic (Malek et al., 2015).

The antagonistic activity of isolate A3 against *C. acutatum*, shown in Figure 2, indicates rapid growth and spread compared to the fungus, suggesting space and nutrient competition between A3 and *C. acutatum* (Malek et al., 2015; Marsaoli et al., 2020). Competition for space and nutrients allows biocontrol agents to grow faster than pathogens (Sriyanti et al., 2015). When multiple microorganisms occupy the same space simultaneously, they compete for nutrients to grow and reproduce, leading to suppressed growth of some microorganisms (Rifai

et al., 2020). The inhibition process by isolate T2 against *C. acutatum*, shown in Figure 2, indicates direct contact between the isolate and the fungus. According to Purnomo et al. (2017), direct contact between a pathogen and bacterial isolate can further inhibit pathogen growth. Direct contact also enables bacteria to perform hyperparasitism (mycoparasitism) (Adeleke et al., 2022). *Streptomyces* species have demonstrated significant biocontrol potential through mycoparasitism, as shown by *Streptomyces* sp. MBCN152-1, which effectively inhibits *Alternaria brassicicola* by parasitizing its mycelium (Shimizu et al., 2022).

Isolates other than A3 and T1 also exhibited inhibitory effects, with inhibition percentages below 41%. The inhibition percentages of the other eight isolates varied, reflecting the different capabilities of each isolate in inhibiting the growth of *C. acutatum*. This variability is attributed to differences in the types of Actinomycetes bacteria and the amounts of inhibitory secondary metabolites produced by each isolate (Ouchari et al., 2019). The mechanism by which pathogens' growth is inhibited through the production of metabolic compounds is known as antibiosis (Khan et al., 2020). The findings indicate that Actinomycetes isolated from strawberry plants and their rhizosphere have significant potential as biocontrol agents against fungal pathogens. The effective isolates, particularly A3 and T2, demonstrate strong antagonistic activity, making them promising candidates for developing biocontrol strategies in agriculture. Utilizing such natural biocontrol agents can reduce the reliance on chemical pesticides, promoting sustainable and eco-friendly farming practices.

4. Conclusion

The research on Actinomycetes isolated from strawberry plants and their rhizosphere revealed their significant potential for agricultural biocontrol. Over 10 Gram-positive Actinomycetes strains were successfully isolated, with two isolates showing the highest antagonistic activity against *C. acutatum*, indicating their potential efficacy as biocontrol agents. These strains used mechanisms such as competition, antibiosis, and hyperparasitism to inhibit fungal growth, offering a sustainable alternative to chemical pesticides. The variability in inhibitory effects suggested that these Actinomycetes could be tailored for specific pathogens or conditions, enhancing their practical use in agriculture. This research highlighted the promising role of Actinomycetes in managing fungal pathogens and supported their application in sustainable farming practices. Future studies should focus on optimizing these strains for broader biocontrol use.

Acknowledgements

We would like to express our sincere gratitude to all those who supported and contributed to this research.

References

Adeleke, B. S., Ayilara, M. S., Akinola, S. A., & Babalola, O. O. (2022). Biocontrol mechanisms of endophytic fungi. In *Egyptian Journal of Biological Pest Control* (Vol. 32, Issue 1). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1186/s41938-022-00547-1

Alimuddin, Widada, J., Asmara, W., & Mustofa. (2011). Antifungal Production of a Strain of Actinomycetes spp Isolated from the Rhizosphere of Cajuput Plant: Selection and Detection of Exhibiting Activity Against Tested Fungi. *Indonesian Journal of Biotechnology*, *16*(1), 1–10. https://doi.org/https://doi.org/10.22146/ijbiotech.7829

Ara, I., Rizwana, H., Al-Othman, M. R., & Bakir, M. A. (2012). Studies of actinomycetes for biological control of Colletotrichum musae pathogen during post harvest anthracnose of banana. *African Journal of Microbiology Research*, *6*(17), 3879–3886. https://doi.org/10.5897/ajmr12.088

Aristya, G. R., Kasiamdari, R. S., Setyoningrum, R., & Larasati, B. (2019). Genetic variations of strawberry cultivars of Fragaria x ananassa and Fragaria vesca based on RAPD. *Biodiversitas*, *20*(3), 770–775. https://doi.org/10.13057/biodiv/d200322

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2016). Taxonomy, Physiology, and Natural Products of Actinobacteria. *Microbiology and Molecular Biology Reviews*, *80*(1), 1–43. https://doi.org/10.1128/mmbr.00019-15

de los Santos García de Paredes, B., & Romero Muñoz, F. (2002). Effect of different fungicides in the control of Colletotrichum acutatum, causal agent of anthracnose crown rot in strawberry plants. *Crop Protection*, *21*(1), 11–15. https://doi.org/10.1016/S0261-2194(01)00054-0

De Simeis, D., & Serra, S. (2021). Actinomycetes: A never-ending source of bioactive compounds—an overview on antibiotics production. In *Antibiotics* (Vol. 10, Issue 5). MDPI AG. https://doi.org/10.3390/antibiotics10050483

El-Saadony, M. T., Saad, A. M., Soliman, S. M., Salem, H. M., Ahmed, A. I., Mahmood, M., El-Tahan, A. M., Ebrahim, A. A. M., Abd El-Mageed, T. A., Negm, S. H., Selim, S., Babalghith, A. O., Elrys, A. S., El-Tarabily, K. A., & AbuQamar, S. F. (2022). Plant growth-promoting microorganisms as biocontrol agents of plant diseases: Mechanisms, challenges and future perspectives. In *Frontiers in Plant Science* (Vol. 13). Frontiers Media S.A. https://doi.org/10.3389/fpls.2022.923880

Elshafie, H. S., & Camele, I. (2022). Rhizospheric Actinomycetes Revealed Antifungal and Plant-Growth-Promoting Activities under Controlled Environment. *Plants*, *11*(14), 1–11. https://doi.org/10.3390/plants11141872

Fatmawati, U., Santosa, S., Rinanto, Y., & Probosari, R. M. (2014). Aktivitas Antibakteri Actinomycetes yang Diisolasi dari Rizosfer Tanaman Solanaceae. *Jurnal Farmasi Indonesia*, *11*(1), 54–68. http://farmasiindonesia.setiabudi.ac.id/

Forcelini, B. B., Seijo, T. E., Amiri, A., & Peres, N. A. (2016). Resistance in Strawberry Isolates of *Colletotrichum acutatum* from Florida to Quinone-Outside Inhibitor Fungicides. *Plant Disease*, *100*(10), 2050–2056. https://doi.org/10.1094/PDIS-01-16-0118-RE

Istifadah, N., Ayuningtyas, A., & Nasahi, C. (2018). Efek Pencampuran Bahan Pestisida Nabati Terhadap Keefektifannya Dalam Menekan Colletotrichum sp. In Vitro Serta Penyakit Antraknosa Pada Stroberi. *Agrologia*, *6*(1), 26–36. https://doi.org/10.30598/a.v6i1.177

Janardhan, A., Kumar, A. P., Viswanath, B., Saigopal, D. V. R., & Narasimha, G. (2014). Production of Bioactive Compounds by Actinomycetes and Their Antioxidant Properties. *Biotechnology Research International*, *2014*, 1–8. https://doi.org/10.1155/2014/217030

Karina, A. R., Trisnowati, S., & Indradewa, D. (2012). Pengaruh Macam dan Kadar Kitosan Terhadap Umur Simpan dan Mutu Buah Stroberi (Fragaria x ananassa Duch.). *Vegetalika*, *1*(3). https://doi.org/https://doi.org/10.22146/veg.1366

Khan, R. A. A., Najeeb, S., Mao, Z., Ling, J., Yang, Y., Li, Y., & Xie, B. (2020). Bioactive secondary metabolites from Trichoderma spp. Against phytopathogenic bacteria and root-knot nematode. *Microorganisms*, *8*(3). https://doi.org/10.3390/microorganisms8030401

Khucharoenphaisan, K., Rodbangpong, K., Saengpaen, P., & Sinma, K. (2016). Exploration on soil actinomycetes against phytophthora sp. Causing root rot of cassava and plant growth promoting activities. *Journal of Plant Sciences*, *11*(1), 38–44. https://doi.org/10.3923/jps.2016.38.44

Lelana, N. E., Anggraeni, I., & Mindawati, N. (2015). Uji Antagonis Aspergillus sp. dan Trichoderma spp.Terhadap Fusarium sp. Penyebab Penyakit Rebah Kecambah pada Sengon. *Jurnal Penelitian Hutan Tanaman*, *12*(1), 23–28. https://doi.org/10.20886/jpht.2015.12.1.23-28

Lestari, W. (2017). Isolasi dan Uji Antifungal Bakteri Endofit dari Akar Tanaman Karet (Hevea brasilliensis). *SIMBIOSA*, *6*(1), 48. https://doi.org/10.33373/sim-bio.v6i1.1036

Li, Q., Chen, X., Jiang, Y., & Jiang, C. (2016). Morphological Identification of Actinobacteria. In *Actinobacteria - Basics and Biotechnological Applications*. InTech. https://doi.org/10.5772/61461

Malek, N. A., Zainuddin, Z., Chowdhury, A., & Abidin, Z. (2015). Diversity and Antimicrobial Activity of Mangrove Soil Actinomycetes Isolated From Tanjung Lumpur, Kuantan. *Jurnal Teknologi*, *77*(25), 37–43. https://doi.org/https://doi.org/10.11113/jt.v77.6734

Marsaoli, F., Matinahoru, J. M., & Leiwakabessy, Ch. (2020). Isolasi, Seleksi, dan Uji Antagonis Bakteri Endofit diisolasi dari Salawaku (Falcataria mollucana) dalam Menekan Pertumbuhan Cendawan Patogen Cercospora spp. *Agrologia*, *8*(2). https://doi.org/10.30598/a.v8i2.1009

Nurjasmi, R., & Suryani, D. (2020). Uji Antagonis Actinomycetes terhadap Patogen Colletotrichum capsici Penyebab Penyakit Antraknosa pada Buah Cabai Rawit. *Jurnal Ilmiah Respati*, *11*(1), 1411–7126. https://doi.org/https://doi.org/10.52643/jir.v11i1.843

Nurrohman, E., Rahardjanto, A., & Wahyuni, S. (2016). Keanekaragaman Makrofauna Tanah di Kawasan Perkebunan Coklat (Theobroma cacao L.) Sebagai Bioindikator Kesuburan Tanah dan Sumber Belajar Biologi. *JPBI (Jurnal Pendidikan Biologi Indonesia)*, *1*(2). https://doi.org/10.22219/jpbi.v1i2.3331

Olano, C., Méndez, C., & Salas, J. A. (2009). Antitumor compounds from marine actinomycetes. In *Marine Drugs* (Vol. 7, Issue 2, pp. 210–248). MDPI AG. https://doi.org/10.3390/md7020210

Ouchari, L., Boukeskasse, A., Bouizgarne, B., & Ouhdouch, Y. (2019). Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. *Biology Open*, *8*(2). https://doi.org/10.1242/bio.035410

Passari, A. K., Mishra, V. K., Saikia, R., Gupta, V. K., & Singh, B. P. (2015). Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their in vitro antimicrobial biosynthetic potential. *Frontiers in Microbiology*, *6*(APR), 1–13. https://doi.org/10.3389/fmicb.2015.00273

Purnomo, E., Mukarlina, & Rahmawati. (2017). Uji Antagonis Bakteri Streptomyces spp. terhadap Jamur Phytophthora palmivora BBK01 Penyebab Busuk Buah pada Tanaman Kakao. *Jurnal Protobiont*, *6*(3), 1–7.

Rebello, C. S., Baggio, J. S., Forcelini, B. B., & Peres, N. A. (2022). Sensitivity of Colletotrichum acutatum Species Complex from Strawberry to Fungicide Alternatives to Quinone-Outside Inhibitors. *Plant Disease*, *106*(8), 2053–2059. https://doi.org/10.1094/PDIS-09-21-1934-RE

Rifai, M. R., Widowati, H., & Sutanto, A. (2020). Sinergisme dan Antagonisme Beberapa Jenis Isolat Bakteri yang Dikonsursiumkan. *BIOLOVA*, *1*(1), 19–24. https://doi.org/10.24127/biolova.v1i1.31

Sari, I. M., Prawanto, A., Sari, K. N., Hartawan, W., & Ansiska, P. (2021). Aplikasi Ozonisasi Dalam Upaya Pengawetan Produk Hortikultura Tomat (Lycopersicum esculentum Mill.). *Jurnal Ilmiah Fakultas Pertanian*, *3*(1), 1–7. https://doi.org/https://doi.org/10.54895/lansium.v3i1.1238

Sastrahidayat, I. R. (2014). *Peranan Mikroba bagi Kesehatan Tanaman dan Kelestarian Lingkungan*. Universitas Brawijaya Press.

Sembiring, L., Ward, A. C., & Goodfellow, M. (2000). Selective isolation and characterisation of members of the Streptomyces violaceusniger clade associated with the roots of Paraserianthes falcataria. *Antonie van Leeuwenhoek*, *78*, 353–366. https://doi.org/https://doi.org/10.1023/A:1010226515202

Shimizu, M., Naznin, H. A., & Hieno, A. (2022). The Significance of Mycoparasitism by Streptomyces sp. MBCN152-1 for Its Biocontrol Activity against Alternaria brassicicola. *Microbes and Environments*, *37*(3). https://doi.org/10.1264/jsme2.ME22048

Sriyanti, N. L. G., Suprapta, D. N., & Suada, I. K. (2015). Uji Keefektifan Rizobakteri dalam Menghambat Pertumbuhan Jamur Colletotrichum spp. Penyebab Antraknosa pada Cabai Merah (Capsicum annuum L.). *Jurnal Agroekoteknologi Tropika*, *4*(1), 53–65. http://ojs.unud.ac.id/index.php/JAT53

Sulistyani, N., & Akbar, A. N. (2014). Aktivitas Isolat Actinomycetes dari Rumput Laut (Eucheuma cottonii) sebagai Penghasil Antibiotik terhadap Staphylococcus aureus dan Escherichia coli. *Jurnal Ilmu Kefarmasian Indonesia*, *12*(1), 1–9.

Susilowati, D. N., Hastuti, R. D., & Yuniarti, E. (2007). Isolasi dan Karakterisasi Aktinomisetes Penghasil Antibakteri Enteropatogen Escherichia coli K1.1, Pseudomonas pseudomallei 02 05, dan Listeria monocytogenes 5407. *Jurnal AgroBiogen*, *3*(1), 15. https://doi.org/10.21082/jbio.v3n1.2007.p15-23

Suwan, N., Boonying, W., & Nalumpang, S. (2012). Antifungal activity of soil actinomycetes to control chilli anthracnose caused by Colletotrichum gloeosporioides. *Journal of Agricultural Technology*, *8*(2), 725–737. http://www.ijat-aatsea.com

Syafriani, E., Riwany, F., Handayani, R., Kamelia, R., Ferita, I., Fatchiyah, F., & Jamsari, J. (2019). Studi Awal Empat Isolat Bakteri Antagonis Terhadap Jamur Colletotrichum gloeosporioides. *BERKALA ILMIAH AGROTEKNOLOGI - PLUMULA*, *6*(2), 109–122. https://doi.org/10.33005/plumula.v6i2.9

Trinidad-Cruz, J. R., Rincón-Enríquez, G., Evangelista-Martínez, Z., Guízar-González, C., Enríquez-Vara, J. N., López-Pérez, L., & Quiñones-Aguilar, E. E. (2021). Actinobacteria from avocado rhizosphere: Antagonistic activity against Colletotrichum gloeosporioides

and Xanthomonas sp. *Terra Latinoamericana*, *39*. https://doi.org/10.28940/TERRA.V39I0.802

Urtgam, S., Kodsueb, R., Puengtang, C., Sumpradit, T., & Thurnkul, N. (2024). Isolation and Identification of Actinobacteria against Colletotrichum, the Causal Agent of Anthracnose Disease of Mangifera indica L. *Universal Journal of Agricultural Research*, *12*(2), 233–241. https://doi.org/10.13189/ujar.2024.120203

Van, N., Mai, D., Yen, L., Minh, N., & Nguyen, T. (2020). Investigation of antagonistic activities of actinomycetes isolated from Cuc Phuong and Ba Be National Parks against four plant pathogenic fungi. *Vietnam Journal of Biotechnology*, *17*, 527–535. https://doi.org/10.15625/1811-4989/17/3/12542

Wu, J., Hu, S., Ye, B., Hu, X., Xiao, W., Yu, H., & Zhang, C. (2022). Diversity and Resistance to Thiophanate-Methyl of Colletotrichum spp. in Strawberry Nursery and the Development of Rapid Detection Using LAMP Method. *Agronomy*, *12*(11), 2815. https://doi.org/10.3390/agronomy12112815

Zunairoh, A., Syauqi, A., & Rahayu, T. (2019). Isolasi dan Analisis Koloni Bakteri Rizosfer Untuk Agen Pengendali Penyakit Layu Fusarium Pada Tanaman Stroberi (Fragaria sp) Isolation and Analysis of Rhizosphere Bacterial Colony for Controlling Agents of Fusarium Wilt in Strawberry Plants (Fragaria sp). *Jurnal Ilmiah BIOSAINTROPIS (BIOSCIENCE-TROPIC*, *5*(1), 45–52. https://doi.org/https://doi.org/10.33474/e-jbst.v5i1.234