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# Antagonistic Ability and Fungicide Tolerance of Actinomycetes from the Rhizosphere of Maize (*Zea mays* L.) Plants in Gorontalo

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## Abstract

Actinomycetes are a group of microbes that live in the rhizosphere of plants and play an important role in soil microbial dynamics. This study aims to describe the antagonistic ability against pathogenic molds and tolerance to fungicides in actinomycetes from the rhizosphere of maize plants in Gorontalo. This research is descriptive uses a quantitative approach and employs a purposive sampling technique. Rhizosphere soil samples were collected from maize fields in the hilly area of Bontula Village, Asparaga District, Gorontalo Regency. The results showed the presence of 7 actinomycetes isolates with different morphological characteristics, namely RFZm-PG, RFZm-PLG, RFZm-PW, RFZm-PB, RFZm-PR, RFZm-PY, and RFZm-PO. Antagonistic tests showed that four isolates (RFZm-PB, RFZm-PG, RFZm-PLG, RFZm-PW) inhibited the growth of *Fusarium oxysporum* and *Neocosmospora* solani with an inhibition percentages of 66.38%-89%. The fungicide tolerance test showed that the seven isolates were tolerant to thiram, dimethomorph and benomyl, with the highest inhibitory effect by thiram. Isolates RFZm-PG and RFZm-PLG had the highest tolerance up to 500 ppm, while other isolates were tolerant only up to 400 ppm. Molecular analysis showed that RFZm-PG belongs to the genus Streptomyces (100% similarity with *Streptomyces enissocaesilis*), while RFZm-PLG belongs to Nocardiopsis (100% similarity with *Nocardiopsis synnemataformans*).

**Keywords:** Actinomycetes; Antagonistic; Fungicide; Nocardiopsis; Streptomyces.

## INTRODUCTION

Maize is an important food crop and the third source of carbohydrates after wheat and rice. This cereal crop is grown and spread almost all over the world to be used as a source of food, animal feed and in other uses (Degani, 2021). Maize farming systems in Indonesia, especially in Gorontalo are in high demand and bring considerable benefits in the food security of local communities (Kandowangko *et al.*, 2020). This is supported by maize farming systems dominated by monoculture and sustainable farming systems, this practice has become the main approach in crop cultivation in various regions in Gorontalo (Lapolo & Iqbal, 2019). The monoculture system allows farmers to optimize yields by planting one crop type on a large scale, making it easier to manage land, as well as fertilizer use (Rotasouw *et al.*, 2020). However, long-term application risks cause a decrease in soil nutrient quality that triggers the emergence of various diseases due to pathogens that attack plant roots (Widnyana, 2023). Various soil-borne pathogens, including fungi, bacteria, nematodes, oomycetes, protozoa, and viruses, threaten maize root systems (Dutta *et al.*, 2023; Kandowangko, 2019). Notable pathogens affecting maize roots include *Rhizoctonia solani*, *Phytium* spp, *Phytophthora* spp, and *Fusarium* spp, which cause cob, stem, and root rot (Terna *et al.*, 2022). Favorable environmental conditions such as temperatures of 25-30ºC, 95% humidity, and pH levels between 5.0 and 7.0 contribute to pathogen survival and proliferation (Anderson *et al.*, 2020). Pathogen attacks often occur during the vegetative stage, with *Fusarium* spp. causing wilt symptoms at 21 days after sowing and *Helminthosporium* spp. causing leaf blight at 30-45 days (Hamidson *et al.*, 2020; Rampersad, 2020). To control these pathogens, farmers widely use synthetic fungicides (Muslim & Suwandi, 2023). However, the overuse of fungicides negatively impacts soil health, leading to water pollution, reduced fertility, and microbial imbalances (Tripathi *et al.*, 2020). Active ingredients such as tebuconazole, prothioconazole and difeconazole alter microbial diversity and reduce populations of beneficial soil microbes (Schaeffer & Wijntjes, 2022). In addition, fungicides containing benomyl, mancozeb, captan, and thiram suppress 30-50% of the microbial activity responsible for phosphate solubilization, plant growth promotion, and pathogen antagonism (Meena *et al.*, 2020). These effects disrupt natural antagonistic interactions in the soil, although certain microbial groups, such as actinomycetes, exhibit resistance to fungicides and continue to support soil health (Khmelevtsova *et al*., 2024).

Actinomycetes are filamentous bacteria with unique morphological and biochemical properties that allow them to survive in diverse environments (Sahur, 2021). Unlike other bacteria, actinomycetes form mycelial-like filaments similar to fungi, although they differ in genetic and sequence classification (Dede *et al*., 2020). They thrive in diverse ecosystems with stable populations at temperatures between 5-7ºC and 45-70ºC and pH levels of 6.5-7.0 (Chukwuneme *et al*., 2021). Actinomycetes play essential roles in the degradation of complex organic compounds, the production of extracellular hydrolytic enzymes, and the enhancement of soil nutrient cycling. Their interactions with plant rhizospheres promote phytohormone synthesis, siderophore production, and antimicrobial enzyme secretion, thereby improving plant health and productivity (Chukwuneme *et al*., 2020). Many studies highlight the antagonistic properties of actinomycetes, particularly their ability in inhibit plant pathogens. The genus Streptomyces, commonly found in soil, has demonstrated significant antifungal activity. For instance, Streptomyces isolates from terrestrial environments inhibited *Fusarium solani* mycelial growth by 69% (Ayed *et al*., 2021). Similarly, marine-derived Streptomyces strains suppressed *Penicillium digitatum*, Aspergillus niger, and *Fusarium solani* by 92%, 73%, and 72%, respectively (Uba *et al*., 2019). Actinomycetes antimicrobial activity is linked to their resistance genes, which protect them from synthetic pesticides and other chemical stressors. Specific resistance genes, including those encoding macrolides (*ermB*), sulfonamides (*sul2*), tetracyclines (*tetO*), glycopeptides (*vanA* and *vanB*), carbapenems (*blaVIM-1*), and aminoglycosides (*aasA2*), enable actinomycetes to persist in fungicide-treated soils (Khmelevtsova *et al*., 2024). Based on this, it is expected that this aims to describe the antagonistic ability against pathogenic molds and tolerance to fungicides in actinomycetes from the rhizosphere of maize (*Zea mays* L.) plants in Gorontalo.

## MATERIALS AND METHODS

### Study area

This research was conducted in Bontula village, Asparaga subdistrict, Gorontalo district, on maize plantation land at coordinates 0°48'27.4"N 122°26'12.2"E, to use rhizosphere soil as a source for isolating actinomycetes that have antagonistic abilities against pathogenic molds and tolerance to fungicides (Figure 1).

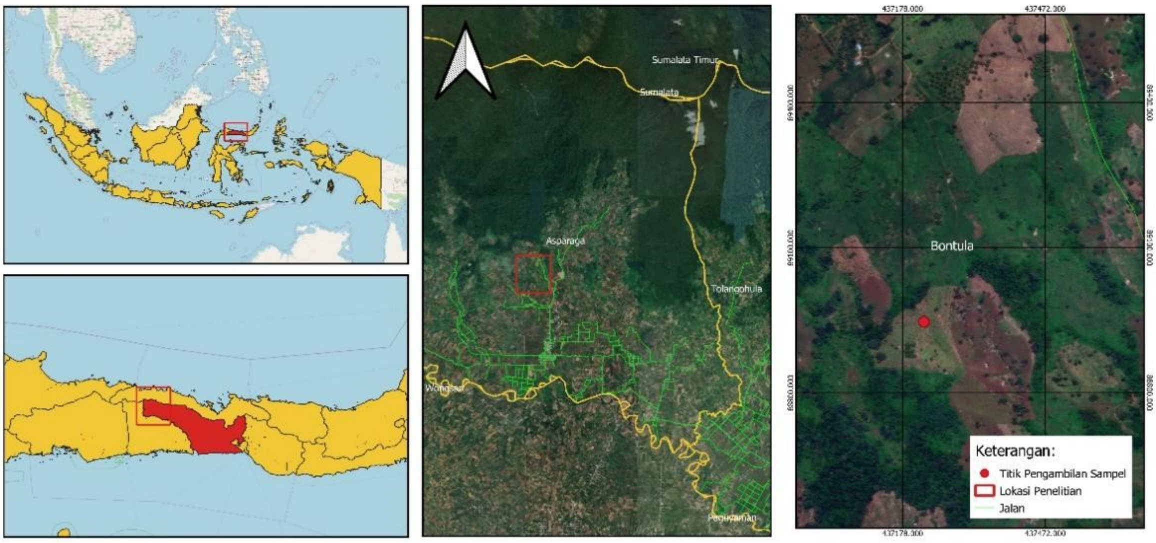

**Figure 1.** Location of rhizosphere sampling in Bontula village, Asparaga subdistrict, Gorontalo district in maize plantation land at coordinates 0°48'27.4"N 122°26'12.2"E.

Rhizosphere soil sampling of maize plants was carried out by identifying several specific points and establishing sampling plots with a distance of ± 10 meters between plots. The location of rhizosphere soil sampling was at an altitude of 274 meters above sea level in the upper, middle and lower sloping hilly areas planted with maize to represent the condition of the soil rhizosphere in the area. Rhizosphere soil samples were taken from hybrid maize plants aged ± 30 day with soil characteristics that tend to be acidic, with a pH of about 5.1, and have relatively low moisture, which is about 4.7%.

### Procedures

**Rhizospheric soil sample.** Sampling activities are determined based on 3 sampling points on the hills of maize land in Bontula village, Asparaga sub-district, Gorontalo district using the Purposive Sampling method, namely by determining the sampling point based on the slope of the land (top, middle and bottom), each soil sampling point consists of 3 plots (distance of each plot ± 10 m). The rhizosphere soil sampling process is carried out using a cetok in the rhizosphere (soil around the roots) of plants with a depth of 15-30 cm, then placed in a sterile plastic clip bag and labeled according to the location of the point using a paper label and then stored in a cool box to keep it awake.

### Isolation and purification of rhizospheric Actinomycetes.

Approximately 5 gr of rhizosphere soil was dissolved in 45 mL of ringer solution and heated at 65°C for 15 minutes to reduce unwanted microbes. The processed sample was stirred at 250 rpm for 30 minutes to obtain a uniform suspension, which was then sequentially diluted to a concentration of 10-5. A total of 100 µL of the dilution was spread onto casein agar supplemented with cycloheximide (50 mg $L^{-1}$) (Retnowati et al., 2018). The plates were incubated at 30°C for two weeks, after which colonies with actinomycetes like characteristics were isolated, purified, and stored as working cultures for further analysis.

### Antagonistic ability of Actinomycetes isolates against pathogenic molds in vitro.

Antagonistic tests of Actinomycetes against pathogenic molds were conducted in vitro. Pathogenic mold inoculums Fusarium oxysporum (IPBCC 070525) and Neocosmospora solani (IPBCC 880012) were obtained from the IPB Culture Collection. In vitro antagonism testing was performed using the cross 'y' method (Oskay, 2009). Pure isolates of actinomycetes bacteria were inoculated individually on Potato Dextrose Agar and then incubated at 30 ºC for 7 days in an incubator. After the actinomycetes incubation period of 7 days, the pathogenic isolate was inoculated on the actinomycetes isolate in one stroke at a 90º angle in the same petri dish and then incubated at 30 ºC for 3 days (Pathalam et al., 2017). The percentage inhibition of actinomycetes against pathogenic molds was calculated using the formula of Oyedoh et al. (2024) as follows:

Inhibition % = (control mold diameter – treated diameter) / (control mold diameter) × 100

### Fungicide tolerance of isolate Actinomycetes in vitro.

The tolerance test was conducted using the poisoned food technique (Simanjuntak et al., 2017). Actinomycetes isolates aged 14 days on SCA medium were picked with a diameter of 5 mm and then attached to a new SCA growth medium containing synthetic fungicidal chemicals, namely; Benomyl (Benlate®, Du Pont, Indonesian; Batch No. D07-006; Purity 50%), Dimetomorph (Captive® 200 SC, Agriculture Construction, Indonesian; Batch No. 1601DD1631; Purity 200g/L) and Thiram (Thiramo 80WP®, Multi Sarana Indotani, Indonesian; Batch No. 23AN090; Purity 80%) at different ppm (parts per million) concentrations (25, 50, 75, 100, 200, 300, 400 and 500) and incubated at 30ºC for 7 days. After incubation, the tolerance category of isolates to fungicide active ingredients is determined based on the percentage category of the RIR value (Relative Inhibition Rate), namely very strong tolerance (RIR <40%), strong tolerance (40-60%), moderate tolerance (60-75%), weak tolerance (>75-90%) and very weak tolerance (>90%) (Mahendra et al., 2022).

RIR % = (control colony – treatment colony) / (control colony) × 100

### Molecular identification of Actinomycetes isolates potential antagonistic and fungicide tolerance.

The isolates were cultured in Starch Casein Broth (SCB) medium with stirring at 200 rpm for seven days to stimulate cell growth. After incubation, the cultures were centrifuged at 12,000 rpm for 10 minutes to obtain cell pellets. Genomic DNA was extracted from the harvested biomass using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005), and only DNA of sufficient quality was used for 16S rRNA gene amplification (Retnowati et al., 2024). The amplification used universal primers 27F and 1492R with 2X MyTaq HS Red Mix (BIO-25048). The PCR process consisted of initial denaturation at 95°C for 3 minutes, followed by 35 cycles at 95°C for 15 seconds, 52°C for 30 seconds, and 72°C for 45 seconds, ending with a final extension at 72°C for 2 minutes (Heuer et al., 1997). The resulting product was purified using the Zymoclean™ Gel DNA Recovery Kit (Zymo Research). Two-way sequencing of the 16S rRNA amplicon was performed, and the sequences were compared with the NCBI nucleotide database using BLAST. Isolates showing ≥99% sequence similarity were considered identified, and their sequences were collected for further validation and analysis (Retnowati et al., 2024).

### Data analysis

Data on the antagonistic ability and fungicide tolerance of Actinomycetes were analyzed using descriptive quantitative methods. Molecular identification was evaluated based on Actinomycetes isolates that have potential antagonistic ability and fungicide tolerance. The 16S rRNA sequence data of the Actinomycetes isolates were analyzed by conducting a comparative assessment with the nucleotide sequences stored in the NCBI GenBank database to determine their genetic similarity and taxonomic classification.

## RESULTS AND DISCUSSION

### Description of Actinomycetes isolates in the rhizospheric of maize plants in Gorontalo.

Actinomycetes are a group of microbes that live in the rhizosphere of plants and play an important role in soil microbial dynamics. They also have the potential to suppress plant pathogens through antagonistic mechanisms, antimicrobial compound production, and stimulation of plant defenses (Javed *et al.*, 2021; Oyedoh *et al.*, 2024). Retnowati *et al.* (2024) stated that most actinomycetes have high diversity and are widely distributed in various soil ecosystems, with a dominant presence in the rhizosphere. In this study, seven actinomycetes isolates were successfully isolated and purified from the rhizosphere of ±30 days old hybrid maize plants. Actinomycetes found in the rhizosphere of maize plants were isolated using the surface plate method on SCA medium supplemented with 25 ppm cyclohexamide as an antifungal agent. Isolates showing different morphological characteristics were then purified to obtain pure isolates. The purification results obtained seven pure isolates coded RFZm-P (Rhizosphere Zea mays Pahu) then followed by color coding (Figure 2).

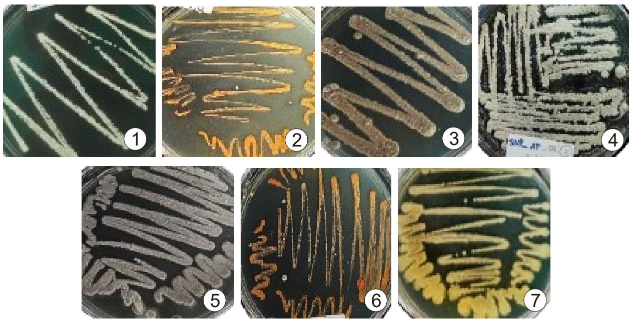

**Figure 2.** Morphology of actinomycetes isolated from the rhizosphere of maize plants in Gorontalo: 1) RFZm-PW; 2) RFZm-PO; 3) RFZm-PG; 4) RFZm-PLG; 5) RFZm-PB; 6) RFZm-PR; and 7) RFZm-PY.

In line with the research by Fatmawati *et al.* (2018), 12 actinomycetes isolates were found in the rhizosphere of maize plants. Furthermore, Wahyudi *et al.* (2019) also found similar results, identifying 30 actinomycetes isolates from the rhizosphere of maize plants. In this study, although actinomycetes were successfully isolated, the number of isolates obtained was generally lower than in previous studies. This difference indicates that the variation in actinomycetes isolates depends on plant variety, plant age, and the physicochemical conditions of the rhizosphere soil (Fan *et al.*, 2023). However, Johnston-Monje *et al.* (2016) found that the distribution of microbes in hybrid maize plants aged 30, 60, and 90 days showed a high degree of similarity in their microbial communities, indicating that plant variety and plant growth period do not have a significant impact on the microbial composition in the maize plant rhizosphere. Meanwhile, the number of actinomycetes isolates obtained is believed to be influenced by the physicochemical conditions of the soil, especially factors such as pH and soil moisture levels, which affect the growth, composition, and activity of soil rhizosphere microbes in maize crop ecosystems. Observations in the rhizosphere area of maize plantations show that the soil pH in this environment is around 5.1. This value indicates that the soil conditions are generally acidic and can affect nutrient availability and microbial diversity, including actinomycetes, in the rhizosphere of maize plants. According to Singh *et al.* (2018), actinomycetes generally require environmental conditions with an optimal pH of around 7.0 for ideal growth and metabolic activity. However, Devanshi *et al.* (2022) found that actinomycetes can be active in acidic soils with a pH below 5.0 and can still be isolated from outside their habitat. These conditions indicate that the low soil pH in the rhizosphere of maize plantations in Bontula village contributes to the low actinomycete diversity and distribution. Therefore, it is suspected that the actinomycetes found in the rhizosphere soil of maize plantations in Bontula village are actinomycetes that are tolerant to acidic soil conditions. In addition, the relatively low soil moisture content, around 4.7%, indicates that the rhizosphere environment of maize plantations in Bontula village tends to be dry, which can affect the availability of water for plants and microbes in the soil. According to Skariah *et al.* (2023), the optimal moisture content for the growth of actinomycetes communities is in the range of ±0.86%. This indicates that actinomycetes are able to grow well in relatively dry environmental conditions. This finding is reinforced by Mohammadipanah & Wink (2016), who found that actinomycetes are not affected by dry environmental conditions like other soil microbes, and that these microbes prefer relatively low moisture levels.

### Antagonistic ability of Actinomycetes isolates against pathogenic molds in vitro.

The antagonistic ability of actinomycetes against pathogenic molds showed a resistance response to the growth of *Fusarium oxysporum* and *Neocosmospora solani*, which was characterized by the formation of an inhibition zone around the growth of actinomycetes. The antagonistic ability of actinomycetes against pathogenic molds was further classified according to the percentage of inhibition. According to Oyedoh *et al.* (2024), the percentage of microbial growth inhibition is divided into several categories, namely very weak (1-25%), weak (26-50%), strong (51-75%), and very strong (76-100%). The results showed that 4 out of 7 actinomycetes isolates had antagonistic ability against pathogenic molds in the inhibition percentage range of 66.38-89% with strong and very strong categories. Meanwhile, the other three isolates showed no antagonistic ability (Table 1, Figure 3).

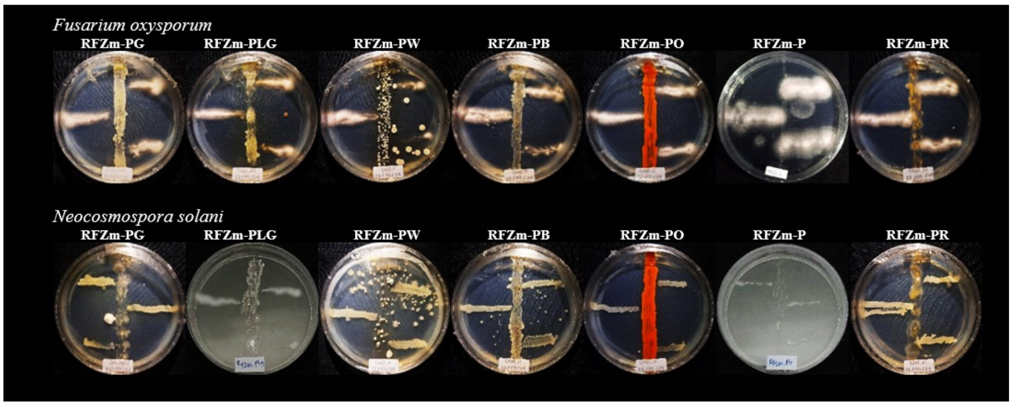

**Figure 3.** In vitro antagonistic activity of Actinomycetes against Fusarium oxysporum and Neocosmospora solani after 3 days of incubation at 30 °C.

**Table 1.** Antagonistic ability of actinomycetes against *Fusarium oxysporum* and *Neocosmospora solani* in vitro.

| **Actinomycetes isolate** | **Percentage inhibition of pathogenic mold isolates (%)** | | **Inhibitory ability category** |
|---|---|---|---|
| | ***F. oxysporum*** | ***N. solani*** | |
| RFZm-PG | 88,93 | 88,89 | very strong |
| RFZm-PLG | 84,89 | 88,33 | very strong |
| RFZm-PW | 66,38 | 71,83 | strong |
| RFZm-PB | 88,18 | 89 | very strong |
| RFZm-PY | 0 | 0 | not inhibition formed |
| RFZm-PR | 0 | 0 | not inhibition formed |
| RFZm-PO | 0 | 0 | not inhibition formed |

Four actinomycetes isolates exhibited strong antagonistic properties, demonstrating their ability to synthesize various bioactive metabolites that can inhibit the growth of pathogenic mold mycelium. According to Vurukonda *et al.* (2018), members of this microbial group are not only known as producers of antimicrobial substances but also as natural agents that enhance plant growth. In fact, more than 60% of the secondary metabolites produced by actinomycetes exhibit antimicrobial activity. Supporting this, Boukaew *et al.* (2017) highlight that the antagonistic action of actinomycetes is largely due to their ability to release antimicrobial metabolites, including siderophores that deprive pathogens of essential iron, as well as hydrolytic enzymes such as cellulase, chitinase, β-1,4-N-acetylglucosaminidase, protease, and β-1,3-glucanase. These enzymes work by degrading the structural components of fungal cell walls and membranes, reducing the viability of pathogenic fungi by approximately 20%, 50%, and even 60% in some cases. In addition, Mohan *et al.* (2024) emphasized that the biosynthesis of these antimicrobial compounds is controlled by the expression of polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) genes, which are crucial in the formation of polyketides and non-ribosomal peptides two classes of molecules that form the backbone of various powerful bioactive compounds. This perspective was further confirmed by Xu *et al.* (2023), who found that actinomycetes possess a robust genetic framework for the production of diverse secondary metabolites, highlighting their tremendous potential as a source of antimicrobial agents.

**Fungicide tolerance of isolate Actinomycetes in vitro.** Actinomycetes are a group of soil microbes known for their diverse biological capabilities, including tolerance to fungicides. In vitro research on fungicide tolerance in actinomycetes aims to evaluate the extent to which actinomycetes isolates are able to survive and continue to grow in conditions containing fungicide active ingredients. This study was conducted by observing the growth diameter of actinomycetes colonies after 7 days of incubation, the percentage of relative inhibition rate, and the tolerance category of isolates to fungicides. In general, all actinomycetes isolates showed tolerance to the three fungicides tested. The observed response pattern showed that the relative inhibition rate increased with increasing fungicide concentration. This indicates that the presence of fungicides at certain concentrations can inhibit the growth of isolates. In addition, among the three types of fungicides tested, the fungicide thiram showed the highest relative inhibition rate against the growth of actinomycetes isolates, followed by benomyl and dimetomorph. These findings are supported by the report by Campanale *et al.* (2023) which shows that the main metabolites of thiram fungicide are copper dimethyl-dithiocarbamate, dithiocarbamate, dimethylamine, and carbon disulfide. According to Kostov & Van Cleemput (2001), thiram fungicide is a copper-based fungicide, such as copper sulfate (copper(II) tetraoxosulfate), which can inhibit the growth of actinomycetes, as evidenced by the inhibition of the structural flexibility of glutathione, which ultimately inhibits enzymes that require a specific form of glutathione to function optimally. Patyka *et al.* (2016) found that the thiram fungicide group exhibits antimicrobial activity that can inactivate sulfhydryl enzyme groups that play a role in many biosynthesis and substance transport reactions, and can inactivate enzymes responsible for ATP synthesis, the conversion of glucose to pyruvate, amino acids, and fatty acids to acetyl coenzyme A in the respiration process. Although most actinomycetes isolates were able to survive up to a concentration of 500 ppm, the RFZm-PB isolate showed higher sensitivity and experienced earlier inhibition. However, based on the results, the actinomycetes isolates that showed the best tolerance to the three types of fungicides were RFZm-PG and RFZm-PLG, which remained viable at various levels of applied fungicide concentrations (Table 2).

**Table 2.** Relative inhibition rates of actinomycetes isolates against the active ingredients of fungicides in vitro.

| **Fungicide tolerance** | **Relative inhibition rate of actinomyces isolates (%)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **RFZm-PG** | **RFZm-PLG** | **RFZm-PW** | **RFZm-PB** | **RFZm-PR** | **RFZm-PY** | **RFZm-PO** |
| Dimetomorph (25 ppm) | -32 | -41 | 37 | -45 | 33 | 33 | 10 |
| Dimetomorph (50 ppm) | -34 | -17 | 36 | -2 | 30 | 28 | 22 |
| Dimetomorph (75 ppm) | -7 | -14 | 23 | -23 | 33 | 21 | 13 |
| Dimetomorph (100 ppm) | -5 | -8 | 43 | -44 | 30 | 33 | 6 |
| Dimetomorph (200 ppm) | 20 | 16 | 35 | -10 | 55 | 46 | 56 |
| Dimetomorph (300 ppm) | 25 | -5 | 32 | 19 | 62 | 33 | 65 |
| Dimetomorph (400 ppm) | 44 | 1 | 45 | 62 | 63 | 32 | 59 |
| Dimetomorph (500 ppm) | 62 | 32 | 50 | 83 | 58 | 32 | 36 |
| Thiram (25 ppm) | -34 | -28 | 22 | -6 | 20 | 18 | 2 |
| Thiram (50 ppm) | -6 | 8 | -6 | 42 | 21 | 11 | 17 |
| Thiram (75 ppm) | -44 | -41 | -5 | -16 | 14 | 32 | -2 |
| Thiram (100 ppm) | -62 | -38 | -50 | 32 | 10 | 9 | 6 |
| Thiram (200 ppm) | 31 | 44 | 50 | 100 | 39 | 36 | 59 |
| Thiram (300 ppm) | 64 | 42 | 42 | 100 | 46 | 64 | 27 |
| Thiram (400 ppm) | 56 | 42 | 70 | 100 | 44 | 36 | 36 |
| Thiram (500 ppm) | 67 | 100 | 73 | 100 | 100 | 100 | 100 |
| Benomyl (25 ppm) | -30 | -30 | 25 | 20 | 12 | 26 | 10 |
| Benomyl (50 ppm) | -28 | -16 | 2 | -15 | 15 | 39 | 10 |
| Benomyl (75 ppm) | -47 | -50 | -3 | -26 | 1 | 29 | 8 |
| Benomyl (100 ppm) | -41 | -47 | -9 | -4 | 2 | 14 | 0 |
| Benomyl (200 ppm) | 53 | 50 | 51 | 50 | 56 | 58 | 41 |
| Benomyl (300 ppm) | 56 | 52 | 38 | 54 | 52 | 54 | 25 |
| Benomyl (400 ppm) | 56 | 57 | 70 | 81 | 70 | 74 | 71 |
| Benomyl (500 ppm) | 48 | 54 | 68 | 77 | 63 | 65 | 55 |

### Molecular identification of Actinomycetes isolates potential antagonistic and fungicide tolerance.

Molecular identification of actinomycetes isolates with antagonistic and fungicide tolerance abilities was analyzed using a molecular approach that included genomic DNA isolation and extraction, 16S rRNA gene amplification, and phylogenetic tree reconstruction based on the neighbor-joining algorithm. The results of the screening test for antagonistic ability and tolerance to fungicides produced two potential actinomycetes isolates, namely RFZm-PG and RFZm-PLG. Molecular identification began with the extraction of genomic DNA using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) (B/7.2.1/IKP/001) method, then the purity of the extraction was determined using nanodrop quantification at an A260/A280 nm wavelength ratio, with DNA extract purity varying between 1.92 and 2.00. According to van de Pol *et al.* (2010), a genomic DNA purity level above 1.8 is relatively free of contamination, meaning that genomic DNA that has reached a good level of purity and meets the requirements will proceed to the gene amplification stage. In the gene amplification stage, primers 27F and 1492R were used, resulting in a DNA band with a length of approximately 1,500 bp for each sample tested. According to Heuer *et al.* (1997), the combination of primers 27F and 1492R is capable of amplifying almost the entire 16S rRNA gene with a length of approximately 1,500 bp, thus providing more complete phylogenetic information compared to primers that only target part of the gene (Figure 4).

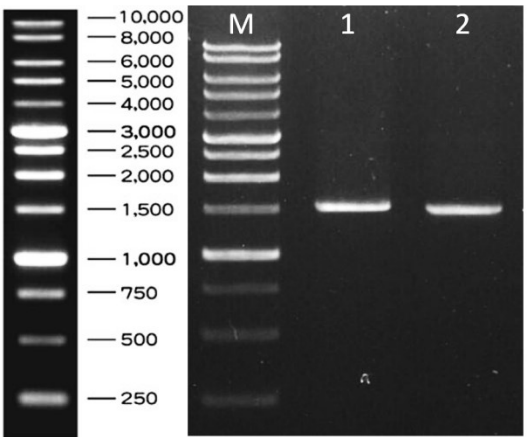

**Figure 4.** 16S rRNA Gene Electrogram Profile on Agarose Gel: M (DNA Marker); 1 (RFZm-PG Isolate); and 2 (RFZm-PLG Isolate).

The DNA sequence produced from the isolate was further analyzed using the BLAST (Basic Local Alignment Search Tool) program to match it against sequences in the GenBank database and evaluate its taxonomic placement. Based on the BLAST results, the RFZm-PG isolate was identified as a member of the genus Streptomyces and showed 100% sequence similarity with *Streptomyces enissocaesilis*. Similarly, the RFZm-PLG isolate was classified into the genus Nocardiopsis and showed a 100% similarity score with *Nocardiopsis synnemataformans*. These findings indicate a very close phylogenetic relationship between the isolates and their respective reference species. As explained by Hagstrom *et al.* (2000), sequence similarity values between 98% and 100% generally indicate species-level similarity, while values between 93% and 97% are more consistent with genus-level resolution (Table 3).

**Table 3.** The nearest phylogenetic relative of actinomycetes isolate based on the 16S RNA sequencing.

| Code of Actinomycetes isolate | Nearest Phylogenetic Neighbor | Homology (%) | *E-value* | Genus |
|---|---|---|---|---|
| RFZm-PG | *Streptomyces enissocaesilis* (PV636605.1) | 100 | 0 | *Streptomyces* |
| | *Streptomyces rochei* (NR_116078.1) | 99,86 | 0 | *Streptomyces* |
| | *Streptomyces plicatus* (NR_112357.1) | 99,86 | 0 | *Streptomyces* |
| | *Streptomyces geysiriensis* (NR_043818.1) | 99,78 | 0 | *Streptomyces* |
| RFZm-PLG | *Nocardiopsis synnemataformans* (PV931908.1) | 100 | 0 | *Nocardiopsis* |
| | *Nocardiopsis dassonvillei* (NR_074635.1) | 99,36 | 0 | *Nocardiopsis* |
| | *Nocardiopsis halotolerans* (NR_112744.1) | 98,64 | 0 | *Nocardiopsis* |
| | *Nocardiopsis flavescen* (NR_108853.1) | 98,64 | 0 | *Nocardiopsis* |

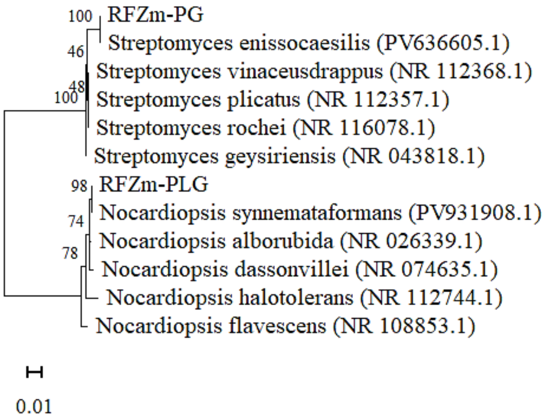

**Figure 5.** Phylogenetic tree reconstruction of actinomycetes isolates based on 16S rRNA sequence analysis.

The results of phylogenetic tree reconstruction (Figure 6) show that the actinomycetes isolate RFZm-PG forms a clear cluster with the species *S. enissocaesilis*, so this isolate was then identified and named *S. enissocaesilis* strain RFZm-PG (PV636605). 1), while the actinomycetes isolate RFZm-PLG showed an evolutionary relationship with the species *N. synnemataformans*, indicating that this isolate belongs to that species, so this actinomycetes isolate was classified as *N. synnemataformans* strain RFZm-PLG (PV931908.1).

## CONCLUSIONS

This study showed that four actinomycetes isolates (RFZm-PB, RFZm-PG, RFZm-PLG and RFZm-PW) were able to inhibit the growth of *F. oxysporum* and *N. solani* with 66.38%-89% inhibition rate. The fungicide tolerance test showed that isolates RFZm-PG and RFZm-PLG had the highest tolerance up to 500 ppm, while other isolates were variable. Molecular analysis identified isolates RFZm-PG as S. enissocaesilis and RFZm-PLG as *N. synnemataformans*, indicating their potential for biological control.

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