HAYATI
Journal of Biosciences

Vol. 32 No. 1, January 2025 241-253
DOI:10.4308/hjb.32.1.241-253
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Endophytic Actinomycetes of Liliaceae Plants as Biocontrol Agents of *Fusarium oxysporum* f.sp. *cepae* Causes of Basal Plate Rot Disease on Shallots

Lisa Marianah[1,2], Abdjad Asih Nawangsih[2*], Abdul Munif[2], Giyanto[2], Efi Toding Tondok[2]

[1]*Agricultur Training Center of Jambi, Agricultural Extension and Human Resource Development Agencies, Indonesian Ministry of Agriculture. Pasar Minggu, Jakarta 12550, Indonesia*
[2]*Department of Plant Protection, Faculty of Agriculture, IPB University, Kampus IPB Dramaga, Bogor 16680, Indonesia*

ARTICLE INFO

Article history:
Received July 1, 2024
Received in revised form August 23, 2024
Accepted September 4, 2024

KEYWORDS:
biological control,
endophytic,
induce resistance,
peroxidase,
Streptomyces,
twisting

ABSTRACT

Basal plate rot disease is one of the critical diseases in shallot plants. Control using synthetic chemical fungicides is still unable to overcome this problem, and it hurts the environment and reduces the population of essential microbes in plants. Endophytic actinomycetes have potential as biocontrol agents. They are reported to be able to inhibit the growth of pathogens, induce plant resistance, produce cell wall degrading enzymes, and promote growth. This research aims to obtain endophytic actinomycete isolates that have the potential to inhibit the growth of the fungus *Fusarium. oxysporum* f.sp. cepae causes of basal plate rot disease in shallot, and evaluate its inhibitory mechanism. Endophytic actinomycetes were isolated from tubers and roots of Liliaceae plants collected from shallots-production center area. The isolate obtained was tested for biosafety and continued with its inhibitory effectiveness against the fungus *F. oxysporum* f.sp. *cepae in vitro*, ability to induce resistance, and plant growth promotion test. The six best isolates were selected based on weighting using AHP and identified molecularly. The endophytic actinomycetes of Liliaceae plants can inhibit the growth of *F. oxysporum* f.sp. *cepae* up to 63.49% with an antibiosis mechanism, producing chitinase enzymes that cause lysis, induce resistance, and produce growth hormones such as IAA. *Streptomyces* sp. can inhibit the fungus *F. oxysporum* f.sp. *cepae* causes basal plate rot disease on shallot by producing antifungal compounds and chitinase enzymes, inducing resistance, and producing growth hormone.

1. Introduction

Shallots are a crucial horticultural crop listed as the commodity with the most significant contribution to national inflation. Indonesia is one of the largest shallots exporting countries, with production centers across six provinces: Central Java, East Java, West Nusa Tenggara, South Sulawesi, West Java, and West Sumatra. National shallot production in 2022 will experience a decrease from the previous year of 30,298.7 tons (BPS 2023). National shallot productivity is currently still low, ranging between 9.6 and 10.7 tons/ha, and this can be increased considering the potential productivity of shallots can reach 20 tons/ha (BPS 2023). The low productivity of shallots in Indonesia is due to several factors, one of which is plant disease. One of the diseases that is a major problem for shallot production in Indonesia and worldwide is basal plate rot, caused by the fungus *F. oxysporum* f.sp. *cepae* (Bektas and Kusek 2019; Wijayanti *et al.* 2021; Le *et al.* 2021a). This pathogen not only attacks plants in the field but also storage (pre- and post-harvest), causing yield loss and reduced quality of shallot bulbs and other types of onions (Haapalainen *et al.* 2016; Le *et al.* 2021a). Basal plate rot disease is also a problem in onion cultivation in several onion-producing countries, such

* Corresponding Author
E-mail Address: ryuntania@apps.ipb.ac.id

as Mexico, India, China, and Vietnam (Quyent *et al.* 2017; Shamyuktha *et al.* 2020; Tirado-Ramirez 2021; Le *et al.* 2021b).

Control efforts at the farmer level currently still rely on synthetic chemical pesticides with a very high frequency but have not been able to control the disease. The results of Degani and Kalman's *et al.* (2020) research that tested ten types of commercial fungicides in Israel (Sportak, Celest 100FS, Signum W.G., Topaz W.P., Mythos 300 SC, Orius 25, Delsene, Azimut, Amistar, and Vibrance) with active ingredients Prochloraz, Fludioxonil, Boscalid 2.6% and Pyraclostrobin 6.7%, Thyophanate-Methyl, Pyrimethanil, Tebuconazole, Carbendazim, Azoxystrobin 12% and Tebuconazole 20%, Azoxystrobin, Fludioxonil 2.5% and Sedaxane 2.5%, only 1 type (Prochloraz) can inhibit the growth of *F. oxysporum* f. sp. *cepae* and unable to inhibit the growth of *F. acutatum* fungi. According to Shin *et al.* 2023, no fungicide can effectively control basal plate rot disease. The results of the research tested seven types of fungicides with the active ingredients Fludioxonil, Hexaconazole, Mandestrobin, Penthiopyrad, Prochloraz-manganese, Pydiflumetofen, and Tebuconazole; only the fungicide with the active ingredient Prochloraz-manganese showed 100% inhibition against *F. oxysporum* f.sp. *cepae*. This study shows that synthetic chemical fungicides are not the right solution to control tuber blight; they can even negatively impact the environment. This is very risky for the sustainability of agriculture in the future. In addition, the excessive use of chemical pesticides can harm human health. Efforts to reduce synthetic chemical pesticides can be made if there are environmentally friendly control alternatives so sustainable agriculture can be realized.

One alternative environmentally friendly control that has potential is using biocontrol agents. One potential biocontrol agent reported to have much potential is actinomycetes. There is much evidence that actinomycetes, which are gram-positive bacteria, make bioactive compounds that are antifungal and antibacterial. These bioactive compounds contain antibiotics (Chanthasena and Nantapong 2016; Shan *et al.* 2018). Actinomycetes can also make enzymes that break down fungal cell walls, like chitinase, cellulase, and catalase (Chaiharn *et al.* 2019; Wijayanti *et al.* 2021). Actinomycetes are also reported to have the ability to produce growth-promoting hormones, such as indole acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC), dissolve phosphates, fix nitrogen, and have potential as plant growth boosters (Shan *et al.* 2018; Fatmawati *et al.* 2019; Wijayanti *et al.* 2021).

Actinomycetes have been reported to inhibit the growth of several plant pathogens such as *Drechslera halodes, Alternaria alternata, A. sesame, Macrophomina phaseolina, M. oryzae, F. proliferatum, F. oxysporum, F. oxysporum* f.sp. *ciceri, F. oxysporum* f.sp. *cepae, F. graminearum, F. oxysporum* f.sp. *lycopersici, F. oxysporum* f.sp. *cubense, F. verticillioides, F. moniliform, Colletotrichum* sp., *Pestalotiopsis* sp., *Diaporthe* sp., *Xylaria* sp., *Rhizoctonia solani, Helminthosporium oryzae* and *Rigidoporus lignosus* (Hussein *et al.* 2014; Passari *et al.* 2017; Shan *et al.* 2018; Mingma and Duangmal 2018; Chaiharn *et al.* 2019; Wijayanti *et al.* 2021). Actinomycetes are also reported to inhibit the Growth of several bacteria, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis*, and *Escherichia coli* (Shan *et al.* 2018).

Endophytic actinomycetes play a unique role, such as protecting host plants from soil-borne pathogens. The presence of *Streptomycetes* in root tissue has a vital role in plant development and fitness, either by helping to provide nutrients to plants or by producing secondary metabolites that stimulate or suppress plant vegetative development (Sardi *et al.* 1992). Endophytic actinomycetes are also reported to induce plant resistance. This is evidenced by the results of the research of Vijayabharathi *et al.* (2018) on endophytic actinomycetes of chickpea plants capable of inducing chickpea plant resistance to Botrytis cinerea by producing antibiotic molecules such as HCN, ammonia, siderophores, β-1,3-glucanase, chitinase, methylalaninliase (PAL), and the number of penolic compounds. Endophytic actinomycetes are potentially biocontrol agents due to their antifungal activity and colonization characteristics in host plants (Subhashini & Singh 2014; Vurukonda *et al.* 2018). Endophytic actinomycetes perform an essential role in plant protection using bioactive compounds, which, in addition to biocontrol agents, can reduce plant stress and promote plant growth (Singh *et al.* 2017). The mechanism of endophytic actinomycetes mainly focuses on the release of bioactive compounds, namely antibiotics and cell wall degrading enzymes, as well as inducing host plant resistance (ISR) (El-Tarabily and Sivasithamparam 2006; Ansari *et al.* 2020). The interaction of endophytic actinomycetes with plants produces bioactive compounds, which have great

potential as an efficient and potential biocontrol agent or bio-fungicide for plant defense (Ansari *et al.* 2020).

These potentials will be obtained by exploring biocontrol agents from the host plant. Excessive application of synthetic chemical pesticides in onion cultivation leads to reduced microbial populations. This condition causes us difficulties in exploring biocontrol agents in onion plants and requires other plants as a source of microbes. Other plants that allow microbes to adapt quickly are those in the same family as onion plants, namely plants of the Liliaceae family. Information regarding the ability of endophytic actinomycetes of Liliaceae plants to control basal plate rot disease in shallots, as well as its species, has never been reported. That became the novelty of this study, which aims to find endophytic actinomycete isolates that can stop the growth of *F. oxysporum* f.sp. *cepae* fungi that cause basal plate rot disease of shallots and evaluate its inhibitory mechanism.

2. Materials and Methods

2.1. Exploration of Endophytic Actinomycetes

2.1.1. Sampling

Samples in tubers and roots from plants of the Liliaceae family were collected from four regions, namely Brebes, Bogor, Cianjur, and Bengkulu. Samples were collected using the purposive sampling method. All plant samples are wrapped in paper bags and then put in a cooler box to maintain the freshness of the samples. Next, the sample is taken to the laboratory for processing.

2.1.2. Isolation of Endophytic Actinomycetes

Isolation of endophytic actinomycetes was carried out using procedures based on Shan *et al.* (2018), which were modified with three types of growth media: The SCA mixture has 10 g of starch, 0.3 g of casein, 2 g of KNO3, 2 g of NaCl, 2 g of K_2HPO_4, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.02 g of $CaCO_3$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 18 g of agar, and 1 liter of sterile distilled water. The WYA mixture has 0.25 g of yeast extract, 0.5 g of K_2HPO_4, 18 g of agar, and 1 liter of distilled water. The YEMA mixture has 20 g of agar, 3 g of dextrose, 3 g of malt extract, 5 g of peptone, 3 g of yeast extract, and 1 liter of sterile distilled water with pH 7.2 and 20 g of Nystatin added. Sterilize the medium by autoclaving at a temperature of 121°C and a pressure of 0.1 MPa for 15 minutes. The roots and tubers of healthy plants are washed thoroughly using running water and dried in the air. The samples were surface sterilized using 3% NaOCl for 5 minutes, 70% alcohol for 30 seconds, rinsed with sterile distilled water three times and dried in the air. 10 g of each sample was ground until smooth by adding 10 ml of 0.9% sodium chloride (NaCl), then diluted to 10^{-3}. The 10^{-2} and 10^{-3} dilutions were spun at 5,000 rpm for 15 minutes, and the pellet was mixed with 100 µl of distilled water. It was then spread on SCA, WYA, and YEMA growth media. Next, incubate for 2-4 weeks at 28°C. On the ISP2 growth medium, colonies that looked like typical actinomycetes (flaming growth, aerial mycelium, hard, dusty, and often colored colonies) were moved and cleaned up. The pure culture was observed under a microscope, stored in a 20% glycerol solution, and put in the refrigerator at -20 °C as stock. The selected isolates would be frozen-dried at -80 °C. To ensure the surface sterilization process worked, sterilized tuber samples were rubbed on the ISP2 growth medium (4 g yeast extract, 10 g malt extract, 4 g dextrose, 20 g agar, and 1 L sterile distilled water). Unsterileized tubers were used as a control, and the mixture was kept at 28°C. If no microbes grow, then surface sterilization is considered adequate.

2.2. Biosafety Test

2.2.1. Hypersensitivity Reaction Test

Hypersensitivity reaction tests were carried out using a variety of Garut tobacco plants. The diameter of two borer endophytic actinomycete isolates was ±0.8 mm. They were grown on 10 ml of liquid ISP2 medium and put on an incubator shaker at 120 rpm for seven days at room temperature (±27°C). A sterile 1 ml syringe (without needles) was used to inject 1 ml of endophytic actinomycete suspension into the underside of tobacco leaves. The reaction is positive if necrosis occurs in the injected part 48 hours after the injection. *Xanthomonas campestris* bacteria (IPB bacteriology laboratory collection) were used as positive controls, and sterile aquades as negative controls (Fatmawati *et al.* 2019).

2.2.2. Blood Hemolysis Test

Actinomycetes isolated from ISP2 media and had been there for seven days were grown on 5% sheep blood agar and kept at 28°C for five days. *Staphylococcus aureus* ATCC (American Type Culture Collection) 6538 bacteria as a positive control and *Escherichia coli* DH5α as a negative control (Aris Tri Wahyudi's collection). The growth of endophytic actinomycete isolates is observed through a hemolysis reaction. Isolates that formed clear zones around actinomycete colonies, showing hemolytic

activity, either α (partial hemolysis) or β (complete hemolysis), were not used in subsequent tests.

2.3. Potential of Biological Control Agent Tests

2.3.1. Endophytic Actinomycete Antagonism Test Against *F. oxysporum* f.sp. *cepae*

Endophytic actinomycete antagonism testing against *F. oxysporum* f.sp. *cepae* uses a dual culture method. Endophytic actinomycetes are scratched on the side of the cup containing PDA media and then incubated for three days. After being incubated, *F. oxysporum* f.sp. *cepae* fungus isolates are put 3 cm to the other side of the petri dish. As controls, fungi that do not have endophytic actinomycetes are also put there. Each treatment is repeated three times and incubated for eight days at room temperature. It was looked at how wide the clear zone was, how resistant actinomycetes were to *F. oxysporum* f.sp. *cepae*, and the number of microscopic fungi.

2.3.2. Volatile Organic Compound (VOC) Production to Inhibit *F. oxysporum* f.sp. *cepae* Test

A test of the effect of volatile organic compounds produced by endophytic actinomycetes on the growth of *F. oxysporum* f.sp. *cepae* fungi was carried out by growing *F. oxysporum* f.sp. *cepae* fungi on PDA media and endophytic actinomycetes on ISP2 media. The two petri dishes were cupped in the same position as the petri dish containing *F. oxysporum* f.sp. *cepae* fungus at the top, and the petri dish containing endophytic actinomycetes at the bottom are glued together using plastic wrap. Petri dishes containing the *F. oxysporum* f.sp. *cepae* fungus without endophytic actinomycetes were used as controls. Incubation was done for eight days at room temperature, and observations were made by calculating the percentage of inhibition.

2.3.3. Effects of Actynomycetes on Peroxidase Enzyme (POD) Activity in Shallots

Actinomycete suspension was prepared by growing actinomycete isolates on ISP2 media for three weeks. One petri dish isolated endophytic actinomycetes and their media added 20 ml of 0.9% NaCl and then chopped them using an ultra-turax shredding machine until smooth. The suspension of endophytic actinomycete isolate was centrifuged at a speed of 5,000 rpm for 15 minutes. The pellets were washed using sterile aqueducts and re-centrifuged. The pellets were suspended with 0.5% carboxymethyl cellulose (CMC) until a 10^8 cfu/ml density was obtained. The test uses shallot seeds of the Bima Brebes variety ready for planting. Tubers are surface sterilized by soaking them in a 1% solution of NaOCl for 5 minutes, 70% alcohol for 1 minute, and being rinsed three times with sterile aquades, then dried in a sterile incubator for 12 hours. As a treatment, tubers were soaked in a suspension of endophytic actinomycete isolate for two days. As a control, 0.5% CMC was used. Next, the bulbs are planted in plastic pots with a diameter of 10 cm containing a 1/2 sterile planting medium combination of soil, manure, and sand in a 1:1:1 ratio. Each pot contains two plants, and the treatment is repeated three times. After the plants were two weeks old, they were uprooted and washed thoroughly with running water for further activity analysis of the POD enzyme. The activity of the POD enzyme was analyzed using the Zhang *et al.* (2016) method. Five grams of root tissue and tubers from each sample were mashed using a cooled mortar. 10 ml of 100 mmol sodium phosphate buffer (pH 6.8–8.8) containing 1% polyvinylpyrrolidone (PVP) is added. The sample solution was put into a 15-ml tube and centrifuged at 15,000 rpm for 15 minutes at 4°C. The supernatant was then collected to check the activity of the PO enzyme and the amount of protein in it. PO activity is determined using guaiacol as a substrate. The reaction mixture had 100 ml of crude extract and 1.5 ml of guaiacol (10 mmol in 100 mmol sodium phosphate buffer solution pH 6.8). It was left to sit at room temperature for 30 minutes. Before analysis using a spectrophotometer at 470 nm absorbance, the reaction mixture included 1 ml of H_2O_2 (24 mmol). One unit of POD activity is defined as the amount of enzyme required for an increased absorbance of 470 nm/s.

2.3.4. Chitinolityc Activity Assay of Endophytic Actinomycetes Isolates

The test of the production ability of chitinase enzyme endophytic actinomycete isolates was carried out by growing endophytic actinomycete isolates on chitin gelatinous media (Hsu and Lockwood 1975) containing colloidal chitin 0.4%, 0.3 g KH_2PO_4, 0.7 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 5H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$, 0.001 g $MnCl_2 \cdot 4H_2O$, and 20 g/L agar with pH 8.0–8.5. The isolates were incubated at room temperature (±27°C) for four days. Testing is carried out in duplicate. A 6% Congo Red solution (0.6 g in 100 ml of aquades) of 5 ml was poured into the culture and left for 15 minutes, then removed and rinsed with 1% NaCl three times. Washing is done to dispose of Congo red that does not bind to polysaccharides. The culture was then incubated at 4°C for 48 hours to

perfect the clear zone formation. Clear zones formed around actinomycete colonies indicate chitinolytic activity. Chitin degradation power is classified based on chitinolytic index values, with high categories when IK values ≥ 2, medium categories when IK values 1-2, and low categories when IK values ≤ 1 (Choi *et al.* 2005). When comparing the diameter of the clear zone to the diameter of the actinomycete colony, get the Chitinolytic Index (IS) value (Nababan *et al.* 2019).

2.3.5. The Ability of Endophytic Actinomycetes to Produce IAA Test

The colorimetric method based on Wahyudi *et al.* (2019) and filtratation analysis using a spectrophotometer measured the ability of endophytic actinomycetes to produce IAA hormones. Two plugs of endophytic actinomycetes (diameter ±0.8 mm) were grown on 20 ml of ISP2 liquid media plus 0.2 ml of 0.2% L-tryptophan (L-trp). The mixture was then put in an incubator shaker at 120 rpm for ten days at room temperature (±27°C). The suspension was centrifuged at 5,000 rpm for 15 minutes at 4°C. In a test tube, 1 ml of supernatant was mixed with 4 ml of Salkowsky reagent (Gordon & Weber 1951) (1 ml $FeCl_3$ 0.5 M + 50 ml $HClO_4$ 50%). The tube was left to sit at room temperature (±27°C) in a dark room for 30 minutes. Observations were made by looking at the color change in the suspension, where the suspension that changed color to pink showed that the isolate was able to produce the hormone IAA. IAA hormone concentrations were measured using a spectrophotometer with a wavelength of 530 nm and calculated using IAA standard curves. IAA standard curves are created using synthetic IAA. The stock solution is prepared by dissolving synthetic IAA (98%) as much as 2.5 mg in 50 ml methanol (concentration 50 ppm). The concentration of IAA solution used was one µl of aquades (0 ppm) as a control, 10 µl (0.5 ppm), 20 µl (1 ppm), 30 µl (1.5), 40 µl (2 ppm), 50 µl (2.5 ppm), 100 µl (5 ppm), 150 µl (7.5 ppm), 200 µl (10 ppm), and liquid ISP2 media (control +).

2.4. The Analytic Hierarchy Process (AHP)

Endophytic actinomycete isolates that have potential as biocontrol agents of *F. oxysporum* f.sp. *cepae* were determined based on the AHP results. Weighting was based on the priority rank of criteria and sub-criteria in each observation category, calculated based on weighting (Saaty 2008). The two best isolates of endophytic actinomycetes from AHP will be used to see how well endophytic actinomycetes can stop the growth of fungi that cause basal plate rot disease on shallots of greenhouse.

2.5. Molecular Identification of Endophytic Actinomycete

Molecular identification was carried out on selected endophyte actinomycete isolates. Pure isolates were cultured in MYG medium for 7–10 days at 30°C. Genomic DNA was extracted from pure isolates using the Quick-DNATM Fungal/Bacterial Miniprep Kit from Zymo Research Corp. The 16S rRNA gene was amplified using primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3) and 16Sact1114R (5'-GAG TTG ACC CCG GCR GT-3') based on Wijayanti *et al.* (2021). The PCR mixture reaction was carried out in a total volume of 25 µL, including Bioline MyTaq HS Red Mix 2x 12.5 µl, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 9.5 µL nuclease-free water (NFW), and 1 µl DNA template (100 ng/µL). PCR conditions begin with a pre-denaturation process (95°C for 4 minutes), denaturation (95°C for 30 seconds), annealing (55°C for 30 minutes), extension (72°C for 1 minute), and post-extension (72°C for 7 minutes). The cycle from denaturation to extension is 30 cycles. PCR products were visualized using gel electrophoresis on a 1% agarose gel and migration at a voltage of 50 V for 50 minutes and observed under a UV transilluminator. PCR results are sequenced through a sequencing service. Sequencing results were aligned with GenBank data using the Basic Local Alignment Search Tool-Nucleotide (BlastN) program from the National Center for Biotechnology Information (NCBI) website. Phylogenetic trees were constructed using MEGA.

2.6. Statitistical Analyses

Data were tabulated using Microsoft Excel 2019 and analyzed using the analysis of variance (ANOVA). Significantly different treatments were tested using Tukey tests at the 5% level using SPSS 27.0 software.

3. Results

3.1. Population of Endophytic Actinomycetes in Liliaceae Plants

There were 11 types of plants from the Liliaceae group that were collected from Brebes, Cianjur, Bogor, and Bengkulu, namely shallots (*Allium cepa* L. var. aggregateum), spring onions (*A. fistulosum*), chives (*A. tuberosum*), chives (*A. schoenoprasum*), Dayak onions (*Eleutherine bulbosa* Mill.), rain lilies (*Zephyranthes*

minuta), spider lilies (*Hymenocallis littoralis*), and swamp lilies (*Crinum asiaticum*). The isolation results from eight types of Liliaceae plants obtained 64 isolates of endophytic actinomycetes. Most isolates were obtained from Brebes, namely 27 isolates, 19 isolates from Bengkulu, 11 isolates from Cianjur, and seven isolates from Bogor. Liliaceae plants in their natural habitat isolated more endophytic actinomycetes than Liliaceae plants around the home yard. Only a few endophytic actinomycetes were obtained from shallot plants cultivated with intensive fungicide application.

The results of hypersensitivity tests on 64 isolates showed that 28 isolates were positive, causing necrotic spots on tobacco leaves, and this indicated that these isolates had the potential to be pathogenic on plants, and 36 isolates were negative. The results of the blood hemolysis test on 64 isolates showed that 15 isolates were positive and could grow and form a clear zone on blood agar media, which means these isolates had the potential to be pathogens in mammals, and 49 isolates were negative. Only 24 isolates were negative for hypersensitivity reactions and blood hemolysis, so they were safe to use as biocontrol agents. The isolates came from Brebes, 11 isolates, five isolates from Bengkulu, and four isolates from Bogor, as in Table 1. The colony characteristics of the endophyte actinomycete isolates obtained from Liliaceae plants are shown in Figure 1.

3.2. Antagonism Ability and Inhibitory of Volatile Organic Compounds of Endophytic Actinomycete Isolates Against *Fusarium oxysporum* f.sp *cepae*

The endophytic actinomycetes of Liliaceae plants were able to inhibit the growth of the fungus *F. oxysporum* f.sp. *cepae* with an inhibition percentage of up to 63.49%. Test results of the inhibitory power of endophytic actinomycetes of Liliaceae plants against the fungus *F. oxysporum* f.sp. *cepae* showed that of the 24 isolates, 11 isolates had an inhibitory power above 50%. Several endophytic actinomycete isolates could inhibit fungal growth to a greater extent on day six

Table 1. Inhibition percentage of endophytic actinomycetes of Liliaceae plants and VOC inhibition of the fungus *Fusarium oxysporum* f.sp. *cepae*

Isolate code	Percentage of inhibition of endophytic atinomycete (%)	Isolate code	Percentage of inhibition of VOC (%)
LBP1	0.00^{a}	LKP1	0.00^{a}
SGLK7	0.00^{a}	LBP1	1.27^{ab}
SGLK8	1.59^{a}	BM SAM	4.09^{abc}
LBP2	3.17^{a}	BM SNDG	4.20^{abc}
SGLK CSA1	6.35^{a}	LKP4	5.06^{abc}
SGLK4	8.73^{a}	BOR1	5.10^{abc}
SGLK5	36.51^{b}	BK BLSR	5.69^{abcd}
SDLO8	38.10^{bc}	LKP2	7.31^{abcd}
SDLO6	41.27^{bcd}	SGLK1	7.76^{abcd}
LKP4	45.24^{bcde}	LBP2	8.10^{abcd}
LKP1	47.67^{bcdef}	BOR4	8.25^{abcd}
SGLK2	49.21^{bcdefg}	SGLK CSA1	8.36^{abcd}
LKP2	50.00^{cdefgh}	SDLO6	9.56^{abcd}
SDLO9	50.00^{cdefgh}	SGLK2	9.60^{abcd}
SGLK1	53.17^{defghi}	SGLK7	10.01^{abcd}
SDLO2	56.35^{efghi}	SDLO9	11.29^{abcd}
BM SAM	57.93^{efghi}	SGLK4	11.51^{abcd}
BM SNDG	58.73^{fghi}	SDLO2	11.62^{abcd}
BOR7	58.73^{efghi}	SGLK8	12.30^{abcd}
BOR1	59.52^{fghi}	BOR7	13.61^{bcd}
BK BLSR	60.32^{fghi}	BOR5	15.41^{de}
BKKRTBSK	61.91^{ghi}	SDLO8	16.31^{de}
BOR4	62.70^{hi}	SGLK5	17.70^{e}
BOR5	63.49^{i}	BKKRTBSK	17.77^{e}

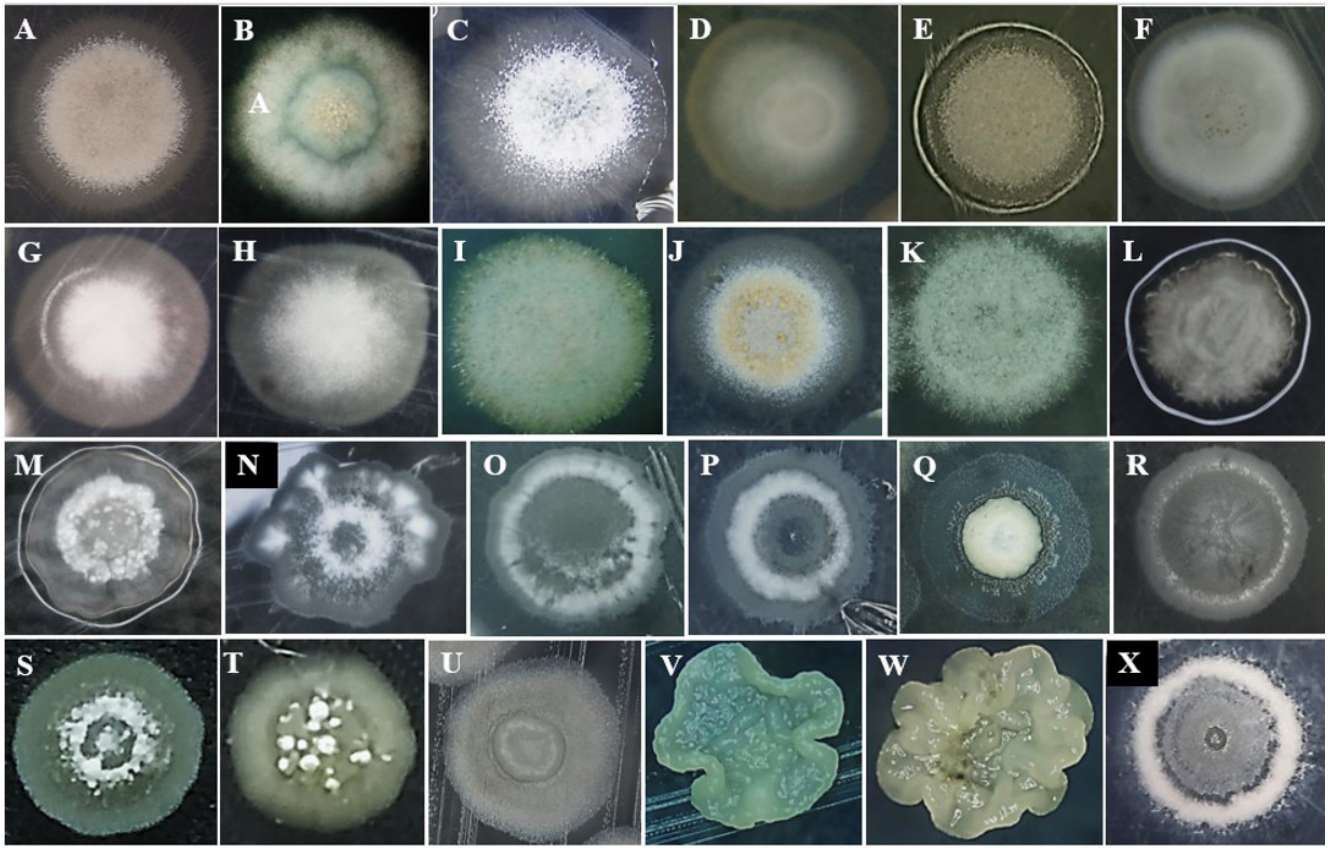

Figure 1. Characteristics of Liliaceae plant endophytic actinomycete isolates that are safe as biocontrol agents: (A) BM SAM isolate, (B) BK BLSR, (C) BK KRT BSK, (D) BM SNDG, (E) BOR1, (F) BOR4, (G) BOR5, (H) BOR7, (I) LKP1, (J) LKP2, (K) LKP4, (L) LBP1, (M) LBP2, (N) SDLO2, (O) SDLO6, (P) SDLO8, (Q) SDLO9, (R) SGLK1, (S) SGLK2, (T) SGLK4, (U) SGLK5, (V) SGLK7, (W) SGLK8, and (X) SGLK CSA

and decreased after that, and only six isolates had an inhibitory ability below 40% (Table 1). A total of 15 isolates showed a clear zone (Figure 2), indicating that the endophyte actinomycete isolates produce antifungal bioactive compounds. The endophytic actinomycetes of Liliaceae plants also produce volatile organic compounds which were able to inhibit the growth of the fungus *F. oxysporum* f.sp. *cepae*, although the inhibitory power is low, ranging from 1-17.77% (Table 1).

3.3. Peroxidase (POD) and Chitinase Enzyme Activities of Endophytic Actinomycete

Plants induced by endophyte actinomycete isolates showed peroxidase enzyme activity, although the level of enzyme activity was relatively low (Table 2). Of the 24 isolates, six isolates showed peroxidase enzyme activity that exceeded the peroxidase enzyme value in control plants, namely isolates BK BLSR, BOR4, LKP1, SGLK2, SGLK4, and SGLK7. Endophyte actinomycetes are also capable of producing chitinase enzymes, where the chitinolytic index value in the test for the ability to produce chitinase enzymes shows values in the range of 1-1.5. Moreover, this value is in the medium category. The two indicators above show that the endophytic actinomycetes of Liliaceae plants have the potential to induce host plant resistance.

3.4. The Ability to Produce The IAA Hormone of Endophytic Actinomycete

Several isolates of endophytic actinomycetes of Liliaceae plants were capable to producing IAA hormones, as shown in Table 2. Two isolates produced IAA hormones with high concentrations of up to 4 ppm, namely isolates BOR1 and SGLKCSA1; 9 isolates produced IAA with moderate concentration levels ranging from 0.4-1.4 ppm, and the remainder produced IAA with low concentrations (<0.4 ppm).

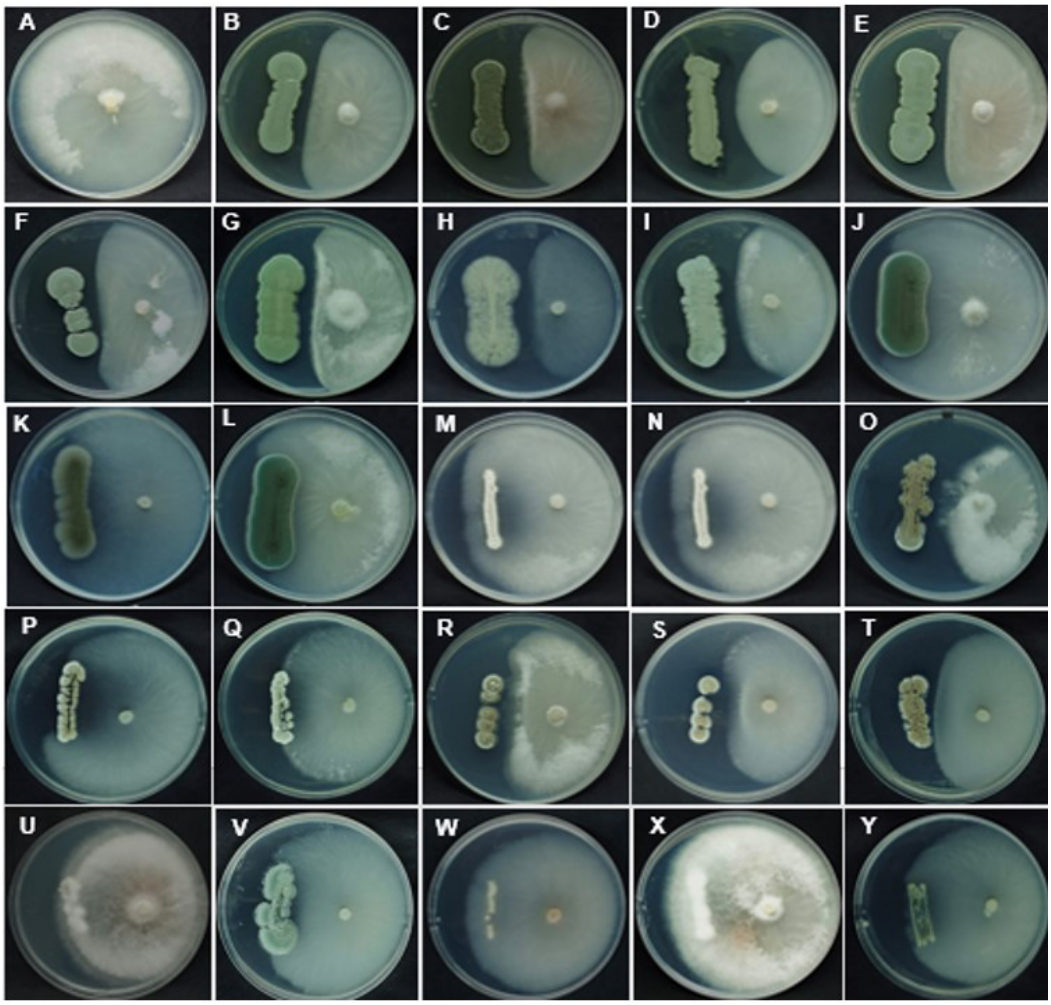

Figure 2. Endophytic actinomycete inhibition test against the fungus *F. oxysporum* f.sp. *cepae in vitro*: (A) Control, (B) BM SAM isolate, (C) BK BLSR, (D) BK KRT BSK, (E) BM SNDG, (F) BOR1, (G) BOR4, (H) BOR5, (I) BOR7, (J) LKP1, (K) LKP2, (L) LKP4, (M) LBP1, (N) LBP2, (O) SDLO2, (P) SDLO6, (Q) SDLO8, (R) SDLO9, (S) SGLK1, (T) SGLK2, (U) SGLK4, (V) SGLK5, (W) SGLK7, (X) SGLK8, and (Y) SGLK CSA

3.5. AHP and Identity of Liliaceae Endophytic Aktinomycete

The AHP results showed that the 24 endophytic actinomycete isolates of Liliaceae plants had potential as biocontrol agents against the fungus *F. oxysporum* f.sp *cepae*. The six best isolates were SGLK2, BOR1, SDLO6, SDLO9, SGLK1 and SDLO2. The five isolates were identified molecularly based on the 16sRNA gene, and the results are shown in Figure 3. Based on the results of DNA sequencing, the five isolates are the *Streptomyces* sp. species with a homology of 99-100%, and 98,29-99,20% to *Streptomyces graminisoli*. One isolate only had 78.36% homology with *Streptomyces* sp.

4. Discussion

The results of a field survey that asked directly to shallot farmers who were met showed that shallot cultivation in several production centers, especially in Brebes, tends to use pesticides with high frequency, namely 2-3 times a week. The doses used exceed the doses stated on the packaging. Some farmers even combine several types of insecticides and fungicides in one application, and the results of this survey have also been reported by Joko *et al.* (2017), who conducted research from 2013-2016. Susanti's research results (2018) show the perception of farmers who think

Table 2. Endophytic actinomycete's ability to produce peroxidase, chitinase enzymes, and IAA hormone

Isolate code	PO activity (UAE)	Chitinolityc indexs	IAA (ppm)
Control	0.00412	0.00	0.00
BM SAM1	0.00299	1.11	0.67
BK KRTBSK	0.00379	1.14	0.37
BK BLSR	0.00428	1.25	0.20
BMSNDG1	0.00416	1.02	0.20
BOR1	0.00247	1.12	4.00
BOR4	0.00552	1.00	0.20
BOR5	0.00321	1.04	0.70
BOR7	0.00310	1.04	1.10
LKP1	0.00546	1.13	0.28
LKP2	0.00186	1.05	0.46
LKP4	0.00365	1.09	0.22
LBP1	0.00305	1.26	0.30
LBP2	0.00353	1.23	0.20
SDLO2	0.00397	1.25	0.30
SDLO6	0.00342	1.33	0.21
SDLO8	0.00388	1.31	0.30
SDLO9	0.00323	1.36	0.07
SGLK1	0.00191	1.18	1.30
SGLK2	0.00575	1.50	0.35
SGLK4	0.00461	1.11	0.40
SGLK5	0.00235	1.09	0.50
SGLK7	0.00515	1.11	0.40
SGLK8	0.00412	1.12	0.05
SGLK CSA1	0.00352	1.09	3.90

that mixing several types of pesticides will make pest control efforts more effective and can save time (efficiency) in using pesticides.

The results of the isolation of endophytic actinomycete isolates in shallot plants cultivated with intensive application of synthetic chemical pesticides showed that only a few isolates were found, as were the isolation results in leek samples taken from Cianjur. Based on survey results, these two areas are horticultural agricultural areas with a high pesticide use intensity. The negative impact of excessive use of pesticides can kill important microbes in plants and cause their population to decline. According to Yadav *et al.* (2022), systemic chemical fungicides are a potential threat to non-target endophytes that affect their diversity and function, and plants become susceptible to disease as their populations decline. Excessive use of systemic insecticides hurts the development of endophytic bacterial communities on host plants, and increasing doses can reduce the diversity and abundance of endophytic bacteria (Salam and Kataoka 2023).

Endophytic microbes are microorganisms that can colonize plant vascular tissue. They are known to be initially harmless to the host plant and can be obligate

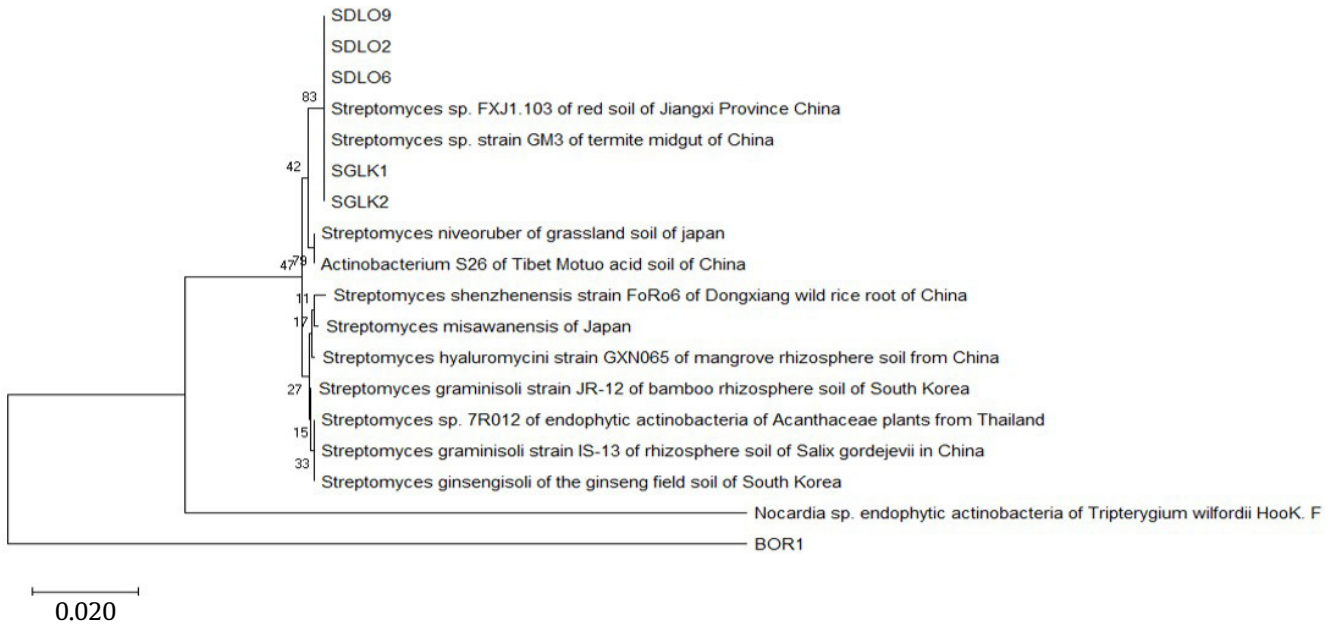

Figure 3. Phylogenetic tree of Liliaceae endophyitic actinomycete isolate based on the Neighbor-Joining method used MEGA 7 with 1,000 bootstrap replications

or facultative in plants (Nair and Padmavathy 2014). Some endophyte species can be pathogenic or beneficial. Most endophytes do not show harmful effects on some plant species, but they may be pathogenic when tested on other plants (Fadiji and Babalola 2020). Endophytic actinomycetes can be pathogenic, commensal and non-pathogenic, so it is necessary to carry out biosafety tests on biocontrol agent candidates so that the candidates obtained are genuinely safe for both pathogens and mammals. This is evident from the isolation results. Most endophytic actinomycetes have the potential to be pathogens in plants and humans.

Endophytic actinomycetes of Liliaceae plants can potentially be biocontrol agents that control the pathogenic fungus *F. oxysporum* f.sp. *cepae*. These can be seen from the results of *in vitro* tests showing that endophytic actinomycetes can inhibit the growth of this fungus by up to 63%. The results of this study certainly add to the evidence that endophytic actinomycetes have great potential as biocontrol agents to suppress the development basal plate rot disease in shallots, as also reported by Wijayanti *et al.* 2021. The results of research by Alblooshi *et al.* (2022) show that the endophytic actinomycetes of palm plant roots are very effective in inhibiting the growth of the fungus *F. solani,* which causes the disease sudden decline syndrome on date palm in the UAE, which can reduce the severity of the disease from 4.75 to 2.25.

The inhibitory mechanism of endophyte actinomycetes is antibiosis and the production of chitinase enzymes. The results of microscopic observations in the dual culture test showed that the pathogenic fungus hyphae experienced lysis, shape malformations such as swelling and rosettes and hyphae growth that tended to move away from the endophyte actinomycete isolates (Figure 4). Endophytic actinomycetes are also thought to produce antifungal bioactive compounds, although the types have yet to be analyzed. Several strains of the genus *Streptomyces* can produce antibiotics such as vancomycin, erythromycin, tetracycline, streptomycin, and chloramphenicol (Hasani *et al.* 2014). Bioactive compounds antifungal against *F. oxysporum* and *F. proliferatum* produced by *Streptomyces* sp. are fluconazole, ketoconazole, and miconazole (Passari *et al.* 2017). This compound can damage and disrupt the function of cell walls, as well as disrupt nucleic acid synthesis in pathogenic fungi (Purnomo *et al.* 2017). The research results of Shan *et al.* (2018) showed that crude extracts of endophytic actinomycetes from tea plants have the potential to produce bioactive compounds that are antifungal against nine pathogenic fungi including several species

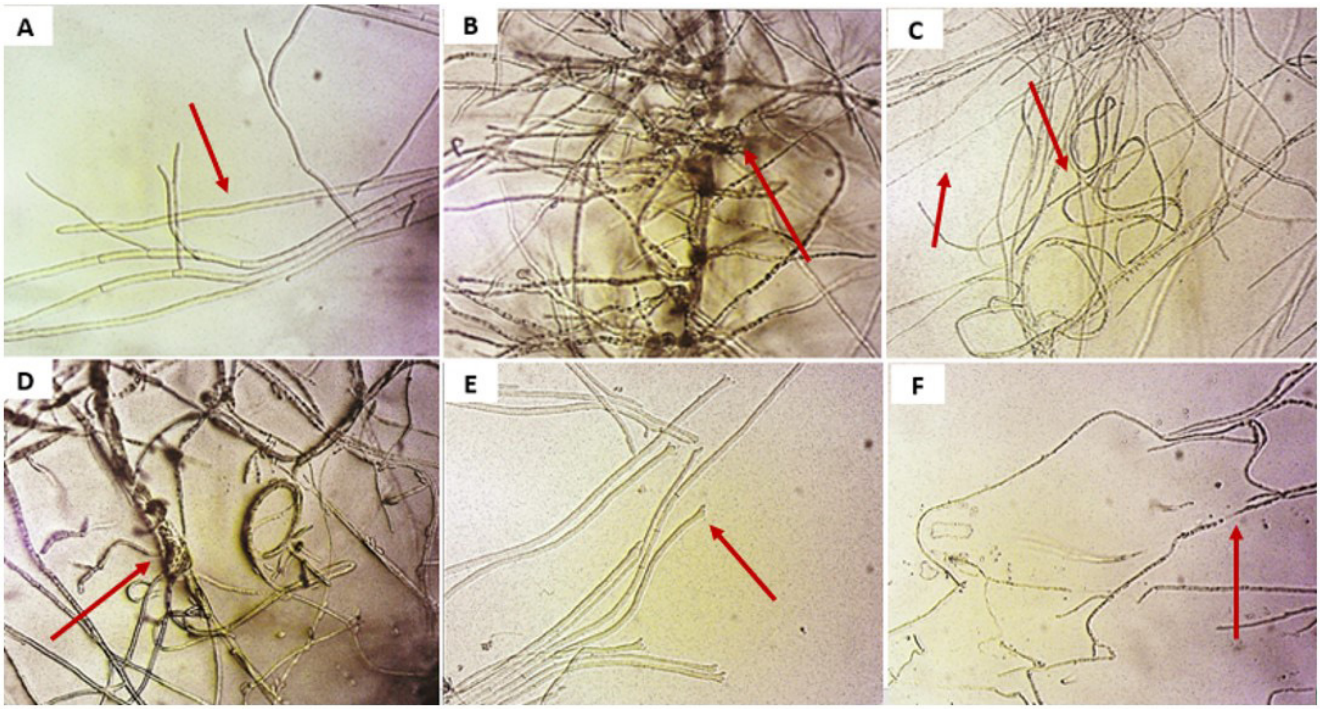

Figure 4. Appearance of the fungus hyphae *F. oxysporum* f.sp. *cepae* due to inhibition of endophytic actinomycetes of Liliaceae plants; (A) healthy hyphae, (B) rosette hyphae, shape malformations, (C) lysis and tangles, (D) swelling of the hyphae, (E) blunt tip of the hyphae like a cut, (F) lysis

of Fusarium fungi (*F. graminearum, F. oxysporum* f.sp. *lycopersici*, *F. oxysporum* f.sp. *cubense* and *F. verticillioides*).

Endophytic actinomycetes of Liliaceae plants also have the potential to be agents inducing resistance in shallot plants. The test results showed that some plants inoculated with endophytic actinomycete isolates produced peroxidase enzymes in higher quantities than control plants, and all isolates produced chitinase enzymes. These two enzymes are indicators of the resistance of host plants inoculated with biocontrol agents. As reported by Vijayabharathi *et al.* (2018), the endophyte actinomycete of chickpea plants can induce resistance of chickpea plants to Botrytis cinerea by producing antibiosis molecules such as phenylalanine and chitinase enzymes. Wheat endophyte actinomycete *Streptomyces* sp. can induce resistance in Arabidopsis plants to the pathogenic fungus *F. oxysporum* mainly through the NPR1 pathway but also requires salicylic acid (Conn *et al.* 2008). Actinobacteria, such as *Streptomyces* spp., influence soil fertility by involving many components and function as nutrient enhancers. They produce various enzymes, including chitinase, cellulase, peroxidase and pectinase, which convert complex nutrients into simple mineral forms (Vurukonda *et al.* 2018). The low concentration of the peroxidase enzyme in plants inoculated with endophytic actinomycetes was probably because the plants were not inoculated with pathogenic fungi, so the induced resistance was also low. This is reinforced by the results of research by Conn *et al.* (2008) that wild-type Arabidopsis thaliana plants inoculated with endophytic actinobacterial strains were able to strengthen the defence pathway by inducing low levels of SAR and JA/ET gene expression; however, after being infected with a pathogen, the defence genes were strongly regulated.

Endophytic actinomycetes of Liliaceae plants can also act as growth-promoting agents by producing high amounts of the hormone IAA (Table 2). Some plants inoculated with endophyte actinomycete isolates showed better growth than control plants. Actinomycetes, as reported, can produce growth-promoting hormones, such as indole acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC), dissolve phosphate, fix nitrogen and have the potential to stimulate plant growth (Shan *et al.* 2018; Wijayanti *et al.* 2021). The ability of endophytic actinomycetes of Liliaceae plants to produce IAA is relatively lower compared to other endophytic actinomycetes. Tomato plant endophyte actinomycetes can produce IAA at concentrations of 7.4-46.3 µg/ml (Passari *et al.* 2016). The endophyte actinomycete of the *Jatropha* sp. plant can produce IAA 17.65-66.84 µg/ml (Ali *et al.* 2021). Likewise, rice endophyte actinomycetes can produce an IAA of 3.18-53.43 µg/ml (Mingma and Duangmal 2018). IAA production is a common characteristic of antagonistic *Streptomyces* species (Sreevidya *et al.* 2016).

The results of molecular identification of the six best isolates showed that five of them were actinomycetes from the species *Streptomyces* sp. This species has been widely reported and has potential as a biocontrol agent. The research results of Chaiharn *et al.* (2019) showed that *Streptomyces* sp. produces bioactive compounds consisting of the enzymes catalase, chitinase and cellulase so that it can suppress the growth of white root fungus (JAP) on rubber seedlings and is no different from the application of chemicals.

The results of this test show that endophytic actinomycetes can inhibit the growth of the fungus *F. oxysporum* f.sp *cepae* with an antibiosis mechanism and produce the enzyme chitinase which can cause hyphae lysis and malformation. In addition, endophytic actinomycetes can induce resistance of shallot plants to *F. oxysporum* f.sp *cepae* directly in the presence of the indicator enzymes peroxidase and chitinase. Indirectly, they can stimulate the growth of shallot plants by producing the hormone IAA, thereby increasing plant growth. According to Shutsrirung *et al.* (2013), the concentration of IAA produced by *Streptomyces* spp. ranged from 1.05-60.95 µg/ml. Actinomycetes have been reported to have the ability to produce growth-promoting hormones, such as indole acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC), dissolve phosphate, fix nitrogen, and have the potential to promote plant growth (Shan *et al.* 2018). IAA production is the only mechanism that increases the growth of rice seedlings inoculated with endophytic actinomycetes (Mingma and Duangmal 2018). The positive impact of IAA depends on the amount of IAA produced, and excess IAA production is considered detrimental to plants (Etesami *et al.* 2014). That shows that the endophyte actinomycete Liliaceae has great potential for controlling the fungus that causes basal plate rot disease on shallot.

Math Formulae

$$\text{Percentage of inhibition (P)} = \frac{R1 - R2}{R1} \times 100\%$$

Where:

P : percentage of inhibition
R1 : colony radius at control
R2 : colony radius at endophytic actinomycete treatment

$$\text{Relative inhibition (RI)} = \frac{D1 - D2}{D1} \times 100\%$$

Where:

D1 : diameter of pathogenic fungus colony at negative control (cm)
D2 : diameter of pathogenic fungus colony at treatment (cm)

$$\text{Chitinolytic index} = \frac{\text{Diameter of clear zone}}{\text{Diameter of actinomycete colony}}$$

Acknowledgements

Part of this research was funded by the National Collaborative Research Scheme (Ri-Na) for the 2023-2024 fiscal year within the scope of IPB based on Rector's Decree no 461/IT3.D10/PT.01.03/P/B/2023. The author would also like to thank the Ministry of Agriculture of Indonesia for the support of the study funding and also for Agricultural Bogor University.

References

Alblooshi, A.A., Purayil, G.P., Saeed, E.E., Ramadan, G.A., Tariq, S., Altaee, A.S., El-Tarabily, K.A., AbuQamar, S.F., 2022. Biocontrol potential of endophytic actinobacteria against *Fusarium solani*, the causal agent of sudden decline syndrome on date palm in the UAE. *Journal of Fungi*. 8, 1-24. https://doi.org/10.3390/jof8010008

Ali, A., Kurnia, N., Ulfa, A.A.N., Damayanti, P., Rante, H., Jumadi, O., 2021. Diversity of endophytic actinomycetes producing indole-3acetic acid and *in vitro* evaluation of plant growth-promoting activity on *Brassica oleracea* L. *Journal Tropical Agricultural Science.* 44, 275-292. https://doi.org/10.47836/pjtas.44.2.02

Ansari, W.A., Krishna, R., Zeyad, M.T., Singh, S., Yadav, A., 2020. Endophytic actinomycetes mediated modulation of defense and systemic resistance confers host plant fitness under biotic stress conditions, in: Singh, R., Manchanda, G., Maurya, I., Wei Y. (Eds.), *Microbial Versatility in Varied Environments*. Springer, Singapore. pp. 167-180. https://doi.org/10.1007/978-981-15-3028-9_10

Bektast, I., Kusek, M., 2019. Phylogenetic and morphological characterization of *Fusarium oxysporum* f.sp. *cepae* the causal agent of basal rot on onion isolated from Turkey. *Fresenius Environ Bull.* 28, 1733-1742.

[BPS] Badan Pusat Statistik, 2023. *Vegetable Crop Production. Central Statistics Agency.* Central Bureau of Statistics, Indonesia.

Chaiharn, M., Nikhom, S., Wasu, P., Saisamorn, L., 2019. Biological control of *Rigidoporus microporus* the cause of white root disease in rubber using PGPRs *in vivo. Chiang Mai Journal of Sciences.* 46, 850-866.

Chantasena, P., Nantapong, N., 2016. Biodiversity of antimicrobial-producing actinomycetes strains isolated from dry dipterocarp forest soil in Northeast Thailand. *Brazillian Archives of Biology and Technology*. 59, 1-11. https://doi.org/10.1590/1678-4324-2016150674

Choi, Y.W., Hodgkiss, I.J., Hyde, K.D., 2005. Enzyme production by endophytes of *Brucea javanica. Journal of Agricultural Technology.* 1, 55-66.

Conn, V.M., Walker, A.R., Franco, C.M.M., 2008. Endophytic actinobacteria induce defense pathways in *Arabidopsis thaliana. Molecular Plant-Microbe Interactions*. 21, 208-218. https://doi.org/10.1094/MPMI-21-2-0208

Degani, O., Kalman, B., 2021. Assessment of commercial fungicides against onion (*Allium cepa*) basal rot disease aused by *Fusarium oxysporum* f.sp. *cepae* and *Fusarium acutatum. Journal of Fungi.* 7, 1-17. https://doi.org/10.3390/jof7030235

El-Tarabilya, K.A., Sivasithamparam, K., 2006. Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Soil Biology and Biochemistry.* 38, 1505-1520. https://doi.org/10.1016/j.soilbio.2005.12.017

Etesami, H., Hosseini, H.M., Alikhani, H.A., 2014. In planta selection of plant growth promoting endophytic bacteria for rice (*Oryza sativa* L.). *Journal Soil Science and Plant Nutrition*. 14, 491-503. DOI:10.4067/S0718-951620 14005000039

Fadiji, A.E., Babalola, O.O., 2020. Exploring the potentialities of beneficial endophytes for improved plant growth. *Saudi Journal of Biological Sciences.* 27, 3622-3633.https://doi.org/10.1016/j.sjbs.2020.08.002

Fatmawati, U., Meryandini, A., Nawangsih, A.A., Wahyudi, A.T., 2019. Screening and characterization of actinomycetes isolated from soybean rhizosphere for promoting plant growth. *Biodiversitas.* 20, 2970-2977.https://doi.org/10.13057/biodiv/d201027

Gordon, S.A., Weber, R.P., 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol. 26, 192-195. DOI: 10.1104/pp.26.1.192

Haapalainen, M., Latvalab, S., Kuivainena, E., Qiua, Y., Segerstedtband, M., Hannukkala, A.O., 2016. *Fusarium oxysporum, F. proliferatum* and *F. redolens* associated with basal rot of onion in Finland. *Plant Pathology*. 65, 1310-1320. https://doi.org/10.1111/ppa.12521

Hasani, A., Kariminik, A., Issazadeh, 2014. Streptomycetes: characteristics and their antimicrobial activities. *Inlternational Journal of Advanced Biological and Biomedical Research*. 2, 63-75.

Hsu, S.C., Lockwood, J.L., 1975. Powdered chitin agar as a selective medium for enumeration of *Actinomycetes* in water and soil. *Applied Microbiology, American Society for Microbiology.* 29, 422-426. https://doi.org/10.1128/am.29.3.422-426.1975

Hussein , A.A.E,. Rihab, E.M.A., Suhair, A.A., Marmar, A.E.S., 2014. Isolation and identification of *Streptomyces rochei* strain active against phytopathogenic fungi. *British Microbiology Research Journal.* 4, 1057-1068.https://doi.org/10.9734/BMRJ/2014/11074

Joko , T., Anggoro, S., Sunoko, H.R., Rachmawati, S., 2017. Pesticides usage in the soil quality degradation potential in Wanasari Subdistrict, Brebes, Indonesia. *Applied and Environmental Soil Science.* 2017, 1-7. https://doi.org/10.1155/2017/5896191

Kalman, B., Abraham, D., Graph, S., Perl-Treves, R., Harel, Y.M., Degani, O., 2020. Isolation and identification of *Fusarium* spp., the causal agents of onion (*Allium cepa*) basal rot in Northeastern Israel. *Biology.* 9, 1-19.https://doi.org/10.3390/biology9040069

Le, D., Audenaert, K., Haesaert, G., 2021a. Fusarium basal rot: profile of an increasingly important disease in *Allium* spp. *Tropical Plant Pathology.* 46, 241-253. https://doi.org/10.1007/s40858-021-00421-9

Le, D., Ameye, M., Boevre, M.D., Saeger, S.D., Audenaert, K., Haesaert, G., 2021b. Population, virulence, and mycotoxin profile of *Fusarium* spp. associated with basal rot of *Allium* spp. in Vietnam. *Plant Disease*. 105, 1942-1950. https://doi.org/10.1094/PDIS-08-20-1850-RE

Mingma, R., Duangmal, K., 2018. Characterization, antifungal activity, and plant growth promoting potential of endophytic actinomycetes isolated from rice (*Oryza sativa* L.). *Chiang Mai Journal of Sciences.* 45, 2652-2665.

Nababan, M., Gunam, I.B.W., Wijaya, I.M.M., 2019. Produksi enzim selulase kasar dari bakteri selulolitik. *Jurnal Rekayasa dan Manajemen Agronomi*. 7, 190-199. https://doi.org/10.24843/JRMA.2019.v07.i02.p03

Nair, D.N., Padmavathy, S., 2014. Impact of endophytic microorganisms on plants, environment and humans. *The Scientific World Journal.* 22, 1-11. https://doi.org/10.1155/2014/250693

Passari, A.K., Chandra, P., Zothanpuia Mishra, V.K., Leo, V.V., Gupta, V.K., 2016. Detection of biosynthetic gene and phytohormone production by endophytic actinobacteria associated with *Solanum lycopersicum* and their plant-growth-promoting effect. *Res Microbiol*. 167, 692–705. DOI: 10.1016/j.resmic.2016.07.001

Passari, A.K., Vineet, K.M., Garima, S., Pratibha, S., Brijesh, K., Vijai, K.G., Rupak, K.S., Ratul, S., Anthonia, O.D., Bhim, P.S., 2017. Insights into the functionality of endophytic actinobacteria with a focus on their biosynthetic potential and secondary metabolites production. *Journal of Scientific Reports.* 7, 11809. https://doi.org/10.1038/s41598-017-12235-4

Purnomo, E., Mukarlina, Rahmawati, 2017. Uji antagonis bakteri *Streptomyces* spp. terhadap jamur Phytophthora palmivora bbk01 penyebab busuk buah pada tanaman kakao. *Protobiont.* 6, 1-7.

Quyen, T.V., Chau, H.T.T., Nguyen, D.K., 2017. Disease-reducing effects of antagonistic soil bacteria on fusarium basal rot of shallot caused by *Fusarium oxysporum* in Vinh Chau, Soc Trang. *Can Tho University Journal of Sciences*. 6, 31-37. https://doi.org/10.22144/ctu.jen.2017.024

Saaty, T.L., 2008. Decision making with the analytic hierarchy process. *International Journal of Services Sciences.* 1, 83-98. https://doi.org/10.1504/IJSSCI.2008.017590

Salam, M.T.B., Kataoka, R., 2023. Changes in the endophytic bacterial community of *Brassica rapa* after application of systemic insecticides. *International Journal of Molecular Sciences*. 24, 1-15. https://doi.org/10.3390/ijms242015306

Sardi, P., Saracchi, M., Quaroni, S., Petrolini, B., Borgonovi, G.E., Merli, S., 1992. Isolation of endophytic streptomyces strains from surface-sterilized roots. *Applied And Environmental Microbiology.* 58, 2691-2693. https://doi.org/10.1128/aem.58.8.2691-2693.1992

Shan, W., Zhou, Y., Liu, H., Yu, X., 2018. Endophytic actinomycetes from tea plants (*Camellia sinensis*): isolation, abundance, antimicrobial, and plant-growth-promoting activities. *BioMed Research International*. 2018, 1-12. https://doi.org/10.1155/2018/1470305

Shamyuktha, J., Sheela, J., Rajinimala, N., Jeberlinprabina, B., Ravindran, C., 2020. Survey on onion basal rot disease incidence and evaluation of aggregatum onion (*Allium cepa* L. var. *aggregatum* Don.) genotypes against *Fusarium oxysporum* f.sp. *cepae. International Journal of Current Microbiology and Applied Sciences*. 9, 529-536. https://doi.org/10.20546/ijcmas.2020.907.058

Shin, J.H., Lee, H.K., Backa, C.G., Kang, S.H., Han, J.W., Lee, S.C., Han, Y.K., 2023. Identification of fusarium basal rot pathogens of onion and evaluation of fungicides against the pathogens. *Mycobiology.* 51, 264-272. https://doi.org/10.1080/12298093.2023.2243759

Singh, R.P., Manchanda, G., Li, Z.F., Rai, A.R., 2017. Insight of proteomics and genomics in environmental bioremediation, in: Bhakta, J.N. (Eds.), *Handbook of Research on Inventive Bioremediation Techniques*. IGI Global, Hershey, pp. 46. https://doi.org/10.4018/978-1-5225-2325-3.ch003

Subhashini, D.V., Singh, R.P., 2014. Isolation of endophytic actinomycetes from roots and leaves of tobacco (*Nicotiana tabacum* L.). *Annals of Plant Protection Sciences*. 22, 458-459.

Shutsrirung, A., Chromkaew, Y., Pathom-Aree, W., Choonluchanon, S., Boonkerd, N., 2013. Diversity of endophytic actinomycetes in mandarin grown in northern Thailand, their phytohormone production potential and plant growth promoting activity. *Soil Science and Plant Nutrition.* 59, 322-330. https://doi.org/10.1080/00380768.2013.776935

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

Susanti, A.D., 2018. Evaluasi aplikasi pestisida pada pertanaman bawang merah di kabupaten brebes [Skripsi]. Bogor, Indonesia: IPB University.

Tirado-Ramirez, M.A., Lopez-Urquidez, G.A., Amarillas-Bueno, L.A., Retes-Manjarrez, J.E., Vega-Gutiérrez, T.A., Avendaño, J.E.L., Lopez-Orona, C.A., 2021. Identification and virulence of *Fusarium falciforme* and *Fusarium brachygibbosum* as causal agents of basal rot on onion in Mexico. *Canadian Journal of Plant Pathology.* 43, 722-733. https://doi.org/10.1080/07060661.2021.1894241

Vijayabharathi, R., Gopalakrishnan, S., Sathya, A., Srinivas, V., Sharma, M., 2018. Deciphering the tri-dimensional effect of endophytic *Streptomyces* sp. on chickpea for plant growth promotion, helper effect with *Mesorhizobium ciceri* and host-plant resistance induction against *Botrytis cinerea. Microbial Pathogenesis*. 122, 98-107. https://doi.org/10.1016/j.micpath.2018.06.019

Vurukonda, S.S., Giovanardi, D., Stefani, E., 2018. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. *International Journal of Molecular Sciences*. 19, 952. https://doi.org/10.3390/ijms19040952

Wahyudi, A.T., Priyanto, J.A., Afrista, R., Kurniati, D., Astuti, R.I., Akhdiya, A., 2019. Plant growth promoting activity of actinomycetes isolated from soybean rhizosphere. *OnLine Journal of Biological Sciences*. 19, 1-8. https://doi.org/10.3844/ojbsci.2019.1.8

Wijayanti, E., Nawangsih, A.A., Tondok, E.T., 2021. Penapisan aktinomiset rizosfer tanaman Liliaceae sebagai agens pengendali hayati *Fusarium oxysporum* f. sp. *cepae*. *Jurnal Fitopatologi Indonesia*. 17, 225-232. https://doi.org/10.14692/jfi.17.6.225-232

Yadav, U., Bano, N., Bag, S., Srivastava, S., Singh, P.C., 2022. An insight into the endophytic bacterial community of tomato after spray application of propiconazole and *Bacillus subtilis* strain NBRI-W9. *Microbiology.* 10, 1-16. https://doi.org/10.1128/spectrum.01186-22

Youssef, K., Sanzani, S.M., Ligorio, A., Ippolito, A., Terry, L.A., 2014. Sodium carbonate and bicarbonate treatments induce resistance to postharvest green mould on citrus fruit. *Postharvest Biology and Technology.* 87, 61-69. https://doi.org/10.1016/j.postharvbio.2013.08.006

Zhang, Q., Yong, D., Zhang, Y., Shi, X., Li, B., Li, G., Liang, W., Wang, C., 2016. *Streptomyces rochei* A-1 induces resistance and defense-related responses against *Botryosphaeria dothidea* in apple fruit during storage. *Postharvest Biology and Technology*. 115, 30-37. https://doi.org/10.1016/j.postharvbio.2015.12.013