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EXPLORATION OF *ACTINOBACTERIA* INDIGENUS AS BIOLOGICAL CONTROL AGENT OF BACTERIAL LEAF BLIGHT (*XANTHOMONAS AXONOPODIS PV. ALLII*) AND INCREASING PRODUCTION OF SHALLOT

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A B S T R A C T

Bacterial leaf blight, caused by *Xanthomonas axonopodis pv. allii*, poses a substantial risk to shallot crops. *Actinobacteria* are promising candidates for biological control because of their ability to generate bioactive compounds. This research aimed to identify the most effective *Actinobacteria* isolates for managing bacterial leaf blight and boosting the growth and yield of shallot plants. The study was conducted in three phases: 1.) Isolation and characterization of *Actinobacteria* and *X. axonopodis* pv. *allii*, 2.) Selection of *Actinobacteria* to control *X. axonopodis* pv. *allii* and boost shallot plant growth with 21 treatments and three replications, and 3.) Molecular identification and testing of selected *Actinobacteria* for controlling *X. axonopodis* pv. *allii*. This research was carried out in the microbiology laboratory and experimental farm of the Faculty of Agriculture at Andalas University in West Sumatra, Indonesia. The study identified several indigenous *Actinobacteria* isolates (AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP6Y, and AS1aP7Y) with high phosphate solubilizing indices, strong antagonistic activity, and significant abilities in nitrogen fixation and phytohormone (IAA) production. These isolates were identified as *Streptomyces* spp. and *Micromonospora shersina* R-Ac134 through 16sRNA sequencing. They demonstrated potential for effectively controlling bacterial leaf blight and enhancing the growth and yield of shallot plants. In conclusion, the indigenous *Actinobacteria* isolates from the regions of Solok, Tanah Datar, and Agam in West Sumatra show promise as biological control agents against bacterial leaf blight in shallot plants, while also promoting plant growth and productivity.

Keywords: *Actinobacteria*, shallots, exploration, *Xanthomonas axonopodis* pv. *allii*.

INTRODUCTION

Leaf blight disease in shallots, caused by *Xanthomonas axonopodis* pv. *allii*, represents a major threat to shallot crops (Robène-Soustrade *et al.,* 2010). Its severity has been observed in various regions of Indonesia, reaching alarming levels of 50-100% in areas such as Cirebon, Tegal, Ngajuk, Bantul, and Sigi Regencies in Central Sulawesi (Robène *et al.,* 2015). Recent studies by Yanti *et al.* (2023) reported a severity rate of 70% in West Sumatra province. The primary target of this pathogen is the leaves of shallot plants, where initial symptoms manifest as pale white spots with water-soaked areas. As the disease progresses, these spots spread throughout the leaves, leading to dieback and causing a reduction in bulb size and weight (Yanti, 2015).

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Various control measures have been implemented, including crop rotation with non-host plants, technical cultivation practices, ensuring the use of healthy seeds, and chemical interventions utilizing biological agents (Schwartz and Gent, 2006 ; Rabnawaz *et al.,* 2023). However, the long-term reliance on chemical pesticides poses environmental risks, including the development of pathogen resistance, toxic effects on crop yield, and potential harm to human health (Kumar *et al.,* 2014). In response to these challenges, the utilization of biological control agents, particularly microbes, has emerged as a promising alternative. Microbes offer high efficacy in disease management while being environmentally friendly and safe for humans, as they do not target non-pathogenic organisms (Bonaterra *et al.,* 2022).

In light of these challenges, there is a pressing need for alternative control methods that utilize microorganisms as biological agents to mitigate the development of bacterial diseases, which are both effective and environmentally safe. Several studies

have demonstrated the efficacy of biological control microbes in reducing the reliance on agricultural chemical compounds (Selim *et al.,* 2021). The soil serves as a rich source of biological control microbes for plant pathogens, among which *Actinobacteria* have shown promising potential (Boukhatem *et al.,* 2022). *Actinobacteria* have the capacity to combat a range of diseases caused by fungi, bacteria, and viruses, and they are safe for both plants and the environment. However, despite their potential, there is still limited knowledge about using *Actinobacteria* to control bacterial leaf blight in shallot plants.

The use of biological agents like *Actinobacteria* offers a practical solution to reduce the risks linked to the extensive use of bactericides by farmers and communities. Emphasizing the non-destructive and environmentally friendly nature of biological agents, their incorporation into agricultural practices aligns with sustainable principles (Doolotkeldieva *et al.,* 2018). *Actinobacteria*, as one of the dominant bacterial groups in soil, play a crucial role in protecting plants from pathogen attacks due to their ability to overgrow. Additionally, they often act as producers of plant growth hormones, further enhancing plant development. Gram-positive and filamentous in nature, *Actinobacteria* are known for their production of bioactive compounds with antimicrobial properties (Goudjal *et al.,* 2016).

These bacteria are known to produce enzymes such as chitinase and β-1.3-glucanase, which aid in breaking down microbial cell walls. Additionally, *Actinobacteria* possess various beneficial properties, including phosphate solubilization, siderophore production, Indole Acetic Acid (IAA) synthesis, ammonia production, and the secretion of lytic enzymes (Myo *et al.,* 2019). These capabilities enable *Actinobacteria* to inhibit the growth of plant pathogens. Referred to as Plant Growth Promoting Rhizobacteria (PGPR) or Plant Growth Promoting *Actinobacteria* (PGPA), they are recognized for their role in boosting plant growth and productivity (Rani *et al.,* 2018; Yanti, *et al.,* 2023).

Research has shown that *Actinobacteria* can greatly enhance plant growth by increasing plant height, root length, dry weight, and levels of photosynthetic pigments. This effect is due to their production of phytohormones like auxins, gibberellins, and cytokinins, along with siderophores, ammonia, phosphate solubilization, and hydrogen cyanide production (Soumare *et al.,* 2021).The main goal of this study is to identify the most effective *Actinobacteria* isolates for controlling bacterial leaf blight while also promoting the growth and yield of shallot plants. By leveraging the diverse benefits of *Actinobacteria*, the study aims to develop sustainable methods for disease management and crop improvement in shallot cultivation.

MATERIALS AND METHODS

Isolation of *Actinobacteria* from the Shallot Rhizosphere: Sampling Method: Soil samples were collected using the purposive sampling method from three locations in each of the districts Agam, Solok, and Tanah Datar which are known shallot production centers located at elevations of 1000 to 1500 meters above sea level. Samples were taken from the roots of healthy shallot plants growing among those showing symptoms of bacterial leaf blight four weeks after planting. Approximately 100-150 grams of soil were placed in plastic bags and transported to the laboratory.

Sample Processing: The soil samples were homogenized, and one gram was transferred into a test tube containing 10 ml of sterile distilled water. After thoroughly mixing with a vortex mixer, the samples were diluted to a concentration of 10^{-7}.Dilutions of 10^{-6} and 10^{-7} were then inoculated onto ISP2 media (International Streptomyces Project 2) in test tubes, while 1 ml of liquid SCA (Starch Casein Agar) medium was poured into Petri dishes. *Actinobacteria* colonies exhibiting clear zones, rapid growth, and dominance were subcultured as pure isolates onto separate Petri dishes. The media utilized included ISP2 media (Aeny *et al.,* 2018) and SCA, specifically tailored for *Actinobacteria*.

Biosafety test: Hypersensitive Reaction Test: The Hypersensitive Reaction Test is utilized to differentiate between pathogenic and non-pathogenic *Actinobacteria*. Initially, all *Actinobacteria* isolates were suspended in sterile distilled water and then diluted until the spore density reached 10^6 spores/ml, as measured using a hemocytometer (Kawuri *et al.,* 2018). Following this, the *Actinobacteria* suspension was injected into the lower surface of the leaves of four o'clock flowers using a syringe needle. The infiltrated area was then covered and incubated for 48 hours. The presence of necrosis in the infiltrated leaf region indicates pathogenicity, whereas the absence of necrosis suggests non-pathogenicity, making the *Actinobacteria* suitable for further experimentation (Schaad *et al.,* 2001).

Pathogenicity Test: The Pathogenicity Test, based on the method by Yanti *et al.* (2022), aimed to determine

whether the *Actinobacteria* isolates obtained were pathogenic to shallot plants, specifically the Medan cultivar. In this test, the leaf tissue of the shallot plants was wounded, and then a suspension of *Actinobacteria* at a density of 10^{-6} spores/ml was applied using a cotton swab. The presence of necrotic symptoms on the shallot leaves indicates pathogenicity, making the *Actinobacteria* unsuitable for further evaluation.

Hemolysis Test: The Hemolysis Test was conducted to assess the hemolytic activity of *Actinobacteria* isolates, which indicates their potential pathogenicity to humans and mammals. *Actinobacteria* were cultivated on blood agar medium and subsequently placed in an incubator at 37°C for 24 hours. Isolates that produce hemolytic toxins will form a clear zone around the colony, indicating potential harm to human and mammalian health. Consequently, bacterial groups exhibiting hemolytic activity were excluded from further testing.

Selection of *Actinobacteria* to Control X. axonopodis pv. allii and Increasing Red Onion Plant Production in Planta: *Actinobacteria* isolates were selected for controlling *X. axonopodis* pv. *allii* on shallot plants using a Completely Randomized Design comprising 21 treatments with three replications. These treatments included 18 *Actinobacteria* isolates, a Positive control (without *X. axonopodis* pv. *allii* inoculation and *Actinobacteria* introduction for comparison with plant growth parameters), a negative control (*X. axonopodis* pv. *allii* inoculation treatment for biocontrol test comparison), and a bactericidal treatment containing streptomycin as the active ingredient.

Propagation of *Actinobacteria*: *Actinobacteria* multiplication was conducted in liquid culture. Initially, pure isolates of *Actinobacteria* from microtubes were revitalized using the scratch method on ISP2 and SCA medium, followed by an incubation period of 96 hours. Subsequently, one colony of *Actinobacteria* was transferred into 25 ml of ISP2 Broth and SC Broth liquid medium in a scotch bottle and then placed on a rotary shaker at a speed of 70 rpm for 336 hours (Kawuri *et al.,* 2018). The population density was determined by diluting the *Actinobacteria* suspension until the spore density reached 10^{-6} spores/ml, which was quantified using a hemocytometer. A population with a density of 10^{-6} spores/ml was utilized for further inoculation.

Planting Shallot Seeds: Medan cultivar shallot bulbs (susceptible to HDB) were used. Before planting, the top 1/3 of the bulb was cut off, and then the cut shallot bulbs were surface sterilized by soaking them in distilled water - 70% alcohol - distilled water for 15 minutes, followed by air-drying. Following this, the shallot bulbs were immersed in the *Actinobacteria* suspension for a duration of 15 minutes. Subsequently, the treated bulbs were planted in polybags with a capacity of 10 kg.

Rejuvenation and Propagation of *X. axonopodis* pv. *Allii:* Rejuvenation and propagation of *X. axonopodis* pv. *allii* were conducted utilizing isolates obtained from Dr. Yumira Yanti, SSi. MP at the Microbiology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Andalas. The rejuvenation and multiplication processes involved the etching technique on Yeast Peptone Glucose Agar (YPGA) media, followed by an incubation period of 48 hours. Subsequently, the culture was suspended in sterile distilled water at a density of 10^{-7} cells/ml, and the suspension density was assessed against McFarland's solution on a scale of 7.

Inoculation of *Xanthomonas axonopodis* pv. *allii*: *X. axonopodis* pv. *allii* was introduced to shallot plants that were 14 days old after planting. Five leaves per shallot polybag were chosen for inoculation by gently puncturing the tip of each shallot leaf with a sterile needle. Subsequently, a suspension of *X. axonopodis* pv. *allii*, with a population density of 10^{-7} cfu/ml, was applied to the wounded area using cotton wool. The plants were then monitored daily until symptoms became evident.

Selection of *Actinobacteria* to Control *Xanthomonas axonopodis* pv. *allii* and Increasing Red Onion Crop Production: Disease Progression: Incubation Mass (Days after Inoculation): The incubation mass was monitored daily following inoculation until the initial signs of bacterial leaf blight appeared on the plants.

Disease Occurrence: Shallots observed were conducted to determine the incidence of bacterial leaf blight one week after inoculation. The disease incidence formula was used. The formula for measuring disease incidence was:

$$I = \frac{n}{N} \times 100$$

Information,
I: Disease incidence
n: Number of infected plant leaves
N: Total number of plant leaves observed.

Disease Severity: The severity of bacterial leaf blight disease on shallot plants was observed one week after inoculation using the disease severity formula.

A	B	C	D	E	F
Scale 0 (No attack)	Scale 1 (Spots on fruit 1-10%)	Scale 2 (Spots on fruit 10-20%)	Scale 3 (Spots on fruit 20-30%)	Scale 4 (Spots on fruit 30-50%)	Scale 5 (Spots on fruit >50%)
Immune	Stand	Somewhat resistant	Somewhat Vulnerable	Prone to	Very Vulnerable

Figure 1. Scale of damage to shallot leaves from scale 0 – 5, (a) healthy plants, (b) scale 1,(c) scale 2, (d) scale 3, (e) scale 4, (f) scale 5 (Roumagnac *et al.* 2004).

The formula for measuring the severity of the disease was:

$$S = \frac{\sum(ni \times vi)}{N \times V} \times 100\%$$

Information

S: Disease severity

N: Number of leaves from each attack category

v: Scale value for each attack category

N: Total number of leaves observed

V: Highest scale value in the attack category

Plant Growth (Generative Phase): Plant Height (cm): Plant height was observed by measuring the plant from the base of the leaf to the main growing point. Measurement using a ruler. Observations were made once a week, starting when the tubers were planted in polybags.

Number of Leaves: Leaf counting commenced when the plants reached seven days old. Observations were conducted weekly thereafter.

Harvest: Harvest yield was determined by weighing the weight of the tubers that have been harvested. The harvest criteria were that the plant is 70-80 days old, the leaves have started to wilt, the base of the stem has hardened, and some of the tubers have appeared above the ground. The harvested shallot bulbs were weighed using an analytical balance.

Antagonism test of selected *Actinobacteria* isolates against *Xanthomonas axonopodis* pv. *allii:* The antagonism test of selected *Actinobacteria* isolates against *Xanthomonas axonopodis* pv. *allii* was conducted using the Kirby-Bauer method. Initially, a suspension of *X. axonopodis* pv. *allii* was cultured on the media. Sterile filter paper discs with a diameter of 5 mm were then soaked in an *Actinobacteria* suspension with concentrations ranging from $1x10^{-7}$ cells/ml to $1x10^{-8}$ cells/ml, or equivalent to 1 McFarland, for 10 minutes (Nuria, 2010; Tarabily *et al.,* 2003). Following this, the soaked filter paper discs were inoculated onto the media, with the center of the paper disc positioned 4 cm from the edge of the Petri dish. The Petri dishes were subsequently incubated at room temperature for 24 hours, and observations were made regarding the diameter of the inhibition zone formed around the paper disc (Kumar *et al.,* 2014).

Test the ability of *Actinobacteria* to produce HCN, Siderophores, Biosurfactants, Ammonia, and Hydrogen Cyanide Production Protease (HCN): Cyanide (HCN) production was assessed using cyanide detection agar, which consisted of TSA (Hymedia®) supplemented with 0.44% glycine. Detection was achieved by placing a 1 cm^2 filter paper soaked in CDS solution (comprising 2 g picric acid and 8 g Na2CO3 dissolved in 200 mL distilled water) on the agar. A change in color from yellow to brownish orange on the filter paper indicated HCN production.

Siderophore Production: Siderophore production was assessed by applying a dotted line in the center of Chrome Azurol S agar (60.5 mg). Chrome Azurol S was dissolved in 50 mL of distilled water and combined with 10 mL of FeCl3.6H2O solution (1 mM FeCl3.6H2O + 10 mM HCl). Additionally, 72.9 mg of HDTMA was dissolved in 40 mL of distilled water, and this solution was added to 900 mL of King's B agar (Hymedia®, containing per liter: 20 g protease peptone, 1.5 g K2HPO4, 1.5 g MgSO4, 15 g agar, and adjusted to pH 7.2 with glycerol). The mixture was then incubated at 28°C for five days. A change in the medium's color from blue to orange indicated the presence of siderophore activity.

Biosurfactant test: Biosurfactant production involves the utilization of Nutrient Broth (NB) media (Hymedia®, 1 L, comprising 5 g peptone, 5 g NaCl, 1.5 g beef extract, and 1.5 g yeast extract, adjusted to pH 7.2). Selected *Actinobacteria* were cultured in culture bottles containing 20 mL NB and incubated for 48 hours without agitation. The presence of biofilm formation on the medium's surface indicates biosurfactant production.

Ammonia Test: Each chosen *Actinobacteria* isolate was inoculated into a medium containing 10 mL of 1% peptone water and incubated for 48-72 hours at 28 ± 2°C. Subsequently, 0.5 ml of Nessler's reagent (SigmaAldrich® - HgI4K2 0.09M) was introduced. This addition induced a color change to yellow. The transition to brown coloration indicated the presence of ammonia production.

Protease activity: Protease activity was assessed on Luria Bertani Broth media (composition per liter comprising 10 g casein enzyme hydrolyzate, 5 g yeast extract, 10 g NaCl, pH 7.5 (HiMedia®)), supplemented with 2% skim milk powder (Nestle) and 15 g agar. A transparent zone forms around the bacterial colony, indicating protease activity.

Test the ability of selected *Actinobacteria* to produce chitinase enzymes, dissolve phosphate, produce IAA, and fix nitrogen

The selected *Actinobacteria* were subsequently evaluated for their chitinase enzyme production using the method described by Hsu and Locwood (1975), phosphate solubilization capability using the Pikovskaya method and media, indole acetic acid (IAA) production using the Gordon and Weber method (1951), and nitrogen fixation ability using the Dobereiner method described by Day (1976).

Table 1. Diversity in Morphology of *Actinobacteria* Isolates and Biosafety Testing

No	Isolate	Mycelium Bottom View	Mycelium Top View	Form Elevation	Form Spores	Hyphae Shape	HR Test	Pathogenicity Test	Hemolysis Test
1	AS1aP6Y	Cream	Green	Convex	Chain	Not partitioned	-	-	-
2	AS1aP7Y	Yellow	White	Convex	-	Not partitioned	-	-	-
3	AS1bP6Y	Cream	Chocolate	Convex	Chain	Not partitioned	-	-	-
4	AS1cA7Y	Cream	White	Convex	-	Not partitioned	-	-	-
5	AS2aA6Y	White	White	Convex	Chain	Not partitioned	-	-	-
6	AS2bA6Y	Chocolate	Cream	Flat	Chain	Not partitioned	-	-	-
7	AS2cP7Y	Yellow	Chocolate	Convex	Chain	Not partitioned	-	-	-
8	AS3aA7Y	Yellow	Gray	Convex	Chain	Not partitioned	-	-	-
9	AS3bP7Y	Green	Cream	Convex	Chain	Not partitioned	-	-	-
10	AA1bP7Y	Cream	White	Convex	Chain	Not partitioned	-	-	-
11	AA1cP7Y	Yellow	Gray	Convex	Chain	Not partitioned	-	-	-
12	AA2aP7Y	Yellow	Green	Convex	-	Not partitioned	-	-	-
13	AA3aA7Y	Yellow	Yellow	Convex	-	Not partitioned	-	-	-
14	AA3bP7Y	Cream	Chocolate	Convex	Chain	Not partitioned	-	-	-
15	AT1bP7Y	Cream	White	Convex	-	Not partitioned	-	-	-
16	AT2aP7Y	Cream	Green	Convex	Chain	Not partitioned	-	-	-
17	AT2cP6Y	Gray	Gray	Flat	-	Not partitioned	-	-	-
18	AT3cP6Y	Cream	Cream	Convex	Chain	Not partitioned	-	-	-
19	AT1aA6Y	Red	Yellow	Flat	Chain	Not partitioned	+	+	+
20	AT3aP7Y	Green	Gray	Flat	-	Not partitioned	+	+	+
21	AS3cA7Y	Green	Chocolate	Flat	-	Not partitioned	+	+	+
22	AS1bP7Y	Cream	Gray	Convex	Chain	Not partitioned	+	+	+
23	AA3cA7Y	Chocolate	Cream	Convex	Chain	Not partitioned	+	+	+
24	AA1bA6Y	Gray	Cream	Convex	-	Not partitioned	+	+	+

Molecular identification of selected *Actinobacteria*: The genomic DNA of the selected *Actinobacteria* was extracted using the Genomic DNA Mini Kit (Blood/Cultured Cell) extraction kit. The 16S rRNA gene sequence was amplified utilizing universal 16S rRNA gene primers for bacterial domains: 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 387r (5'-GGG CGG WGT GTA CAA GGC-3') (Marchesi *et al.,* 1998), targeting an amplicon length of approximately 1300 bp. The PCR reaction mixture, with a total volume of 50 µL, comprised GoTaq Green (25 µL), reverse and forward primers (4 µL each), DNA (8 µL), and nuclease-free water (9 µL). The PCR process began with an initial denaturation step of 4 minutes at 94°C, followed by 30 cycles consisting of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 55°C, and elongation for 1 minute at 72°C. A final elongation step was carried out for 7 minutes at 72°C. Genomic DNA and PCR products were subjected to electrophoresis on a 1% agarose gel at 80 V for 45 minutes. Subsequently, the gel was immersed in Ethidium Bromide (EtBr) for 15 minutes and visualized under a UV transilluminator. The PCR products were then sequenced using the First Base sequencing service. Sequencing results were aligned with GenBank data using the Basic Local Alignment Search Tool-Nucleotide (BlastN) program on the National Center for Biotechnology Information (NCBI) website. Phylogenetic analysis was conducted using the MEGA 6.0 program employing the Neighbor Joining (NJ) method with 1000X bootstrap.

DATA ANALYSIS

Data analysis was performed using analysis of variance (ANOVA), followed by the Duncan's Multiple Range Test (DNMRT) at a significance level of 5%, using Statistics 9 software, in cases where significant differences were observed.

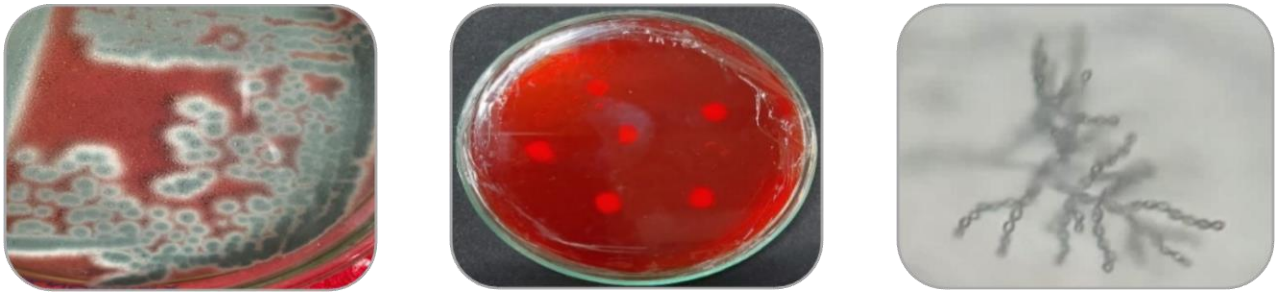
RESULTS AND DISCUSSION

A total of 24 *Actinobacteria* isolates were obtained from the roots of shallot plants. The underside view of the mycelium displayed a range of colors, including cream, white, green, brown, yellow, and gray. Conversely, the upper side of the mycelium exhibited colors such as white, cream, yellow, green, brown, and gray. Each of the *Actinobacteria* isolates exhibited distinct morphological and physiological characteristics (refer to Table 1).

Based on the results of biosafety tests, six isolates showed positive hypersensitive reactions to four o'clock flower plants, pathogenicity, and hemolysis tests. Furthermore, 18 other isolates showed adverse reactions in the pathogenicity and hemolysis tests (Table 1 and Figure 2). The hemolysis test showed no clear zone around the *Actinobacteria* isolates, indicating that the isolate was negative or not dangerous to human or mammal health. *Actinobacteria* isolates that show an adverse reaction in the biosafety test are used for the next stage, while isolates with a positive hypersensitive reaction and cause necrosis on four o'clock flower leaves cannot be used in the next stage.

In Table 2, it is indicated that out of the total 18 *Actinobacteria* isolates, none were capable of producing cyanic acid (HCN) or protease enzymes. Eleven isolates demonstrated siderophore production, while biosurfactants were produced by 18 isolates. Additionally, 10 isolates exhibited the ability to produce ammonia.

Disease Progression: Incubation Period: Shallot plants treated with *Actinobacteria* revealed that five isolates were able to delay the incubation period compared to the control. *Actinobacteria* isolates bearing the codes AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP7Y, and AS1aP6Y exhibited incubation periods ranging from 21.33 to 31.66 days, as detailed in Table 3.



A B C

Figure 2. Morphology of *Actinobacteria* (a) circular colony shape and gray-green (b) Microscopic, as indicated by the arrow, the chain spores are oval, and the hyphae are straight(c) The hemolysis test for the *Actinobacteria* isolate showed a negative result with no clear zone around it

Table 2. *Actinobacteria* isolates produce HCN, siderophores, protease enzymes, biosurfactants, and ammonia

A. No.Isolate	B. HCN	C. siderophore	D. Biosurfactant	E. Ammonia	F. Proteases
G. 1 AS1aP6Y	H. -	I. +	J. +	K. ++	L. -
M. 2 AS1aP7Y	N. -	O. +	P. +	Q. +++	R. -
S. 3 AS1bP6Y	T. -	U. +	V. +	W. -	X. -
Y. 4 AS1cA7Y	Z. -	AA. +	BB. +	CC. -	DD. -
EE. 5 AS2aA6Y	FF. -	GG. -	HH. +	II. +	JJ. -
KK. 6 AS2bA6Y	LL. -	MM. -	NN. +	OO. +	PP. -
QQ. 7 AS2cP7Y	RR. -	SS. +	TT. +	UU. -	VV. -
WW. 8 AS3aA7Y	XX. -	YY. +	ZZ. +	AAA. -	BBB. -
CCC. 9 AS3bP7Y	DDD. -	EEE. -	FFF. +	GGG. +	HHH. -
III. 10 AA1bP7Y AA1bP7Y	JJJ. -	KKK. +	LLL. +	MMM. +++	NNN. -
OOO. 11 AA1cP7Y	PPP. -	QQQ. +	RRR. +	SSS. -	TTT. -
UUU. 12 AA2aP7Y AA2aP7Y	VVV. -	WWW. +	XXX. +	YYY. -	ZZZ. -
AAAA. 13 AA3aA7Y	BBBB. -	CCCC. -	DDDD. +	EEEE. +	FFFF. -
GGGG. 14 AA3bP7Y	HHHH. -	IIII. -	JJJJ. +	KKKK. +	LLLL. -
MMMM. 15 AT1bP7Y AT1bP7Y	NNNN. -	OOOO. +	PPPP. +	QQQQ. -	RRRR. -
SSSS. 16 AT2aP7Y AT2aP7Y	TTTT. -	UUUU. +	VVVV. +	WWWW. -	XXXX. -
YYYY. 17 AT2cP6Y	ZZZZ. -	AAAAA. -	BBBBB. +	CCCCC. +	DDDDD. -
EEEEE. 18 AT3cP6Y	FFFFF. -	GGGGG. -	HHHHH. +	IIIII. +	JJJJJ. -

+ Positive Reaction

- Negative Reaction

Disease Occurrence: Shallots treated with *Actinobacteria* exhibited a lower percentage of disease incidence compared to the control group. *Actinobacteria* isolates labeled as AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP7Y, and AS1aP6Y displayed disease incidence percentages ranging from 7.27% to 23.72%, as outlined in Table 3.

Disease Severity: Shallots treated with *Actinobacteria* displayed reduced disease severity percentages compared to the control group. *Actinobacteria* isolates identified as AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP7Y, and AS1aP6Y exhibited disease severity percentages ranging from 6.74% to 21.64%. The negative control, representing the treatment with the highest severity of bacterial leaf blight disease and categorized as very susceptible, recorded a disease severity value of 84.25%, as detailed in Table 3.

Table 3. Selection of *Actinobacteria* indigenous isolates for controlling bacterial leaf blight

Treatment	Incubation period	Disease incidence (%)	Disease severity (%)
AT2aP7Y	31.66 a	7.27 k	6.74 k
AS3aA7Y	28.66 ab	12.97 ij	10.00 ij
AA2aP7Y	26.33 abc	14.04 ij	11.22 hij
AS1aP7Y	23.33 cd	17.07 ghi	11.82 hij
AS1aP6Y	21.33 de	16.26 hij	11.62 hij
AS1bP6Y	18.33 ef	23.16 efgh	15.92 ghi
AT2cP6Y	16.33 fg	21.25 efghi	15.29 ghi
AS2aA6Y	15.33 fg	22.27 efgh	16.00 fghi
AA1cP7Y	15.00 fg	23.72 defgh	15.00 fg
AA3aA7Y	13.00 gh	24.53 cdefg	17.12 efgh
AS2cP7Y	12.66 ghi	24.52 cdefg	17.70 efgh
AS3bP7Y	10.33 hij	26.31 cdefg	18.40 defg
AA3bP7Y	9.33 hij	26.28 cdefg	19.11 def
AS1cA7Y	9.00 ij	29.01 cde	20.84 de
AT1bP7Y	9.00 ij	28.78 cdef	20.21 de
AS2bA6Y	8.33 ij	29.92 cde	21.37 d
AA1bP7Y	7.66 j	30.33 cd	21.64 d
AT3cP6Y	7.66 j	41.19 b	37.35 b
Streptomycin	8.00 j	31.47 c	29.15 c
Control -	4.00 k	97.60 a	84.25 a
K.K	8.39	8.24	10.35

*Numbers with the same lowercase letters in the same column are not significantly different based on the Duncan's Multiple Range Test (DNMRT) at a significance level of 5%.

Plant Growth: Plant height: Shallot seeds treated with *Actinobacteria* demonstrated a significant increase in the height of shallot plants compared to the control group. *Actinobacteria* isolates AT2aP7Y, AS3aA7Y, and AA2aP7Y contributed to the enhancement of shallot plant height, which ranged from 53.14 to 65.90 cm, as indicated in Table 4.

Number of Leaves: Shallots treated with *Actinobacteria* demonstrated an increase in the number of shallot leaves compared to the control group. *Actinobacteria* isolates AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP7Y, and AS1aP6Y contributed to this increase, with the number of shallot leaves ranging from 75.00 to 90.33 pieces. A comparison of the number of leaves between shallot plants treated with *Actinobacteria* and the control is illustrated in Figure 3.

A B

Figure 3. Comparison of the growth of shallot plants after the introduction of the *Actinobacteria* isolates (49 DAP) (a) Positive control shallot plants (without the introduction of *Actinobacteria*) (b) Red onion plants introduced with the AT2aP7Y isolate

Weight of shallot bulbs: Shallots treated with *Actinobacteria* exhibited an increase in the weight of shallot bulbs compared to the control group. *Actinobacteria* isolates identified as AT2aP7Y, AS3aA7Y, AA2aP7Y, and AS1aP6Y contributed to this increase, with the weight of shallot bulbs ranging from 186.00 to 223.00 grams. A comparison of the bulb weight between shallot plants treated with *Actinobacteria* and the control is depicted in Figure 4.

A B

Figure 4. Comparison of the weight of shallot bulbs after the introduction of *Actinobacteria* isolates (70 days old) (a) Positive control shallot plants (without the introduction of *Actinobacteria*) (b) *Actinobacteria* isolate code AT2aP7Y.

Table 4. Plant height, number of leaves, and weight of shallot bulbs introduced with *Actinobacteria* isolates

Treatment	Plant height (cm)	Number of leaves (pieces)	Tuber weight (gr)
AT2aP7Y	65.90 a	82.66 ab	223.00 a
AS3aA7Y	61.48 ab	90.33 a	212.33 ab
AA2aP7Y	58.35 ab	79.00 abc	198.33 bc
AS1aP7Y	53.55 c	76.00 abcd	177.00 de
AS1aP6Y	53.14 c	75.00 abcd	186.00 cd
AS1bP6Y	45.34 cde	64.66 cdef	161.33 efg
AT2cP6Y	46.47 cd	70.00 bcde	167.67 def
AS2aA6Y	44.20 cde	68.66 bcde	157.00 fg
AA1cP7Y	40.90 cdef	62.33 defg	153.00 fgh
AA3aA7Y	40.24 cdef	59.00 efgh	143.00 ghi
AS2cP7Y	37.55 defg	57.33 fghi	137.33 hij
AS3bP7Y	37.08 defg	51.66 fghij	132.33 ijk
AA3bP7Y	36.29 efgh	51.66 fghij	124.00 jkl
AS1cA7Y	32.99 ghi	47.33 ghij	114.00 klm
AT1bP7Y	34.57 fghi	48.66 ghij	120.33 jkl
AS2bA6Y	32.48 ghi	43.66 hij	109.00 lmn
AA1bP7Y	31.37 ghi	42.00 ijk	100.00 mn
AT3cP6Y	28.01 ij	36.33 ijk	95.00 mn
Streptomycin	29.34 ghij	35.00 jkl	93.66 no
KONTROL -	24.55 k	31.33 lm	75.66 p
Coeficient of Variance (CV)	7.96	16.59	4.22

*Numbers with the same lowercase letters in the same column are not significantly different based on the Duncan's Multiple Range Test (DNMRT) at a significance level of 5%.

Antagonism test of selected *Actinobacteria* isolates against *Xanthomonas axonopodis pv. allii:* *Actinobacteria* isolates were subjected to an antagonist test against *Xanthomonas axonopodis pv. allii*. *Actinobacteria* capable of inhibiting the growth of X. axonopodis pv. *allii* and producing antibiotics were identified (refer to Table 5), with an average area of inhibition zone ranging from 2.90 to 19.98 mm.

Table 5. Diameter of the inhibition zone of indigenos *Actinobacteria* against *Xanthomonas axonopodis pv. allii*

Treatment	Time		Average Inhibition Zone Diameter (mm)
	24 hours	48 Hours	
AT2aP7Y	20.27±2.99	19.68±3.76	19,98
AS3aA7Y	6.95±0.62	7.58±0.83	7,27
AA2aP7Y	8.43±1.64	8.87±1.58	8,65
AS1aP6Y	7.23±0.71	7.85±0.96	7,42
AS1aP7Y	9.42±1.73	10.31±1.92	9,28
AS1bP6Y	4.16±1.03	5.45±0.65	4,81
AT2cP6Y	4.15±0.47	5.37±0.6	4,76
AS2aA6Y	4.18±0.11	5.72±0.29	4.95
AA1cP7Y	4.06±0.03	5.13±0.2	4,60
AA3aA7Y	3.5±0.92	4.8±0.1	4,15
AS2cP7Y	3.81±0.31	4.6±0.1	4,21
AS3bP7Y	3.62±0.17	4.47±0.15	4,05
AA3bP7Y	3.4±0.17	4.37±0.15	3,89
AS1cA7Y	3.27±0.15	4.1±0.1	3,69
AT1bP7Y	3.17±0.04	3.93±0.15	3,55
AS2bA6Y	3.09 ± 0.06	3.73 ± 0.2	3.41
AA1bP7Y	2.88 ± 0.21	3.37 ± 0.15	3.13
AT3cP6Y	2.62 ± 0.47	3.17 ± 0.25	2.90

*Numbers with the same lowercase letters in the same column are not significantly different based on the Duncan's Multiple Range Test (DNMRT) at a significance level of 5%.

A range of capabilities in *Actinobacteria* was observed, including the production of chitinase enzymes, phosphate solubilization, indole acetic acid (IAA) production, and nitrogen fixation. Among the 18 *Actinobacteria* isolates,

chitinase enzymes were produced with a chitinolytic index ranging from 0.02 to 1.44. The phosphate solubilization ability varied, with isolate AT3cP6Y showing the lowest value at 0.21 and AS1bp6Y displaying the highest at 3.79. *Actinobacteria* isolates exhibited IAA production with concentrations ranging from 2.20 to 82.80. Additionally, five *Actinobacteria* isolates demonstrated nitrogen fixation capability exclusively, as outlined in Table 6.

Table 6. The ability of *Actinobacteria* to produce chitinase enzymes, dissolve phosphate, produce IAA, and nitrogen fixation

No	Isolate	Produces chitinase enzyme with chitinolytic index	Phosphate Dissolving Index	IAA concentration (ppm)	Fixing Nitrogen
1	AT2aP7Y	1.44	3.18	80.56	+
2	AS3aA7Y	1.45	2.31	57.85	+
3	AA2aP7Y	1.15	2.41	53.74	+
4	AS1aP6Y	1.21	3.02	67.12	+
5	AS1aP7Y	1.70	3.79	82.80	+
6	AS1bP6Y	0.78	1.91	22.12	-
7	AT2cP6Y	0.66	1.42	25.95	-
8	AS2aA6Y	0.51	1.81	31.78	-
9	AA1cP7Y	0.42	1.01	27.57	-
10	AA3aA7Y	0.40	1.10	19.32	-
11	AS2cP7Y	0.59	1.46	10.59	-
12	AS3bP7Y	0.31	1.54	12.44	-
13	AA3bP7Y	0.23	1.12	13.67	-
14	AS1cA7Y	0.18	1.02	6.09	-
15	AT1bP7Y	0.10	0.83	5.93	-
16	AS2bA6Y	0.05	0.57	4.13	-
17	AA1bP7Y	0.06	0.44	2.67	-
18	AT3cP6Y	0.02	0.21	2.20	-

+ React positively
- React negatively

Five top-performing *Actinobacteria* isolates were identified based on their ability to suppress the growth of *Xanthomonas axonopodis pv. allii* both in planta and in vitro, as well as their capacity to produce various beneficial compounds including hydrogen cyanide (HCN), siderophores, protease enzymes, biosurfactants, ammonia, indole acetic acid (IAA), nitrogen fixation, chitinase enzymes, and phosphate solubilization. These isolates, namely AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP6Y, and AS1aP7Y, were selected as the most promising candidates, as shown in Table 7.

Table 7. 16sRNA sequence homology of 5 selected isolates with GenBank accessions

Isolate Code	GenBank Isolate	Query Cover (%)	Homology (%)	Number of accessions
AT2aP7Y	*Streptomyces luteogriseus* 3-7	100	99	KJ571036.1
AS3aA7Y	*Micromonospora shersina* R-Ac134	100	99	FN64951.1
AA2aP7Y	*S. olivaceus* TB2210	100	99	MH411235. 1
AS1aP6Y	*S. pseudogsceulus* 158	100	99	MN199546. 1
AS1aP7Y	*S. griseoflavus* CB16	100	99	MK929476.1

DISCUSSION

Twenty-four indigenous *Actinobacteria* isolates were obtained from shallot roots collected from three districts in West Sumatra. Biosafety testing revealed that six isolates exhibited hypersensitive reactions when exposed to four o'clock flower plants, while the remaining 18 isolates displayed adverse reactions during pathogenicity and hemolysis tests. The distribution of isolates varied among the districts, with the lowest number obtained from Tanah Datar District (5 isolates), followed by Agam District (7 isolates), and the highest from Solok District (12 isolates). This discrepancy in isolation numbers is attributed to the extensive use of pesticides in Tanah Datar Regency, as communicated directly with farmers, which has led to a decline in the *Actinobacteria* population in the soil. This finding aligns with previous research by Newman *et al.* (2016) indicating that soil exposure to pesticides can diminish

the populations of microorganisms such as *Actinobacteria*, mycorrhiza, and soil fungi.. According to Murugan *et al.* (2013) also reported that total pesticide residues in soil can reduce the *Actinobacteria* population by 32% compared to soil without residues. The morphological characterization of *Actinobacteria* isolates involved examining the color of the mycelium both above and below, microscopic shape, Gram stain result, and conducting biosafety tests including hypersensitive reaction, hemolysis test, and pathogenicity test. Observation of the underside of the mycelium revealed a spectrum of colors including cream, white, green, brown, yellow, and gray, while the mycelium above displayed colors such as white, cream, yellow, green, brown, and gray. Each of the *Actinobacteria* isolates exhibited distinct morphological and physiological characteristics. The wide-ranging morphological and physiological diversity observed in *Actinobacteria* can be attributed to variations in environmental conditions. This finding aligns with the findings of Sapkota *et al.* (2020) who reported that diverse environmental factors impact the growth and diversity of *Actinobacteria*. Such environmental variations lead to discrepancies in physiological characteristics, morphology, sporulation patterns, biochemical profiles, and the synthesis of antimicrobial metabolites among organisms. The distinct colony colors exhibited by *Actinobacteria* are attributed to variations in pigment content within each cell comprising the *Actinobacteria*. The introduction of *Actinobacteria* isolates into shallot plants resulted in enhanced growth and yield compared to the positive control. Parameters such as plant height, number of leaves, and weight of shallot bulbs exhibited superior performance in plants treated with *Actinobacteria* isolates, particularly AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP6Y, and AS1aP7Y. These five *Actinobacteria* isolates are identified as having significant potential in enhancing the growth and bulb weight of shallot plants compared to other isolates. This potential is attributed to their ability to fix nitrogen and produce phytohormones, specifically indole acetic acid (IAA), with concentrations ranging from 53.74 to 82.80. This observation aligns with the findings of Chaiyasen *et al.* (2017) indicating that *Actinobacteria* can synthesize plant growth hormones such as IAA, with varying concentrations influenced by the environmental conditions in which the *Actinobacteria* reside.

According to observation Kamal *et al.* (2014), *Actinobacteria* possess the capability to produce phytohormones such as indole acetic acid (IAA), which serves as a pivotal hormone in plant physiology. IAA production facilitates plant growth by stimulating cell division and elongation. In addition to promoting the growth and weight of shallot bulbs, indigenous *Actinobacteria* introduced into shallot plants demonstrated the ability to mitigate the onslaught of *Xanthomonas axonopodis pv. allii*, the causal agent of bacterial leaf blight in shallot plants. All *Actinobacteria* isolates exhibited the capacity to delay the incubation period and alleviate the incidence and severity of leaf blight in shallots. All *Actinobacteria* isolates exhibited superior potential in suppressing the development of leaf blight compared to both the negative control and streptomycin. Among them, five *Actinobacteria* isolates demonstrated the highest phosphate solubilizing index, namely AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP6Y, and AS1aP7Y, with respective values of 3.18, 2.31, 2.41, 3.02, and 3.79. *Actinobacteria* employ an indirect mechanism to control plant pathogens, primarily through the induction of resistance activity. This mechanism involves the activation of Induced Systemic Resistance (ISR) and Plant Growth-Promoting (PGP) pathways in host plants, particularly through the jasmonate or ethylene pathways, triggering a defensive response. Salicylic acid, jasmonic acid, and ethylene serve as key compounds regulating signals involved in inducing plant resistance, mainly by enhancing the levels of pathogenesis-related proteins (Sharma and Salwan, 2018 ; Haq *et al.,* 2021).

Eighteen *Actinobacteria* isolates exhibited the potential to suppress the development of *Xanthomonas axonopodis pv. allii*, attributed to the variation in both the type and quantity of antibacterial compounds effective against the pathogen. The antagonist test results revealed that five isolates, namely AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP6Y, and AS1aP7Y, displayed the largest average diameter of the inhibition zone, with values ranging from 7.27 to 19.98 cm. As noted Raval *et al.* (2021) diverse environmental factors can influence the inhibitory potency of *Actinobacteria*, including the nature, abundance, and efficacy of antibacterial compounds produced. This aligns with the findings of Yanti *et al.* (2022) indicating that *Actinobacteria* are proficient in generating antibacterial agents such as hydrogen cyanide (HCN) and can function as Plant Growth Promoting *Actinobacteria* (PGPA) by inducing Systemic Resistance (ISR), thereby eliciting defense signals in plants. The

utilization of Plant Growth-Promoting Rhizobacteria (PGPR) offers substantial benefits to plants, as it not only stimulates phytohormone synthesis but also induces plant defense mechanisms against pathogens through the production of various compounds such as IAA, HCN, antibacterials, salicylic acid, and actinomycin. IAA serves as the primary auxin for plant growth, also contributing to enhanced photosynthesis and transpiration rates. Additionally, HCN, although toxic, exhibits the capability to suppress the growth of plant pathogens. The findings underscored the potential of *Actinobacteria* isolates in controlling the pathogen *Xanthomonas axonopodis pv. allii* and enhancing the growth and yield of shallot plants, particularly isolates AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP6Y, and AS1aP7Y.

Actinobacteria possess the capability to suppress pathogenic microorganisms that hinder plant growth, which aligns with the assertion made by Omran and Kadhem. (2016) regarding the diverse array of secondary metabolites, commonly known as antibiotics, produced by *Actinobacteria*. These antibiotics encompass antitumor agents like doxorubicin and bleomycin, antifungals such as amphotericin B and nystatin, and immunosuppressive compounds like FK-506 and rapamycin (Lo Grasso *et al.,* 2016). *Actinobacteria* also function as plant growth promoters (PGPR), as highlighted Nozari *et al.* (2021), indicating their potential as Plant Growth-Promoting Rhizobacteria (PGPR). Some of the roles attributed to *Actinobacteria* in PGPR activity include the production of the hormone Indole Acetic Acid (IAA) or auxin, synthesis of siderophore compounds and plant growth hormones (Plant Growth Regulators), stimulation of mycorrhizal fungi mycelium growth, facilitation of organic and inorganic phosphate dissolution, and beneficial bacterial interactions.The findings of this study demonstrate the capacity of *Actinobacteria* to manage bacterial leaf blight in onion plants in planta and enhance the growth and yield of shallot plants, particularly isolates designated by the codes AT2aP7Y, AS3aA7Y, AA2aP7Y, AS1aP6Y, and AS1aP7Y. Following the 16sRNA sequence homology assessment, the most promising *Actinobacteria* isolates were identified as *Streptomyces* spp. and *Micromonospora shersina* R-Ac134.

CONCULSION

This study highlights the promising role of *Actinobacteria* as biological agents for controlling bacterial leaf blight in shallot plants and enhancing crop production. *Actinobacteria* demonstrate significant potential in inhibiting *Xanthomonas axonopodis pv. allii*, the pathogen responsible for bacterial leaf blight. The findings underscore the environmentally friendly approach offered by *Actinobacteria*, reducing reliance on chemical pesticides with potential environmental hazards. Future research should focus on elucidating the mechanisms of action of *Actinobacteria*, identifying the most effective strains for disease control in large-scale agriculture.

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Yulmira Yanti	:	Conceptualization and designing of the research work
Hasmiandy Hamid	:	wrote the manuscript
Nurbailis	:	Supervised the project, helped in data collection and analysis of data
Yaherwandi	:	Execution of field/lab experiments and data collection
Yenny Liswarni	:	Execution of field/lab experiments and data collection
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Sisxri Selviana	:	helped in data collection in laboratorium