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Diversity of phyllospheric Actinomycetes in Liliaceae plants and their potential as growth inhibitors of *Alternaria porri*

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Abstract. *Wati C, Nawangsih AA, Wahyudi AT, Wiyono S, Munif A. 2023. Diversity of phyllospheric Actinomycetes in Liliaceae plants and their potential as growth inhibitors of* Alternaria porri. *Biodiversitas 24: 5234-5242.* Actinomycetes are microorganisms belonging to a group of Gram-positive bacteria, that have the potential to act as biological control agents against various plant pathogens. Phyllospheric Actinomycetes particularly, can inhibit growth of various microorganisms, such as the fungus *Alternaria porri* Ell. Cif., which causes purple blotch disease in the shallot plants. These microbes thrive on the surface of leaves, particularly in plants belonging to Liliaceae family. Environmental conditions have been proven to greatly affect their diversity. Present investigation aims to determine diversity of phyllospheric Actinomycetes from Liliaceae plants and their potential to inhibit growth of *A. porri.* The methods used were to investigate, characterize, and quantify isolates from Liliaceae plants. An antagonistic test was conducted on the isolates to examine the proportion of *A. porri* fungus growth inhibition. The results showed that plants species *Allium fistulosum* and *A. tuberosum* were the most common hosts for phyllospheric actinomycetes. Morphological differences were found in shape, color, elevation, edges, surface, and hyphae size, as well as the type of spore violation. The surface texture of colonies was smooth, rough, powdery, and opaque, usually appearing after 7-12 days of isolation. The isolates also inhibited growth of *A. porri,* causing the mycelium thins, and a clear zone of inhibition between pathogens and Actinomycetes forms. Isolates BCW9 caused the highest suppression of fungus growth 57.78%. This study is the first experiment to evaluate the diversity of phyllosphere Actinomycetes from Liliaceae plants, which are able to control purple blotch disease caused by *A. porri* in Indonesia. The phyllospheric Actinomycetes were isolated from the same habitat of *A. porri*. These investigation show the prospect of phyllospheric Actinomycetes as biocontrol agents against another fungal diseases on leaves of another plants.

Keywords: *Allium cepa*, antagonism, inhibition level, leave diseases, purple blotch

INTRODUCTION

Actinomycetes are Gram-positive bacteria with high guanine and cytokinin content, belonging to the phylum Actinobacteria, which have a widespread distribution. The phylum Actinobacteria has five subclasses, six orders, and 14 suborders, with enormous diversity in terms of morphology, physiology, and metabolism (Anandan et al. 2016; Barka et al. 2016). One of the common habitats of Actinomycetes is the phyllosphere, which refers to the surface of plants [caulosphere (stems), phylloplane (leaves), anthosphere (flowers), and carposphere (fruits)]. Environmental conditions greatly affect the diversity of actinomycetes, such as biological, chemical, and physical factors.

Actinomycetes are unicellular microorganisms that share general characteristics with bacteria and fungus groups but also have distinct features such as the absence of cell walls. The mycelium (air and substrate) are not insulated and have a slimmer size compared to those of fungus. Air mycelium has a flat-convex surface and is powdery, while substrate mycelium adheres firmly to the surface of the media (Anandan et al. 2016; Li et al. 2016; Sukmawaty et al. 2020). Furthermore, Actinomycetes colonies are generally round in shape with prominent and convex elevations, irregularly flat edges, with smooth, rough, or wrinkled surfaces (Li et al. 2016). As the colony grows, characteristic clumps of aerial, granular, and powdery hyphae are formed (Sulistyani and Akbar 2014; Sukmawaty et al. 2020). The powdery surface is a hyphae collection with many asexual spores for reproduction. Mature Actinomycetes colonies have a powdery appearance, while the young ones consist only of hyphae, resembling bacteria with a round, convex, and smooth surface firmly attached to the agar medium.

The Actinomycetes acquired in this study are biocontrol agents isolated from the phyllosphere of different plants in the Liliaceae family. The actinomycetes have the ability to inhibit the growth of *Alternaria porri* Ell. Cif. Phyllosphere is the portion of plants above the Earth, specifically the leaves, which serves as a habitat of numerous microorganisms (Sivakumar et al. 2020). The pathogen *A. porri* is a fungus that affects the leaves of shallot plants, which are members of the Liliaceae family. No research has been conducted regarding the diversity of phyllospheric Actinomycetes of Liliaceae and their potential to suppress

purple blotch disease on shallot. Nanda et al. (2016) reported that this disease affects plant leaves and interferes with photosynthesis, especially when tubers are forming, resulting in yield losses that can range from 2.5% to 97%. The results of this study are interesting because they show that the phyllospheric Actinomycetes used as biocontrol agents isolated from the same habitat with the target pathogen.

Fardiyanti et al. (2021), reported 11 species of Actinomycetes from the rhizosphere of Liliaceae plants, but without evaluation the potential as biocontrol agents. Studied conducted by Wijayanti et al. (2021) found that 43 isolates of Actinomycetes were successfully isolated from the rhizosphere of Liliaceae plants. Among them, 14 isolates were able to inhibit the growth of *Fusarium oxysporum* f. sp. *cepae* by 3.67-53.67%. Yanti et al. (2023) reported that 12 rhizosphere actinomycete isolates from shallot were able to suppress *A. porri* with range of 4.87-63.77%. The phyllospheric Actinomycetes isolated from the leaves of another plants shown the potential as biocontrol agents of *Pyricularia oryzae*, *Xanthomonas oryzae* pv. *oryzae*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Vibrio* sp. (Ilsan et al. 2016; Harsonowati 2017; Ilsan et al. 2018).

Most biological control microorganisms that have been isolated from soil are unable to be applied directly on leaves. The failure of root colonizers like *Rhizobium* and *Azospirillum* to settle on the leaves is proof that the bacterial communities of the roots and leaves have different compositions (Lindow and Brandl 2003). This discovery has expanded the possibilities of using phyllospheric Actinomycetes as biocontrol agents against fungal diseases that attack leaves.

Actinomycetes serve various roles in the environment, such as producing phytohormones, acting as phosphate solubilizers, fixing nitrogen, producing indole-3-acetic acid (IAA), and generating siderophores (Kunova et al. 2016; Fatmawati et al. 2019; Wahyudi et al. 2019). Other functions include producing secondary metabolites, competing with other microorganisms, acting as a parasite, and inducing plant resistance. Actinomycetes generate antibacterial, antifungus, and hydrolytic enzymes such as chitinase, lipase, protease, and β-1.3 glucanase (Gopalakrishnan et al. 2013; Sreevidya et al. 2016; Fatimah et al. 2022). *Streptomyces griseus* and *S. albolongus* are phyllospheric Actinomycetes that produce polyketide and peptide bioactive compounds with antifungus activity (Harsonowati 2017). These bioactive compounds and hydrolytic enzymes can inhibit fungus growth by causing cell wall damage, cytoplasmic coagulation, inhibition of conidia germination, and elongation of the mycelium (Helal 2017). Therefore, this study aims to determine the diversity of phyllospheric Actinomycetes from Liliaceae plants and their potential as a biocontrol agent capable of inhibiting the growth of *A. porri*, which causes purple blotch disease in shallot plants.

MATERIALS AND METHODS

Isolation of Actinomycetes from Liliaceae plants

Actinomycetes were isolated from the leaves of healthy Liliaceae plants among diseased plants. The Liliaceae plant leaves weighing 10 g were heated for 15 min at 70°C. After being heated, leaves of Liliaceae plants are inserted in a bottle with 90 mL of sterile physiological salt, and homogenized for an hour. Isolation was carried out using the spread plate technique, with three different media namely humic acid vitamin agar (HVA), water yeast extract (WYE), and starch casein agar (SCA). HVA was composed of g/L: $CaCO_3$ 0.02 g, Na_2HPO_4 0.5 g, KCl 1.71 g, $MgSO_4$ 0.05 g, $FeSO_4$ 0.01 g, agar 20 g, humic Acid 40 mL (1 g of humic acid is added to 40 mL of NaOH 0.4%), Vitamin B 5 mL (Vit. B 0.25 g mixed into 200 mL of distilled sterile water) (Hayakawa and Nonomura 1987). WYE was composed of g/L: yeast extract 0.25 g, K_2HPO_4 0.5 g, and agar 18 g (Jiang et al. 2016), while SCA was composed of g/L: starch 10 g, casein 0.3 g, KNO_3 2 g, NaCl 2 g, K_2HPO_4 2 g, $MgSO_4.7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4.7H_2O$ 0.01 g, and agar 18 g (Kuster and Williams 1964). Nalidixic acid and cycloheximide were added at a concentration of 50 ppm to all isolation media. The growth of isolates was observed after 1-2 weeks of incubation.

Actinomycetes isolates with different colors and colony shapes were purified on ISP2 media (International *Streptomyces* Project 2). The following composition g/L: yeast extract 4 g, malt extract 10 g, glucose 4 g, and agar 20 g, incubated at 28°C. Samples of Actinomycetes were purified until a single colony was obtained (Ni et al. 2021).

Characterization of phyllospheric Actinomycetes of Liliaceae plants

The macroscopic morphological characteristics of single colonies of Actinomycetes were observed, which included colony color, colony shape, height, and colony surface. Additionally, using a light microscope with 1000x magnification, the microscopic features of Actinomycetes were also observed, including the mycelium form of actinomycete isolates. The diversity of Actinomycetes populations was obtained through the exploration of various Liliaceae plants, which were differentiated based on the morphospecies of the Actinomycetes. The environment around the sampling location was also observed. The Shannon-Wiener (H'), evenness (E), and dominance index (C) were used to calculate the diversity of actinomycete populations.

Hypersensitivity assay on tobacco

Hypersensitivity assay on tobacco leaves to determine whether it was a general plant pathogen. Actinomycetes isolates were grown on liquid ISP2 media with an incubation period of 7 days at 28°C on a shaker with a speed of 150 rpm. The liquid culture was injected into the underside of the tobacco leaf, which was the part between the two major veins, using a sterile syringe (Wiraswati et al. 2019). The positive control was *Xanthomonas oryzae* p.v. *oryzae*, while the negative was *Streptomyces rameus*. The observation was carried out by looking out for necrosis

in the tissue of the injected leaf. Symptoms of necrosis indicated that Actinomycetes isolates injected had the potential to be plant pathogens.

Pathogenicity assay on shallot

The Actinomycetes were evaluated for their pathogenic character ability through hypersensitivity assay on shallot leaves. Actinomycetes isolates were grown on a liquid ISP2 medium with a colony density of 10^6 CFU/mL, and inoculated on the leaves of shallot, utilizing the spray technique (Hersanti et al. 2019). The shallot plants that were two weeks old had their leaves punctured with sterile needles and then each plant was sprayed with 5 mL of Actinomycetes suspension. The plants were incubated for 14 days in the greenhouse. Observations were made by examining the presence or absence of necrosis. The symptoms of necrosis that appeared on the leaf tissue indicated that the actinomycete isolate tested was pathogenic to shallot.

Blood hemolysis assay

Seven days old of Actinomycetes were inoculated to the blood agar medium which contained 5% sheep blood and 2.5% NaCl, and then incubated for three days at 28°C. The formation of a clear zone around the colony indicated hemolytic activity (hemolysin production), demonstrating that Actinomycetes were able as pathogenic to humans and animals (Bernal et al. 2015).

Actinomycetes antagonism assay against *Alternaria porri*

The Actinomycetes antagonists assay in vitro against *A. porri* was conducted using the dual culture method according to Bonaldi et al. (2014) with slight modifications. The Actinomycetes isolated seven days old were scratched on the edge of potato dextrose agar (PDA) media, 2.12 cm from the center of the Petri dish. After three days, *A. porri* isolates seven days old, were inoculated into the center of the media, then incubated for four days at 28°C. Each treatment was repeated three times, then the percentage inhibition of the fungus radial growth was calculated using the formula according to Dikin et al. (2006) with slight modifications as follows:

$$Inhibition\ persentage\ (\%) = \frac{(B - A)}{B} x100\%$$

Where:

A = diameter mycelium of the fungal (treatment)

B = diameter mycelium of the fungal (control)

Data analysis

Research was conducted using randomised complete design with three replication. The parameter observed was the percentage inhibition of fungal mycelium growth. Data were analyzed using analysis of variance (ANOVA). If the data is significantly different, it is continued with an honestly significant difference (HSD) at the 5% level.

RESULTS AND DISCUSSION

Diversity of phyllospheric Actinomycetes of Liliaceae plants

The ten efficient Actinomycetes were selected based on the 10 highest results in the inhibition test using the dual culture method, from the total of 26 isolates tested. Several isolates were found to have morphological differences in color, shape, height, edges, colony surface, size, and type of spore chain (Figure 1). Different morphological characteristics include wrinkled, L-shaped, complex, and round colonies with stringy edges. There are also colony borders such as curved, whole, filiform, and wavy. The elevation forms include umbonation, flat, medium growth, like a water drop, and hilly. The surface is irregular, wavy, smooth, hairy, branched, and pitted, while the color ranges between orange, white, and blackish gray. Apart from that, the size is between 1.65 mm and 6.09 mm with a scale of 2 mm (Table 1).

Table 2. Micromorphological characteristics of phyllospheric Actinomycetes of Liliaceae

Code	Diameter of Hyphae (µm)	Spore chains form
BBW12	1.01	Rx
BBW14	1.36	Rx
BCW9	1.15	Rx
CES25	0.71	Rt
CFS28	0.90	Rx
AHS199	1.60	Rx
AHS190	2.30	Rx
AHW173	1.01	Sp
AHW161	2.05	Rx
AHS176	1.28	Rx

Note: spiral spore chain shape (Sp), retinaculiaperti (Rt), and rectiflexibles (Rx), using 200x magnification

Table 1. Macromorphological characteristics of phyllospheric Actinomycetes of Liliaceae

Code	Colony color	Form	Colony edge	Elevation	Colony surface	Size (mm)
BBW12	Orange	Wrinkled	Curled	Umbonate	Irregular	3.51
BBW14	Orange	Wrinkled	Curled	Umbonate	Wavy	3.32
BCW9	White	Round	Entire	Flat	Delicate	4.28
CES25	Blackish grey	L shape	Filiform	In growing into medium	Wool	2.11
CFS28	Orange	Wrinkled	Curled	Umbonate	Irregular	2.38
AHS199	White	Complex	Undulate	Umbonate	Wavy	6.09
AHS190	White	Round with stringy edges	Filiform	Drop-like	Branch	1.65
AHW173	White	Wrinkled	Filiform	Umbonate	Wavy	5.39
AHW161	White	Complex	Curled	Hilly	Lobat	3.38
AHS176	White	Complex	Filiform	Drop-like	Wavy	2.10

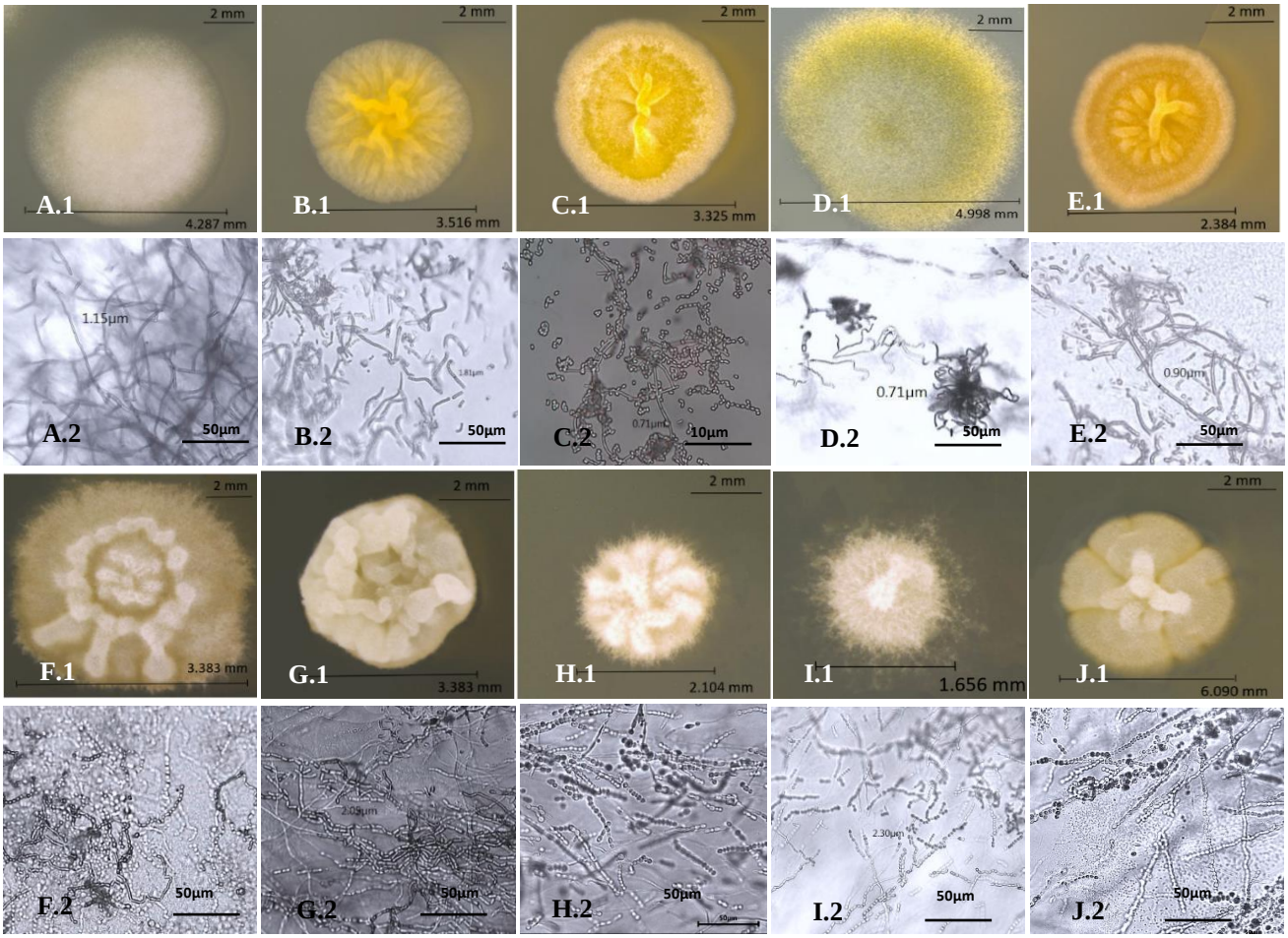

Figure 1. Macromorphological and micromorphological characteristics of phyllospheric Actinomycetes of Liliaceae on ISP2 media for seven days of incubation period based on diversity of shapes, colony colors, and types of spore chains: rectiflexibles (Rx), spirals (Sp), and retinaculiaperti (Rt). Isolates: A. BCW9, B. BBW12, C. BBW14, D. CES25, E. CFS28, F. AHW161, G. AHW173, H. AHS176, I. AHS190, J. AHS199

Actinomycetes had hyphae sizes ranging from 0.71 µm-2.30 µm, as well as three forms of spore chains such as spiral, retinaculiaperti, and rectiflexibles at 200x magnification (Table 2).

Actinomycetes obtained had a variety of spore chain types including spiral, retinaculiaperti, and rectiflexibles (Figure 1).

Actinomycetes were isolated from twelve plant species of the Liliaceae family, including *Allium cepa*, *Hymenocallis littoralis*, *H. occidentalis*, *Lilium longiflorum*, *A. tuberosum*, *A. fistulosum*, *Eleutherine bulbosa*, *Hippeastrum vittatum*, *H. puniceum*, *Agapanthus umbellatus*, *Zephyranthes candida*, and *Z. rosea*. The most common population was found in *A. fistulosum* and *A. tuberosum* reaching 1.8×10^8 CFU/gram, while shallots (*A. cepa*) showed a slightly lower abundance reaching 2.1×10^6 CFU/gram. Furthermore, the five plant species isolated were *L. longiflorum*, *H. puniceum*, *A. umbellatus*, *Z. candida*, and *Z. rosea* (Figure 2A). Actinomycetes were isolated using three different media and the highest abundance was found in WYE of 1.80×10^8 CFU/gram, followed by SCA and HVA with 1.8×10^8 CFU/gram, and 1.39×10^5 CFU/gram respectively (Figure 2B).

Three places make up the plant sampling area: specifically, the lowland region in the Brebes region of Central Java, which is the hub of shallot plantations; the medium-land region in the Bogor region of West Java; and the highland region in the Cianjur region of West Java. Each of the 11 locations was taken from the Brebes area; four locations were in the Bogor area; and two locations were taken from the Cianjur area. Actinomycetes were not found in several locations in the Wanasari District, Brebes Regency, including Tanjung Sari, Duku Waringin, Sawojajar, Pebatan, Klampok, Sigentong, Pesantunan Village. Samples were also not found in Larangan District, such as Siandong, and Forbidden Village. The highest population abundance was identified in leek plants originating from the Bogor Regency, namely Pasireurih Village. Tamansari District (-6°38'13" S. 106°45'43" E. 276°), of 1.8×10^8 CFU/gram, and chives from Cibereum Village, Cigenang District, Cianjur Regency (-6°47'27" S.107°4'13' E. 863.7m. 154°), of 1.8×10^8 CFU/ gram, followed by the fountain lily plants isolated from Gunung

Malang Village, Tenjolraya District Bogor Regency (-6°39'27" S. 106°42'39" E. 628.7m. 177°), of 3.3×10^6 CFU/gram (Figure 2C). The highest population based on the Shannon-Wiener diversity index analysis obtained H'=0.13, the Pielou evenness index E= 0.04, and, the Simpson dominance index C=0.96. This implied that the phyllospheric Actinomycetes diversity index in Liliaceae plants was categorized as low (H'<1), the distribution evenness index was uneven (0.00-0.25), and the dominance index was high (0.75<C≤1).

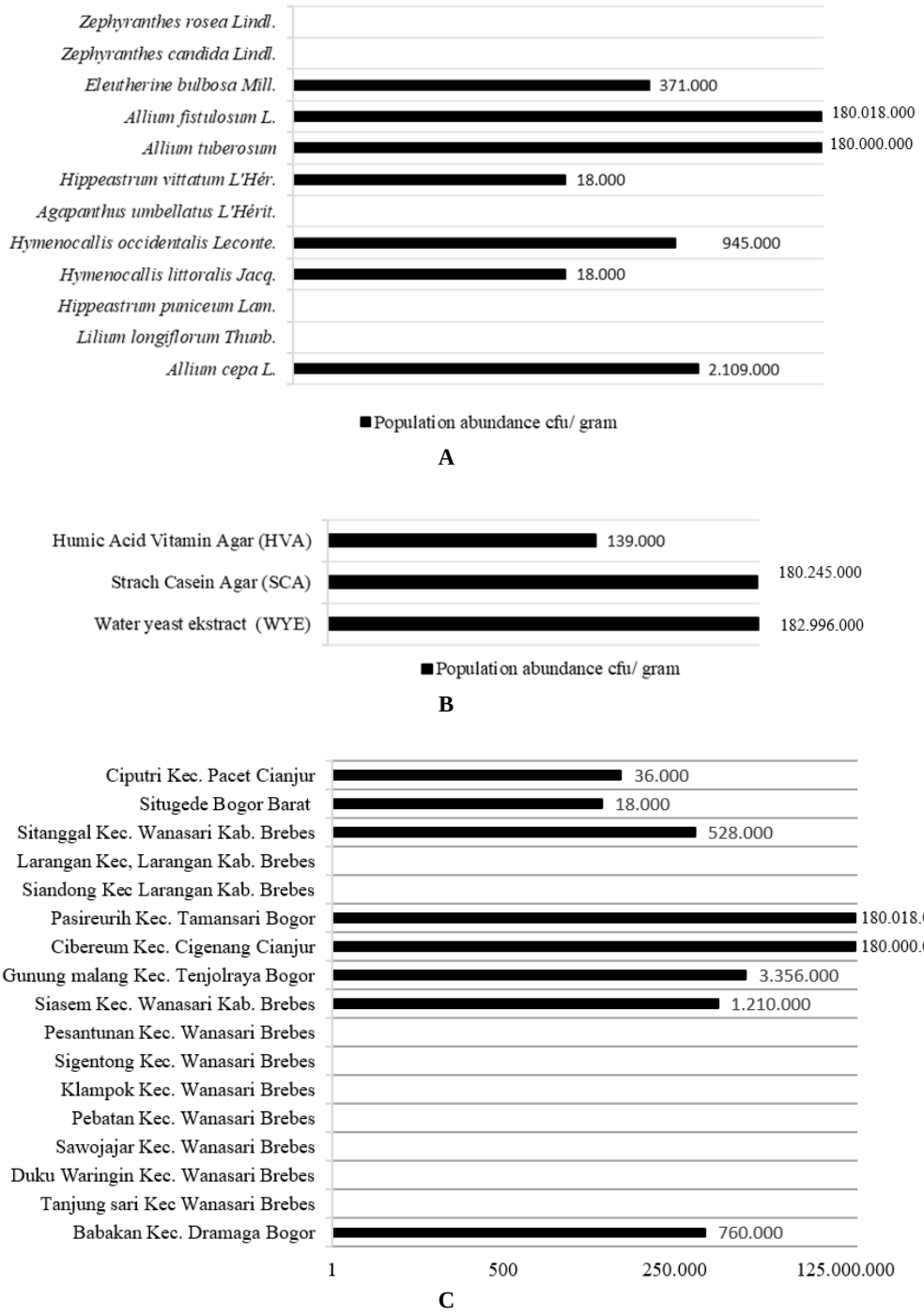

Figure 2. Population abundance of phyllospheric Actinomycetes of Liliaceae plants, (A) population abundance of Actinomycetes on various Liliaceae plants, (B) population abundance of Actinomycetes based on the media type, (C) population abundance of Actinomycetes based on region

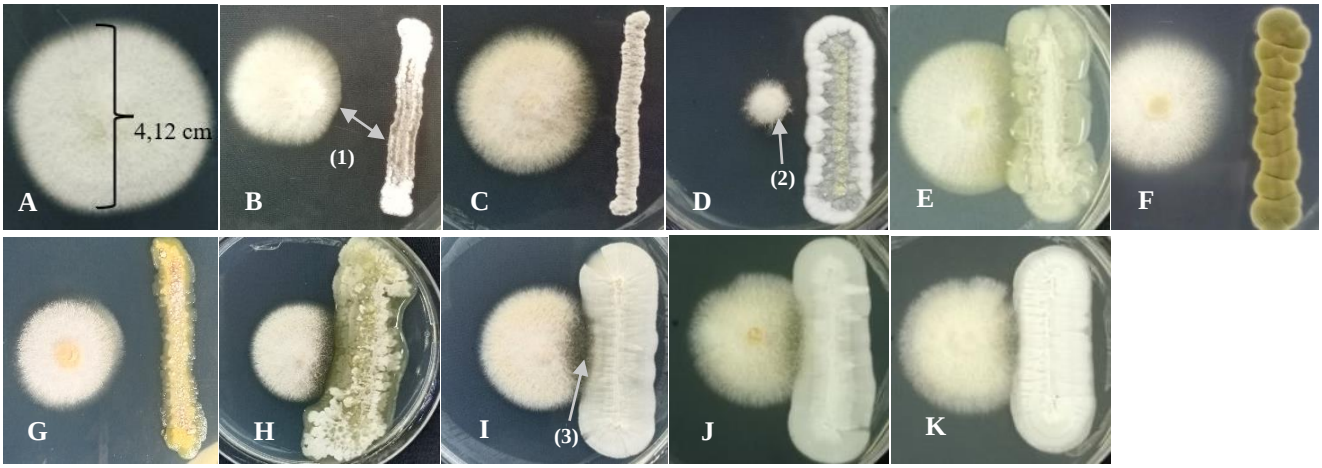

Figure 3. Growth inhibition of the mycelium of *A. porri*: A. control, B. BBW12, C. BBW14, D. BCW9, E. AHW173, F. CES25, G. CFS28, H. AHS199, I. AHS190, J. AHW161, K. AHS176. 1. forming inhibition zone, 2. mycelia *A. porri* growing upwards, 3. mycelia *A. porri* thinning due to Actinomycetes activity in the shallot phyllospheric which was incubated for seven days on PDA media.

Table 3. Results of the dual culture Actinomycetes assay on the *Alternaria porri*

Code	Colony diameter (cm)	Inhibition rate (%)	Inhibition index*
	($\bar{x}$ ± SDV)	($\bar{x}$ ± SDV)	
Control	4.5 ± 0.10^{c}	0.00 ± 0.00^{a}	-
BBW12	2.43 ± 0.15^{ab}	45.93 ± 3.39^{bc}	+++
BBW14	2.60 ± 0.10^{ab}	42.22 ± 2.22^{b}	+++
BCW9	1.90 ± 0.66^{a}	57.78 ± 14.60^{c}	+++
CES25	2.83 ± 0.21^{b}	37.04 ± 4.63^{b}	+++
CFS28	2.40 ± 0.10^{ab}	46.67 ± 2.22^{bc}	+++
AHS199	2.62 ± 0.15^{b}	41.48 ± 3.39^{b}	+++
AHS190	2.72 ± 0.06^{b}	39.26 ± 1.28^{b}	+++
AHW173	2.70 ± 0.10^{b}	40.00 ± 2.22^{b}	+++
AHW161	2.73 ± 0.15^{b}	39.26 ± 3.39^{b}	+++
AHS176	2.80 ± 0.30^{b}	37.78 ± 6.66^{b}	+++

Note: the numbers in the same column follow the same letters, showing that they are not significantly different based on HSD test at the level of α = 5%. *Inhibition zone + = < 20%; ++ = 20 - 30%; +++ = > 30%

Hypersensitivity, hemolytic and pathogenicity reactions phyllospheric Actinomycetes of Liliaceae

The results of hypersensitivity, hemolytic, and pathogenicity assay on 60 Actinomycete isolates showed that 24 isolates were positive for hypersensitivity to tobacco. This isolate caused necrosis in tobacco leaves after three days of inoculation. Infected leaves turn yellow around the inoculation site, followed by the formation of necrotic lesions, which change color to dark brown within 5-6 days after inoculation. Ten types of positive isolates were pathogenicity, which caused the leaves of shallot to become necrotic 5-6 days after inoculation. Furthermore, among the isolates tested, five were positive for hemolysis, which causes color changes or the formation of clear zones around the colonies within 3-4 days after inoculation.

Antagonism ability of Actinomycetes in inhibiting growth of *Alternaria porri*

The ten isolates displayed were the best isolates selected based on the results of inhibiting the ten highest isolates using the dual culture assay, which can inhibit the growth of *A. porri* in vitro. Its antagonistic ability causes the *A. porri* mycelium to thin, grow upwards, and form an inhibitory zone between *A. porri* and Actinomycetes (Figure 3).

The diameter of the inhibited colonies ranged from 1.90-2.83 cm, and it was significantly different compared to the control, which was 4.50 cm. The smallest colony diameter was 1.90 cm in isolate BCW9, but it was not significantly different from isolates BBW12, BBW14, and CFS28. The inhibition level of Actinomycetes isolates against *A. porri* ranged from 37.04 to 57.58% indicating a strong inhibition index (except control). The highest inhibition rate reached 57.78% in isolate BCW9, but it was not significantly different from BBW12 and CFS28 (Table 3).

Discussion

Actinomycetes were found to vary in shape, color, elevation, edges, colony surface, size, hyphae size, and the type of spore chain. The surface texture was smooth, rough, powdery, and opaque. There are three different kinds of spore chains: spiral, retinaculiaperti, and rectiflexibles. Sulistyani and Akbar (2014) successfully isolated Actinomycetes which had a rounded morphology with raised and convex elevations, flat and irregular edges, as well as smooth and rough or wrinkled surfaces. Astuty (2017) also found samples with three types of spore chains, namely rectiflexibles, retinaculiaperti, and spirals. Furthermore, the observation results showed varying colony colors due to the production of secondary metabolites, especially pigments, as previously stated by Rante et al. (2020). The differences in the pigment content of the cells led to variations in the color, according to the type of Actinomycetes strain.

The purification test results of isolates aged 1-3 days did not show any powder or flour on the surface and had a slippery appearance resembling bacteria. However, old colonies aged 4-7 days developed a rough texture, and showed visible powder or flour on their surface, extending up to 14 days, which indicated the production of spores. Actinomycetes colonies with a mealy appearance at this age indicated that their nutrients were running out, leading to the formation of mycelia growing vertically on the surface (Olanrewaju and Babalola 2019). The isolates obtained had a character resembling the smell of soil. Ananda et al. (2016) argued that Actinomycetes have a distinctive odor similar to freshly plowed soil due to their important role in the decomposition of organic matter or humus in the soil.

Actinomycetes were isolated from twelve families of Liliaceae, particularly from healthy plants among diseased ones. Plants and environmental conditions influenced the abundance and diversity of phyllospheric Actinomycetes. The factors that influence the abundance of Actinomycete populations are secondary data, namely observing the conditions of the area around the planting area. According to Sukmawaty et al. (2020), their diversity was strongly influenced by environmental, chemical, physical, and biological factors. Phyllospheric Actinomycetes were isolated from the bakung flower (*H. occidentalis*) in Sigentong area, Brebes Regency, with the coordinates (6°53'56" S. 108°59'32" E) and an altitude of 31.4 meters above sea level. The position of plants were also near the highway, which had a relatively high level of air pollution. Moreover, the Actinomycetes population was found in the Gunung Malang area, Tenjolraya District, Bogor Regency, with coordinates (6°39'27" S. 106°42'39" E), and an altitude of 628.7 meters above sea level (masl).

Altitude, air pollution, and the temperature of an area, as well as cultivation techniques, can influence the abundance of phyllospheric Actinomycetes. The Sigentong area in Brebes Regency is located in the lowlands, while Mount Malang, Tenjolraya District, Bogor Regency, is in the medium plains area. Long et al. (2021) suggested that temperature, humidity, and altitude factors can influence the diversity and abundance of phyllospheric microorganisms. For example, the species *Arundinaria spanostachya* is more commonly found in the highlands than in the lowlands. The diversity and abundance of phyllospheric Actinomycetes found were also influenced by the cultivation techniques used. The highest abundance of phyllospheric Actinomycetes was found to come from organically cultivated plants. The level of use of synthetic pesticides can affect their populations. Phyllospheric Actinomycetes were not found in leeks (*A. fistulosum*) from the Ciputri area, Pacet District, Cianjur Regency; this was due to the application of synthetic pesticides for 2-3 days. On the other hand, leeks isolated from the Pasireurih area of Tamansari District, which did not use synthetic pesticides, showed the presence of Actinomycetes.

At several locations in the Brebes Regency, Central Java, Actinomycetes were also not found, particularly in onion center areas, such as Wanasari District, including Tanjung Sari (-6°54'55" S. 108°59'44" E. 38.9 masl), Duku Waringin (-6°54'49" S. 108°59'17" E. 25.4 masl), Sawojajar (-6°52'36" S. 109°0'20" E. 28.7 masl), Pebatan (-6°52'36" S. 109°0'20" E. 28.7 masl), Klampok (-6°51'47" S. 109°1'2" E. 19.0 masl), Sigentong (-6°53'56" S. 108°59'32" E. 31.4 masl), and Pesantunan (-6°52'15" S. 109°0'29" E. 30.7 masl). Actinomycetes were also not found in Larangan District, such as Siandong (-6°57'47.07" S. 108°58'18.74" E), and Larangan Village (-6°58'50.73" S. 108°57'36.51" E). The majority of the isolated plant species were shallots. Based on the results, shallots isolated from several areas in Brebes did not have Actinomycetes populations. This was presumably due to the intensive use of pesticides, which were applied every 2-3 days. The farmers in Brebes Regency primarily use a synthetic fungicide containing the active ingredient propineb to control *A. porri* on shallots.

The results also showed that the abundance of Actinomycetes was influenced by the type of media used. Among the media used. WYE supported the highest growth, followed by SCA, and HVA. The three media were designed specifically for Actinomycetes. Good growth was obtained with spores formation when the media was prepared using inositol, sucrose, mannitol, rhamnose, fructose, raffinose, and cellulose as a carbon source after incubation at room temperature and incubation for 7 days. Additionally, the presence of xylose, arabinose, and glucose will induce the formation of hyphae and substrate mycelium. Air mycelium and spores were also formed when Actinomycetes were inoculated in media containing carbon sources in the form of sucrose, fructose, raffinose, cellulose, and rhamnose (Utarti et al. 2020).

The abundance of phyllospheric Actinomycetes populations in Liliaceae plants was categorized as low, the distribution evenness index was uneven, and the dominance index was high. This can be attributed to the nutrient-poor habitats in which these epiphytic microbes were found, directly exposed to the atmosphere, the diurnal cycle, as well as sunlight, and indirectly affecting plant metabolism (Vorholt 2012). Several environmental factors can affect the abundance of phyllospheric microbial populations, such as solar radiation, hot or cold temperatures, and air pollution. Biotic factors also have a significant influence including plants' age and competition between other microorganisms in the phyllosphere. The phyllosphere is an aerial habitat of plants dominated by leaves and colonized by various types of microbes.

The selected isolates were subjected to the biosafety assay, with 24 showing hypersensitivity, leading to necrosis in tobacco leaves. This indicated that the isolates were plant pathogens, with ten causing necrosis in shallot leaves. The hypersensitivity response was characterized by rapid cell death at the point of pathogen entry. Plants recognize specific signaling molecules called elicitors produced by the pathogen, and this hypersensitive reaction was reportedly related to pathogen resistance (Balint and Kurti 2019; Kalungi 2022).

Five hemolysis-positive Actinomycetes isolates caused discoloration or the formation of clear zones around the colonies on blood agar media, indicating their pathogenicity to animals and humans. Phyllospheric

Actinomycetes used as biological control agents must be microorganisms safe for plants, animals, and humans. The ability of microbes to degrade red blood cells is divided into three categories, namely beta (β), alpha (α), and gamma hemolysis (γ). Beta or true hemolysis results in the formation of clear zones around bacterial colonies. Alpha hemolysis causes a discoloration of the blood agar medium surrounding the colony when red blood cells are reduced to methemoglobin and form a greenish or brownish color. Meanwhile, gamma hemolysis does not cause lysis reactions and discoloration (Mogrovejo et al. 2022).

Phyllospheric Actinomycetes isolated from Liliaceae plants act as antagonistic agents, inhibiting the growth of the fungus *A. porri* mycelium by 37.04-57.78%. Inhibition was evidenced by thinning of the mycelium, slender upward growth, and the formation of an inhibitory zone (Figure 3). Several studies have found that Actinomycetes produce compounds that can inhibit the growth of pathogens, such as chitinase, protease, amylase, lipase, and cellulose enzymes (Fatmawati et al. 2018; Wibowo et al. 2020; Kishani et al. 2022). The chitinase enzyme is able to degrade chitin, which is the main component of fungal cell walls. Actinomycetes have chitinolytic abilities, enabling the degradation of chitin (Hartanto and Krestini 2016). These antifungus activities are crucial for suppressing the growth of the fungus mycelium, culminating in the use of Actinomycetes as a biocontrol agent due to their antibiosis activity (Djebaili et al. 2021). Ohike et al. (2018) tested the inhibition effect of Actinomycetes against eight types of phytopathogenic fungi and found that all isolates exhibited positive results on the mycelium. Fadhilah et al. (2021) successfully isolated marine Actinomycetes from mangrove ecosystems. The isolates demonstrated antagonistic activity against *Colletotrichum* sp., with an inhibition percentage of 84.94%.

In conclusion, a total of ten isolates were selected from 26 actinomycete isolates based on the highest inhibitory percentage. Several isolates were found to have morphological differences in color, shape, height, edges, colony surface, size, and spore chains. The diversity level of phyllospheric Actinomycetes of Liliaceae plants is low, the distribution evenness index is uneven, and the dominance index is high. The abundance of Actinomycetes can be influenced by altitude, media used, type of host plant, and conditions around the sampling location. Actinomycetes have the potential to inhibit the development of *A. porri* by 37.0-47.58%. This discovery expands the possibility of phyllospheric Actinomycetes as potential antagonistic agents against fungal diseases that attack leaves. Therefore, we recommend further testing of phyllospheric Actinomycetes as biocontrol agents on the field scale to increase shallot yields and support the sustainable agricultural systems.

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