RESEARCH PAPER

The effectiveness of Liliaceae phyllospheric Actinomycetes as biocontrol agent of purple blotch disease (*Alternaria porri* Ell. Cif) on shallot

Cheppy Wati[1,] Abdjad Asih Nawangsih[2], Aris Tri Wahyudi[3], Suryo Wiyono[2], & Abdul Munif[2]

ABSTRACT

Purple blotch, caused by *Alternaria porri*, affects leaves and tubers, leading to reduced yields. Traditionally, synthetic chemical fungicides were heavily relied upon for control. As an alternative, biocontrol agents like actinomycetes have gained attention. Some actinomycetes can suppress plant pathogens by producing antifungal compounds. This research aimed to investigate the efficacy of phyllosphere actinomycetes from Liliaceae plants as biocontrol agents against purple blotch disease on shallots in the greenhouse. Conducted at the IPB University Plant Bacteriology and Mycology Laboratory and the greenhouse at Bogor Agricultural Development Polytechnic, West Java, the research involved several stages, including pathogen identification, preparation of actinomyces inoculum and *A. porri*, and application of actinomycetes biocontrol agents to shallots in the greenhouse. *A. porri* isolates were obtained from shallot production centers in the Brebes area, Central Java. The use of the actinomycetes from the phyllosphere effectively suppressed purple blotch disease, with the lowest area under the disease development curve (AUDPC) recorded at 635.9% for the CFS28 isolate. Lower AUDPC values indicated slower disease progression. Actinomycetes biocontrol agents showed promising efficacy, with the CFS28 isolate achieving the highest efficacy percentage of 78.37%. Additionally, plant growth was significantly enhanced by actinomycete application, with tuber sizes ranging from 1.44 to 2.06 g, fresh weights from 17.63 to 24.72 g, and dry weights of shallot bulbs from 5.43 to 17.96 g. The incubation period for *A. porri* could be extended by actinomycetes, ranging from 5.43 to 8.5 days for purple blotch symptoms to manifest on shallots. The use of Actinomyces phyllosphere biocontrol agents holds promise for disease control on other plants' leaves, contributing to environmentally friendly and sustainable agricultural practices.

Key words: control, disease development, Liliaceae, sustainable agricultural system

INTRODUCTION

One of the economically important horticultural crops in Indonesia is the shallot (*Allium cepa*). Shallots are widely used as a seasoning and in traditional medicine because they contain alliin, which has antibacterial activity. In addition, shallots contain carbohydrates, fatty acids, sugar, protein, and various other minerals needed by the body (Waluyo & Sinaga, 2015).

The demand for shallots continues to increase as the population grows. Efforts to boost shallot production face challenges, one of which is plant disease. One of the important diseases in shallots is purple blotch, caused by *Alternaria porri* Ell. & Cif. This pathogen infects many kinds of plants in the Liliaceae family, especially the *Allium* genus. Purple blotch has been reported across Java, Sumatra, Sulawesi, and Nusa Tenggara. *A. porri* causes purple blotch disease, also known as *trotol,* which can infect the leaves of shallot plants down to the bulbs. Black et al. (2012) reported that purple blotch can reduce leaf quantity by 62–92%. The formation of necrosis on the leaves can interfere with the process of tuber formation. Severe infection can cause plants to wilt and die. The severity of purple blotch disease ranges from 29.5% to 60.8%, and this disease can cause yield losses of 3–57%. Yield losses due to purple blotch disease can reach 30–100% (Yadav et al., 2013; Shree et al., 2020).

The control of purple blotch in several regions of Indonesia still relies on the use of synthetic fungicides. Based on observations of onion farmers in the Brebes area of Central Java, the frequency of pesticide spraying on shallot plants is about 2–3 times

Corresponding author:
Abdjad Asih Nawangsih (ryuntania@apps.ipb.ac.id)

[1]Study Program of Horticulture Agribusiness, Department of Agriculture, Bogor Agricultural Development Polytechnic. Jl. Aria Surialaga. Pasir Jaya West Bogor District, Bogor Indonesia 16119
[2]Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Meranti, IPB University, Campus Dramaga, Bogor, Indonesia 16680
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Meranti, IPB University, Campus Dramaga, Bogor, Indonesia 16680

per week. There are concerns that the use of synthetic fungicides could cause resistance in pathogens, so it is recommended to use environmentally friendly controls. The use of biological agents to control plant diseases has been proven to be effective and to increase crop yields. Actinomycetes are a group of bacteria that have potential as biocontrol agents because they can produce antifungal, antibacterial, antinematode, and antiviral compounds (Fatmawati et al., 2018; Jakubiec-Krzesniak et al., 2018; Park et al., 2020; Zou et al., 2021). Actinomycetes can also enhance plant growth and production. Anwar et al. (2016) reported that *Streptomyces,* a member of the actinomycete group, was able to increase the growth and yield of wheat due to its ability to produce indole acetic acid (IAA), HCN, ACC deaminase, siderophores, and to solubilize phosphate.

The use of actinomycetes to control pathogenic fungi has been widely reported, but specific types of phyllospheric actinomycetes have not been extensively studied, especially those from Liliaceae plants. Therefore, in this study, actinomycetes from the phyllosphere of Liliaceae plants were tested for their effectiveness as biocontrol agents against purple blotch of shallots.

MATERIALS AND METHODS

Research Site. This research was conducted in the Plant Mycology Laboratory, the Plant Bacteriology Laboratory, the Plant Protection Department at IPB University, and the greenhouse at the Bogor Agricultural Development Polytechnic in Bogor, West Java, Indonesia.

Experimental Design and Treatment. The greenhouse experiment used a completely randomized design, with each treatment repeated three times and ten plants per replication. The treatments used were: positive control sprayed only with *A. porri* (control 1), negative control sprayed only with sterile distilled water (control 2), treatment inoculated with ten actinomycetes and *A. porri*, and inoculated with the fungicide propineb + *A. porri* (fungicide + AP).

Isolation of *Alternaria porri*. Isolation was carried out using the tissue implantation method. Diseased plant samples were taken from the Brebes, Central Java (Lat S 6.89°, Long E 108.99°). A 1 cm section of the leaf between the diseased and healthy areas was cut, surface-sterilized using 1% NaOCl for 1 min, rinsed three times with sterile distilled water, dried with sterile blotting paper, and then placed on potato dextrose agar (PDA) media. The isolates that grew were identified macroscopically, microscopically, and through molecular tests.

Macroscopic, Microscopic, and Molecular Identification of the *Alternaria porri*. Macroscopic identification was performed by examining the color and shape of the colony on both the upper and reverse side. Macroscopic observations included colony color (upper and reverse) and colony surface shape, while microscopic observations included the shape and size of conidia, hyphae, and mycelium of *A. porri* fungus.

DNA amplification used the primer set ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Singha et al., 2016), targeting ±600 bp. The PCR mix consisted of 12.5 µL Bioline MyTaq HS Red Mix 2×, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 9,5 µL Nuclease-Free Water (NFW), and 1 µL template DNA (100 ng/µL) making a final reaction volume of 25 µL. The DNA was amplified in a GeneAmp® PCR System 9700 with an initial pre-heating step of 5 min at 94 ºC, followed by 35 cycles of pre-denaturation (2 min at 94 ºC), denaturation (50 s at 94 ºC), annealing (60 s at 55 ºC), extension (60 s at 72 ºC), and post-extension (5 min at 72 ºC). Amplicons were then visualized on 1% agarose gel, migrated at a voltage of 50 V for 50 min using Mupid-Exu Submarine electrophoresis in TAE 0.5× buffer, and observed with a UV transilluminator. PCR products were sequenced through the First Base Sequencing Service (Malaysia). The sequencing results were aligned with GenBank data using the Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The ORF Finder program (https://www.ncbi.nlm.nih.gov/orffinder/), and Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) were used to examine the nucleotide sequence data. Phylogenetic analysis was carried out using the MEGA 8.0 program for Windows 10 (Kumar et al., 2018), with the Neighbor Joining (NJ) method and 1000× bootstrapping.

Inoculum Preparation of Actinomycetes and *A. porri* Isolates. The shallot bulbs used were of the Bima Curut variety from Brebes, Central Java. The tubers were surface-sterilized with 1% NaOCl and rinsed three times with sterile distilled water. The tubers were then planted in 25 × 20 cm polybags, with one tuber per polybag. The planting medium consisted of soil, drum fertilizer, and sand in a volume ratio of 1:1:1.

The preparation method of the actinomycete suspension following the method by Fatmawati et al. (2019). The actinomycetes used in this research were the result of exploration by Wati et al. (2023). They were BCW9, BBW12, BBW14, CES25, CFS28, AHW161, AHW173, AHS176, AHS190, and AHS199. These strains were 10 days old and were soaked in a 0.85% NaCl solution to release actinomycete spores. The actinomycete suspension was centrifuged and washed twice with sterile distilled water. The resulting spore pellets were re-suspended in 0.5% CMC (carboxy methyl cellulose). *A. porri* was grown on PDA media and harvested after three weeks, by adding distilled water to the *A. porri* isolate then rubbing it using a sterile brush to release the spores.

Application Suspension of Actinomycetes and *A. porri* on Shallot. Inoculation of *A. porri* suspension was carried out on plant leaves two weeks after planting by wounding the leaves with a sterile needle. Then, 15 mL per plant of *A. porri* suspension with a density of 10^5 conidia per mL was sprayed. Meanwhile, the application of actinomycete was carried out at the age of 1 WAP or one week before *A. porri* inoculation, by spraying 15 mL per plant of actinomycete suspension with density of 10^8-10^9 cfu mL^{-1}.

Observation. The variables observed were the incubation period, disease severity, disease incidence, plant height, number of tubers, tuber fresh weight, and tuber dry weight. The incubation period was observed from one day after inoculation (dai) of the pathogen until the first symptoms appeared. Plant height, disease severity, and disease incidence were observed at 5 dai. Meanwhile, the number of tubers was counted at harvest. Fresh weight and dry weight measurements were carried out by weighing. Fresh weight was measured after harvest, while dry weight was measured after drying in an oven at a temperature of 60 ºC.

The percentage of disease severity, disease incidence, AUDPC, and control efficiency were calculated using the following formula:

Disease severity. The disease severity was calculated based on the formula of Pathak et al. (1986). The score for calculating disease severity was based on a 0–5 scale, namely scale 0 = disease severity ranging from 0–5%, scale 1 = 6–10%, scale 2 = 11–20%, scale 3 = 21–40%, scale 4 = 41–60%, and scale 5 = ≥ 61%.

$$IP = \sum \frac{n \times v}{N \times V} \times 100\%$$

IP = Disease severity;

n = Number of infected plants in the score category;
v = Score in each infected category;
N = Number of all plants observed;
V = Highest infected category.

Disease incidence. Disease incidence was calculated based on the formula by Allen (1983):

$$DI = \frac{n}{N} \times 100\%$$

DI = Disease incidence;
n = Number of plants with symptoms;
N = Total number of plants observed.

Area under the disease progress curve (AUDPC). The effect of treatment was shown on the disease progression curve over time using the area under the disease progress curve (AUDPC) model, according to Simko & Piepho (2012):

$$AUDPC = \sum_{i=1}^{N-1} \left(\frac{y_i + y_{i+1}}{2} \right) \times \left(t_{i+1} - t_i \right)$$

y_i = Percentage of disease severity at observation i;
t_i = Time at observation i;
N = Total number of observations.

The effectiveness of control on plants was determined by the formula (Cooke, 2006), which was modified:

$$E = \frac{S_K - S_P}{S_K} \times 100\%$$

E = Control effectiveness;
S_K = AUDPC value in the positive control;
S_P = AUDPC value in the treatment.

Data Analysis. Data were analyzed using analysis of variance (ANOVA). The effect of each treatment was evaluated using the Tukey test at a significance level of 5%. All data were analyzed using SPSS version 27 software (https://www.ibm.com/docs/en/SSLVMB_27.0.0/pdf/en/IBM_SPSS_Statistics_Base.pdf). Editing of *A. porri* DNA sequence was performed using the Bioedit application version 7.2.5.0 (Hall, 2011).

RESULTS AND DISCUSSION

Alternaria porri was observed under the microscope. The hyphae and branched mycelium were brown and had partitions (Figure 1A). The brown club-shaped conidia of *A. porri* showed partitions and transverse lines in the centre. Conidia had 5–10 horizontal partitions and 1–6 vertical partitions in the

centre and were 16.38–36.80 μm long and 5.78–6.84 μm wide (Figure 1B). Meanwhile, the macroscopic morphology of the *A. porri* isolates had a cotton-like texture, thick without concentric circles, light to dark grey in colour, with a slightly yellowish to orange hue. The reverse of the isolate was brownish-red with orange edges (Figures 1C and 1D). Consistent with the work of Risdiyanti et al. (2023), isolates of *A. porri* on shallot were characterized by blackish-grey colonies with regular colony shapes and no concentric circles, spreading evenly. The conidia of *A. porri* were shaped like inverted sticks with brown mycelium. The length of *A. porri* conidia ranged from 17.90–76.15 μm. *A. porri* had 6–12 horizontal partitions and 1–3 vertical partitions. Genetic changes in pathogens and different environmental conditions can affect the morphological form of *A. porri* (Chethana et al., 2018; Priya et al., 2018).

The sequencing results indicated that isolate AA1 was *Alternaria porri* with GenBank accession number LC440611.1, a query coverage value of 100%, and a similarity level of 99.67% (Table 1). Based on the results of phylogenetic tree analysis, isolate AA1 is closely related to *A. porri*. AC32 [LC440611.1] and *Alternaria* sp.YZU [ON129509.1] (Figure 2).

Treatment with actinomycete microbes can act not only as a biocontrol agent but also as a biofertilizer. Actinomycete isolates have been reported to produce indole-3-acetic acid (IAA), dissolve phosphate, fix nitrogen, and produce siderophores (Wijayanti et al., 2021; Fatmawati et al., 2019). Tuber size and weight showed a significantly different effect compared to the positive control. However, crown length and number of tubers showed no significant effect compared to the control (Table 2). According to Carretero et al. (2011), pathogen infection in plant leaves can affect tuber formation by reducing the area of green leaves and leaf chlorophyll content, thus disrupting plant function, sunlight absorption, and CO_2 uptake. Purple blotch can disrupt the bulb formation process and affect the weight of onion bulbs. According to Risdiyanti et al. (2023), *A. porri* infection can damage the physical properties of bulbs, which may affect the nutritional and vitamin content of shallot bulbs.

The use of biocontrol agents from the actinomycetes group was effective in controlling *A. porri* in greenhouses, as evidenced by the relatively high percentage control values for the treatment with isolates CFS28, BWW12 and BWW14. The incubation period for *A. porri* can be extended by the use of actinomycetes, where the time required for *A. porri* to cause purple blotch symptoms on shallot ranging from 5.43 to 8.5 days (Table 3). Disease severity was influenced by the time taken for the pathogen to incubate. The longer the incubation period of the pathogen, the lower the disease intensity and the more effective the control. The shortest incubation period was 4.20 dai in the control. The incubation period
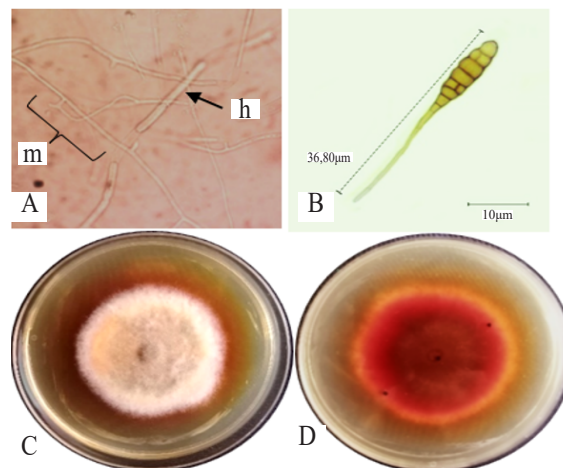

Figure 1. Identification of *Alternaria porri*. A. Hyphae and mycelium of *A. porri*; h=hyphae, m=mycelium. B. Conidia form of *A. porri*; C. Upper view of *A. porri* colony; D. Reverse view of *A. porri* colony.

Table 1. Sequence alignment of *A. porri* isolates with GenBank accessions

Homologous species (Genbank)	Query Cover	E-value	Similarity	Accession number
A. porri AC32	100%	0,0	99.67%	LC440611.1
A. porri AC16	100%	0,0	99.67%	LC440609.1
A. porri AC6	100%	0,0	99.67%	LC440607.1

of a pathogen is the time it takes for the pathogen to cause symptoms. Marlitasari et al. (2016) stated that symptoms of infection caused by *A. porri* generally start to appear 4–7 dai, specifically between 19 and 22 dai.

Symptoms of purple blotch caused by *A. porri* on shallot plants include pseudo-purple concentric circular spots with reddish-yellow margins. Purple blotch disease can interfere with physiological processes such as photosynthesis, which occurs in the leaves. When these processes are disrupted, plant growth is reduced, leading to lower production yields. Purple blotch disease can reduce shallot production by damaging the leaves, the neck of the stem base, and the bulbs. According to observations by Shree et al. (2020) and Korlina et al. (2021), symptoms of purple blotch disease on leaves begin with small white to grey spots, which then enlarge and become concave, forming
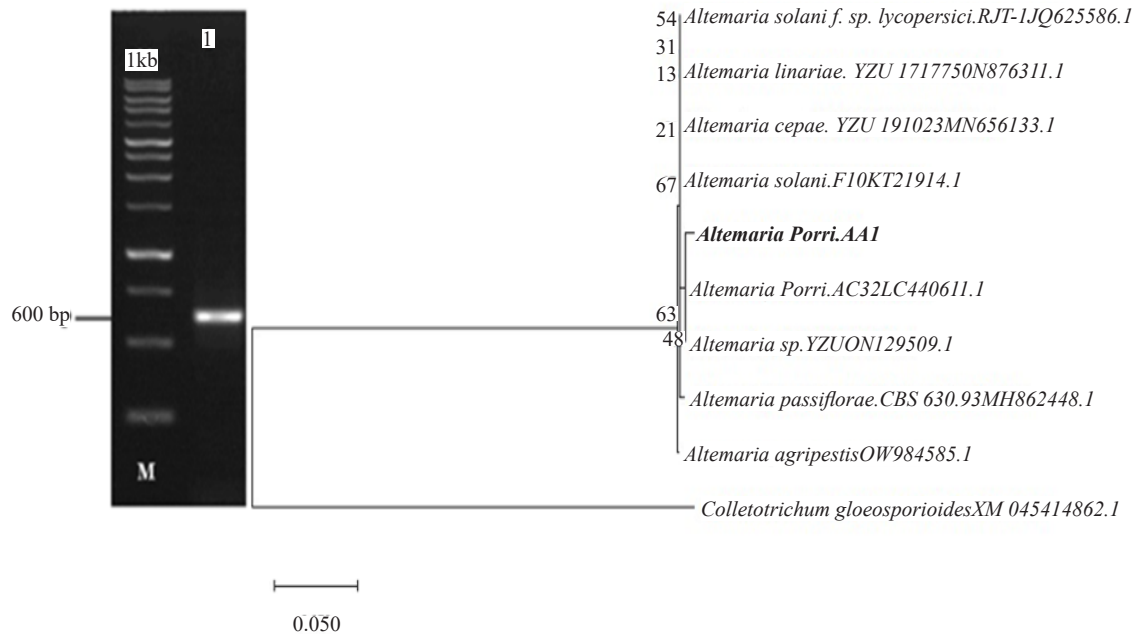

Figure 2. Identification of *A. porri*. Left: 600-bp DNA band amplified using primers ITS1 and ITS4, marker (M). Right: phylogenetic tree of *A. porri*.

Table 2. Effect of Actinomycete treatment on the growth of shallot in the greenhouse

Treatment	Header length (cm)	Number of tubers	Tuber size (cm)	Fresh weight (g)	Dry weight (g)
Positive Control	34.15 a	5.80 a	1.37 a	15.39 a	4.10 a
Negative Control	35.93 a	5.87 a	1.47 a	17.80 abc	6.50 bc
Fungicide + AP	35.80 a	5.50 a	1.41 ab	17.63 ab	6.31 bc
BCW9 + AP	35.28 a	6.60 a	1.60 ef	19.61 bc	6.26 bc
BBW12 + AP	38.40 a	6.10 a	1.91 g	21.64 cd	10.89 f
BBW14 + AP	36.85 a	6.30 a	1.83 g	21.25 bcd	9.87 ef
CES25 + AP	34.58 a	5.03 a	1.55 def	19.23 bc	6.88 c
CFS28 + AP	36.77 a	5.87 a	2.06 h	24.72 d	17.96 g
AHW161 + AP	35.43 a	6.00 a	1.46 abcd	17.85 abc	8.87 de
AHW173 + AP	36.38 a	5.73 a	1.44 abc	17.63 ab	8.07 d
AHS176 + AP	34.37 a	5.40 a	1.49 bcd	18.07 abc	5.43 b
AHS190 + AP	35.05 a	5.40 a	1.62 f	19.65 bc	8.92 de
AHS199 + AP	35.13 a	5.10 a	1.51 cde	17.81 abc	8.18 d

Numbers in the same column followed by the same letter are not significantly different based on the 5% level on the Tukey test. AP= *A. porri*.

purple concentric rings surrounded by a yellow halo. Symptoms of *A. porri* infection usually begin to appear when the plant is 16 days old. Purple blotch disease, which infects shallot bulbs, can occur through the base of the stem neck, with symptoms appearing within 5 days, leading to bulb rot (Dar et al., 2020). Purple blotch causes severe damage to the surrounding environment. The development of purple blotch occurs under favourable climatic conditions, specifically humidity of 80–90% and an optimum temperature of 24 ± 2 ºC (Hadisutrisno et al., 1996).

The development of disease severity and incidence caused by *A. porri*, when observed weekly, shows differences due to the application of actinomycetes biocontrol agents on shallots in the greenhouse. Figure 3 shows that all treatments had lower disease incidence and severity compared to the positive control. The peak incidence of purple blotch in the positive control was at 12 dai of the pathogen, while the peak disease severity was at 47 dai. Differences in the severity of disease progression can be seen by how far the AUDPC value is below the disease progression curve. The CFS28 treatment had the lowest AUDPC, followed by BWW12 and

Table 3. Effect of Actinomycete treatment on incubation time, AUDPC value, and effectiveness of controlling *A. porri*

Treatment	Incubation period (days)	AUDPC (%/week)	Control effectiveness (%)
Positive Control	4.20 a	2950.61 d	0.00
Fungicide + AP	6.83 bcde	2001.66 cd	31.08
BCW9 + AP	5.43 ab	2132.53 cd	26.25
BBW12 + AP	7.30 bcde	762.69 a	73.31
BBW14 + AP	7.43 cde	810.94 ab	72.70
CES25 + AP	5.80 abc	2147.15 cd	25.60
CFS28 + AP	8.50 e	635.90 a	78.37
AHW161 + AP	6.03 abc	1535.33 abc	45.80
AHW173 + AP	5.70 abc	1922.59 bcd	33.31
AHS176 + AP	6.40 bcd	2180.01 cd	23.54
AHS190 + AP	8.27 de	1338.37 abc	53.25
AHS199 + AP	6.47 bcd	1731.70 abc	39.74

Numbers in the same column followed by the same letter are not significantly different based on the 5% level on the Tukey test. AP= *A. porri*.
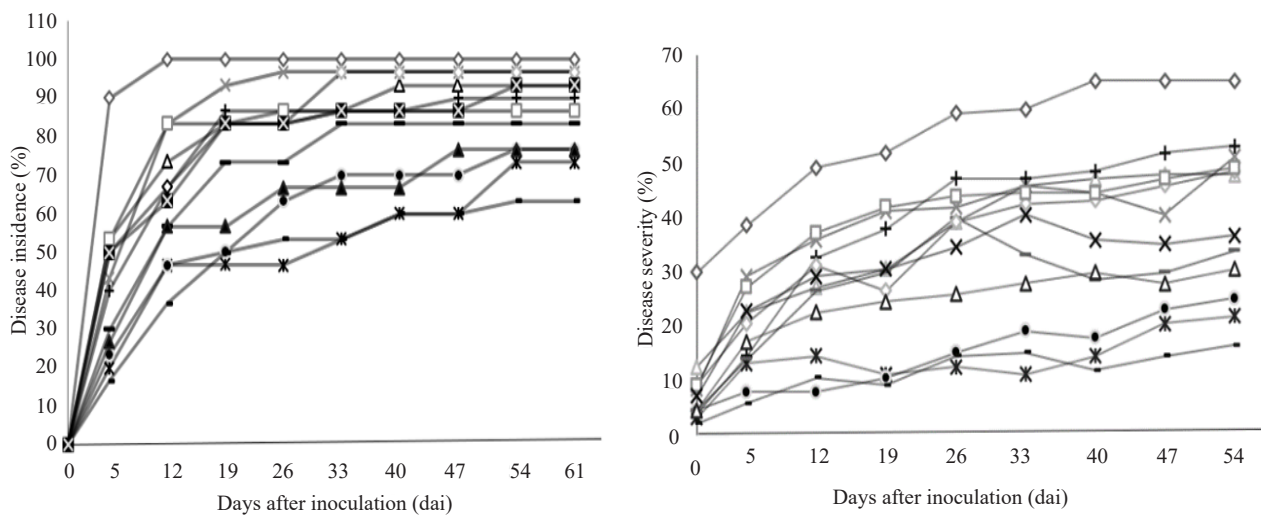

Figure 3. Development of purple blotch disease, *A. porri*, on shallot. ◇ positive control, □ negative control, △ fungisida + AP, ✕ BCW9 + AP, ✳ BBW12 + AP, ○ BBW14 + AP, + CES25 + AP, -•- CFS28 + AP, ▬ AHW161 + AP, ◆ AHW173 + AP, ▫ AHS176 + AP, ▲ AHS190 + AP, ✖ AHS199 + AP.

BWW14, while the control had the highest AUDPC (Table 3). The lower the AUDPC, the more effective the Actinomyces biocontrol agent was in controlling purple blotch on shallots.

The application of the actinomycetes isolated from the phyllosphere effectively suppressed the severity of *A. porri*, with the lowest disease severity value being 1.13%. In line with research by Saeed et al. (2021), the application of *Streptomyces hydrogenans* to radish plants reduced the severity of *A. brassicaola* by 22.18% (Risdiyanti et al., 2023).

Based on the results of partial sequence alignment of the 16S rRNA gene of actinomycete strains, in the GenBank database using the BLASTIN program, it produced a query cover value of 99%, the highest similarity was 97.78%. The species name of the best actinomycete isolate CFS28 is *Streptomyces enissocaesilis*.

The actinomycetes group of biological agents has many advantages, especially as biocontrol agents for plant diseases. In addition to producing antifungal compounds, actinomycetes have been reported to produce antibacterial, antinematode, and antiviral compounds (Jakubiec-Krzesniak et al., 2018; Zou et al., 2021; Park et al., 2020; Fatmawati et al., 2018). Actinomycetes are also capable of producing hydrolytic enzymes such as protease, cellulase, and chitinase, which are antagonistic to plant pathogens (Song et al., 2020).

CONCLUSION

The time required for *A. porri* to cause purple blotch symptoms on shallot ranges from 5.43 to 8.5 days. The use of the actinomycete phyllosphere biocontrol agent was able to suppress the development of purple blotch on shallots. The lowest area under the disease development curve (AUDPC) value was 635.9% for the CFS28 isolate and the lowest disease severity level was 1.13%. The highest percentage of control efficiency was 78.37% in the CFS28 isolate treatment. While, plant growth can be significantly enhanced by the application of actinomycetes, such as tuber size ranging from 1.44 to 2.06 g, fresh weight ranging from 17.63 to 24.72 g, and dry weight of shallot bulbs ranging from 5.43 to 17.96 g.

ACKNOWLEDGMENTS

FUNDING

AUTHORS' CONTRIBUTIONS

CW and AAN prepared the research manuscript; ATW, SW, and AM considered and reviewed the research flow plan. CW conducted the isolation and identification of the *A. porri*, including macroscopic, microscopic, and molecular identification, as well as the application of actinomycete biocontrol agents in the greenhouse. CW calculated the incubation time of the pathogen, disease severity, and disease incidence, and observed the growth of shallot in the greenhouse. AAN and ATW helped analyze and interpret plant damage and growth data. SW and AM provided input on the results and discussion of the research. All authors contributed to the research flow, analysis and interpretation of the data, and manuscript preparation. All authors have read and approved the final manuscript.

COMPETING INTEREST

There is no competing interest regarding our publication.

REFERENCES

Allen NS. 1983. Degradation and Stabilisation of Polyolefins. *Applied Science Publishers.* England.

Anwar S, Ali B, & Sajid I. 2016. Screening of rhizospheric actinomycetes for various *in-vitro* and *in-vivo* plant growth promoting (PGP) traits and for agroactive compounds. *Front. Microbiol.* 7: 1334. https://doi.org/10.3389/fmicb.2016.01334

Black L, Conn K, Gabor B, Kao J, & Lutton J. 2012. Purple blotch. In: Conn K, Lutton J, & Rosenberger SA (eds.). *Onion Disease Guide.* pp. 29. Seminis Vegetable Seeds Inc. St. Louis. MO.

Carretero R, Bancal MO, & Miralles DJ. 2011. Effect

of leaf rust (*Puccinia triticine*) on photosynthesis and related processes of leaves in wheat crop grown at two contrasting sites and with different nitrogen level. *Eur. J. Agron*. 35(4): 237–246. https://doi.org/10.1016/j.eja.2011.06.007

Chethana BS, Ganeshan G, Rao AS, & Bellishree K. 2018. Morphological and molecular characterization of *Alternaria* isolates causing purple blotch disease of onion. *Int. J. Curr. Microbiol. App. Sci.* 7(4): 3478–3493. https://doi.org/10.20546/ijcmas.2018.704.394

Cooke BM. 2006. Disease assessment and yield loss. In: Cook BM, Jones DG, & Kaye B (Eds.). The Epidemiology of Plant Disease. Springer. Dordrecht. pp. 43–80. https://doi.org/10.1007/1-4020-4581-6_2

Dar AA, Sharma S, Mahajan R, Mushtaq M, Salathia A, Ahamad S, & Sharma JP. 2020. Overview of purple blotch disease and understanding its management through chemical, biological and genetic approaches. *J. Integr. Agric*. 19(12): 3013–3024. https://doi.org/10.1016/S2095-3119(20)63285-3

Fatmawati U, Lestari Y, Meryandini A, Nawangsih AA, & Wahyudi AT. 2018. Isolation of actinomycetes from maize rhizosphere from Kupang, East Nusa Tenggara Province, and evaluation of their antibacterial, antifungal, and extracellular enzyme activity. *Indones. J. Biotechnol*. 23(1): 40–47. https://doi.org/10.22146/ijbiotech.33064

Fatmawati U, Meryandini A, Nawangsih AA, & Wahyudi AT. 2019. Screening and characterization of actinomycetes isolated from soybean rhizosphere for promoting plant growth. *Biodiversitas*. 20(10): 2970–2977. https://doi.org/10.13057/biodiv/d201027

Hadisutrisno B, Sudarmadi, Subandiyah S, & Priyatmojo A. 1996. Peranan faktor cuaca terhadap infeksi dan perkembangan penyakit bercak ungu pada bawang merah. [The role of climatic factors on the infection and development of the purple blotch of shallot]. *Indones. J. Plant Prot*. 1(1): 56–64.

Hall T, Biosciences I, & Carlsbad C. 2011. BioEdit: an important software for molecular biology. *GERF Bull Biosci.* 2: 60–61.

Korlina E, Hasyim A, & Hermanto C. 2021. Efficacy of different dose of fungicide Mancozeb against

purple blotch complex (Alternaria porri) of Shallot. *IOP Conf. Ser.: Earth Environ. Sci.* 653: 012150. https://doi.org/10.1088/1755-1315/653/1/012150

Jakubiec-Krzesniak K, Rajnisz-Mateusiak A, Guspiel A, Ziemska J, & Solecka J. 2018. Secondary metabolites of actinomycetes and their antibacterial, antifungal, and antiviral properties. *Pol. J. Microbiol.* 67(3): 259–272. https://doi.org/10.21307/pjm-2018-048

Kumar S, Stecher G, Li M, Knyaz C, & Tamura K. 2018. Mega X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Marlitasari E, Sulistyowati L, & Kusuma RR. 2016. Hubungan ketebalan lapisan epidermis daun terhadap infeksi jamur *Alternaria porri* penyebab penyakit bercak ungu pada empat varietas bawang merah [The relationship between the thickness of the leaf epidermis layer and the fungal infection *Alternaria porri*, which causes purple spot disease in four shallot varieties]. *J. HPT*. 4(1): 8–16. https://jurnalhpt.ub.ac.id/index.php/jhpt/article/view/223

Park EJ, Jang HJ, Park CS, Lee SJ, Lee S, Kim KH, Yun BS, Lee SW, & Rho MC. 2020. Evaluation of nematicidal activity of *Streptomyces yatensis* KRA-28 against *Meloidogyne incognita*. *J. Microbiol. Biotechnol*. 30(5): 700–707. https://doi.org/10.4014/jmb.1908.08038

Pathak CS, Singh A, Despande A, & Sridar TT. 1986. Source of resistance to purple blotch in onion. *Veg. Sci.* 13(2): 300–303.

Priya RU, Sataraddi AR, & Kavitha TR. 2018. Studies on cultural and physiological variability of *Alternaria porri* (Ellis) Cif.–a causative of purple blotch of onion (*Allium cepa* L.). *Int. J. Curr. Microbiol. App. Sci*. 7(8): 3284–3291. https://doi.org/10.20546/ijcmas.2018.708.350

Risdiyanti RL, Rahmadhini N, Suryaminarsih P, & Mujoko T. 2023. Study of *Streptomyces* spp, to control purple blotch disease caused by *Alternaria porri* in shallot plant. *Cropsaver*: *Journal of Plant Protection.* 6(1): 56–61. https://doi.org/10.24198/cropsaver.v6i1.43647

Saeed I, Khan SH, Rasheed A, Jahangir MM, Jabbar A, Shaheen HMF, Din WU, & Mazhar K.

2021. Assessment of antagonistic potential of bacteria as biocontrol agent against Alternaria leaf spot of turnip. *Pak*. *J. Phytopathol*. 33(2): 401–409. https://doi.org/10.33866/phytopathol.033.02.0727

Shree RTN, Kalpana K, Ebenezar EG, Theradimani M, Thiruvudainambi S, & Vellaikumar S. 2020. Survey for purple blotch disease of onion (*Allium cepa* L.) in cited by *Alternaria porri* (Ellis) Cifin different regions of Tamil Nadu. Int. *J. Curr. Microbiol. App. Sci*. 9(12): 323–329. https://doi.org/10.20546/ijcmas.2020.912.042

Simko I & Piepho HP. 2012. The area under the disease progress stairs: calculation, advantage, and application. *Phytopathology*. 102(4): 381–389. https://doi.org/10.1094/PHYTO-07-11-0216

Song L, Jiang N, Wei S, Lan Z, & Pan L. 2020. Isolation, screening, and identification of actinomycetes with antifungal and enzyme activity assays against *Colletotrichum dematium* of *Sarcandra glabra. Mycobiology*. 48(1): 37–43. https://doi.org/10.1080/12298093.2020.1716604

Waluyo N & Sinaga R. 2015. *Bawang Merah yang diRilis oleh Balai Penelitian Sayuran* [*Shallots released by the Vegetable Research Institute*]. IPTEK Tanaman Sayuran No. 004. Bandung.

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(10): 5234–5242. https://doi.org/10.13057/biodiv/d241003

Wijayanti E, Nawangsih AA, & Tondok ET. 2021. Penapisan Aktinomiset rizosfer tanaman liliaceae sebagai agens pengendali hayati *Fusarium oxysporum* f.sp. *cepae.* [Screening of liliaceae rhizosphere actinomycetes as biological control agents of *Fusarium oxysporum* f.sp. *cepae*]. *Jurnal Fitopatologi Indonesesia*. 17(6): 225–232. https://doi.org/10.14692/jfi.17.6.225-232

Yadav PM, Rakholia KB, & Pawar KB. 2013. Evaluation of bioagents for management of the onion purple blotch and bulb yield loss assessment under field conditions. *The Bioscan* 8(4): 1295–1298.

Zou N, Zhou D, Chen Y, Lin P, Chen Y, Wang W, Xie J, & Wang M. 2021. A novel antifungal actinomycete *Streptomyces* sp. Strain H3-2 effectively controls banana Fusarium wilt. *Front. Microbiol.* 12: 706647. https://doi.org/10.3389/fmicb.2021.706647