



Antagonistic Test of Endophytic Bacteria Against *Fusarium oxysporum* f.sp. *cepae* Causes of Moler Disease on Shallots

Afri Wihayyu¹, Zurai Resti^{2*}, Eri Sulyanti², Darnetty², & Ujang Khairul²

¹Student on Department of Plant Protection, Faculty of Agriculture, Andalas University
²Department of Plant Protection, Faculty of Agriculture, Andalas University Kampus Unand Limau Manis, Padang 25163, Indonesia

*Corresponding Author: zurairesti@agr.unand.ac.id

Received April 13, 2024; revised June 13, 2024; accepted June 20, 2024

ABSTRACT

Fusarium oxysporum f.sp. *cepae* (FOCe) is a pathogenic fungus causing moler disease in shallots. One method of controlling this disease is by using endophytic bacteria. Endophytic bacteria are bacteria that live in plant tissues without causing disease symptoms. This research aims to obtain the most effective endophytic bacteria in inhibiting the growth of the pathogenic fungus FOCe. The study employs a Completely Randomized Design (CRD) consisting of 8 treatments with 3 replications each. The treatments include *Bacillus* sp. HI, *Bacillus* sp. SJI, *Bacillus cereus* P14, *Bacillus cereus* Se07, *Bacillus subtilis*, *Serratia marcescens* JB1E3, *Serratia marcescens* ULG1E4, and control. Tests conducted include the antibiosis test of endophytic bacterial cells using the dual culture method and the secondary metabolite test using media poisoning method. The observed parameters include inhibition zone, effectiveness of secondary metabolites, fresh weight, and dry weight of the FOCe fungus. All endophytic bacteria were capable of inhibiting the growth of FOCe. The most effective endophytic bacteria in inhibiting the growth of FOCe were found to be *Serratia marcescens* ULG1E4 and *Serratia marcescens* JB1E3. In the antibiosis test of endophytic bacterial cells, *Serratia marcescens* ULG1E4 exhibited an inhibition zone of 64.44%, while *Serratia marcescens* JB1E3 showed an inhibition zone of 61.11%. In the secondary metabolite compound test, the effectiveness values for *Serratia marcescens* ULG1E4 and *Serratia marcescens* JB1E3 were 95.31% and 95.03%, respectively.

Keywords: Antibiosis, Dual culture, Inhibition zone, Secondary metabolites

Uji Antagonis Bakteri Endofit terhadap *Fusarium oxysporum* f.sp. *cepae* Penyebab Penyakit Moler Pada Bawang Merah

ABSTRAK

Penelitian ini bertujuan untuk mendapatkan bakteri endofit yang paling efektif dalam menghambat pertumbuhan jamur patogen *Fusarium oxysporum* f.sp. *cepae* (FOCe) penyebab penyakit busuk moler pada bawang merah. Metode yang digunakan adalah Desain Acak Lengkap (CRD) dengan 8 perlakuan dan masing-masing perlakuan diulang sebanyak 3 kali. Perlakuan tersebut meliputi *Bacillus* sp. HI, *Bacillus* sp. SJI, *Bacillus cereus* P14, *Bacillus cereus* Se07, *Bacillus subtilis*, *Serratia marcescens* JB1E3, *Serratia marcescens* ULG1E4, dan kontrol. Pengujian dilakukan melalui uji antibiosis sel bakteri endofit menggunakan metode kultur ganda dan uji senyawa metabolit sekunder menggunakan metode pemakaian media. Parameter yang diamati meliputi zona hambat, efektivitas senyawa metabolit sekunder, berat segar, dan berat kering jamur FOCe. Hasil penelitian menunjukkan bahwa semua bakteri endofit mampu menghambat pertumbuhan FOCe. Bakteri endofit yang paling efektif dalam menghambat pertumbuhan FOCe adalah *Serratia marcescens* ULG1E4 dan *Serratia marcescens* JB1E3. Pada uji antibiosis sel bakteri endofit, *Serratia marcescens* ULG1E4 memiliki zona hambat sebesar 64,44%, sedangkan *Serratia marcescens* JB1E3 memiliki zona hambat sebesar 61,11%. Pada uji senyawa metabolit sekunder, nilai efektivitas untuk *Serratia marcescens* ULG1E4 dan *Serratia marcescens* JB1E3 masing-masing adalah 95,31% dan 95,03%. Temuan ini menunjukkan potensi penggunaan bakteri endofit *Serratia marcescens* ULG1E4 dan *Serratia marcescens* JB1E3 sebagai agen pengendali biologis yang efektif terhadap penyakit busuk moler pada bawang merah yang disebabkan oleh FOCe.

Kata Kunci: Antibiosis, Dual culture, Inhibition zone, Secondary metabolites

INTRODUCTION

Fusarium oxysporum f.sp. *cepae* (FOCe) is a pathogen that causes moler or basal rot disease in

shallots. This pathogen infects the roots and bulbs, with symptoms including the bending of leaves instead of growing upright due to the elongation of

pseudo-stems, pale green or yellowish leaf color, and wilting (Prakoso *et al.*, 2016). Plants become easily uprooted as a result of disrupted root growth and decay. This pathogen not only attacks onion plants in the field but can also infect bulbs during the storage process (Isniah & Widodo, 2015).

Control measures against moler disease include cultural control technique, the use of resistant varieties, and chemical control. However, the common practice among farmers is chemical control using synthetic fungicides. Continuous use of synthetic fungicides leads to the death of beneficial microorganisms, the development of pathogen resistance, and environmental damage. Therefore, environmentally friendly control methods are crucial as ecologically safe alternatives (Zulkipli *et al.*, 2018). One such alternative control method for moler disease is biological control. One of the biological agents that can be used is endophytic bacteria.

Endophytic bacteria are bacteria that live in plant tissues without causing disease symptoms (Ginting *et al.*, 2020). Endophytic bacteria employ two mechanisms to suppress the growth of plant pathogens, namely direct and indirect mechanisms. Direct mechanisms include antibiosis, competition, lytic enzyme secretion, siderophore production, indolic compound production, and phosphate solubilization. Antibiosis is a mechanism that hinders the development of plant pathogens by producing toxic antibiotic compounds (Leiwakabessy *et al.*, 2019). Competition is a competitive mechanism used by endophytic bacteria to obtain space and nutrients in preventing pathogens from entering host tissues (Martinuz *et al.*, 2012). Indirectly, endophytic bacteria can induce plant resistance.

The endophytic bacteria used in this study have been reported for their success in inhibiting the growth of pathogens. According to the research by Resti *et al.*, (2013) *Bacillus* sp. H1, *Bacillus cereus* P14, *Bacillus cereus* Se07, *Serratia marcescens* JB1E3, *Serratia marcescens* ULG1E4, isolated from healthy shallots tissues, were capable of suppressing the attack of *Xanthomonas axanopodis* pv *allii* (bacterial leaf blight disease) with a percentage of 65.06%. These endophytic bacteria also demonstrated inhibition against pathogenic fungi *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, and *Fusarium oxysporum* f.sp *cubense* Resti *et al.*, (2017). Resti *et al.*, (2022) stated that *Bacillus* sp. HI, *Bacillus* sp. SJI, *B. cereus* P14, *B. cereus* Se07, *S. marcescens* strain JB1E2, *S. marcescens* strain JB1E3, *S. marcescens* ULG1E2 and *S. marcescens* ULG1E were capable of inhibiting the growth of *Culvularia. oryzae* with inhibitory effects ranging between 58.50% to 75.00% and the percentage of inhibitory power of endophytic bacterial consortium against the growth of *C. oryzae* between 38.00% to 77.00%.

The aim of this study is to identify the most effective endophytic bacteria in inhibiting the growth

of FOCE, the causative agent of moler disease in shallots plants.

MATERIALS AND METHODS

This research employed a Completely Randomized Design (CRD) consisting of 8 treatments with 3 replications each. The treatments included *Bacillus* sp. HI, *Bacillus* sp. SJI, *B. cereus* P14, *B. cereus* Se07, *B. subtilis*, *S. marcescens* JB1E3, *S. marcescens* ULG1E4, and control. The testing involved the antibiosis test of endophytic bacterial cell suspensions using the dual culture method and the secondary metabolite test of endophytic bacteria using the media poisoning method.

Preparation of FOCE

Isolation and Purification of FOCE Fungus

The FOCE fungus was isolated from shallots plants affected by moler disease in Alahan Panjang, Solok Regency, West Sumatera. The isolation process involved cutting symptomatic and asymptomatic shallots plant leaves into approximately 1 x 1 cm pieces. These pieces were then surface-sterilized using sterile distilled water, 70% alcohol for 3 minutes, and rinsed with sterile distilled water. Subsequently, the sample pieces were cultured on Potao Dextrosa Agar (PDA) media and incubated for 7 days at room temperature (Latifah *et al.*, 2011). The grown fungus was transferred to PDA medium in sterile petri dishes and repeated until obtaining a pure culture of the fungus.

Pathogenicity Test

Pure cultures of FOCE successfully isolated from infected (diseased) plant tissues in the field were inoculated into healthy 4-week-old shallots by applying a 20 ml suspension (10^6 conidia/ml) of FOCE to the soil around the roots of shallots plants in polybags. Subsequently, they were incubated until symptoms appeared. A suspension of FOCE spores was made by adding 20 ml of sterile distilled water to a petri dish containing pure cultures of 7-day-old FOC. The spores were then released using a small sterile brush. The suspension was transferred to a test tube and homogenized using a vortex for approximately 1 minute, and then diluted to 10^{-2} . The density was then counted using a haemocytometer.

Preparation of Bacteria

Regeneration and Purification of Endophytic Bacteria

Endophytic bacterial isolates were obtained from the collection of Dr. Zurai Resti, SP, MP. Endophytic bacteria were regenerated using the quadrant streak method on Nutrient Agar (NA) media and incubated for 48 hours. Purification was carried out by taking a single bacterial colony using an inoculation needle, then streaking it on a sterile petri

dish containing NA media and incubating it for 48 hours.

Confirmation of Endophytic Bacteria

Gram staining was performed using the method described by Schaad *et al.*, (2001). A 3% KOH solution was dropped onto a glass slide, and a single colony of endophytic bacteria was placed. If mucus is present when the inoculation needle is lifted, the bacterium is classified as Gram-negative; otherwise, if no mucus is present, it is classified as Gram-positive. To determine whether endophytic bacteria are plant-pathogenic, a hypersensitivity test was conducted using the method described by Klement *et al.*, (1990). Endophytic bacteria were suspended in sterile distilled water, then diluted to a density of 10^8 cells/ml, homogenized with a vortex, and compared with a McFarland scale 8 solution. One ml of the bacterial suspension was infiltrated onto the lower surface of healthy *Mirabilis jalapa*. It was then covered with plastic and incubated for 48 hours. If the leaf area did not undergo necrosis, indicating a non-hypersensitive reaction, the isolate could be used for further testing. The expected reaction is the absence of necrosis.

Antagonistic Ability Test of Endophytic Bacteria against FOCE

Test of Endophytic Bacterial Cell Suspension Antibiosis Against FOCE

Antibiosis testing was conducted on a mixed NA and PDA (1:1) medium using the dual culture method (Sutariati & Wahab, 2010). FOCE fungal mycelium was taken and placed 3 cm from the edge of a 9 cm diameter petri dish. Each endophytic bacterium was streaked longitudinally at a distance of 3 cm from the edge of the petri dish in the opposite direction to the FOCE fungus. For the control, FOCE mycelium was placed at the edge of the petri dish without endophytic bacterial treatment. Observations were made every day from the first day after inoculation until the control petri dish was fully covered with fungus (7 days). The percentage of inhibition of endophytic bacteria against the FOCE pathogen was calculated using the formula:

$$DH = \frac{R1-R2}{R2} \times 100\% \quad \dots (1)$$

Description:

- DH = Inhibition rate
- R1 = Pathogen radius towards the edge of the petri dish in control
- R2 = Pathogen radius towards the endophytic bacteria

Test of Endophytic Bacterial Secondary Metabolite Against FOCE

The potential of secondary metabolite compounds was tested using the method described by

(Rustam, 2011). The production of endophytic bacterial supernatant refers to Resti *et al.*, (2017), where endophytic bacteria were cultured on NB media by taking a single colony and placing it in a 10 ml NB media culture bottle. Incubation was carried out on a shaker (150 rpm) at room temperature for 3x24 hours. Then, the endophytic bacterial suspension was centrifuged at a speed of 10.000 rpm for 10 minutes. The resulting supernatant was then filtered using a 0.22 μ m millipore filter. One ml of the supernatant was taken and placed into a reaction tube containing 9 ml PDA media, then homogenized with a vortex and immediately poured into a petri dish. After the media solidified, FOCE fungal mycelium was taken using a 5 mm cork borer and placed in the center of the media, then incubated at room temperature. As a control, 1 ml of sterile distilled water was added to a reaction tube containing 9 ml of PDA media. Observations were made every day from the first day after inoculation until the control petri dish was fully covered with fungus (7 days). Colony area calculations were made by outlining the colony area on millimeter plotting paper. To measure the effectiveness of each treatment against the FOCE fungus colony area, the formula is as follows:

$$E = \frac{Lk-Lp}{Lk} \times 100\% \quad \dots (2)$$

Description:

- E = Effectiveness
- Lk = Colony area of the pathogen in control
- Lp = Colony area of the pathogen in treatment

Fresh Weight and Dry Weight of FOCE Fungal Mycelium

The fresh weight of the fungal colony was calculated on the last day of observation by adding 10 ml of 1% HCL to each treatment petri dish, then heated until the agar dissolved. Next, it was filtered using a funnel and filter paper until there were no more water droplets left, then weighed using an analytical balance to obtain the initial weight. The fresh weight was then calculated by subtracting the initial weight from the weight of the filter paper. The percentage effectiveness of the wet weight was calculated using the formula:

$$E = \frac{BSK-BSP}{BSK} \times 100\% \quad \dots (3)$$

Description:

- E = Effectiveness
- BSK = Fresh weight of control
- BSP = Fresh weight of treatment

After obtaining the fresh weight of the fungus, it was then dried using an oven at 60°C until a constant weight was achieved. It was then weighed using an analytical balance. The effectiveness of the

dry weight of the fungus can be calculated using the formula:

$$E = \frac{BKK - BKP}{BKK} \times 100\% \quad \dots (4)$$

Description:

E = Effectiveness
BKK = Dry weight of control
BKP = Dry weight of treatment

Data Analysis

The obtained data were analyzed by analysis of variance and followed by Duncan's Multiple Range Test (DNMRT) at the 5% significance level.

RESULT AND DISCUSSIONS

Identification of Pathogenic Fungi

Identification of pathogenic fungi is carried out through macroscopic and microscopic

observations. Observations are conducted on the 7 days day after incubation. Macroscopic observation results show colonies of FOCE that are apund and cotton-like in appearance. The colonies are pinkish-red and the unside is also pinkish-red. Microscopically the macroconidia are crescent shaped with pointed ends, divided into 3-5 septate. Microconidia are oval with blunt ends and no septate. The hyphae are septate and branched. At the end of the hyphae there are rounds shaped chlamydospores.

Pathogenicity Test

The symptoms of moler disease in shallot plants appear 15 days after inoculation. Shallot leaves pale green and yellowish. Other symptoms include wilting and twisting of shallot leaves.

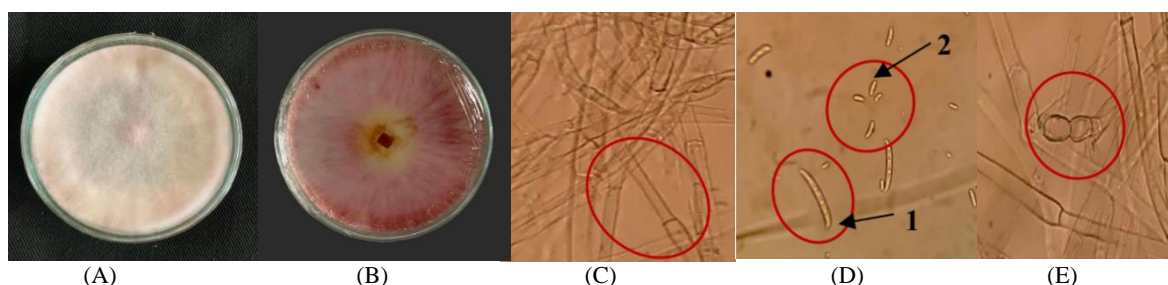


Figure 1. Morphology of FOCE fungus on PDA media 7 days after incubation. (A) Macroscopic characteristics of FOCE observed from the top, (B) Macroscopic characteristics of FOCE observed from the bottom, (C) Septate and branched hyphae (400x magnification), (D1) Macroconidia (400x magnification), (D2) Microconidia (400x magnification), (E) Chlamydospores (400x magnification).

Pathogenicity Test

The symptoms of moler disease in shallot plants appear 15 days after inoculation. Shallot leaves

pale green and yellowish. Other symptoms include wilting and twisting of shallot leaves

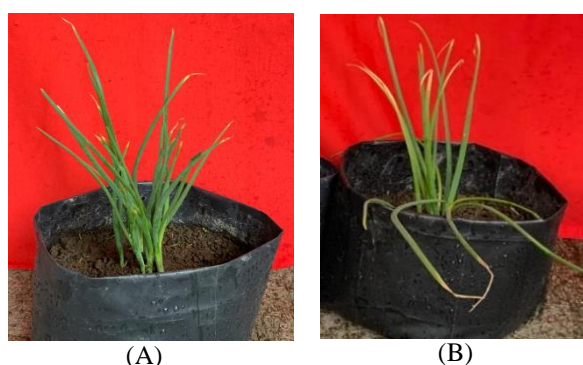


Figure 2. Symptoms of moler rot disease in shallots plants. (A) Shallot plant without inoculation (asymptomatic), (B) Symptoms of yellowing leaves, wilting, and bending after FOCE inoculation (15 days after inoculation)

Antibiosis Test of Endophytic Bacterial Cell Suspension Against FOCE

The results of the observation of the inhibitory ability of endophytic bacteria indicate that endophytic bacteria can inhibit the growth of FOCE through a competition mechanism, marked by the cessation of

FOCE fungal growth and its failure to grow maximally when cultured on the same medium as endophytic bacteria (Figure 2). The percentage inhibitory effect of endophytic bacteria against FOCE can be seen in Table 1.

Table 1. Percentage inhibitory effect of endophytic bacteria against FOCe (Test of Endophytic Bacterial Cell Suspension Antibiosis Against FOCe and Test of Endophytic Bacterial Cell Suspension Against FOCe)

Treatment	Inhibition rate (%)	Colony area of FOCe (cm)	Effectiveness (%)
<i>Serratia marcescens</i> ULG1E4	64,44 a	2,976 e	95,31
<i>Serratia marcescens</i> JB1E3	61,11 b	3,156 e	95,03
<i>Bacillus</i> sp. SJI	59,62 c	5,910 d	90,70
<i>Bacillus cereus</i> Se07	57,03 d	7,383 d	88,38
<i>Bacillus cereus</i> P14	55,18 d	13,02 b	79,50
<i>Bacillus</i> sp. HI	48,88 d	7,426 d	88,31
<i>Bacillus subtilis</i>	39,62 e	9,803 c	84,57
Control	00,00 e	63,58 a	00,00

Numbers followed by the same lowercase letters in the same column are not significantly different according to DMRT test at the 5% significance level.

In Table 1, it can be observed that the percentage of inhibitory effect indicates that all treatments of endophytic bacteria are capable of inhibiting the growth of FOCe. *S. marcescens* ULG1E4 is significantly different from *S. marcescens* JB1E3. *S. marcescens* ULG1E4 is significantly different from *Bacillus* sp. SJI, *B. cereus* Se07, *B. cereus* P14, *Bacillus* sp. HI, and *B. subtilis*.

Endophytic bacteria with high inhibitory ability are *S. marcescens* ULG1E4 (64.44%) and *S. marcescens* JB1E3 (61.11%). As shown in Figures 5A and 5B, treatment with endophytic bacteria *S. marcescens* ULG1E4 and *S. marcescens* JB1E3 resulted in the cessation of fungal growth, and the fungus was unable to grow maximally compared to fungal growth in the control (Figure 2H).

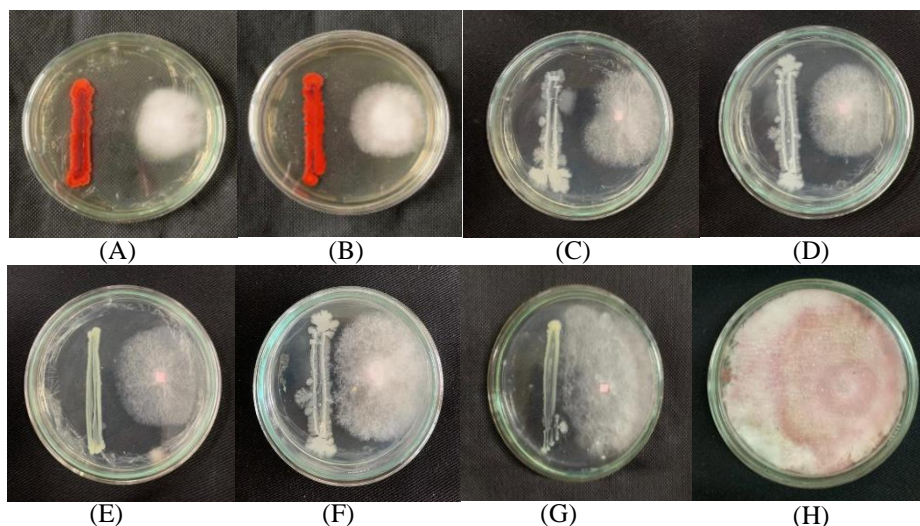


Figure 3. Test of Endophytic Bacterial Cell Suspension Antibiosis Against FOCe (7 days after inoculation) (A) *S. marcescens* ULG1E4 (B) *S. marcescens* JB1E3 (C) *Bacillus* sp. SJI (D) *B. cereus* Se07 (E) *B. cereus* P14 (F) *Bacillus* sp. HI (G) *B. subtilis* (H) Control.

Colonial Area of FOCe and the Effectiveness of Endophytic Bacterial Secondary Metabolites Against FOCe

The results of observations on the effectiveness of endophytic bacterial secondary metabolites in inhibiting FOCe indicate that the secondary metabolites produced by endophytic bacteria can suppress the growth of FOCe through an antibiosis mechanism, as marked by the inhibited growth of FOCe and its failure to grow and develop maximally. The effectiveness of endophytic bacterial secondary metabolites against FOCe can be seen in Table 1.

Table 1 shows that all endophytic bacterial treatments are capable of inhibiting the growth of FOCe with varying effectiveness. *Serratia*

marcescens ULG1E4 shows non-significant differences with *Serratia marcescens* JB1E3, and significantly differs from other endophytic bacterial treatments. *Bacillus* sp. SJI and *Bacillus cereus* Se07 show non-significant differences with *Bacillus* sp. HI. Meanwhile, *Bacillus subtilis* shows a significant difference from *Bacillus cereus* P14.

Based on the colonial area, *S. marcescens* ULG1E4 (95,31%) and *S. marcescens* JB1E3 (95,03) have the highest effectiveness compared to other treatments. As seen in Figure 3A, the fungal colony area is very small compared to the control, which has the largest colony area (Figure 3H). The growth of FOCe mycelium in the *S. marcescens* ULG1E4 treatment also appears thinner compared to other treatments and the control (Figure 3H).

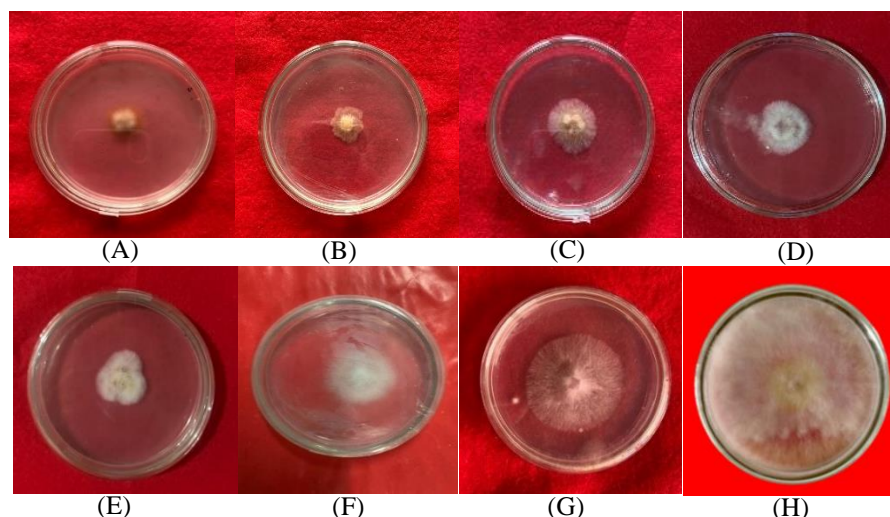


Figure 4. Test of Endophytic Bacterial Cell Suspension Against FOCe (7 days after inoculation) (A) *S. marcescens* ULG1E4 (B) *S. marcescens* JB1E3 (C) *Bacillus* sp. SJI (D) *B. cereus* Se07 (E) *Bacillus* sp. HI (F) *B. subtilis* (G) *B. cereus* P14 (H) Control

Fresh Weight and Dry Weight of FOCe

The influence of endophytic bacterial secondary metabolite treatments on the fresh weight and dry weight of FOCe shows significant differences

compared to the control. The resulting fresh weight ranged from 2.70 g to 4.28 g, and for dry weight, it ranged from 0.270 g to 0.413 g.

Table 2. Influence of endophytic bacterial secondary metabolites on the fresh weight and dry weight of FOCe

Treatment	Fresh weight (gr)	Effectiveness (%)	Dry weight (gr)	Effectiveness (%)
Control	5,900 a	00,00	0,550 a	00,00
<i>Bacillus cereus</i> P14	4,286 b	27,34	0,413 b	24,84
<i>Bacillus subtilis</i>	4,253 b	27,92	0,350 bc	36,36
<i>Bacillus</i> sp. HI	4,010 bc	32,03	0,333 cd	39,39
<i>Bacillus cereus</i> Se07	3,603 bcd	38,92	0,326 cd	40,60
<i>Bacillus</i> sp. SJI	3,423 bcd	41,97	0,313 cd	43,02
<i>Serratia marcescens</i> JB1E3	3,033 cd	48,53	0,290 cd	47,26
<i>Serratia marcescens</i> ULG1E4	2,700 d	54,23	0,270 d	50,90

Numbers followed by the same lowercase letters in the same column are not significantly different according to DMRT test at the 5% significance level.

Table 2 shows that, in terms of fresh weight, the treatments with *S. marcescens* ULG1E4 and *S. marcescens* JB1E3 show non-significant differences with *Bacillus* sp. SJI and *B. cereus* Se07, and significantly differ from *Bacillus* sp. HI, *B. subtilis*, and *B. cereus* P14. For dry weight, *S. marcescens* ULG1E4 and *S. marcescens* JB1E3 show non-significant differences with *Bacillus* sp. SJI, *B. cereus* Se07, and *Bacillus* sp. HI, and significantly differ from *B. subtilis* and *B. cereus* P14.

Discussion

All tested endophytic bacterial treatments exhibited antagonistic abilities in inhibiting the growth of FOCe. This is marked by the slower growth of the fungus compared to the control (Figure 3 and Figure 4). In the antibiosis test of endophytic bacterial cell suspensions against FOCe, the fungal growth was slower and suboptimal (Figure 5). This phenomenon occurs due to the presence of antibiotic compounds leading to competition between the fungus and

endophytic bacteria for space and nutrients. This statement aligns with (Astuti, 2008) suggesting that bacterial inhibition of fungi involves various inhibitory mechanisms, including competition for space and nutrients.

In the antibiosis test, the inhibition percentages ranged from 39.62% to 64.44% (Table 1). The endophytic bacterial treatments with the highest inhibitory effect were *S. marcescens* ULG1E4 (64,44%) and *S. marcescens* JB1E3 (61,11%). According to Prastya *et al.*, (2014) the categories for bacterial endophyte inhibition percentage are strong (>40%); moderate (40%<n>30%); weak (<30%), and no inhibitory ability (0%). Based on the research results, all endophytic bacteria demonstrated strong abilities in inhibiting FOCe, except for *B. subtilis*, which exhibited moderate inhibition against FOCe.

The ability of endophytic bacteria to inhibit FOCe growth is also demonstrated in the secondary metabolite effectiveness test, with effectiveness ranging from 79.50% to 95.31% (Table 2). The

endophytic bacterial treatments with the highest effectiveness were *S. marcescens* ULG1E4 (95.31%) and *S. marcescens* JB1E3 (95.03%). In the secondary metabolite effectiveness test, all endophytic bacterial treatments were capable of inhibiting FOCE growth. The secondary metabolite compounds produced are produce enzymes lipase, protoase, salicylic acid, and siderophores.

This is attributed to endophytic bacteria producing secondary metabolite compounds. The administration of secondary metabolites in the media also influenced the fresh weight and dry weight of FOCE (Table 2). The reduction in the fungal colony's area is a result of the antimicrobial compounds present in the endophytic bacterial supernatant.

In the results of the observations on the inhibitory activity of endophytic bacteria and the effectiveness of secondary metabolites, varied values of inhibitory activity and effectiveness were obtained. This diversity is presumed to stem from the distinct content of each endophytic bacterium among isolates, with variations in the produced compounds. This notion finds support in the opinions of Pitasari & Ali, (2018), asserting that differences in the types and quantities of compounds produced by each isolate play a role in inhibiting pathogens. Resti *et al.*, (2017) stated that the tested endophytic bacteria (*Bacillus* sp., *Bacillus* sp. SJI, *B. cereus* Se07, *B. cereus* P14) could produce enzymes, salicylic acid, and siderophores. *Bacillus* sp. SJI, and *B. cereus* Se07 could produce salicylic acid, lipase, and protease. Different capabilities were exhibited by *B. cereus* P14, which could not produce siderophores but could generate salicylic acid, lipase, and protease. *S. marcescens* could solubilize phosphate and produce siderophores, lipase, and protease.

In this study, the endophytic bacteria demonstrating superior abilities in suppressing FOCE were *S. marcescens* ULG1E4 and *S. marcescens* JB1E3, with *S. marcescens* exhibiting higher inhibitory percentages and effectiveness compared to other treatments. *S. marcescens* produced antifungal compounds, and the strain used in this research could produce lipase and protease capable of lysing the fungal cell walls. Nasiroh *et al.*, (2015) reported that *S. marcescens* produces prodigiosin, which has antifungal properties. This is corroborated by the study of Febbiyanti, (2012) indicating that *Serratia* sp. has a better inhibitory effect on fungal colony growth compared to other bacterial inhibitors.

CONCLUSIONS

All tested endophytic bacteria could inhibit the growth of the FOCE fungus. The endophytic bacteria that excelled in inhibiting FOCE growth were *S. marcescens* ULG1E4 and *S. marcescens* JB1E3. In the antibiosis test with endophytic bacterial cell suspensions, *S. marcescens* ULG1E4 exhibited an inhibitory activity of 64.44%, and *S. marcescens* JB1E3 showed 61.11% inhibition. In the secondary

metabolite effectiveness test, *S. marcescens* ULG1E4 demonstrated an effectiveness of 95.31%, and *S. marcescens* JB1E3 showed an effectiveness of 95.03%.

Acknowledgement

The Faculty of Agriculture at Andalas University provided funding for this study under Research Contract number: 6/PL/SPK/PNP/FAPERTA-Unand/2023 for the fiscal year 2023.

REFERENCES

- Astuti RP. (2008). Rizobakteria *Bacillus* sp. Asal Tanah Rizosfer Kedelai Yang Berpotensi Sebagai Pemacu Pertumbuhan Tanaman. Tesis. Institut Pertanian Bogor.
- Febbiyanti. (2012). Penapisan Jamur dan Bakteri Antagonis Terhadap Jamur Akar Putih (*Rigidoporus microporus*) Dari Rizosfer Tanaman Lidah Mertua (*Sansevieria trifasciata* Prain). *Jurnal Penelitian Karet*, 30(1), 1–11.
- Ginting L, & Kusdiyantini E. (2020). Isolasi Bakteri Endofit Tanaman Pepaya (*Carica papaya* L.) Dan Uji Aktivitas Enzim Amilase. *Berkala Bioteknologi*, 3(2), 1–7. <https://ejournal2.undip.ac.id/index.php/bb/article/view/9654>.
- Isniah & Widodo. (2015). Eksplorasi Fusarium Nonpatogen untuk Pengendalian Penyakit Busuk Pangkal pada Bawang Merah. *Jurnal Fitopatologi Indonsia*, 11(1), 14–22. <https://doi.org/10.14692/jfi.11.1.14>.
- Klement Z, Rudolph K, & Sand, D.C. (1990). Methode in Phytobacteriology. Academic Kiado.
- Latifah A, Kustantinah, & Soesanto L. (2011). Pemanfaatan Beberapa Isolat *Trichoderma harzianum* Sebagai Agensia Penegndali Hayati Penyakit Layu Fusarium Pada Bawang Merah In Planta. *Eugenia*, 17(2), 86–95. https://www.researchgate.net/publication/256981452_pemanfaatan_beberapa_isolat_Trichoderma_harzianum_sebagai_agensia_pengendali_hayati_penyakit_layu_fusarium_pada_bawang_merah_in_planta.
- Leiwakabessy C, Yatni C, Uruilal C, Ririheana RE, & Rimalatu FJ. (2019). Kemampuan Antagonis Bakteri Endofit Asal Tanaman Sagu (*Metroxylon* spp.) Dalam Menekan Pertumbuhan *Rhizoctonia solani* Kuhn. Secara In Vitro. *Agrinimal*, 7(2), 48–52. DOI:10.30598/ajitt.2019.7.2.48-52.
- Martinuz A, Schouten A, & Sikora RA. (2012). Systemically Induced Resistance and Microbial Competitive Exclusion: Implications on Biological Control. *Phytopathology*, 102(3), 260–266. DOI: 10.1094/PHYTO-04-11-0120
- Nasiroh U, Isnawati, & Trimulyono G. (2015). Aktivitas Antifungi *Serratia marcescens* Terhadap *Alternaria porri* Penyebab Penyakit

- Bercak Ungu Secara *In Vitro*. *Lentera Bio: Berkala Ilmiah Biologi*, 4(1), 13–18. <https://ejournal.unesa.ac.id/index.php/lenterabi/article/view/10883>.
- Pitasari A, & Ali M. (2018). Isolasi Dan Uji Antagonis Bakteri Endofit Dari Tanaman Bawang Merah (*Allium ascalonicum* L.) Terhadap Jamur *Alternaria porii* Ellis Cif. *JOM Faperta*, 5(1), 1–12.
- Prakoso Ega B, Wiyatingsih S, & Nirwanto H. (2016). Uji Ketahanan Berbagai Kultivar Bawang Merah (*Allium ascalonicum*) Terhadap Infeksi Penyakit Moler (*Fusarium oxysporum* f.sp.cepae). *Plumula*, 5(1), 10-20. <https://www.semanticscholar.org/paper/uji-ketahanan-berbagai-kultivar-bawang-merah-moler-Prakoso-Wiyatingsih/53d36057062b1261150e5c32736f28c60754105d>.
- Prastya ME, Supriyadi A, & Kusdiyantini E. (2014). Eksplorasi Rhizobakteri Indigenous Tanaman Cabai Rawit (*Capsicum frutescens* Linn.) Dari Pertanian Semi Organik Desa Batur Kabupaten Semarang Sebagai Agen Hayati Pengendali Pertumbuhan Jamur *Fusarium oxysporum* f.sp. capsici. *Jurnal Biologi*, 3(3), 18–31. <https://ejournal3.undip.ac.id/index.php/biologi/article/view/19449>.
- Resti Z, Habazar T, Putra DP, & Nasrun. (2013). Screening and Identification of Endophytic Bacteria to Control Bacterial Leaf Blight Disease on Shallot. *Jurnal HPT Tropika*, 13(2), 167–178.
- Resti Z, Reflin, & Suardi G. (2017). Antagonistic and Plant Growth Promoting Potentials of Indigenous Endophytic Bacteria of Shallots. *International Journal of Science and Applied Technology*, 2(2), 42–49.
- Resti Z, & Martinius YL. (2022). Kemampuan Antagonis Bakteri Endofit dan Konsorsiumnya terhadap Pertumbuhan Jamur *Culvularia oryzae* Bugnic. *Jurnal Proteksi Tanaman*, 6(1), 35–43. DOI:10.25077/jpt.6.1.34-42.2022.
- Rustam. 2011. Potensi Bakteri Penghasil Metabolit Sekunder untuk Mengendalikan Penyakit Hawar Pelepah Padi yang Disebabkan oleh *Rhizoctonia solani* Kunh. Sekolah Pascasarjana Institut Pertanian Bogor.
- Schaad NW, Jones JB, & Chun W. (2001). Laboratory Guide for Identification of Plant Pathology Bacteria. The American Phytopathology Society.
- Sutariati GAK, & Wahab DA. (2010). Isolasi dan Uji Kemampuan Rizobakteri Indigenous sebagai Agensia Pengendali Hayati Penyakit pada Tanaman Cabai. *Jurnal Hortikultura*, 20(1), 86-95. DOI: 10.21082/jhort.v20n1.2010.p%p
- Zulkipli S, Marsuni Y, & Rosa Helda O. (2018). Uji Lapangan Beberapa Pestisida Nabati Untuk Menekan Perkembangan Penyakit Antraknosa Pada Tanaman Cabai Besar. *Proteksi Tanaman Tropika*, 1(2), 32–34. DOI:10.20527/JPT.V1I2.14.

