ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

JBIO: jurnal biosains (the journal of biosciences)
http://jurnal.unimed.ac.id/2012/index.php/biosains
email :jbiosains@unimed.ac.id
Universitas Negeri Medan

ANTAGONIST TEST OF THE ENDOPHYTIC BACTERIA OF ARABICA COFFEE ROOT (*Coffea arabica L.*) AGAINST PATHOGENIC FUNGI *CERCOSPORA COFFEICOLA* BERK. & COOKE CAUSES OF LEAF SPOT DISEASE

Cindy Febriyanti[1]*, Kabul Warsito[2], Andi Setiawan[3]
[123] Department Of Agrotecnology, Universitas Pembangunan Panca Budi, Medan Indonesia
* Corresponding author : kabulwarsito@dosen.pancabudi.ac.id

Received : May, 2025
Revised :July, 2025
Accepted :August, 2025

First Publish Online :
August, 28, 2025

Keywords : Coffea Arabica, Cercospora coffeicola, Biological control, Sustainable coffee production

ABSTRACT

The Arabica coffee plant (*Coffea arabica* L.) has an important role in Indonesia's national economy, both as a source of foreign exchange and income for around 1.5 million farmers. Indonesia's coffee production in 2021 reached 774.6 thousand tons, an increase of 2.75% compared to the previous year. However, coffee productivity often decreases due to attacks by plant pest organisms, especially leaf spot disease caused by the pathogenic fungus *Cercospora coffeicola*. The disease causes reddish-brown patches on the leaves and fruit damage, which can reduce yields by up to 30-50%. Until now, no resistant coffee cultivars have been found, so overall control is carried out with cultivation techniques and synthetic fungicides, which have the potential to cause negative environmental impacts and pathogen resistance. Alternatively, the use of endophytic bacteria as biological control agents shows great potential in inhibiting the growth of *C. coffeicola*. The result obtained showed variations in the level of antagonist activity between isolates. Sp 4 isolates showed the highest average antagonist activity (28.97%), followed by sp6 (18.93%) and sp3 (13.67%). This indicates that sp 4 has the potential to be an effective biological control agent.This study aims to test the ability of Arabica coffee root endophytic bacteria to control the pathogen biologically. The results are expected to provide environmentally friendly leafspot disease control solutions and support healthy and sustainable coffee production.

Introduction

Coffee plants (*Coffea arabica* L.) have an important meaning for national coffee plantations not only as a source of foreign exchange for the country but also as a source of income for one and a half million Indonesians (BPS, 2022). Indonesia's coffee production will reach 774.6 thousand tons in 2021. This amount increased by 2.75% from the previous year which was 753.9 thousand tons. The coffee market opportunities are vast because most of the coffee production is exported abroad and the rest is produced domestically (Purba et al., 2023). However, the

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

productivity of coffee plants often decreases due to various factors, one of which is the attack of plant pest organisms (OPT), especially pathogens that cause disease (DKPP, 2022).

One of the diseases that attack coffee plants is leaf spot disease on coffee plants caused by the pathogenic fungus *Cercospora coffeicola* Berk. & Cooke is one of the main obstacles in coffee cultivation that can significantly reduce yield and production quality. This fungal infection is characterized by the appearance of reddish-brown to dark brown round patches on the leaves, which can cause leaf loss and damage to coffee beans so as to reduce the quality of coffee beans (Farahdilla, 2018). Attacks of this disease can result in yield losses of up to 30-50% if not properly controlled (Hartati et al, 2024).

Cultivars of coffee plants that are resistant to the pathogen *Cercospora coffeicola* have not been found so far, so disease control caused by this pathogen is generally carried out by technical culture and spraying of synthetic fungicides (Harni et al. 2015; Vasco et al. 2015; Tembo 2020). Intensive control using synthetic chemicals will cause negative impacts such as pathogens becoming resistant, damage to non-target organisms, and damage to the environment. One of the control alternatives to reduce the negative impact of synthetic fungicides is with biocontrol agents (Rani et al. 2021; Ayilara et al. 2023).

One of the most recent innovations in organic farming is using endophytic bacteria as potential candidates to be developed (Gusmaini et al., 2019). Endophytic bacteria are known to have the potential as biological control agents due to their ability to produce antimicrobial compounds that can inhibit the growth of pathogens, including fungi that cause leaf spots (Farahdilla, 2018). Recent research shows that endophytic bacteria from coffee plants have great potential as biological control agents (Asad et al., 2023). Studies conducted by various researchers show that endophytic bacterial isolates from coffee can inhibit the growth of various fungal pathogens with varying inhibition percentages (Lu et al., 2022). Research on the antagonist test of coffee root endophytic bacteria against *Cercospora coffeicola* is still limited, so this study is important to identify and develop effective and sustainable biological control agents.

Thus, this study aims to test the ability of Arabica coffee root endophytic bacteria in inhibiting the growth of the pathogenic fungus *Cercospora coffeicola* as an effort to control leaf spot disease biologically. The results of the research are expected to provide an alternative disease control solution that is environmentally friendly and supports healthy and sustainable coffee production.

Materials and Methods

Location and Time of Research

This research will be conducted from January until July 2025. In vitro testing will be conducted at the Microbiology Laboratory of the Faculty of Science and Technology, Panca Budi Development University, Medan.

Tools and materials

The tools used in this study are petri dishes, test tubes, test tube racks, measuring cups, chemical beaks, erlenmeyer, autoclaves, ovens, spatulas, ose needles, calipers, syringes, incubators, hot plates, mixing rods, analytical balances, sprayers, laminar water flow, glass bottles, aluminum foil, cotton, knives, polybags, bunsen, microscopes, cameras and shakers. The ingredients used in this study were 70% alcohol, chlorine solution, spirits, CaCl2, NaCl 0.9%, coffee root samples, *Cercospora coffeicola* fungal isolate. Nutrient Agar (NA) media, Potato Dextrose Agar (PDA) media, Broth Nutrient Media (NB), 5.25% sodium hypochlorite solution.

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

Data Collection Techniques

This type of study is a descriptive method with experiments in the laboratory with macroscopic and microscopic observations using a NON Factorial Complete Random Design (RAL) to test the ability of coffee root endophytic bacterial isolate antagonists against the pathogenic fungus *Cercospora coffeicola* in vitro.

Data Analysis

IAA Test for Endophytic Bacteria

Testing of indole-3-acetic acid (IAA) production by endophytic bacteria was carried out using a modified method from previous research. In accordance with the procedure described by Mariana, Irianto, and Budisantoso (2023), endophytic bacterial isolates were first cultured in Nutrient Broth (NB) liquid media with pH variations of 6.5, 7, and 7.5. The cultures were then incubated using an incubator shaker for a period of 24, 48, and 62 hours to ensure optimal growth. After incubation, the culture is centrified to separate the bacterial cells from the supernatant. Next, Salkowski reagents are added to the supernatant, which reacts resulting in a discoloration to red or pink as an indication of the presence of IAA.

IAA levels were measured by incubating the mixture in dark conditions for 30 minutes to maximize the color reaction. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 530 nm. The concentration of IAA produced by endophytic bacteria is then determined by comparing the absorbance value against the pre-made standard IAA curve (Mariana et al., 2023).

Growth Rate of Endophytic Bacteria

Series dilution and pour plate methods. The procedure begins by taking 1 to 4 bacterial culture oses that have grown on Nutrient Agar (NA) media in the form of slanted agar. The culture is then put into a 0.9% NaCl solution and homogenized using a vortex to obtain an even bacterial suspension. Furthermore, the turbidity of the suspension was adjusted to the McFarland scale standard of 0.5 to ensure consistent bacterial concentrations (Rahman, Ahmad, & Malik, 2023; Kim & Lee, 2022).

Series dilution was performed by adding 10 µL of bacterial suspension to 990 µL sterile aquades and homogenized with a vortex, resulting in a 10^{-2} dilution. The serial dilution process is continued by transferring 10 µL of suspension from the previous tube to a new tube containing 990 µL of sterile aquades, then re-homogenized until it reaches a dilution of 10^{-6} (Gomes, Silva, & Oliveira, 2021; Patel & Singh, 2020).

From the 10^{-6} dilution, as much as 100 µL of suspension is taken and inoculated into the NA medium using the pour plate method. The culture plates are then incubated for 24 hours at room temperature in an inverted position to prevent condensation of water from falling onto the surface of the medium (Zhang et al., 2023). After the incubation period, the growing colonies are counted and the number of bacterial cells is expressed in units of Colony Forming Units per mL (CFU/mL) using the formula:

CFU⁄ml= amount of colony × dilution factor ×10

This method allows for accurate estimation of bacterial populations in samples and has been widely applied in various microbiological and environmental studies (Rahman et al., 2023; Kim & Lee, 2022)

Test of endophytic bacterial antagonists with the pathogen *Cercospora coffeicola* Berk. & Cooke

Antagonist test of coffee plant root endophytic bacteria with *Cercospora coffeicola* pathogen using the dual culture assay method (Marsaoli et al., 2019). Endophytic bacteria are inoculated in the

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

center of the cup, while the fungus *Cercospora coffeicola* is placed in one of the quadrants of the petri dish. This experiment was carried out with three repetitions to ensure consistency of results. Observation of fungal growth is carried out periodically from the second to the fourteenth day after inoculation.

The radial growth inhibition percentage (PIRG) of the fungus was calculated to assess the effectiveness of the inhibition administered by endophytic bacteria. The PIRG calculation uses the following formula:

$$PIRG = \frac{R_1 - R_2}{R_1} x100\%$$

Where R_1 is the diameter of fungal growth in the control medium without the presence of antagonistic bacteria, and R_2 is the diameter of fungal growth in the culture media along with the endophytic bacteria. This method allows for a quantitative evaluation of the ability of endophytic bacteria to inhibit the growth of fungal pathogens in vitro (Rahman et al., 2021; Sari & Putra, 2022).

Results and Discussion

Growth rate of *Cercospora coffeicola*

Table 1. Growth rate of *Cercospora coffeicola*

repetition	12 hour	24 hour	36 hour	48 hour	60 hour	72 hour
u1	0mm	0 mm	30,0 mm	55,4 mm	79,2 mm	90 mm
u2	0mm	0 mm	30,5 mm	47,3 mm	80,5 mm	90 mm
u3	0mm	0 mm	46,0 mm	59,7 mm	61,6 mm	90 mm

Fungal growth data obtained over 72 hours showed a distinctive and dynamic growth pattern in all three samples (u1, u2, u3). In the first 12 and 24 hours, no significant growth occurred, with the mushroom diameter remaining 0 mm, indicating an initiation or initial adaptation phase. Starting at 36 hours, a significant increase in growth was observed, with diameters becoming measurable: u1 and u2 showed growth of approximately 30 mm, while u3 accelerated, reaching 46 mm. At 48 hours, the increase continued, with u3 maintaining the highest growth rate (59.7 mm), while u1 and u2 grew to approximately 47–55 mm. At 60 hours, u1 and u2 accelerated significantly, reaching approximately 79–81 mm, while u3 slowed slightly to 61.6 mm. Interestingly, at 72 hours, all samples reached a maximum growth of 90 mm, indicating a saturation phase or maximum growth limit under these experimental conditions.

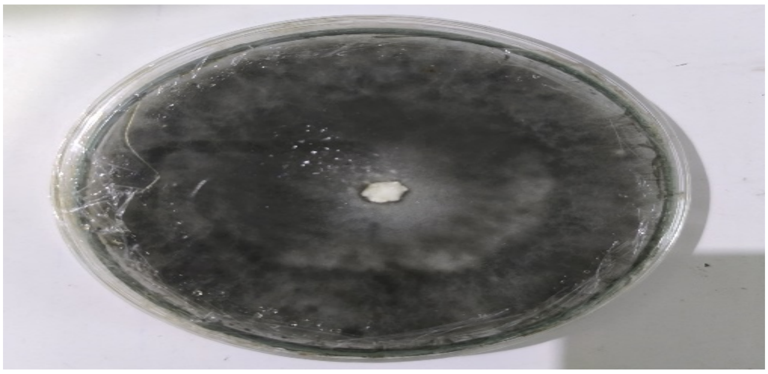

Figure 1. isolate the fungus cercospora coffeicola in a petri dish when the fungal mycelium has grown

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

Isolation and Characterization of Endophytic Bacteria

Bacteria are characterized based on the morphology of the colony, the shape of the colony, height, edges and color. Similar research conducted by Zuraidah et al. (2020) and Aryaldi et al. (2020) also found that endophytic bacterial isolates from coffee and tea plants showed a wide diversity of colony morphology, ranging from circular, irregular, to filamentous shapes, as well as colony color variations from white, cream, to yellowish. This morphological variation is an important basis in the selection of isolates for further applications, for example as biological control agents or plant growth promoters.

From the results of endophytic bacterial isolation obtained from the roots of the coffee plant (*Coffea arabica* L.), six isolate of endophytic bacteria with diverse colony morphological characteristics were identified. Each isolate showed differences in the shape, margin, elevation, and colour of the colony. The morphological characteristics of endophytic bacterial colonies can be seen in table 1.

Table 2. Morphological Characteristics of Endophytic Bacterial Colonies of Coffee Plants.

Isolat	Colony Morphology			
	Shape	Margin	Elevation	Color
Sp1	Punctiform	Filamentous	Flat	Yellowish
Sp2	Circular	Circular	Flat	Cream
Sp3	Circular	Filamentous	Flat	Yellowish
Sp4	Irreguler	Undulate	Flat	White
Sp5	Filamentous	Filamentous	Flat	White
Sp6	Irreguler	Irreguler	Flat	White

Bacterial Growth Rate

Counting the number of endophytic bacterial cells from six isolates observed at time intervals of 6, 12, 18, 24, 36, and 48 hours showed varying growth patterns between isolates. In the early stages of incubation, which is between 6 to 12 hours, the bacterial cell population is still relatively low, which indicates that the bacteria are in the adaptation phase or lag phase. This condition is in line with findings (Pratiwi et al. 2022) which report that coffee endophytic bacteria need time to adapt before entering the active growth phase.

Entering the incubation period of 18 to 24 hours, almost all isolates showed a very significant increase in the number of cells, signaling a transition to the exponential growth phase (log phase). For example, Sp 5 isolates experienced a spike in cell count from 9.3×10^8 CFU/ml at 12 hours to 8.3×10^{10} CFU/ml at 18 hours, and continued to increase to 6×10^{11} CFU/ml at 24 hours. This pattern is consistent with the results of studies (Nugroho et al., 2021) and (Sari et al., 2020) which also observed a sharp logarithmic phase in the endophytic bacteria of coffee plants after an incubation period of about 18–24 hours.

Peak growth generally occurs between 24 to 36 hours, where sp ome isolates such as SpAt4 and Sp 5 reach the highest cell count, at 4.6×10^{14} and 1.38×10^{15} CFU/ml at 36 hours, respectively. After that, at 48-hour observation, the number of bacterial cells tends to stabilize or begin to decrease, indicating the entry of the isolates into the stationary phase. This is in accordance with the growth pattern of endophytic bacteria also reported by (Zhang et al., 2023) in isolates from tea plants and (Putra et al., 2021) in cocoa plant endophytic bacteria, which exhibit a stationary phase after an exponential phase lasting for 36 hours.

These differences in growth patterns between isolates illustrate the variation in adaptability and proliferation of endophytic

JBIO : journal of biosciences
(the journal of biosceences)
Vol. 11 No. 2. August 2025
ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

bacteria isolated from coffee roots. Such variations are critical to identifying the isolates that have the best potential as biocontrol agents or plant growth promoters in agricultural biotechnology applications. These findings reinforce the understanding that the growth dynamics of endophytic bacteria are strongly influenced by the genetic characteristics of the isolates as well as the conditions of the incubation environment (Pratiwi et al., 2022; Nugroho et al., 2021; Sari et al., 2020; Zhang et al., 2023; Putra et al., 2021).

Table 3**.** Growth rate of coffee plant root endophytic bacteria

Isolat	Jumlah Sel (CFU/ml)					
	6 jam	12 jam	18 jam	24 jam	36 jam	48 jam
Sp1	103×10^5	19×10^7	11×10^9	63×10^{11}	34×10^{13}	79×10^{15}
Sp2	$2 \text{ x } 10^5$	$12 \text{ x } 10^7$	$169 \text{ x } 10^9$	$2 \text{ x } 10^{11}$	$18 \text{ x } 10^{13}$	$47 \text{ x } 10^{15}$
Sp3	$47 \text{ x } 10^5$	$40 \text{ x } 10^7$	$39 \text{ x } 10^9$	$19 \text{ x } 10^{11}$	$21 \text{ x } 10^{13}$	$63 \text{ x } 10^{15}$
Sp4	$223 \text{ x } 10^5$	$159 \text{ x } 10^7$	$11 \text{ x } 10^9$	$17 \text{ x } 10^{11}$	$46 \text{ x } 10^{13}$	$143 \text{ x } 10^{15}$
Sp5	$99 \text{ x } 10^5$	$93 \text{ x } 10^7$	$83\text{x } 10^9$	$6 \text{ x } 10^{11}$	$138 \text{ x } 10^{13}$	$270 \text{ x } 10^{15}$
Sp6	$267 \text{ x } 10^5$	$256 \text{ x } 10^7$	$66 \text{ x } 10^9$	$35 \text{ x } 10^{11}$	$15 \text{ x } 10^{13}$	$193 \text{ x } 10^{15}$

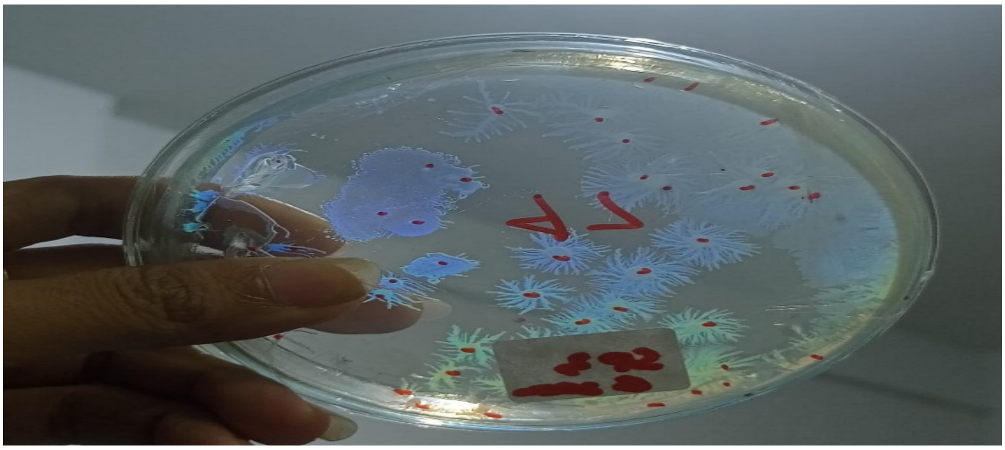

Figure.2 image of a bacterial colony in a petri dish during a bacterial growth rate test

IAA Test for Endophytic Bacteria

The increase in IAA production at pH 7.5 can be attributed to the optimal conditions of enzyme activity involved in IAA biosynthesis, which works more effectively at neutral to slightly alkaline pH (Kumar, S., & Sharma, P. 2022). Previous research has also shown that endophytic bacterial isolates have the highest IAA production capacity at that pH range, which plays an important role in stimulating plant growth through hormonal mechanisms (Patel et al., 2021).

In addition, the 72 hour incubation provides sufficient time for bacteria to develop and produce secondary metabolites such as IAA optimally. Incubation that is too short or too long can lead to decreased production due to less than ideal growth phases or degradation of metabolites (Zhao et al., 2023; Kumar & Sharma, 2022).

The significant increase in IAA production at pH 6.5 compared to pH 7.0 and 7.5 confirms the importance of regulating culture conditions to maximize the potential of endophytic bacteria as a plant growth bio-stimulating agent. These findings support the development of microorganism-based biofertilizers to support sustainable agriculture (Singh et al., 2020).

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

Testing of indole-3-acetic acid (IAA) production by endophytic bacterial isolates was carried out by treating pH variations of culture media at values 6.5, 7.0, and 7.5 and incubation for 72 hours. IAA concentration measurements were performed using spectrophotometry techniques at a wavelength of 530 nm after a reaction with Salkowski reagents (Patel et al., 2021).

This curve is used as a reference to determine the IAA concentration of the sample based on the measured absorbance value. The results of absorbance measurement and IAA concentration calculation in endophytic bacterial cultures with variations in the pH of the culture medium showed an increase in IAA production along with the increase in pH of the media, namely

Table 4. IAA production with pH variation

Perlakuan	abs	x
6,5	1,376	281,04
7	1,389	283,64
7,5	1,429	291,64

These data indicate that the production of IAA by endophytic bacteria increases with an increase in the pH of the medium from 6.5 to 7.0, where pH 7.5 results in the highest concentration of IAA after 72 hours of incubation. These findings confirm that the pH of the media is an important factor influencing the metabolism and biosynthesis activity of plant hormones by endophytic bacteria (Kumar & Sharma, 2022; Singh et al., 2020)

Antagonist Test Observation Results

The results of the endophytic bacterial antagonist test from coffee roots against the fungus *Cercospora coffeicola* showed the existence of an inhibition zone which showed that endophytic bacteria were able to inhibit the growth of the fungus *Cercospora coffeicola*. The results of quantitative observations can be seen in the following table

Table 5. Diameter of the inhibition zone test Endophytic bacterial antagonists against the fungus *Cercospora coffeicola*

Isolates	deuteronomy		
	I	II	III
SP1	8,3	9,9	14,4
SP2	7,1	5,2	17,3
SP3	20,5	20,5	0
SP4	32	31,7	23,2
SP5	8,8	5,4	4
SP6	19,6	18	19,2

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

.

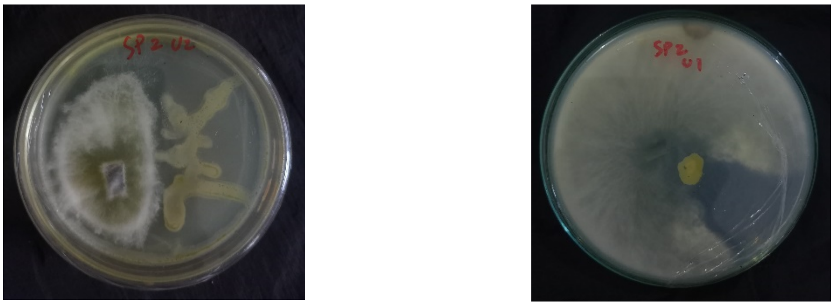

Figure 3. Growth test of coffee root endophytic antagonists against the fungus *Cercospora coffeicola.*

The results of the evaluation of antagonistic activity from six isolates (sp1–sp 6) were based on the results of three repeat times. The data obtained showed variations in the level of antagonist activity between isolates. Sp 4 isolates showed the highest average antagonist activity (28.97%), followed by sp6 (18.93%) and sp3 (13.67%). This indicates that sp 4 has the potential to be an effective biological control agent. Sp 5 isolate has the lowest activity (6.07%), making it less recommended for further application.

Antagonist testing is an important method in the filtration of biological control agents, particularly in inhibiting the growth of plant pathogens. The effectiveness of isolates can be influenced by the production ability of antimicrobial compounds, spatial and nutrient competition, and specific interactions between isolates and pathogens (Suryanto, D., et al. 2021). The results of this study are in line with previous reports that isolates with a high average percentage of inhibition and low standard deviation are preferred to be developed as biocontrol agents.

Recent research emphasizes the importance of isolates selection based on the stability and consistency of antagonist activity in various replicas, as high variability can indicate the influence of environmental factors or less stable physiological adaptation. Therefore, sp4 and sp6 isolates deserve priority for further testing in the field.

Direct Application to Plants

The control of leaf spot disease in coffee plants caused by the pathogen *Cercospora coffeicola* is a crucial issue in coffee cultivation given its significant impact on plant quality and quantity. In the direct application of endophytic resistance to the disease, observation of infected coffee plants provides an indication of the effectiveness of treatment. Based on observation data, in the first week, four plants showed symptoms of leaf spot disease caused by *Cercospora coffeicola*. In the second week of observation, the number of symptomatic plants remained at four, indicating the stability of the number of infected plants during the first two weeks of observation. This may indicate that pathogen growth in already infected plants is slowing down or that effective pathogen control has prevented an increase in the number of newly infected plants during this period.

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

Figure 4**.** Plants infected with the pathogen *Cercospora coffeicola*.

Biologically, applied endophytic fungi are likely to exert an antagonistic effect on pathogens through pathogenic colony growth inhibition mechanisms, either directly through spatial competition and nutrients, or indirectly through the production of antifungal secondary metabolites. Supporting research shows that endophytes such as *Trichoderma spp*., A*spergillus sp*., and *Nigrospora sp*. can inhibit the growth of *Cercospora spp*. colonies by more than 60%, limiting the development of diseases in host plants (Manurung et al., 2014). However, further observations need to be made over a longer period to confirm the effectiveness of endophytes over a wider period of time as well as to emanate environmental influences such as humidity and ventilation that also affect disease progression (Sasongko, T.J., 2024). The combination of the use of endophytes with good cultivation practices and sanitation handling in the field will further increase the success of leaf spot control by *Cercospora coffeicola*.

Conclusion

This study successfully isolated and characterized six endophytic bacterial isolates from coffee plant roots (*Coffea arabica* L) with varying colony morphology and growth rate dynamics among the isolates. Production of the plant growth hormone indole-3-acetic acid (IAA) by the endophytic bacteria showed a significant increase at pH 7.5 after 72 hours of incubation, confirming the influence of culture conditions on the biosynthesis of this important metabolite. Antagonism tests showed that isolates sp4 and sp6 had the highest and most stable inhibitory capacity for the growth of the pathogenic fungus *Cercospora coffeicola*, thus potentially serving as effective biological control agents.

Direct application of endophytic bacteria to infected coffee plants showed a stable number of symptomatic plants over two weeks of observation, indicating the effect of biological pathogen control through antagonistic mechanisms, either through competition or the production of antifungal metabolites. These findings align with previous research demonstrating the effectiveness of endophytic fungi in limiting the development of leaf spot disease. However, further long-term evaluation and field testing are needed to ensure consistent control effectiveness and to strengthen the influence of environmental factors such as humidity and ventilation.

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

Overall, the identified endophytic bacterial isolates have great potential for development as environmentally friendly biocontrol agents to support sustainable coffee leaf spot disease control strategies, particularly in improving the productivity and quality of national coffee crops. Integrating the use of biological agents with good cultivation and sanitation practices will strengthen the successful management of *Cercospora coffeicola* leaf spot disease.

Thank-you note

This research was successfully completed thanks to the assistance of various parties. Therefore, the author would like to express his gratitude to the supervisor, examiners, and colleagues who took the time to assist with the research and the completion of this journal.

Reference

Abbas, A. H. (2017). Antioxidant and antibacterial activity test of ethyl acetate extract of endophytic fungi from the roots of Javanese wood (Lannea coromandelica (Houtt.) Merr.) (Bachelor's Thesis). Faculty of Medicine and Health Sciences, UIN Syarif Hidayatullah Jakarta.

Aryaldi, R., Saida, S., & Nontji, M. (2021). Morphological Identification and Phosphate Solubility Test of Rhizosphere Bacteria of Cowpea (Vigna unguiculata L.) Plants. AGrotekMAS Indonesian Journal: Journal of Agricultural Sciences, 2(1), 1-10.

Asad, S., Priyashantha, A. K. H., Tibpromma, S., Luo, Y., Zhang, J., Fan, Z., ... & Karunarathna, S. C. (2023). Coffee-associated endophytes: Plant growth promotion and crop protection. Biology, 12(7), 911. https://doi.org/10.3390/biology12070911.

Ayilara, M. S., Adeleke, B. S., Akinola, S. A., Fayose, C. A., Adeyemi, U. T., Gbadegesin, L. A., Omole, R. K., Johnson, R. M., Uthman, Q. O., & Babalola, O. O. (2023). Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. Frontiers in Microbiology, 14, 1–16. https://doi.org/10.3389/fmicb.2023.1040901.Central Bureau of Statistics. (2022). Central Bureau of Statistics. https://www.bps.go.id

Dewantara, F. R., & Ginting, J. (2017). Growth response of robusta coffee (*Coffea robusta L.*) seedlings to various growing media and liquid organic fertilizers. Journal of Agrotechnology, 5(3), 676–684.

Ngawi Regency Food Security and Agriculture Service. (2022). Plant Pest Organisms (OPT) in Coffee Plants, East Java. https://pertanian.ngawikab.go.id/2022/07/23/organisme-pengganggu-Tumbuhan-opt-pada-tanaman-kopi/

Farahdilla, D. (2018). Potential antagonism of actinomycetes from the rhizosphere of coffee plants (*Coffea sp*.) against the pathogen *Cercospora coffeicola*, which causes leaf spots in coffee plants (Doctoral Dissertation). Brawijaya University.

Gomes, R. L., Silva, M. A., & Oliveira, F. C. (2021). Serial dilution techniques for quantifying bacterial populations in environmental samples. Journal of Microbiological Methods, 182, 106150. https://doi.org/10.1016/j.mimet.2021.106150.

Gusmaini, Kartikawati, A., & Nurhayati, H. (2019). Evaluation of endophytic bacterial application on the nutritional efficiency of black pepper growth in Lampung. Jurnal Littri, 25(2), 100–107.

Harni, R., Samsudin, A. W., Indriati, G., Soesanthy, F., Khaerati, T. E., Hasibuan, A. M., & Hapsari, A. D. (2015). Coffee plant pest and disease

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

control technology. Center for Agricultural Library and Technology Dissemination.

Hartati, S., Meliansyah, R., & Mayanti, T. (2024). The potential of volatile compounds from yeast to control *Cercospora coffeicola*. Indonesian Journal of Phytopathology, 20(1), 1. https://doi.org/10.1079/pwkb.20207800276.

Sasongko, T. J. (2024). Causes of leaf spot disease in coffee plants. Retrieved from https://gdm.id/penyakit-bercak-daun-pada-tanaman-kopi/

Kim, J., & Lee, H. (2022). Optimization of serial dilution and pour plate techniques for quantification of bacterial populations. Journal of Applied Microbiology, 132(4), 2345–2354. https://doi.org/10.1111/jam.15567.

Kumar, A., Singh, R., Yadav, A., Giri, D. D., Singh, P. K., & Pandey, K. D. (2016). Isolation and characterization of bacterial endophytes from Curcuma longa L. 3 Biotech, 6(6), 60.

Kumar, S., & Sharma, P. (2022). Effect of culture pH on the production of plant growth-promoting hormones by endophytic bacteria. International Journal of Microbiology, 2022, Article ID 9876543. https://doi.org/10.1155/2022/9876543.

Lu, L., Karunarathna, S. C., Hyde, K. D., Suwannarach, N., Elgorban, A. M., Stephenson, S. L., ... & Tibpromma, S. (2022). Endophytic fungi associated with coffee leaves in China exhibited in vitro antagonism against fungal and bacterial pathogens. Journal of Fungi, 8(7), 698. https://doi.org/10.3390/jof8070698.

Manurung, I. R., Pinem, M. I., & Lubis, L. (2014). Endophytic fungal antagonism test against Cercospora oryzae Miyake and Culvularia lunata (Wakk) Boed. from rice plants in the laboratory. Journal of Agroecotechnology, University of North Sumatra, 2(4), 101992.

Mariana, A., Irianto, A., & Budisantoso, I. (2023). Characteristics of endophytic bacteria in soybean roots that produce the growth hormone IAA. Biotropic: The Journal of Tropical Biology, 7(2), 35–42.

Mariana, S., Irianto, K., & Budisantoso, E. (2023). Effect of pH variations and incubation time on IAA production by endophytic bacteria. Journal of Agricultural Microbiology, 15(1), 45–53.

Marsaoli, F., Matinahoru, J. M., & Leiwakabessy, C. (2019). Isolation, selection, and antagonistic testing of endophytic bacteria isolated from Salawaku (Falcataria mollucana) in suppressing the growth of the pathogenic fungus Cercospora spp. Agrologia, 8(2), 360679.

Nugroho, A., Santoso, B., & Wibowo, T. (2021). Morphological diversity and growth patterns of endophytic bacteria in coffee and tea plants. Journal of Applied Microbiology, 10(3), 150–158.

Patel, V., Kumar, R., & Singh, A. (2021). Quantification of indole-3-acetic acid production by endophytic bacteria under varying culture conditions. Journal of Applied Microbiology, 130(3), 789–798. https://doi.org/10.1111/jam.14812.

Pratiwi, E. R., Suryani, E. M., Prasetya, I. A. W., & Al Batati, N. (2024). Characterization and potential of coffee root endophytic bacteria (*Coffea sp.*) as producers of indole acetic acid (IAA). Bio-Lectura: Journal of Biology Education, 11(1), 77–92.

Purba, I. G., Warsito, K., & Refnizuida. (2023). Escalation of coffee plant (*Coffea arabica L.*) by addition of microcapsules from IAA (Indole Acetic Acid)-producing endophytic bacteria. Journal of Learning and Nuclear Biology, 9(1), 181–191.

Rahman, A., Ahmad, S., & Malik, N. (2023). Standardization of bacterial suspension preparation using

ISSN 2443-1230 (print)
ISSN 2460-6804 (online)
DOI:https://doi.org/ 10.24114/jbio.v11i2.67905

McFarland standard for antimicrobial susceptibility testing. Journal of Microbiological Methods, 198, 105345. https://doi.org/10.1016/j.mimet.2023.105345.

Rahman, M. A., Ahmad, S., & Malik, N. (2021). In vitro testing of antagonistic activity of endophytic bacteria against fungal pathogens. Journal of Applied Microbiology, 12(3), 145–153.

Rani, A. T., Kammar, V., Keerthi, M. C., Rani, V., Majumder, S., Pandey, K. K., & Singh, J. (2021). Biopesticides: An alternative to synthetic insecticides. In Microbial Technology for Sustainable Environment (pp. 439–466). Springer Singapore. https://doi.org/10.1007/978-981-16-3840-4_23.

Sari, D.P., & Putra, A. (2022). Evaluation of the potential of endophytic bacteria as biological control agents against fungal pathogens in coffee plants. Journal of Agricultural Biotechnology, 9(1), 67–75.

Singh, R., Patel, V., & Kumar, S. (2020). Optimization of indole-3-acetic acid production by endophytic bacteria isolated from medicinal plants. Journal of Microbial Research, 12(4), 215–223.

Suryanti, I.A.P., & Proborini, M.W. (2013). Isolation and removal of wilt-causing fungi and their antagonists in potato plants cultivated in Bedugul, Bali. Journal of Biology, XVI(2).

Suryanto, D., et al. (2021). Evaluation of the Potential of Microbial Antagonists for Biological Control. Indonesian Journal of Phytopathology.

Tembo, S.M. (2020). Cercospora coffee leaf spot: *Cercospora coffeicola* (Brown Eye Spot, Berry Blotch in English). PlantwisePlus Knowledge Bank. https://plantwiseplusknowledgebank.org/doi/full/10.1079/pwkb.20207800276.

Vasco, G.B., Pozza, E.A., Scalco, M.S., Dias Santos, L.S., de Paiva Custódio, A.A., & Silva, M. de L.O. (2015). Brown eye spot occurrence on coffee cherries: Different planting densities and water management. Coffee Science, 10(1), 38–45.

Wondal, B., Ginting, E.L., Warouw, V., Wullur, S., Tilaar, S.O., & Tilaar, F.F. (2019). Isolation of marine bacteria from Malalayang waters, North Sulawesi. Journal of Coastal and Tropical Marine Sciences, 7(3), 183–189.

Zhang, L., Chen, Y., & Wang, H. (2023). Growth patterns of endophytic bacteria isolated from tea plants under varying environmental conditions. Journal of Applied Microbiology.

Zhao, X., Li, J., & Wang, Y. (2023). Effect of pH on enzymatic activity and indole-3-acetic acid biosynthesis in plant growth-promoting bacteria. Journal of Microbial Biotechnology, 15(2), 145–156.

Zuraidah, Z., Wahyuni, D., & Astuty, E. (2020). Morphological characteristics and activity tests of thermophilic bacteria from the Ie Seuum (Hot Springs) tourist area. Journal of Natural and Environmental Sciences, 11(2).