Menara Perkebunan 2023, 91(1), 72-86
p-ISSN: 0125-9318/ e-ISSN: 1858-3768
http://dx.doi.org/10.22302/iribb.jur.mp.v91i1.522
Accreditation Number: 164/E/KPT/2021

Biocontrol activity of endophytic bacteria from cocoa against *Phytophthora* sp. and *Colletotrichum* sp.

Aktivitas biokontrol bakteri endofit asal tanaman kakao terhadap Phytophthora *sp. dan* Colletotrichum *sp.*

Grace WIJAYA[1)], Agustin Krisna WARDANI[1)] & Deden Dewantara ERIS[2*)]

[1)] Faculty of Agricultural Technology, Brawijaya University, Jl. Veteran, Malang 65145, Indonesia
[2)]Indonesian Oil Palm Research Institute (IOPRI), Jl. Brigjen Katamso No. 51 Kampung Baru, Medan 20158, Indonesia

Received 9 Jan 2023 / Revision 20 Jan 2023/ Accepted 11 Apr 2023

Abstrak

*Kakao (*Theobroma cacao *L.) dan cabai (*Capsicum annuum *L.) banyak dibudidayakan oleh petani, dan oleh beberapa petani keduanya dibudidayakan secara tumpangsari. Patogen utama yang menyerang kakao adalah cendawan* Phytophthora *sp. yang menyebabkan busuk buah, kanker batang, dan hawar daun, sedangkan pada tanaman cabai adalah* Colletotrichum *sp. yang menyebabkan antraknosa. Kemampuan bakteri endofit dalam menghambat perkembangan cendawan dan bakteri patogen tidak diragukan lagi. Pada penelitian ini kemampuan biokontrol terhadap cendawan patogen diujikan, namun untuk mengetahui kemampuan awal biokontrol terhadap bakteri patogen juga dilakukan pengujian potensi penghambatannya pada* C. violaceum *yakni uji anti-quorum sensing. Bakteri endofit diisolasi dari tanaman kakao, sedangkan cendawan Phytophthora dan* Colletotrichum *diisolasi dari tanaman kakao dan cabai yang bergejala busuk buah dan antraknosa. Sebanyak 34 isolat bakteri endofit berhasil diisolasi, 10 isolat dari daun (DK), 12 isolat dari ranting (RK), dan 12 isolat dari akar (KA) tanaman kakao ICCRI 4. Sebanyak 16 isolat menunjukkan kemampuan penghambatan populasi bakteri/ anti-quorum sensing dengan indeks degradasi AHL berkisar antara 0,44 – 1,56. Uji antagonis menunjukkan bahwa 11 dari 16 isolat tersebut memiliki antibiosis kuat terhadap* Phytophthora *sp. dengan zona hambat antara 0,6 – 1,35 cm. Sedangkan 10 dari 16 isolat tersebut memiliki antibiotik yang kuat terhadap* Colletotrichum *sp. dengan zona bening antara 0,6 – 1,1 cm. Isolat bakteri endofit terbaik yang memiliki kombinasi kemampuan antiquorum sensing* C. violaceum *dan biokontrol terhadap* Phytophthora *sp. dan* Colletotrichum *sp. adalah RK11, KA1, dan KA8.*

[Kata kunci: antagonis, ahl laktonase, mikroorganisme unggul tanaman]

Abstract

Cocoa (*Theobroma cacao* L.) and chili pepper (*Capsicum annuum* L.) are commonly cultivated by farmers, and in some cases, both crops are grown together in intercropping systems. The main pathogen infected cocoa is the *Phytophthora* sp. fungus, which causes fruit rot, stem cancer, and leaf blight, while *Colletotrichum* sp. causes anthracnose in chili pepper plants. The ability of endophytic bacteria to inhibit the growth of fungi and bacterial pathogens is well known. In this study, the biocontrol ability of endophytic bacteria against fungal pathogens was tested, and a biocontrol preliminary test was also observed by examining their potential inhibition on *Chromobacterium violaceum* called antiquorum sensing test. Endophytic bacteria were isolated from cocoa plants, while *Phytophthora* and *Colletotrichum* were isolated from cocoa and chili pepper plants that showed symptoms of fruit rot and anthracnose. A total of 34 endophytic bacterial isolates were successfully obtained, with 10 isolates from leaves (DK), 12 isolates from branches (RK), and 12 isolates from roots (KA) of cocoa plant ICCRI 4. Sixteen isolates showed quorum sensing ability, which AHL degradation index ranging from 0.44 – 1.56. Antagonistic tests showed that 11 out of 16 isolates had strong antibiosis against Phytophthora sp., with inhibition zones ranging from 0.6-1.35 cm. Meanwhile, 10 out of 16 isolates had strong antibiotics against *Colletotrichum* sp., with clear zones ranging from 0.6 – 1.1 cm. The three best endophytic bacterial isolates that had a combination of antiquorum sensing ability on *C. violaceum* and biocontrol against *Phytophthora* sp. and *Colletotrichum* sp. were RK 11, KA 1, and KA 8.

[Keywords: antagonist, ahl lactonase, plant beneficial microorganism]

[*)]Corresponding author: dedendewantaraeris@gmail.com

Menara Perkebunan is DOAJ indexed Journal and accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3125)
How to Cite: Wijaya, G., Wardani, A. K., & Eris, D. D. (2023). Biocontrol activity of endophytic bacteria from cocoa against Phytophthora sp. and Colletotrichum sp. *Menara Perkebunan, 91*(1), 72-86. http://dx.doi.org/10.22302/iribb.jur.mp.v91i1.522

Introduction

Cocoa (*Theobroma cacao* L.) is one of several important agricultural commodities in Indonesia. The seeds produced from its fruit are generally used for the manufacture of various food products (Nasamsir, 2014). Indonesia is one of the largest cocoa bean-producing and exporting countries in the world with total production reaching 706,500 tons in 2021 (ISC, 2022). Therefore, cocoa is one of the plantation commodities that is very important for Indonesia. Cocoa plant production can be negatively affected by various diseases, including pod rot caused by *Phytophthora* sp. (Indrawangsa et al*.,* 2017). Black pod rot, a disease caused by *Phytophthora* sp., is particularly harmful to cocoa crops. The disease is characterized by the appearance of black patches on the affected fruits, accompanied by white discoloration, and a wet and flabby texture on the fruits (Widiyatmoko et al*.,* 2019).

Some local farmers cultivated cocoa with other commodities as an intercropping system to increase their income, such as cocoa with chilli (*Capsicum anuum*). However, an anthracnose disease infection in chilli caused by the pathogenic fungus *Colletotrichum* sp. also caused a problem in intercropping cultivation. This pathogen causes a decrease in production by 2-35% in the dry season and by 10-80% in the rainy season (Najah et al*.,* 2016). The characteristics of the anthracnose-infected plants are black spots with a pale margin on the fruit and large basins on the affected areas (Harahap et al*.,* 2013).

Several solutions have been carried out to overcome the disease problem, such as the application of synthetic antibiotics and fungicides (Sharma et al*.,* 2018). However, synthetic antibiotics can cause resistance and the use of fungicides can cause environmental pollution to eutrophication. Therefore, an effective and environmentally friendly solution is needed to overcome the pathogens (Purwanto et al*.,* 2014). A potential solution to overcome this problem is utilizing biocontrol agents in the form of endophytic bacteria.

Endophytic bacteria are bacteria that live in the plant tissue without having a negative impact or can even provide benefits for the host and the surrounding environment, such as producing natural antibiotics and degrading enzymes to suppress the growth of pathogens (Camila et al*.,* 2019). ICCRI 4 Cocoa Plant (*Theobroma cacao* L.) is one of the hosts that can be used as a source of endophytic bacteria. This is because the plant has resistance to pathogens and pests, and has good-quality of cocoa beans (Rubiyo, 2013).

Lately, some researcher was focused on the quorum use of endophytic bacteria as biocontrols for mycotoxin fungal plant pathogen (Bacon et al*.,* 2017). Endophytic metabolites possessing antimicrobial properties can be categorized into two distinct groups based on their mode of action. The first group comprises antibiotic metabolites that can either kill microorganisms or inhibit their growth. The second group consists of endophyte-derived compounds that possess inhibitory activity against quorum sensing. This ability functions by quorum sensing mechanisms that are responsible for communication between pathogenic microbes that cause disease. Quorum sensing is a vital microbial communication system, and the ability to disrupt it has emerged as a new target for antibiotic development. This offers a unique opportunity to combat microbial infections using a novel approach (Joo et al*.,* 2020). While the pathogenic bacteria continue to thrive until the infection appears, anti-quorum sensing helps prevent the spread of disease. In addition to testing for anti-quorum sensing abilities, antagonist ability tests were also conducted to determine the ability of endophytic bacteria to inhibit pathogens (Abidin et al*.,* 2015; Novita et al*.,* 2016). The tests were carried out such as the ability of anti-quorum sensing using *C. violaceum* bacteria and its antagonistic ability to pathogenic fungi *Phytophthora* sp. and *Colletotrichum* sp. Therefore, the test aims to assess the potency of endophytic bacteria derived from cocoa plants in suppressing fungal phytopathogens of intercropping plants and to evaluate their ability as anti-quorum sensing bacteria. Apart from identifying their anti-quorum sensing abilities, the study also aims to identify other functions of endophytic bacteria, such as nitrogen fixation and phosphate dissolution.

Materials and Methods

The research was conducted from January to August 2022 at the Indonesian Oil Palm Research Institute Unit Bogor (IOPRI Unit Bogor) at the Research Microbiology Laboratory, Bogor, West Java.

Isolation of Colletotrichum *sp.*

Samples were collected from the fruit of chili plants (*Capsicum annuum* L.) at the Ciomas Experimental Field of the Oil Palm Research Center Bogor Station. The samples were taken from the seeds of infected fruits, targeting the *Colletotrichum* sp. pathogen (Sudirga et al., 2016). Surface steriliza-

tion was conducted by soaking in 70 % alcohol for one minute, followed by two minutes in 1 % Sodium Hypochlorite (NaOCl) and rinsing with sterile water three times. The surface-sterilized fruit parts were cultured on PDA and Water Agar media, each supplemented with Chloramphenicol, and incubated at a temperature of 28 °C (room temperature) for 7 days (Suryanti et al., 2019). The results of macroscopic and microscopic observations were identified using identification key books (Barnett & Hunter, 1972; Alexopoulos, 1996).

Isolation of Phytophthora *sp.*

Samples were taken from the fruit parts of cocoa plant (*Theobroma cacao* L.) infected with fruit pod rot, obtained from the Cibodas experimental field, IOPRI Unit Bogor. The sample fruits were cut into small pieces of 1 cm between the healthy and infected parts of the pathogen. Surface sterilization was then carried out by soaking them in 70 % alcohol for one minute, 1 % sodium hypochlorite (NaOCl) for 2 minutes, and rinsing with sterile water 3 times. The surface-sterilized fruit parts were then inoculated on PDA, V8, and Water Agar media, each of which has been supplemented with chloramphenicol, and incubated at a temperature of 28 °C (room temperature) for 7 days (Suryanti et al., 2019).

Isolation of endophytic bacteria

Samples were collected from the adult leaves, twigs, and roots of ICCRI 04 cocoa plants (*Theobroma cacao* L.). Leaves, twigs, and roots were cut into 2 cm pieces and surface-sterilized using 70 % alcohol for one minute, 1 % sodium hypochlorite (NaOCl) for 2 minutes, 70 % alcohol for 30 seconds, and rinsed three times with sterile water. After surface sterilization, the samples were inoculated by swabbing them on NA media as a control and incubated at room temperature for 2 – 7 days. Successful surface sterilization was confirmed by the absence of living bacteria on the control NA agar media after 24 hours. Bacteria living on the inoculated NA plating media with plant tissues were categorized as endophytic bacteria. Pure cultures of endophytic bacteria were then stored for cultures stock (Aryani et al*.,* 2020).

Gram staining of bacteria

Gram staining of endophytic bacteria refers to the standard methods that use crystal violet, iodine, and safranin as staining reagents.

Hypersensitivity test

One-milliliter suspension of endophytic bacteria that had been incubated for 24 hours was injected into 3-month-old tobacco plants (*Nicotiana tabacum* L.) between the leaves' bones. The treated leaf tissue was observed after 48 hours. If there were symptoms of tissue death such as necrosis or chlorosis, the isolates of endophytic bacteria tested have the potency to be plant pathogens (Murtado et al., 2020).

Hemolysis test

A twenty microliter of suspension of endophytic bacteria was dripped onto a sterile paper disc in 5 % blood agar (v/v) and incubated for 2 days at room temperature. Observations were made, and if a clear zone was formed, or if there was a change in color due to blood lysis around the colony, the isolates of endophytic bacteria tested were potentially pathogenic to animals and humans (Eris et al., 2017).

Phosphate dissolution

Isolates of endophytic bacteria (20 µL) were dripped onto a sterile paper disc placed in a Petri dish containing Pikovskaya agar media and incubated at room temperature for 7 days. If a clear zone was formed around the bacterial colony, phosphate dissolution activity occurred, and the clear zone's width was measured. The endophytic bacteria were collected, and the test was repeated three times (Ouattara et al., 2019).

Nitrogen-fixing test

A total of 100 µL of endophytic bacteria isolates were inoculated into screw test tubes containing nitrogen-free media or semi-solid nitrogen-free broth. The inoculated tubes were incubated for two weeks at room temperature. If discoloration and pellicles were formed, it indicates that the endophytic bacteria can fix nitrogen, and the bacteria with firm pellicles were collected (Puri et al., 2020).

Anti-quorum sensing ability test of endophytic bacteria

Antiquorum sensing (AQS) testing in endophytic bacteria is a physiology and biochemistry testing by using *Chromobacterium violaceum* bacteria as a positive indicator of the occurrence of quorum sensing since this bacteria can produce the pigment violacein (purple)-as influenced by *N-hexanoyl homoserine lactone* (C_6HSL) as an autoinducer. *C. violaceum* was cultured on Nutrient Agar (NA) media using the spread plate method.

Next, a sterile paper disc (6 mm) was placed around the Petri dish that has been spread with *C. violaceum*. Then, each isolate of endophytic bacteria was inoculated with 20 µL and one of the paper discs was inoculated with 20 µL of sterile NB media as a negative control (no degradation of AHL compounds occurs). The plates were then incubated for 24 hours at room temperature. If a non-purple zone appears around the paper disc, it indicates AHL degradation, and the endophytic bacteria were collected as those with anti-quorum sensing ability (Satwika et al., 2017). The diameter of the non-purple zones was also measured (Suryanti et al., 2019).

The antagonistic test

In this experiment, pathogenic fungi cultures of *Phytophthora* sp. and *Colletotrichum* sp. that had been purified and grown aseptically on a growth medium were used. The isolates of *Phytophthora* sp. were grown for 24 hours, and isolates of *Colletotrichum* sp. were grown for 3 days. The selected endophytic bacteria were then tested on the left and right sides of the fungus culture, at 2.5 cm from the edge, as shown in Figure 1. Inoculation of endophytic bacteria on the PDA plate was done 3 days earlier before *Colletotrichum* and *Phytophthora* were placed. Then the test was incubated for 7 days, and the ability of the isolates to inhibit the growth of mycelium of pathogenic fungi *Colletotrichum* sp. and *Phytophthora* sp. were observed (Eris et al., 2017).

Result and Disscusion

Isolation and identification of pathogenic fungi

The pathogenic fungus *Phytophthora* sp. isolated from cocoa fruits showed symptoms such as brown spots, rotting on the surface, and was covered by mycelium in some parts of the pod. Upon touching, the affected fruit feels flabby, wet, and slightly starchy, with the presence of secondary fungus spores and *Phytophthora* sp. sporangium (Nurfianti, 2019). Figure 2 shows the characteristic features of cocoa fruit infected with the *Phytophthora* sp. fungus.

Colonies of the fungus *Phytophthora* sp. exhibited macroscopic morphological characteristics such as a white color on the surface and bottom of the colony. In Figure 2, the bottom of the colony appears white with a slightly yellowish hue. Another observed characteristic besides the color was the cotton-like (smooth) texture with a layered growth shape, resembling a flower crown with a diameter of 8 cm after 10 days of incubation at room temperature (Figure 3).

The morphology of macroscopic colonies in *Phytophthora* sp. fungi was characterized by a cottony texture and a petaloid pattern resembling flower petals, as reported by Das et al. (2016). Fungus *Phytophthora* sp. has a white mycelium forming a floral pattern, sometimes with a faded star pattern. Furthermore, *Phytophthora* sp. fungus colonies exhibited relatively fast growth, ranging from 0.7 – 0.8 cm per day, as noted by Wartono et al. (2021).

$$\text{AHL degradation index} = \frac{(\text{average nonpurple diameter zone} - \text{paper disc diameter})}{\text{paper disc diameter}}$$

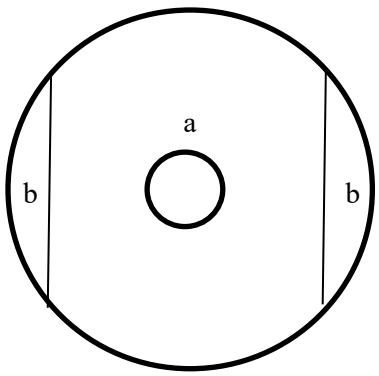


Figure 1. Antagonist test illustration: a) isolates of pathogenic fungi; b) isolates of endophytic bacteria tested

Gambar 1. Ilustrasi uji antagonis: a) isolat cendawan pathogen uji; b) isolat bakteri endofit yang diuji

Figure 2. Fruits of cocoa plant TSH 858 (*Theobroma cacao* L.) the half-stricken with fruit rot, cut longitudinally

*Gambar 2. Buah tanaman kakao TSH 858 (*Theobroma cacao *L) bergejala busuk buah yang di potong secara membujur*

(a) (b)

Figure 3. Colonies of fungi isolated from cocoa fruit with symptoms of *Phytophthora* sp. infection (a) the upper part of the colony and (b) the lower part of the colony

Gambar 3. Koloni cendawan hasil isolasi dari buah kakao bergejala terserang Phytophthora *sp. (a) bagian atas koloni dan (b) bagian bawah koloni*

(a) (b)

Figure 4. Magnified image of fungi *Phytophthora* sp. under the microscope (magnification 400x) (a) hyphae, (b) 1. sporangium, and 2. chlamydospores

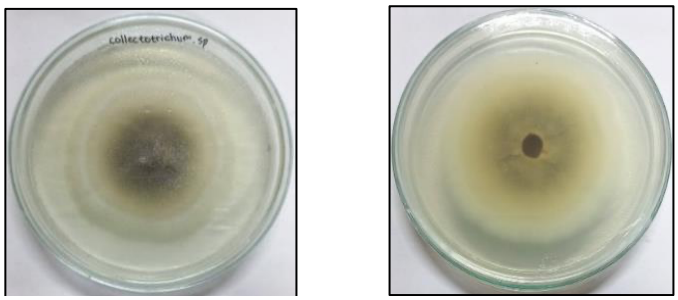
Gambar 4. Perbesaran gambar Phytophthora *sp. dibawah mikroskop (perbesaran 400x) (a) hifa, (b) 1. sporangium dan 2. klamidospora*

Microscopic analysis revealed that the fungi exhibit branched hyphae, produce ovoid sporangia resembling pears, and have round chlamydospores. Figure 4 shows the results of these observations. This pathogenic fungus is characterized by sporangia that are oblong with a protrusion at the end resembling a pear, as reported by Perrine-Walker (2020). *Phytophthora* sp. has non-septate hyphae that are hyaline and branched. Additionally, this fungus produces black chlamydospores as asexual spores during dormant phases under unfavorable environmental conditions and can also produce round-shaped zoospores that are greenish-black in color, according to Muzuni et al. (2020). In summary, the isolated fungi exhibited morphological characteristics that are similar to those of *Phytophthora* sp., based on macroscopic and microscopic observations, particularly the special features of mycelium forming a pattern of flower petals, ovoid-shaped sporangia, and spherical chlamydospores (Das et al., 2016).

The isolated fungi from infected fruits exhibited macroscopic morphological characteristics, such as a colony with a white-greyish color on the upper surface, a cotton-like texture on the colony surface, a circular shape, and an uneven colony edge with a diameter of 6.5 cm after a one-week incubation period at room temperature (Figure 5).

Colletotrichum gloeosporioides, one of the species of *Colletotrichum* sp., has a characteristic greyish-white colony color with a brownish part at the bottom of the colony. It has a cotton-like colony texture and grows concentrically or in circular and spreading patterns in all directions (Arneti & Edriwilya, 2020). Another species that often infects chili plants in the Asian region is *Colletotrichum acutatum*. In this species, the colony morphological characteristics are white, pink to light orange, and the growth of colonies is relatively slow, about 3.3 – 7.0 mm per day (Ibrahim et al., 2017).

This study did not perform molecular characterization of the *Colletotrichum* sp. However, based on the results of microscopic morphological observations of isolates of the pathogenic fungus *Colletotrichum* sp. in Figure 6, there are morphological features that are consistent with the literature. The common feature of *Colletotrichum*-type fungus is that it has insulated branched hyphae, blackish transparent conidia with an elongated shape, and a rounded end (Sudirga et al., 2016).



(a) (b)

Figure 5. Colonies of fungi resulted from the isolation of chili plants with symptoms of *Colletotrichum sp*. infection. (a) The upper part of the colony and (b) The lower part of the colony

Gambar 5. Koloni cendawan hasil isolasi dari tanaman cabe merah besar yang bergejala serangan Colletotrichum *sp (a) penampakan koloni bagian atas dan (b) penampakan koloni bagian bawah*

Figure 6. Microscopically of fungus *Colletotrichum* sp. (400x magnification) (a) hyphae section and (b) conidia

Gambar 6. Mikroskopis Cendawan Colletotrichum *sp. (perbesaran 400 x) (a) potongan hifa dan (b) konidia*

However, in each species, there are differences in the shape of the spores produced. In *Colletotrichum gloeosporioides*, the spores are cylindrical and gray, while in *Colletotrichum acutatum*, the spores are cylindrical and white-gray to blackish-brown. This is also supported by Anggraeni et al. (2019), stating that if the fungus *Colletotrichum* sp. has a cylindrical conidium with a blunt or rounded tip, the structure of the hyphae is insulated and does not have chlamydospores. According to the analysis carried out, the results of the isolation obtained have characteristics that are very similar to the isolates of the pathogenic fungus *Colletotrichum* sp. based on the results of macroscopic or microscopic morphological observations.

Endophytic bacteria isolates, their morphology, Gram staining, and selected test

The total number of endophytic bacteria isolates successfully isolated from each sample was 10 isolates from leaf samples, 12 isolates from twig samples, and 12 isolates from root samples, resulting in an overall total of 34 endophytic bacterial isolates. The test was carried out on all endophytic bacterial isolates without multistage selection. Grouping of the best endophytic bacteria was conducted at the end of the observation after all the data had been obtained. The shape of the bacteria observed majority were basil, followed by spiral and coccus.

A hypersensitive reaction test on tobacco leaf has resulted that all isolates being negative. No chlorotic and necrotic symptoms on the leaves after 72-hour incubation. Because no necrotic symptoms appeared, it was concluded that all the isolates were not phytopathogenic, similar to Sudewi et al., (2021). A hemolysis test was conducted on all endophytic bacterial isolates, 18 endophyte isolates were found to be positive for hemolysis tests due to the emergence of discoloration or clear zones around the colony. These changes were the same as the research conducted by Sudewi et al., (2020), in which hemolysis tests were indicated by discoloration of blood agar. This indicated that the 18 endophytic bacterial isolates had the potential to become pathogenic to animals or humans. The eighteen positive isolates were DK 4, DK 6, DK 7, DK 8 (1), DK 8 (2), DK 10, RK 1, RK 2, RK 3, RK 4, RK 5, RK 6, RK 10, KA 3, KA 5, KA 6, KA 11, and KA 12. These isolates were then eliminated, and isolates with negative indications were further tested to determine their potential (see Table 1).

The phosphate solubilization test showed that nine isolates could dissolve phosphate, namely DK 8 (2), RK 3, RK 4, RK 7, RK 8, RK10, KA 2, KA7, and KA 8. Clear zones formed around the colonies of DK 8 (2), RK 4, and RK 7 isolates during the observation. Another test, the nitrogen-fixing bacteria test, showed that ten isolates could fix nitrogen, namely DK 3, DK 7, RK 1, RK 4, RK 6, RK 9, KA1, KA 2, KA3, and KA 10. Additionally, some endophytic bacterial isolates were suspected of having the ability to produce acid, as indicated by the change in the color of the media becoming lighter or brighter. This was because when the BTB indicator in the testing media decreases in pH to become acidic, the color of the media changes to lighter or yellowish (Bettencourt et al., 2021). Superior endophytic bacteria have functions not only as biocontrol agents but also in the ability to dissolve phosphates and fix nitrogen, both of which function to support plant growth (Lacava et al., 2022).

Antiquorum sensing (AQS) test of endophytic bacteria

The testing of AQS ability on 34 isolates of endophytic bacteria resulted in 16 isolates that exhibited the ability to inhibit bacterial quorum sensing, as indicated by the absence of a purple zone around the paper disc. This absence of a purple zone suggests that the quorum sensing mechanism failed due to the presence of obstacles, preventing *C. violaceum* from producing purple pigment around the paper disc. The 16 isolates with AQS capabilities were DK 10, RK 1, RK 3, RK 4, RK 9, RK 11, RK 12, KA 1, KA 3, KA 4, KA 5, KA 6, KA 8, KA 9, KA 10, and KA 11 (Figure 7).

Sixteen of these endophytic bacteria might produce the enzyme AHL lactonase which can degrade the compound N-hexanoyl homoserine lactone (C_6HSL) which is an autoinducer to produce the pigment violacein (purple) in *C. violaceum bacteria.* The already degraded compound N-hexanoyl homoserine lactone (C_6HSL) can no longer be recognized by the regulatory protein CviR so the vio ABCD operon cannot be expressed (inactivated). The unexpressed ABCD vio platform causes *C. violaceum* cannot to produce the pigment violacein (purple), forming clear zones around the colonies.

The highest lactonase AHL degradation was observed in KA 6 (Table 2). The increase in the AHL degradation index is also directly proportional to the average of the clear zones formed (Suryanti et al., 2019). In this test, only isolates with the best characteristics, especially the anti-quorum sensing ability, will be selected as candidates for biocontrol agent pathogens. The endophytic bacteria that do not have anti-quorum sensing (negative results) will be eliminated as candidates for biocontrol agent pathogens.

Table 1. Morphological and physiological test of endophytic bacteria
Tabel 1. Morfologi dan fisiologi bakteri endofit pada penelitian ini

Isolate code *Kode isolat*	Gram[a] *Gram*	Shapes[b] *Bentuk*	HR[c]	HE[d]	PF[e]	PN[f]	AQS[g]	ANT. PHY[h]	ANT.COL[i]
DK 3	-	Basil	-	-	-	+	-	+	+++
DK 4	-	Coccus	-	+	-	-	-	-	-
DK 5	+	Basil	-	-	-	-	-	+++	-
DK 6	-	Basil	-	+	-	-	-	++	+
DK 7	-	Basil	-	+	-	+	-	++	++
DK 8 (1)	-	Basil	-	+	-	-	-	++	++
DK 8 (2)	-	Spiral	-	+	+	-	-	+	+++
DK 9	-	Spiral	-	-	-	-	-	++	+
DK 10	-	Spiral	-	+	-	-	+++	++	+
DK 11	-	Spiral	-	-	-	-	-	+	+
RK 1	-	Basil	-	+	-	+	++	+	+
RK 2	-	Spiral	-	+	-	-	-	+++	+++
RK 3	-	Basil	-	+	+	-	+	++	++
RK 4	-	Basil	-	+	+	+	+	++	++
RK 5	-	Coccus	-	+	-	-	-	-	-
RK 6	-	Spiral	-	+	-	+	-	+	-
RK 7	-	Basil	-	-	+	-	-	-	-
RK 8	-	Basil	-	-	+	-	-	-	++
RK 9	-	Basil	-	-	-	+	+	-	-
RK 10	-	Spiral	-	+	+	-	-	++	+
RK 11	-	Basil	-	-	-	-	+	+	++
RK 12	-	Basil	-	-	-	-	++	+	-
KA 1	+	Basil	-	-	-	+	++	++	++
KA 2	-	Coccus	-	-	+	+	-	++	++
KA 3	+	Basil	-	+	-	+	++	+++	+
KA 4	-	Spiral	-	-	-	-	++	++	+
KA 5	-	Basil	-	+	-	-	++	+	+
KA 6	-	Basil	-	+	-	-	+++	-	-
KA 7	-	Coccus	-	-	+	-	-	++	++
KA 8	-	Coccus	-	-	+	-	++	++	+
KA 9	-	Basil	-	-	-	-	++	+	+
KA 10	+	Basil	-	-	-	+	++	+	++
KA 11	-	Basil	-	+	-	-	++	++	++
KA 12	-	Coccus	-	+	-	-	-	++	++

[a]Gram: Gram Staining Test of Endophytic Bacteria
[a]Gram: Uji pewarnaan Gram bakteri
[b]Shape: Microscopically shape observation
[b]Bentuk: Bentuk koloni bakteri di bawah mikroskop
cHR: Hypersensitivity Biosafety Test
[c]HR: Uji hipersensitivitas
[d]HE: Hemolysis Biosafety Testing.
[d]HE: uji hemolisis
[e]PF: Phosphate Dissolving Ability Test
[e]PF: Uji pelarutan fosfat
[f]PN: Nitrogen fixing Ability Test.
[f]PN: uji penambatan Nitrogen
[g]AQS: Antiquorum Sensing Capability Testing
[g]AQS: uji kemampuan antiquorum sensing
[h]ANT.PHY= *Phytophthora* sp. Antagonist Test
[h]ANT.PHY=uji antagonis Phytophthora *sp.*
[i]ANT.COL: Antagonist Test of *Colletotrichum* sp.
[i]ANT.COL: Uji antagonis Colletotrichum *sp.*
(+++): Very strong; (++): Strong; (+): Weak; (+):Gram-positive); (-): None; (-): Gram-negative; (x): cannot be identified
(+++): sangat kuat; (++): kuat; (+): lemah; (+): Gram positif; (-): tidak ada; (-): Gram negatif; (x): tidak dapat diidentifikasi

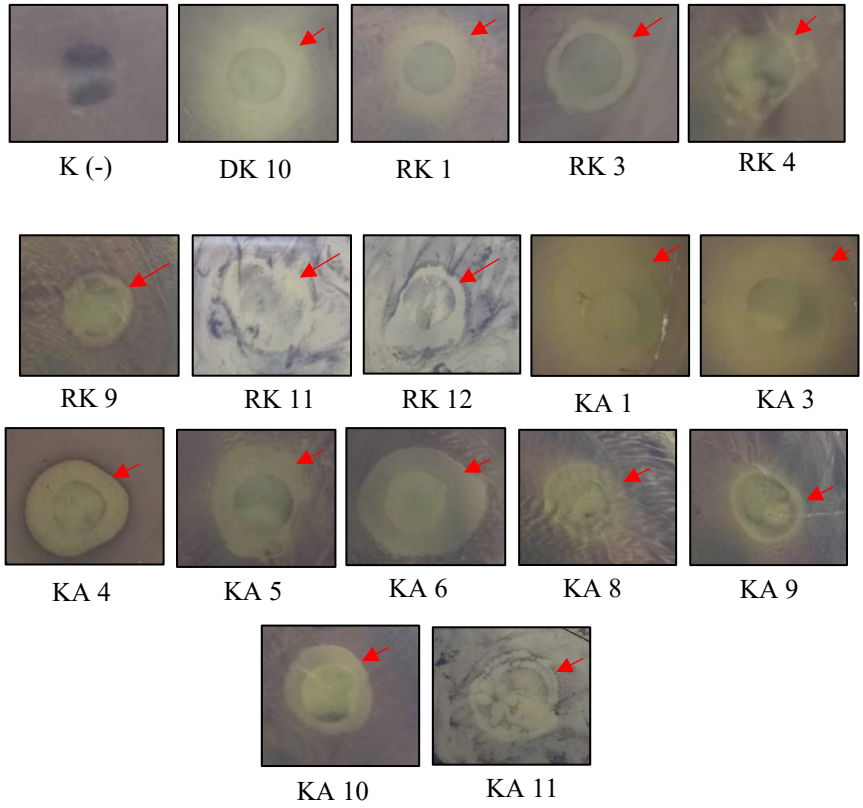


Figure 7. Endophytic bacteria isolates that have anti-quorum sensing ability against *Chromobacterium violaceum*, indicated by a clear zone around the paper disc (red arrow)

Gambar 7. Isolat-isolat bakteri endofit yang memiliki kemampuan antiquorum sensing terhadap Chromobacterium violaceum *yang ditunjukkan dengan zona bening di sekitar kertas uji (ditunjukkan tanda merah)*

Table 2. AHL degradation index of 16 isolates endophytic bacteria

Tabel 2. Indeks Degradasi AHL dari 16 isolat bakteri endofit

No *No*	Isolate code *Kode isolat*	AHL degradation index *Indeks degradasi AHL*	No. *No.*	Isolate code *Kode isolat*	AHL degradation index *Indeks degradasi AHL*
1	KA 6	1.56±0.20	9	KA 9	0.89±0.12
2	DK 10	1.33±0.12	10	KA 11	0.89±0.31
3	KA 4	1.22±0.00	11	RK 12	0.78±0.12
4	KA 1	1.11±0.00	12	RK 3	0.67±0.12
5	KA 5	1.11±0.20	13	RK 4	0.67±0.12
6	RK 1	0.89±0.12	14	RK 9	0.67±0.31
7	KA 3	0.89±0.31	15	KA 10	0.67±0.00
8	KA 8	0.89±0.12	16	RK 11	0.44±0.12

Endophytic bacteria antagonist ability test

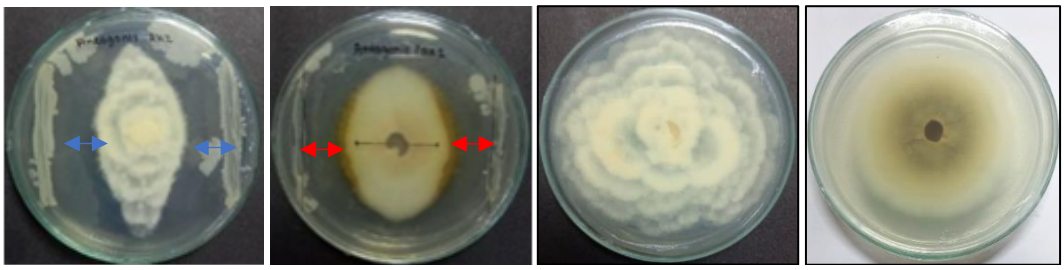
According to the test results, 28 isolates have shown antagonistic abilities against *Phytophthora* sp. The clearest clear zones were found in 11 isolates, namely KA 11, RK 4, KA 8, DK 10, DK 9, DK 7, KA 1, KA 12, KA 3, DK 5, and RK 2. These isolates exhibited inhibition zone ranging from 0.6 to 1.35 cm, as shown in Table 3. This study shows that RK 2 has the strongest antagonistic ability among the tested isolates (Figure 8). This is indicated by its larger clear zone diameter. Antagonistic microorganisms that act as pathogenic biocontrol agents utilize mechanisms such as competition, parasitism, antibiosis, or induction resistance to inhibit the growth of pathogens (Djaenuddin, 2016). This finding is supported by other literature where endophytic bacterial isolates can produce secondary metabolite compounds such as antibiotics, antifungals, HCN, and cell wall-degrading enzymes to inhibit the growth of pathogens (Widiantini et al., 2020).

Table 3. Clear zone characteristics of endophytic bacteria against *Phytophthora* sp. and *Colletotrichum* sp.
Tabel 3. *Karakteristik zona bening dari bakteri endofitik terhadap* Phytophthora *sp. dan* Colletotrichum *sp*.

Isolate code *Kode isolat*	*Phytophthora* sp. inhibition test *Uji penghambatan* Phytophthora *sp.*		*Colletotrichum* sp. inhibition test *Uji penghambatan* Colletotrichum *sp.*	
	Clear zone average (cm) *Rerata zona bening (cm)*	Information *Keterangan*	Clear zone average (cm) *Rerata zona bening (cm)*	Information *Keterangan*
RK 2	1.35±0.21	All the hyphae of the fungus edges facing the bacteria become yellowed.	1.10±0.14	All the hyphae of the fungus edges facing the bacteria become yellowed.
DK 5	1.0±0.00	The peripheral hyphae of the fungus facing the bacteria become thin.	-	No effect (no inhibition)
KA 3	1.0±0.00	The peripheral hyphae of the fungus facing the bacteria become thin.	0.30±0.00	The peripheral hyphae of the fungus facing the bacteria become thin.
KA 12	0.9±0.14	The growth of hyphae of the fungus is inhibited.	0.60±0.14	The hyphae of the fungus edges facing the bacteria have yellowed parts.
KA 1	0.85±0.21	The growth of hyphae of the fungus is inhibited.	0.35±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.
DK 7	0.80±0.14	The growth of hyphae of the fungus is inhibited.	0.60±0.14	The hyphae of the fungus edges facing the bacteria have yellowed parts.
DK 9	0.80±0.28	The hyphae of the fungus edges facing the bacteria have yellowed parts.	-	No effect (no inhibition)
DK 10	0.75±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.	0.25±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.
KA 8	0.70±0.14	The growth of hyphae of the fungus is inhibited.	0.45±0.07	The hyphae of the fungus edges facing the bacteria have yellowed parts.
RK 4	0.65±0.21	The growth of hyphae of the fungus is inhibited.	0.80±0.14	The growth of hyphae of the fungus is inhibited.
KA 11	0.6±0.14	The growth of hyphae of the fungus is inhibited.	0.70±0.00	The growth of hyphae of the fungus is inhibited.
KA 2	0.55±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.	0.85±0.21	The hyphae of the fungus edges facing the bacteria have yellowed parts.

Table 3. Continuation
Tabel 3. Lanjutan

Isolate code *Kode isolat*	*Phytophthora sp.* inhibition test *Uji penghambatan* Phytophthora *sp.*		*Colletotrichum sp.* inhibition test *Uji penghambatan* Colletotrichum *sp.*	
	Clear zone average (cm) *Rerata zona bening (cm)*	Information *Keterangan*	Clear zone average (cm) *Rerata zona bening (cm)*	Information *Keterangan*
DK 6	0.50±0.00	The growth of hyphae of the fungus is inhibited.	0.45±0.21	The peripheral hyphae of the fungus facing the bacteria become thin.
KA 4	0.5±0.00	The peripheral hyphae of the fungus facing the bacteria become thin.	0.10±0.00	The peripheral hyphae of the fungus facing the bacteria become thin.
KA 7	0.5±0.00	The growth of hyphae of the fungus is inhibited.	0.90±0.14	The growth of hyphae of the fungus is inhibited.
DK 3	0.45±0.00	The growth of hyphae of the fungus is inhibited.	1.05±0.07	The hyphae of the fungus edges facing the bacteria have yellowed parts.
DK 8 (1)	0.45±0.07	The growth of hyphae of the fungus is inhibited.	0.60±0.14	The growth of hyphae of the fungus is inhibited.
DK 11	0.45±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.	0.35±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.
RK 10	0.45±0.07	The growth of hyphae of the fungus is inhibited.	-	No effect (no inhibition)
RK 3	0.4±0.14	The growth of hyphae of the fungus is inhibited.	0.40±0.14	The hyphae of the fungus edges facing the bacteria have yellowed parts.
RK 1	0.35±0.07	The growth of hyphae of the fungus is inhibited.	0.15±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.
RK 12	0.35±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.	0.40±0.14	The peripheral hyphae of the fungus facing the bacteria become thin.
RK 6	0.3±0.00	The growth of hyphae of the fungus is inhibited.	-	No effect (no Inhibition)
DK 8 (2)	0.25±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.	1.05±0.07	The hyphae of the fungus edges facing the bacteria have yellowed parts.
RK 11	0.25±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.	0.50±0.00	The peripheral hyphae of the fungus facing the bacteria become thin.
KA 10	0.15±0.07	The peripheral hyphae of the fungus facing the bacteria become thin.	0.35±0.07	The hyphae of the fungus edges facing the bacteria have yellowed parts.
KA 5	0.1±0.00	The peripheral hyphae of the fungus facing the bacteria become thin and turn yellow.	0.10±0.00	The growth of hyphae of the fungus is inhibited.
KA 9	0.1±0.00	The peripheral hyphae of the fungus facing the bacteria become thin.	0.25±0.07	The growth of hyphae of the fungus is inhibited.



(a) (b) (c) (d)

Figure 8. Clear zone (inhibition zone) of *Phytophthora* sp. (blue arrow)(a) and *Colletotrichum* sp (red arrow)(b) as response RK2's antibiotic production after 7 days inoculation, (c) control of *Phytophthora* sp. and (d) control of *Colletotrichum* sp.

Gambar 8. *Zona bening/zona penghambatan pada koloni* Phytophthora *sp. (panah biru)(a) dan* Colletotrichum *sp. (panah merah) (b) sebagai respons terhadap antibiotik yang dihasilkan isolat RK2 pada 7 hari setelah inokulasi, (c) kontrol* Phytophthora *sp., dan (d) kontrol* Colletotrichum *sp*.

Several isolates of endophytic bacteria are suspected to have antibiotic abilities DK 6, DK 7, DK 8 (1), DK 9, RK 2, RK 4, RK 10, KA 7, KA8, KA 11, and KA 12. This is because the 11 isolates produce a firm clear zone and even some isolates cause *Phytophthora* sp. hyphae to turn yellow which indicates the presence of cell death. In addition, other isolates show strong competition ability with *Phytophthora* sp. This causes *Phytophthora* sp. hyphae adjacent to the endophytic bacteria to be thinner which can be caused by a lack of nutrients due to competition (Shan et al., 2018).

As many as 24 isolates have antagonistic abilities against *Colletotrichum* sp. Of the 24 isolates that have antagonistic abilities, the clear zones are found in 10 isolates i.e. KA 12, DK 8 (1), DK 7, KA 11, RK 4, KA 2, KA 7, DK 8 (2), DK 3, and RK 2 with zone capabilities inhibition range from 0.6 – 1.1 cm. In this test, it was known that the isolate with the strongest antagonist ability was RK 2. This is because RK 2 has the largest clear zone diameter and causes death in the hyphae of pathogenic fungi (yellowing/necrosis). This fact also describes by Prihatiningsih et al. (2022) that bacteria consortiums were tested for the pathogen of rice *Rhizoctonia solani*. As before in this analysis, the grouping of endophytic bacteria was carried out according to the antagonistic mechanism (antibiosis ability) consisting of 13 isolates such as DK 3, DK 8 (1), DK 8 (2), RK 2, RK 4, RK 11, KA 1, KA 2, KA 7, KA 9, KA 10, KA 11 and KA 12. Another group has competitive abilities including 11 isolates consisting of DK 6, DK 7, DK 10, DK 11, RK 1, RK 3, RK 8, KA 3, KA 4, KA 5, and KA 8. However, to see the type of secondary metabolites produced by endophytic bacteria when performing antibiosis mechanisms requires further testing.

There is a potential endophytic microbe that produced diverse antimicrobial compounds that have the potential for plant disease management. Endophytic bacteria are known to be able to produce compounds that are antibiotics, which can be direct antibiotic compounds. Several antibiotics produced by endophytic bacteria can be in the form of dibutyl phthalate, eicosane, tetrapentacontane, heneicosane, and hexadecane which show anti-microbial activity (Sharma & Mallubhotla, 2022). Several other antifungal compounds produced by endophytic bacteria include Eicosane, Hexatriacontane, Tetratetracontane, trans-2-Decenoic acid, and 1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro- 1,4a-dimethyl-7-(1-methylethyl) (Alsultan et al., 2019).

In this research, we found several modes of action of endophytic bacteria isolates once as biocontrol, and another in multiple modes such as not only as biocontrol but also the producer of AHL lactonase, as antiquorum sensing bacteria. This fact gives us information about how they live in their ecosystem, and support the health of the plant. Isolates that can act as biocontrol only were DK 3, DK 5, DK 6, DK 7, DK 8, DK 9, DK 11, RK 2, RK 6, RK 8, RK 10, KA 2, KA 7, and KA 12. RK 2 was the strongest biocontrol. Other isolates that have multiple modes of action as biocontrol and AQS promoter were DK10, RK 1, RK 3, RK 4, RK 11, RK 12, KA 1, KA 3, KA 4, KA 5, KA 8, KA 9, KA 10, and KA 11.

Conclusion

Eleven isolates of endophytic bacteria isolated from cocoa have a strong antagonistic ability against *Phytophthora* sp. with an inhibitory zone ability ranging from 0.6 – 1.35 cm. While for the antagonist test of *Colletotrichum* sp. obtained 10 isolates of endophytic bacteria have strong inhibitory zone ranging from 0.6 – 1.1 cm. In the antiquorum sensing ability test for *C. violaceum*, 16 isolates of endophytic bacteria were obtained that have the

ability of AQS to degrade AHL compounds with an AHL degradation index ranging from 0.44 – 1.56. Some of the isolates were found as biocontrol of the fungal such as RK2, but have no ability in producing AHL lactonase. Based on the series of tests, the three best-selected isolates are RK11, KA 1, and KA 8 which have the potency as biocontrol agents against those two pathogens and anti-quorum sensing to *C. violaceum.*

References

Abidin, Z., Aini, L. Q., & Abadi, A. L. (2015). Pengaruh bakteri *Bacillus sp.* dan *Pseudomonas sp.* terhadap pertumbuhan jamur patogen *Sclerotium rolfsii Sacc*. penyebab penyakit rebah semai pada tanaman kedelai. *Jurnal HPT*, 3(1), 1-10. http://repository.ub.ac.id/ id/eprint/129928

Agisha, V. N., Kumar, A., Eapen, S. J., Sheoran, N., & Suseelabhai, R. (2019). Broad-spectrum antimicrobial activity of volatile organic compounds from endophytic *Pseudomonas putida* BP25 against diverse plant pathogens. *Biocontrol Science and Technology*, 29(11), 1069-1089. https://doi.org/10.1080/09583157.2019.1657067

Alexopoulos, C. J., Mims, C. W., & Blackwell, M. (1996). *Introductory mycology*. John Wiley & Sons. Singapore. p. 244 - 324.

Alsultan, W., Vadamalai, G., Khairulmazmi, A., Saud, H. M., Al-Sadi, A. M., Rashed, O., A. K. M. J., & Nasehi, A. (2019). Isolation, identification and characterization of endophytic bacteria antagonistic to *Phytophthora palmivora* causing black pod of cocoa in Malaysia. *European Journal of Plant Pathology*, 155, 1077-1091. https://doi.org/10.1007/s10658-019-01834-8

Anggraeni, W., Elvi, R. P., Wardoyo, & Rahmawati. (2019). Isolation and identification of fungi in cayenne pepper fruit (*Capsicum frutescens* L.) The symptomatic anthracnose from farmland In orange hamlet. *Jurnal Protobiont.* 8(2), 94 – 100. http://dx.doi.org/10.26418/protobiont.v8i2.34058

Arneti, Y. L., & Edriwilya, R. (2020). Effectiveness of papaya leaf extract in vitro against *Colletotrichum gloeosporioides* causing anthracnose disease in chili plants. *Jurnal Proteksi Tanaman (Journal of Plant Protection)*. 4(1), 1 – 10. https://doi.org/10.25077/jpt.4.1.1-10.2020

Aryani, P., Kusdiyantini, E., & Suprihadi, A. (2020). Isolation of endophytic bacteria of reed leaves *(Imperata cylindrica)* and its secondary metabolites that have the potential to be antibacterial. *Jurnal Akademika Biologi*, 9(2), 20-28. https://doi.org/ 10.1186/s12906-020-03095-z

Bacon, C. W., Hinton, D. M., & Mitchell, T. R. (2017). Is quorum signaling by mycotoxins a new risk-mitigating strategy for bacterial biocontrol of *Fusarium verticillioides* and other endophytic fungal species?. *Journal of Agricultural and Food Chemistry*, 65(33), 7071-7080. https://doi.org/10.1021/acs.jafc.6b03861

Barnett, H. L., & Hunter, B. B. (1972). *Illustrated genera of imperf ect fungi*. Third Edition. Burgess Publishing. Company.

De França Bettencourt, G. M., Degenhardt J., Dos Santos G. D., Vicente V. A., & Soccol C. R. (2021). Metagenomic analyses, isolation and characterization of endophytic bacteria associated with *Eucalyptus urophylla* BRS07-01 in vitro plants. *World Journal of Microbiology and Biotechnology*, 37, 1-16. https://doi.org/10.1007/s11274-021-03127-x

Camila, F. S., Temaja, I. G. R., & Khalimi, K. (2019). Potensi bakteri endofit dalam menekan pertumbuhan *Phytophthora sp.* (Butler) Secara *In Vitro*. *Jurnal Agroekoteknologi Tropika*, 8(4), 388-398.

Djaenuddin, & Nurasiah. (2016). Interaksi bakteri antagonis dengan tanaman: Ketahanan terinduksi pada tanaman jagung. *Iptek Tanaman Pangan*, 11(2), 2016.

Eris, D. D., Munif, A., Soekarno, B. P. W., & Purwantara, A.. (2017). Screening and potential of endophytic bacteria of *arecaceae* plant origin as biological control agents of the fungus *Pestalotiopsis* sp. causes of leaf spot disease in coconut kopyor (*Cocos nucifera*). *Menara Perkebunan*, 85(1),19-27. https://doi.org/ 10.22302/iribb.jur.mp.v85i1.235

Harahap, Hakim, T. F., Lubis, L., & Hasanuddin. (2013). Efek temperatur terhadap virulensi jamur *Colletotrichum sp.* Penz. *Sacc*. penyebab penyakit antraknosa pada tanaman kakao (*Theobroma cacao L.*). *Jurnal Online Agroekoteknologi*, 2(1), 411-420. https://doi.org/10.32734/jaet.v2i1.5842

Hidayatun, N., Susilowati, D. N., & Mulya, K. (2011). Identification of 26 isolates of endophytic bacteria and rice filospheres with sequencing analysis16s RDNA. *Berita Biologi*, 10 (4). https://doi.org/10.14203/beritabiologi.v10i2.1978

Ibrahim, R., Hidayat, S. H, & Widodo. (2017). Morphological diversity, genetics, and pathogenicity of *Colletotrichum acutatum* causes chili anthracnose in Java and Sumatra. *Jurnal Fitopatologi Indonesia*,13(1), 9-16. https://doi.org/10.14692/jfi.13.1.9

Indrawangsa, G. D., Sudarma, I. M., & Singarsa, I. D. P. (2017). Endophytic fungus inhibitory test against *Phytophthora sp.* (Butler) butler causes cocoa fruit rot disease *in vitro*. *E-Jurnal Agroekoteknologi Tropika*, 6 (3), 229-238. https://doi.org/10.13057/biodiv/d220519

ISC Indonesian Statistic Center. (2022). Produksi tanaman perkebunan 2019-2021. Pejabat Pengelola Informasi dan Dokumentasi Badan Pusat Statistik [ID].

Joo, H. S., Deyrup, S. T., & Shim, S. H. (2021). Endophyte-produced antimicrobials: a review of potential lead compounds with a focus on quorum-sensing disruptors. *Phytochemistry Reviews*, 20, 543-568. https://doi.org/10.1007/s11101-020-09711-7

Lacava, P. T., Bogas, A. C., & Cruz, F. D. P. N. (2022). Plant growth promotion and biocontrol by endophytic and rhizospheric microorganisms from the tropics: A review and perspectives. *Frontiers in Sustainable Food Systems*, 6, 796113. https://doi.org/10.3389/fsufs.2022.796113

Muzuni, H. & Yanti, N. A. (2020). Morphological characterization of *Phytophthora sp*. Origin of cocoa fruit Olo-oloho village, Konawe county, Southeast Sulawesi. *Jurnal Penelitian Biologi /Journal of Biological Research*, 7 (1), 1064-1069. http://dx.doi.org/10.33772/biowallacea.v7i1.11812

Murtado, A., Mubarik, N. R., & Tjahjoleksono A. (2020). Isolation and characterization endophytic bacteria as biological control of fungus *Colletotrichum* sp. on onion plants (*Allium cepa* L.). In *IOP Conference Series: Earth and Environmental Science*, 457 (1), 012043. https://doi.org/10.1088/17551315/457/1/012043

Nasamsir. (2014). Respons pertumbuhan bibit kakao (*Theobroma cacao L.*) terhadap aplikasi pupuk organik cair pada jenis aksesi buah kakao yang berbeda. *Jurnal Ilmiah Universitas Batanghari Jambi*, 14(3), 91-100. http://dx.doi.org/10.33087/jiubj.v14i3.264

Najah, Naelun, L., Suhartanto, N. M., & Widodo. (2016). Control *of Colletotrichum spp*. carried away chili seeds with microwave exposure. *Jurnal Fitopatologi Indonesia*, 12(4),115-123.

Novita, H., Rusmana, I., Yuhana, M., Pasaribu, F. H., & Lusiastuti, A. M. (2016). In vitro test of bacteria anti quorum sensing degradation acyl homoserine lactone *Aeromonas hydrophila*. *Jurnal Riset Akuakultur*, 11(3), 291-296. https://doi.org/10.15578/jra.11.3.2016.291-296

Nurfianti, U. (2019). Pengamatan gejala infeksi *Phytophthora palmivora* penyebab penyakit busuk buah pada kakao. *Biocelebes*, 13(3), 253-261.

Ouattara, A., Coulibaly, K., Konate, I., Ismaë, B., Tidou, A. S., & Filali-Maltouf, A. (2019). Selection of cocoa tree (*Theobroma cacao* Linn) endophytic bacteria solubilizing tri-calcium phosphate, isolated from seedlings grown on soils of six producing regions of Côte d'Ivoire. *Advances in Microbiology*, 9(9), 842-852. https://doi.org/10.4236/aim.2019.99051

Perrine F., & Walker. (2020). Review *Phytophthora* sp. cocoa interaction. *Journal of Fungi*, 6, 167. DOI: https://doi.org/10.3390/jof6030167

Prihatiningsih, N., Djatmiko, H. A., & Lestari, P. (2022). Antagonistic feature displayed by endophytic bacteria consortium for control rice pathogens. *Jurnal Hama dan Penyakit Tumbuhan Tropika,* 22(2), 154-161. https://doi.org/10.23960/jhptt.222154-161

Puri, A., Padda, K. P., & Chanway, C. P. (2020). Can naturally-occurring endophytic nitrogen-fixing bacteria of hybrid white spruce sustain boreal forest tree growth on extremely nutrient-poor soils?. *Soil Biology and Biochemistry*, 140, 107642. https://doi.org/10.1016/j.soilbio.2019.107642

Purwanto, Safira, U. M., Pasaribu, F. H., & Bintang, M.(2014). Isolasi bakteri endofit dari tanaman sirih hijau (*Piper betle L.*) dan potensinya sebagai penghasil senyawa antibakteri. *Current Biochemistry*, 1(1), 51-57.

Rubiyo. (2013). Inovasi teknologi perbaikan bahan tanam kakao di Indonesia. *Buletin RISTRI*, 4(3), 199-214. https://doi.org/10.21082/jtidp.v4n3.2013.p199-214

Shan, W., Zhou, Y., Liu, H., & Yu, X. (2018). Endophytic actinomycetes from tea plants (*Camellia sinensis*): isolation, abundance, antimicrobial, and plant-growth-promoting activities. *BioMed Research International*, 2018, 1-12. https://doi.org/10.1155/2018/1470305

Sharma, M., & Mallubhotla, S. (2022). Diversity, antimicrobial activity, and antibiotic susceptibility pattern of endophytic bacteria sourced from *Cordia dichotoma* L. *Frontiers in Microbiology*, 13. https://doi.org/ 10.3389/fmicb .2022.879386

Sharma, S., Duveiller, E., Khetarpal, R. K., & Sharma, S. M. (2018). Alternatives to chemical fungicides for the control of fusarium wilt of tomato: challenges and opportunities. *Plant Pathology*, 67(6), 1281-1293.

Sudirga, S. K. (2016). Isolation and Identification of the fungus *Colletotrichum* spp. PCS isolates causing anthracnose disease in large chili fruits (*Capsicum annuum* L.) in Bali. *Jurnal Metamorfosa*. 3(1), 23-30.

Surujdeo-Maharaj, S., Sreenivasan, T. N., Motilal, L. A., & Umaharan, P. (2016). Black pod and other phytophthora induced diseases of cacao: History, biology, and control. *Cacao diseases: A history of old enemies and new encounters*, pp.213-266. https://doi.org/10.1007/978-3-319-24789-2_7

Suryanti, E. (2019). The potential of AHL lactonase-producing bacteria as a control of potato soft rot by *Dickeya dadantii*. *Thesis*. Faculty of Mathematics and Natural Sciences. Institut Pertanian Bogor (IPB).

Vasavi, H. S., Ananthapadmanabha, B. A., & Punchapady, D. R. (2014). Anti-quorum sensing activity of *Psidium guajava* L. flavonoids against *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1. *Microbiology and Immunology*, 58, 286–29. https://doi.org/10. 1111/1348-0421.12150

Wartono & Efi, T. (2021). Cocoa fruit rot disease pathogens: Character and pathogenicity of *Phytophthora Sp.* isolates of Pakuwon Origin, Sukabumi. *Jurnal Tanaman Industri dan Penyegar*, 8(1), 49 – 58. https://doi.org/10.21082/jtidp.v8n1.2021.p49-58

Widiantini, F., Endah, Y., & Kurniawan, A. (2020). Penghambatan pertumbuhan *Rhizoctonia oryzae* dan *Cercospora oryzae* oleh senyawa volatil yang dihasilkan bakteri endofit padi. *Jurnal Agrikultura*, 31(1), 61-67. https://doi.org/10. 24198/agrikultura.v31i1.27323

Widiyatmoko, Eko, W., Christiani, Y., Indrabayu, & Yoga A. H. (2019). Efektivitas antagonisme *Trichoderma virens* terhadap fitopatogen *Phytophthora sp.* pada tanaman kakao (*Theobroma cacao* L.). *Jurnal Pertanian Tropik*, 6(1), 101–107. https://doi.org/10.32734/jpt. v6i1.3046