

Endophytic Bacteria from White Cambodia Stems (*Plumeria acuminata*) Have Strong Inhibition Against *Escherichia coli*

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Keywords: Endophytic Bacteria, *Plumeria acuminata*, *Escherichia coli*, Antibacterial Compounds.

Abstract: The health issues caused by *Escherichia coli* are a primary trigger for infectious diseases in Indonesia. Bacterial infections can be treated with antibiotics, leading to an increase in their usage. The rising use of antibiotics has led to irrational usage, resulting in the development of antibiotic-resistant bacteria. Therefore, there is a need for new antibiotic sources that can combat bacterial infections, especially from various biological resources. Due to its antimicrobial compound properties, white cambodia (*Plumeria acuminata*) is a biological resource with potential as an antibiotic. One way to utilize this potential is by isolating endophytic bacteria. The stem of the white cambodia provides a suitable environment for endophytic bacteria. Thus, this study aims to determine the antibacterial potential of endophytic bacterial isolates from the stem of white Cambodia to inhibit the growth of *E. coli*. This research uses a qualitative data collection method with laboratory experimental research. In the first stage, endophytic bacteria were isolated from the stem of *P. acuminata* using the serial dilution method. Subsequently, the characteristics of the colonies were observed based on their shape, edges, elevation, texture, pigmentation, and the result of Gram staining. In the final stage, the antagonistic test of the endophytic bacterial isolates against *E. coli* was conducted using the double-layer agar method. The isolation of endophytic bacteria from *P. acuminata* resulted in 3,817 colonies. Based on the observation of colony morphology, 93 isolates have different colony morphologies due to the diverse characteristics of the colonies and bacterial shapes. The Gram staining test showed that 83 endophytic bacterial isolates were Gram-positive, and ten were Gram-negative. The antagonistic test revealed that seven positive endophytic bacterial isolates could inhibit the growth of *E. coli*. Endophytic bacterial isolates from *P. acuminata* can produce antibacterial compounds that can inhibit the growth of *E. coli*.

1 INTRODUCTION

Infectious diseases still cause health problems in developing countries like Indonesia. Based on Indonesia's health profile in 2021 still has infectious diseases are the reason death is most frequently post-neonatal. In 2021, pneumonia and diarrhea still

became the reason for death most during the post-neonatal period 14.4 % of deaths were due to pneumonia, and 14% were because of diarrhea. The leading causes of death in the group of child toddlers (12-59 months) are diarrhea by 10.3% and pneumonia by 9.4% (Ministry of Health RI, 2021). Most of these infectious diseases are caused by bacteria.

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One of the most common bacteria that causes infection in the ducts digestion is *Escherichia coli*, which has been diagnosed by personnel health as one reason diarrhea is the highest in Indonesia, reaching 10% (Ministry of Health RI, 2018). *E. coli* is a bacil Gram-negative bacteria with a size range between 1.0-1.5 μm x 2.0-6.0 μm . *E. coli* are many opportunistic bacteria found in the large intestine of humans as a microbiota. Most *E. coli* strains are commensal bacteria that help, but some strains are pathogenic and can cause disease. The disease that *E. coli* can cause is diarrhea, which is caused by consuming contaminated food or water. It also causes stomach cramps, malaise, and fever (Joeginjantoro R, 2019).

Antibiotics are the primary choice for treating bacterial infections due to their significant benefits in reducing pain and death consequences of infectious diseases. Its practical usage of antibiotics has rapidly improved in overcoming and preventing infectious diseases. Unfortunately, its high demand for antibiotics has caused its use to be inappropriate and excessive. The cause is the availability of easy antibiotics obtained by society without instructions or recipes from medical personnel, especially doctors. Improper use of antibiotics can result in the development of resistant bacteria to antibiotics, which later becomes a severe issue in treating bacterial infections (Andiarna et al., 2020).

Resistance to antibiotics is a problem that society needs to handle seriously. When bacteria become resistant to antibiotics, medicine has lost its effectiveness in treating human infections and diseases. Resistance to antibiotics can happen due to mutation or transfer of resistance genes through a horizontal process. Resistance genes can inherited or acquired from an element genetically mobile, like a plasmid that can transferred between bacteria. Mutations known as single-step mutations cause appearance resistance levels quickly and in a short time (Nurjanah et al., 2020). Incident resistance in Indonesia is sporadic, selective, and impossible to overcome fully (Lia Yunita et al., 2021). Based on the problem above, it is vital to research new antibiotics from various sources that are effective in treating bacterial infections.

One example of source life that can utilized is the plant white cambodia (*Plumeria acuminata*), which is frequently used as a source of traditional drugs. *P. acuminata* is a plant originating from Central America that belongs to the Apocynaceae family. *P. acuminata* are often found in Indonesia. Apart from being an ornamental plant, *P. acuminata* also has compounds that have antimicrobial properties. *P. acuminata* is one of the plants with potential as an alternative antibiotic

to treat infections caused by bacteria (Zulkifli et al., 2022).

Endophytic bacteria reside and live inside network plants and then form colonies without harming the host plants (Tangapo et al., 2018). The connection between endophytic bacteria and host plants own mutual relationship, beneficial or symbiotic mutualism. In this connection, plants supply nutrients for bacteria, while bacteria protect plants from seed disease, help produce phytohormones, and stimulate the absorption of minerals, especially nitrogen. *P. acuminata* stems provide a suitable environment for endophytic bacteria that can do nitrogen-fixing. This ability is beneficial in a biological way because it helps plants obtain nitrogen (N). The N elements comprise essential proteins in photosynthesis, increasing plant resistance (Koomnok et al., 2007). The presence of endophytic bacteria in plants is significant because these bacteria can produce bioactive compounds with characteristics similar to those produced by host plants. This is because there is an evolutionary genetic exchange between host and microbe endophyte (Hasan Basri et al., 2021). This compound provides profit for the plant and has potency benefits, especially in matter health.

One of the uses of endophytic bacteria is research conducted by Zulkifli (2022), showing that endophytic bacteria in the bark of *P. acuminata* plants have the potential to be a source of antibacterial substances against the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Further research was carried out by Hidayati (2019), where the results of phytochemical tests showed that endophytic bacteria in white Cambodia stems had secondary metabolites in the form of alkaloids.

Based on the background above, research on the isolation of endophytic bacteria in stems of *P. acuminata* is critical because there is still limited scientific information about the existence of *P. acuminata* endophytic bacteria and their benefits as agents producing potential antibacterial compounds to inhibit the activity of *E. coli*.

2 METHODS

The type of research used by researchers is experimental research. The data collection method used by researchers was qualitative to determine the antibacterial activity of the endophytic bacteria of *P. acuminata* stems against *E. coli*. This research was conducted at the Microbiology Laboratory, Faculty of Medicine and Health Sciences, Bengkulu University.

The *P. acuminata* plant used in this research was taken from Bengkulu City. The samples needed are three stems from three *P. acuminata* plants.

2.1 Isolation of Endophytic Bacteria

Isolation of endophytic bacteria was carried out using dilution methods. The samples that must be prepared are three *P. acuminata* stems from three plants. The *P. acuminata* stems were cut with a size of 2-5 cm, then sterilized surface with running water, and peeled the skin stem. The piece sample was sterilized by soaking in alcohol 70% for 1 minute, then moving it into 5.25% Sodium Hypochlorite (NaOCl) for 5 minutes, then moving it back inside 70% alcohol three times with an interval of 30 seconds (Hidayati, 2019). After that, the sample is crushed or crushed using a sterile mortar and pestle by adding 3-5 ml of sterile distilled water. Prepare 5 test tubes filled with 9 ml of sterile distilled water, then take 1 ml of solution from the first tube containing the sample that has been ground with a micropipette and put it in the second tube, then homogenized using a vortex mixer. Please do the same thing; take another 1 ml sample in the second tube filled with 9 ml of sterile distilled water and put it in the third tube, then homogenize. The same step is done until the fifth tube. After dilution, take 0.1 ml of the suspension from each dilution and distribute it in King's B medium aseptically, then spread it evenly using a spreader, then incubate the isolate for 24 hours in an incubator (Rizqoh et al., 2021).

2.2 Colony Characteristics and Morphology of Endophytic Bacteria

The characterization of bacterial morphology is done in macroscopic and microscopic ways. Macroscopic observation involves elevation, edges, shape, and bacterial colony formation. Meanwhile, microscopic observations were done using Gram staining (Oktavia & Pujiyanto, 2018).

2.3 Antagonist Test

Escherichia coli are cultured into Nutrient Broth media. The media was incubated at room temperature for 24 hours. Then, spectrophotometry will measure turbidity (OD = 0.3 concentration $10^6 - 10^7$ cells/mL).

Antagonist tests of endophyte isolates against target microorganisms are carried out using the

double-layer technique. This procedure involves the use of semi-solid nutrient media and solid nutrient media. *E. coli* put into NB medium. Furthermore, *E. coli* culture is mixed into semi-solid NA media, then placed on previously solid NA media as a layer first on the plate. Isolate endophytic bacteria dotted atop the layer, then incubated for 24 hours at room temperature. Bacterial isolates are said to be positive and produce compound antibacterial if an inhibition zone is formed in the test. The inhibition zone then measured the diameter of the inhibition zone based on Morales category (2003) (Table 1).

Table 1. Inhibitory Power Categories (Morales, 2003)

Inhibition Zone Diameter	Category
$\geq 20 - 30$ mm	Very strong
10-20 mm	Strong
5-10mm	Moderate
≤ 5 mm	Weak

3 RESULTS

3.1 Isolation of Endophytic Bacteria

The results of calculating the number of endophytic bacterial colonies that grow in a total of 3,810 bacterial colonies (Table 2) and after viewing based on characteristics colony 93 isolates were found to have characteristics different colonies.

Table 2: Calculation Results of the Number of Endophytic Bacterial Colonies

Plant Code	Dilution	Number of Colonies
K ₁ E	10 ⁻¹	Plate 1
		209
K ₁ E	10 ⁻²	Plate 1
		181
K ₁ E	10 ⁻³	Plate 1
		122
K ₁ E	10 ⁻⁴	Plate 1
		253
K ₂ E	10 ⁻¹	Plate 1
		267
K ₂ E	10 ⁻²	Plate 1
		273
K ₂ E	10 ⁻³	Plate 1
		269
K ₂ E	10 ⁻⁴	Plate 1
		217
K ₃ E	10 ⁻¹	Plate 1
		137
K ₃ E	10 ⁻²	Plate 1
		15
K ₃ E	10 ⁻³	Plate 1
		26
K ₃ E	10 ⁻⁴	Plate 1
		165
K ₃ E	10 ⁻¹	Plate 1
		33
K ₃ E	10 ⁻²	Plate 1
		56
K ₃ E	10 ⁻³	Plate 1
		265
K ₃ E	10 ⁻⁴	Plate 1
		271
K ₃ E	10 ⁻¹	Plate 1
		TMTC
K ₃ E	10 ⁻²	Plate 1
		226
K ₃ E	10 ⁻³	Plate 1
		TMTC
K ₃ E	10 ⁻⁴	Plate 1
		159

K ₃ E	10 ⁻³	Plate 1	115
		Plate 2	178
K ₃ E	10 ⁻⁴	Plate 1	259
		Plate 2	114
Total Number of Colonies			3,810

*TMTC: too many to count

3.2 Characterization Colonies and Morphology of Endophytic Bacteria

From growing colonies of endophytic bacteria screening between colonies, characteristics between

different colonies were observed by looking at the colony's shape, edges, elevation, texture, and pigment (Table 3). Based on the results of observations of the characteristics of the colony, 93 isolates of endophytic bacteria were obtained, which were grouped into 14 different colony groups.

Gram staining is done to determine the morphology of the bacteria by determining the cell morphology and the Gram type (Table 4). The observation results of microscopic Gram stain show that there were 80 isolates of endophytic bacteria in the coccus Gram-positive bacteria, four isolates of endophytic bacteria were coccus Gram-negative bacteria and nine isolates of bacillus Gram-negative.

Table 3 Characteristics Colony of *P. acuminata* Endophytic Bacterial Isolates

No	Characteristics colony					Isolate code	Number of isolates
	Shape	Margin	Elevation	Texture	Pigment		
1.	Circular	Entire	Convex	Moist	Shiny white	KE 1, KE 5, KE 6, KE 9, KE 10, KE 11, KE 21, KE 29, KE 30, KE 31, KE 32, KE 33, KE 34, KE 35, KE 36, KE 37, KE 38, KE 49, KE 50, KE 39	20
2.	Circular	Entire	Convex	Moist	Yellow	KE 2, KE 8, KE 17, KE 20, KE 78, KE 48, KE 51, KE 53, KE 54, KE 81, KE 69	11
3.	Circular	Entire	Convex	Moist	Shiny white	KE 3, KE 18, KE 19, KE 22, KE 25, KE 62, KE 63, KE 64, KE 65, KE 70, KE 71, KE 72, KE 60, KE 82, KE 84, KE 85	16
4.	Circular	Entire	Convex	Moist	Shiny white	KE 4, KE 23, KE 74, KE 75, KE 52	5
5.	Irregular	Undulate	Convex	Moist	White	KE 7	1
6.	Circular	Entire	Convex	Moist	Shiny white	KE 12, KE 13, KE 14, KE 25, KE 26, KE 27, KE 41, KE 42, KE 43, KE 57, KE 58, KE 61	12
7.	Irregular	Undulate	Flat	Moist	White	KE 15, KE 28, KE 66, KE 83, KE 89, KE 90	6
8.	Circular	Entire	Flat	Moist	White	KE 16, KE 45, KE 46, KE 55, KE 59, KE 67, KE 68, KE 80	8
9.	Irregular	Undulate	Flat	Moist	Shiny white	KE 40	1
10.	Irregular	Undulate	Flat	Moist	Yellow	KE 44, KE 47, KE 76	3
11.	Spindle	Entire	Convex	Moist	White	KE 55	1
12.	Circular	Undulate	Flat	Moist	Yellow	KE 73	1
13.	Irregular	Undulate	Convex	Moist	Yellow	KE 77, KE 79	2
14.	Irregular	Undulate	Convex	Moist	Shiny white	KE 86, KE 87, KE 88	3

Information : KE 1= Endophytic bacterial isolate 1st Cambodia and so on

Table 4: Grouping Isolate Based on Gram Stain

Form	Isolate	Gram	Amount
Cocci	KE 1, KE 2, KE 3, KE 4, KE 5, KE 6, KE 7, KE 8, KE 9, KE 10, KE 11, KE 12, KE 13, KE 14, KE 15, KE 16, KE 18, KE 20, KE 23, KE 24, KE 25, KE 26, KE 27, KE 28, KE 29, KE 30, KE 31, KE 32, KE 33, KE 34, KE 35, KE 36, KE 37, KE 38, KE 39, KE 40, KE 41, KE 42, KE 43, KE 44, KE 45, KE 46, KE 47, KE 48, KE 49, KE 50, KE 51, KE 52, KE 53, KE 54, KE 55, KE 56, KE 57, KE 58, KE 59, KE 60, KE 61, KE 62, KE 63, KE 64, KE 65, KE 66, KE 67, KE 69, KE 70, KE 71, KE 72, KE 73, KE 74, KE 75, KE 76, KE 80, KE 86, KE 87, KE 88, KE 89, KE 90, KE 91, KE 92, KE 93	Positive	80
Cocci	KE 22, KE 17, KE 79, KE 78	Negative	4
Bacil	KE 21, KE 19, KE 68, KE 77, KE 84, KE 81, KE 82, KE 83, KE 85	Negative	9

3.3 Antagonist Test Endophytic Bacterial Isolate to *Escherichia coli*

Of the 93 isolates obtained, an antagonist test was carried out. The antagonist test was carried out using a 2-layer agar method consisting of solid and semi-solid nutrient media. Antagonist test results can be seen in Table 5.

Antagonist test gets that result endophytic bacterial isolates tested on *E. coli*. Seven isolates can inhibit the growth of *E. coli*. Inhibiting activity growth of *E. coli* bacteria can be seen from the clear zone formed around the tested endophytic bacterial isolate (Figure 1).

according to Afzal et al., which exists influencing factors diversity of endophytic bacteria something

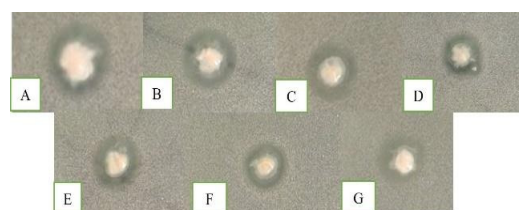


Figure 1. Clear zone formed around endophytic bacterial isolates tested (A) KE 19, (B) KE 44, (C) KE 76, (D) KE 77, (E) KE 78, (F) KE 79, (G) KE 81

Calculating the zone of inhibition and endophytic bacteria that grow using a compass tool shove. The calculation method is by calculating the diameter of the isolate and the zone of inhibition, subtracting the diameter of the isolate, then obtaining a mark of the diameter of the inhibition zone. The value of the diameter of the inhibition zone formed is categorized based on classification Morales (2003). Table 6 shows that isolates with codes KE 19 and KE 44 have power activity hampered by categories strong against *E. coli* bacteria. Isolate others with codes KE 76, KE 77, KE 78, KE 79, and KE 81 have power activity in the moderate category of inhibition against *E. coli* bacteria.

4 DISCUSSIONS

Endophytes are microorganisms in the form of bacteria that live well in the tissues of the plant host. In the network, plant endophytes do not give rise to damage to plants. During co-evolution, endophytic bacteria estimated origin from outside the environment plants, then enter the network plant passed various pathways, such as stomata (pores small on the surface leaves), lenticels (holes in the skin wood), lenticels (holes in the skin stem), wound plant, area of emergence of shoots, roots side (root shoots) and sprouts (Siregar et al., 2020). The number of endophytic bacteria generally ranges between 10³ -10⁵ cfu /g network plants (Tangapo, 2018). In this research, the results of the isolation of endophytic bacteria from stem White Cambodia on King'B agar that has been incubated for 24 hours, 3,810 colonies of endophytic bacteria that grew, are presented.

There are differences in endophytic bacteria colony numbers from the stem. It is in line

plants, besides competent with bacteria to colonize plants as endophytic bacteria, plants host and factors

the environment also has an influence on the diversity of endophytic bacteria from something plant (Afzal et al., 2019). Afzal et al. also mentioned that the total population of endophytic bacteria in plants can vary depending on the type of growth medium used for isolation and the level of dilution when doing isolation. In this study, endophytic bacteria were isolated using King's B media. King's B media consists of glycerol, peptone, dyspotassium phosphate, magnesium sulfate, and agar, which supports the growth of bacteria. King's B media was selected because it has content similar to the situation inside plants and is also non-selective, so it is possible for endophytic bacteria to live and grow (Rizqoh et al., 2021).

Table 5. Antagonist Test Results Endophytic Bacterial Isolate to *Escherichia coli*

Results	Isolate Code	Number of isolates
Positive (+)	KE 19, KE 44, KE 76, KE 81, 78, KE 79, KE 77	7
Negative (-)	KE 1, KE 2, KE 3, KE 4, KE 5, KE 6, KE 7, KE 8, KE 9, KE 10, KE 11, KE 12, KE 13, KE 14, KE 15, KE 16, KE 17, KE 18, KE 20, KE 21, KE 22, KE 23, KE 24, KE 25, KE 26, KE 27, KE 28, KE 29, KE 30, KE 31, KE 32, KE 33, KE 34, KE 35, KE 36, KE 37, KE 38, KE 39, KE 40, KE 41, KE 42, KE 43, KE 45, KE 46, KE 47, KE 48, KE 49, KE 50, KE 51, KE 52, KE 53, KE 54, KE 55, KE 56, KE 57, KE 58, KE 59, KE 60, KE 61, KE 62, KE 63, KE 64, KE 65, KE 66, KE 67, KE 68, KE 69, KE 70, KE 71, KE 72, KE 73, KE 74, KE 75, KE 76, KE 80, KE 82, KE 83, KE 84, KE 85, KE 86, KE 87, KE 88, KE 89, KE 90, KE 91, KE 92, KE 93	86

Note: Positive (+) = potentially produce compound antibiotics by forming an inhibition zone, Negative (-) = no potentially produce compound antibiotics

Endophyte isolates of this research have various types of colonies, including shape, edges, elevation, texture, pigment, and cells. Diversity colonization endophytes are formed and influenced by several factors related to the environment, plants, and bacteria. In addition to the ability of bacteria to

colonize plants as endophytes, the plant's host and the environment in which bacteria grow can influence the growth and size of endophytic bacterial cells in certain plants. The age of the plant host, genotype, location, geographic location, and even analyzed network can determine the type of endophytic bacteria it contains. Apart from that, the growth stages of the host can also determine the diversity of endophytes. Nutrient availability tends to experience enhanced bacterial diversity where the plant stage is enriched. Not only that, but the climate can also influence the colonization of endophytes in plants (Afzal et al., 2019). The isolates obtained were then observed characteristics of the colony. Observation morphology Bacterial colonies are needed to facilitate the identification process of the type of bacteria (Wardhani et al., 2020).

Table 6. Inhibitory Power Category Endophytic Bacterial Isolate

No.	Isolate code	Inhibition zone diameter (mm)	Category
1.	KE 19	13 ± 1.65	Strong
2.	KE 44	10, 55 ± 0.05	Strong
3.	KE 76	7.05 ± 0.4	Moderate
4.	KE 77	7.3 ± 0.6	Moderate
5.	KE 78	7.5 ± 0.6	Moderate
6.	KE 79	7.35 ± 0.6	Moderate
7.	KE 81	6.15 ± 0.7	Moderate

This result of the Gram stain also showed various types of cell morphology. The type of endophytic bacteria found in one plant host is not limited to only types of endophytic bacteria, but consists of various genera and types. Based on the results of research conducted by Zulkifli (2022), skin stem *P. acuminata* produces endophytic bacteria from the genus *Bacillus*, *Pseudomonas*, and *Alcaligenes*. This is in line with the statement Tangapo (2018) stated that the genera *Pseudomonas*, *Bacillus*, *Agrobacterium*, and *Enterobacter* are the most abundant genera found (Tangapo, 2018).

This antagonist test stage tests between endophytic bacterial isolates that have been obtained in the isolation process with the target bacteria, *E. coli*. The purpose of this antagonist test is to see the ability of isolates of these endophytic bacteria to inhibit pathogenic bacteria, namely *E. coli*. Results from the antagonist test of endophytic bacterial isolates to *E. coli* showed that seven positive isolates of endophytic bacteria inhibit the growth of *E. coli*. Seven isolates of this bacteria are KE 19, KE 44, KE

76, KE 77, KE 78, KE 79 and KE 81. These isolates have different cell types; KE 44, KE 76, KE 78, and KE 79 have cocci form, and KE 19, KE 77, and KE 81 have basil form. Every bacterias has a different genetic composition, metabolism pathway, and biochemical ability, so each has produced different compounds and metabolites (Afzal et al., 2019). Endophytic bacterial isolates that showed positive results can be observed by forming a clear zone around the colony of endophytic bacterial isolates. A clear zone is formed because target microorganisms cannot grow around the isolate. This can be interpreted that the isolate from *P. acuminata* can kill and inhibit the growth of pathogenic bacteria.

Endophytic bacteria benefit indirectly; bacteria as biocontrol can role against or as controller microbe pathogen through the production of antipathogen compounds, one of them being antibiotics. Endophyte microbes generally can produce compounds with structures similar to those produced by plant hosts with the help of enzyme activity. Some endophytes also can produce compound antibiotics that can oppose pathogen microbes. Compound antibiotics in the form of metabolites Secondary bacteria, created by endophytic bacteria, act as active substances, antibiotics, or products that help protect plants from attacking insects or pathogen microbes. Hence, endophytic bacteria have the potential to be utilized as biological agents or biocontrol agents to protect plants from pathogens (Tangapo, 2018).

The formation of an inhibition zone around the endophytic bacterial isolate that was inoculated on the test medium indicates that endophytic bacteria from *P. acuminata* have antibacterial activity. This finding is in accordance with the results of research by Yuli (2019), which states that fraction test ethanol from flower *P. acuminata* has an antibacterial effect on *E. coli*. Based on research conducted by Hidayati (2019), endophytic bacteria from *P. acuminata* stems produce secondary metabolites in alkaloids. Additionally, *P. acuminata* stems have content in the form of tannins, flavonoids, alkaloids, and triterpenoids. An alkaloids compound in endophytic bacteria present in *P. acuminata* stem have an antibacterial effect. The alkaloid compound works with mechanisms inhibition that interferes with components shaper peptidoglycan inside bacterial cells, resulting in the layer from bacterial cell wall no longer forming correctly, resulting in cell death. Apart from that, Alkaloids can also prevent protein synthesis, which can influence bacterial metabolism. This alkaloid compound can also prevent the development of Gram-negative bacteria (Anggraini et al., 2019).

5 CONCLUSIONS

Based on the results of the research, the following conclusion is derived. Results of isolation of *P. acuminata* endophytic bacteria obtained as many as 3,810 colony endophytic bacterial isolates from stem of *P. acuminata*. Result of observation of characteristics colonies and morphology of endophytic bacteria of 93 samples of *P. acuminata* endophytic bacterial isolates get various type shapes, edges, elevations, textured, bacterial pigments and also shape bacterial cells. The result of the Gram stain shows that there were 80 isolates in the Gram-positive group and 13 isolates in the Gram-negative group. Antagonist test results of the 93 endophytic bacterial isolates tested against *E. coli* bacteria showed seven endophytic bacterial isolates in *P. acuminata* have the potency to inhibit the growth of *E. coli*.

ACKNOWLEDGEMENTS

Thanks for the support from the Faculty of Medicine, Universitas Bengkulu, laboratory staffs, and all parties who have helped this research process. A grant from the Non-tax Revenue of the Faculty of Medicine, Universitas Bengkulu, supports this research.

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