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Isolation and Characterization of Antibiotic-Producing Endophytic Microbes from Glutinous Taro Tubers (*Colocasia esculenta* L.) Against Pathogenic Bacteria

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ABSTRACT

A research had been done to isolation of endophytic microba producing antibiotics of taro ketan tuber (*Colocasia esculenta* L.) with puspouse to deside activity antibiotics of endophytic microbe of taro ketan tuber (*Colocasia esculenta* L.) toward some microbe test. The result isolation endophytic microbe of taro ketan tuber (*Colocasia esculenta* L.) from dilution 10^{-1} until 10^{-5} result 8 colony bacteria using Nutrien Agar (NA) media and 7 colony fungi using Potato Dextrose Agar (PDA) media. Colony bacteri and fungi purified by quadran method result that 8 isolates bacteria (ISBT1, ISBT2, ISBT3, ISBT4, ISBT4, ISBT5, ISBT6, ISBT7, ISBT8) and 4 isolates fungi (ISJT1, ISJT2, ISJT3, ISJT4) then do fermentation with Maltose Yeast Broth (MYB). Based on activity isolates bacteria and isolates fungi provide activity toward bacteria *Bacillus subtilis*, *Vibrio cholerae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Shigella disentri*, *Eschericia coli* and *Salmonella thypi* with largest inhibition zone diameter was isolates bacteria (ISBT7 = 18.7 mm toward bacteria *Vibrio cholerae* and was isolates fungi (ISJT3) = 23.4 mm toward bacteria *Streptococcus mutans*.

KEYWORDS: Taro Ketan Tuber ((*Colocasia esculenta* L.)), Isolation Endophytic Microbe, Antibiotics.

I. INTRODUCTION

Endophytic microbes are microorganisms that grow in plant tissues. Endophytic microbes can be isolated from root, stem and leaf tissue, and the most commonly found are fungi. Some endophytic microbes can produce bioactive compounds as secondary metabolites that have antimicrobial, antimalarial, anticancer and so on. Endophytic microbes in addition to having an important role in the world of medicine, also have an important role in the industrial world (1).

Endophytic microbes are believed to be able to produce bioactive compounds whose characteristics are similar to or the same as their hosts. This is due to the genetic exchange that occurs between the host and endophytic microbes evolutionarily. The relationship between the host and endophytic microbes is thought to be mutually beneficial (mutualism symbiosis) where plants provide nutrients for microbes, then microbes transform and produce bioactive compounds (2).

In recent years, the exploration of microbial resources found in plant tissues has received much attention. In addition to the chemical content contained in a plant which is a nutritious substance, there are also types of microbes that live in the living tissue of the plant. (4). The increasingly widespread use of antibiotics in the treatment of infectious diseases has become an important concern in exploring the search for antibiotics derived from plant tissues through secondary metabolites produced by their host plants. The developed hypothesis proves that plants that are efficacious as a source of medicine are determined by bioactive compounds that are most likely produced by endophytic microbes.

Plants that can be used as medicine are sticky rice taro (*Colocasia esculenta* L.) which contains various secondary metabolites such as 6-C-glycosylflavonoid and O-glycosylflavonoid, including schaftoside, isoschaftoside, orientin, isovitexin, isoorientin, vitexin and luteolin 7-O-sophoroside. (5). Among the people, the sticky taro tuber (*Colocasia esculenta* L.) is widely used as a medicine for scrofula, purulent skin inflammation, psoriasis, bloody stools, boils and burns. While the stalks and leaves are used for the treatment of urticaria,

diarrhea and wound dressings. This is because the taro plant contains polyphenolic compounds and saponins which by TLC-Bioautography provide an antibacterial effect (9).

This is what underlies the search for endophytic microbes in the tissue of the glutinous taro tuber (*Colocasia esculenta* L.) as a producer of antibiotics.

II. MATERIAL AND METHODS

This research was carried out at the Microbiology Laboratory of the Faculty of Pharmacy, Indonesian Muslim University, Makassar based on laboratory experimental testing of endophytic microbes of glutinous taro tubers against pathogenic bacteria.

1. Materials

The tools used are autoclave (Smic Model YX-280B), petri dish, enkas, erlenmeyer glass (Iwake, Pyrex). beaker (Iwake, Pyrex), incubator (Memmert), micropipette (Huawei), oven (Memmert), Laminar Air Flow (LAF), centrifuge, analytical balance, coarse balance (O'Haus). The materials used were 70% alcohol, 0.9% physiological NaCl solution, Nutrien Agar (NA) medium, Potato Dextrose (PDA) medium, Maltose Yeast Broth (MYB) medium, test microbes (*Bacillus subtilis*, *Shigella dysenter*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans* and *Vibrio cholerae*) and samples of sticky rice taro tubers (*Colocasia esculenta* L.).

2. Procedures

a. Sampling

Samples of glutinous taro tubers (*Colocasia esculenta* L.) were obtained from Pare-pare, South Sulawesi taken at 10.00 am. Samples of glutinous taro tubers (*Colocasia esculenta* L.) were cleaned of adhering impurities using running water for 10 minutes then cut into small pieces and then sterilized by soaking in 70% alcohol for 15 minutes, then rinsed with sterile water. The sample was mashed by means of a blender and then weighed as much as 1 gram then dissolved in 10 ml of sterile water and made a 10-1 dilution to a 10-5 dilution.

b. Isolation of Antibiotic-Producing Endophytic Microbes

The sample suspension from each dilution was pipetted 1 ml aseptically and put into a petri dish according to the level of dilution, then 9 ml of Nutrien Agar (NA) medium and 9 ml of Potato Dextrose Agar (PDA) medium were poured into each petri dish, then incubated at 37°C for 1 x 24 hours for bacteria and at room temperature for 3 x 24 hours for fungi (13,10).

c. Endophytic Microbial Purification

Each different isolate was purified by the quadrant method to obtain a single isolate. The pure culture was then transferred to a slanted agar as stock (10).

d. Microbial Characterization of Glutinous Taro Tubers Endophytic

1) Macroscopic Testing of Endophytic Microbial Isolates

Each isolate was taken one ose was inoculated into Nutrien Agar (NA) medium for bacteria and Potato dextrose Agar (PDA) for fungi. Incubate for 1-5 days at 37°C for bacteria and at 25°C for 1-8 days for fungi. The results from the macroscopic are then transferred to a slanted agar as stock. Macroscopic observations of isolates were carried out by observing the shape of the colony, the periphery of the colony, elevation, color and structure in the colony (6).

2) Gram Painting of Endophytic Microbial Isolates of Glutinous Taro Tubers

Glass objects and glass decks were prepared that had been cleaned and freed from grease with 70% alcohol. With a round loop, a thin film is made on the surface of the glass object. The film was dried in air, then fixed by touching the surface of the glass object to the Bunsen flame. After cooling, the preparations were added with 1-2 drops of A paint, allowed to stand for 1 minute, then washed with running water and dried with a tissue. After drying, the same treatment was carried out with the addition of paint B, paint C, and paint D. The preparations were observed under a microscope by looking at the morphology and color of the endophytic microbial isolates. Purple color indicates gram positive and red color indicates gram negative.

3) Fermentation of Endophytic Microbial Isolates

Pure culture colonies of endophytic microbes were taken one ose, inoculated in 10 ml of MYB medium, then shaken at 200 rpm for 7 days (14).

4) Testing the Antibiotic Activity of Endophytic Microbial Isolates Against Pathogenic Bacteria

10 mL of Nutrient Agar (NA) medium was taken and added with 20 L of microbial suspension, then the blank disc that had been immersed in the fermentation product was then placed on the surface of the solidified medium. Then it was incubated at 37°C for 1x24 hours for bacteria and then observed and measured the inhibition zone formed (11,12).

III. RESULT AND DISCUSSION

1. Isolation of endophytic microbes from glutinous taro tubers (*Colocasia esculenta* L.)

Based on the results of the research that has been carried out, it was obtained that the isolated culture of microorganisms from glutinous taro tubers (*Colocasia esculenta* L.) which showed a zone of inhibition as many as 8 colonies of endophytic bacteria taken at a dilution of 10⁻² there were 4 colonies, 10⁻³ there were 1 colony, 10⁻⁴ there were 2 colonies and 10⁻⁵ there was 1 colony on Nutrien Agar (NA) medium, while in mushrooms 7 colonies of endophytic fungi were obtained which were taken at a dilution of 10⁻¹ there were 2 colonies, 10⁻² had 1 colony, 10⁻³ had 1 colony, 10⁻⁴ there was 1 colony and 10⁻⁵ there were 2 colonies on Potato Dextrose Agar (PDA) medium.

2. Purification of endophytic microbial isolates of glutinous taro tubers

Microbial colonies showing the zone of inhibition were purified by the quadrant method so that pure microbial cultures were obtained, namely isolates containing only one form of the same colony morphology. This method was used with the aim of obtaining pure cultures of isolates obtained using Nutrien Agar (for bacteria) and Potato Dextrose Agar (for fungi) medium and incubation for 1x24 hours at 37°C for bacteria and 3x24 hours at room temperature for fungi. As in the following figure.

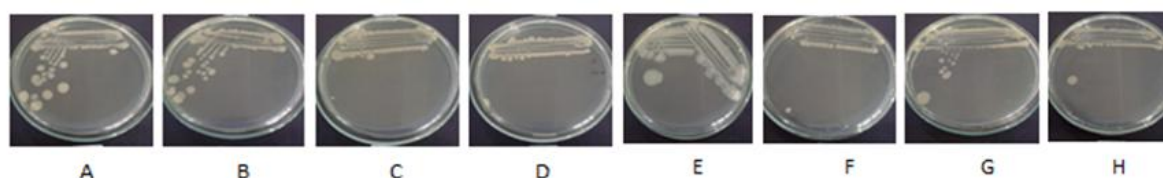


Figure 1. Purification of Microbial Endophytic Tubers of Glutinous Taro (*Colocasia esculenta* L.) on Nutrient Agar (NA) medium. (A) Isolat bakteri endofit ke-1 (SBT1), (B) Isolat bakteri endofit ke-2 (SBT2), (C) Isolat bakteri endofit ke-3 (SBT3), (D) Isolat bakteri endofit ke-4 (SBT4), (E) Isolat bakteri endofit ke-5 (SBT5), (F) Isolat bakteri endofit ke-6 (SBT6), (G) Isolat bakteri endofit ke-7 (SBT7), (H) Isolat bakteri endofit ke-8 (SBT8)

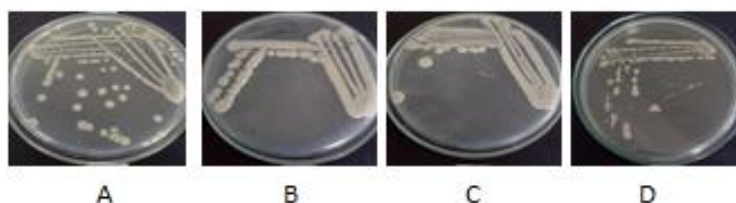


Figure 2. Purification of Microbial Endophytic Tubers of Glutinous Taro (*Colocasia esculenta* L.) on Potato Dextrose Agar (PDA) medium. (A) 1st endophytic fungus isolate (SJT1), B : 2nd endophytic fungal isolate (SJT2), C : 3rd endophytic fungal isolate (SJT3), D : 4th endophytic fungal isolate (SJT4)

Table 1. Results of Purification of Endophytic Bacterial Isolates of Taro Glutinous Tubers (*Colocasia esculenta* L.)

Number	Endophytic Microbial Code	Description
1	ISBT ₁	Endophytic bacteria isolate 1
2	ISBT ₂	Endophytic bacteria isolate 2
3	ISBT ₃	Endophytic bacteria isolate 3
4	ISBT ₄	Endophytic bacteria isolate 4
5	ISBT ₅	Endophytic bacteria isolate 5
6	ISBT ₆	Endophytic bacteria isolate 6
7	ISBT ₇	Endophytic bacteria isolate 7
8	ISBT ₈	Endophytic bacteria isolate 8

Table 2. Purification Results of the Endophytic Fungus of Glutinous Taro Tubers

Number	Endophytic Microbial Code	Description
1	ISJT ₁	Endophytic Fungus Isolate 1
2	ISJT ₂	Endophytic Fungus Isolate 2
3	ISJT ₃	Endophytic Fungus Isolate 3
4	ISJT ₄	Endophytic Fungus Isolate 4

3. Macroscopic Examination of Endophytic Microbial Isolates of Glutinous Taro Tubers (*Colocasia esculenta* L.)

Observations were made by looking at the colony shape, elevation, edge and color of the bacterial and fungal isolates. It can be seen in the chart below and tables 3 and 4.

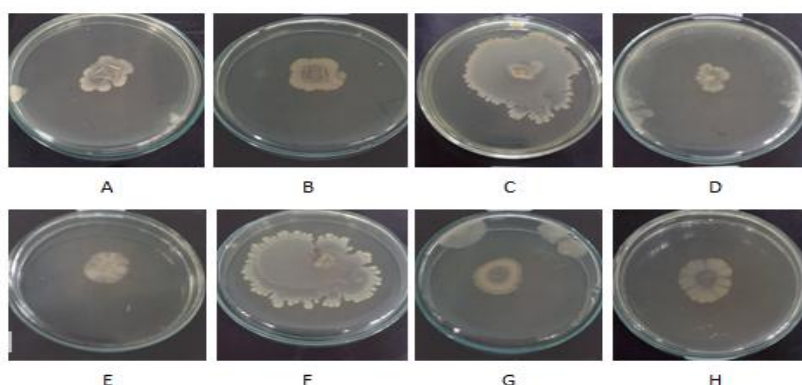


Figure 3. Macroscopic Isolate of Endophytic Bacteria from Tubers of Glutinous Taro (*Colocasia esculenta* L.). (A) 1st endophytic bacterial isolate (SBT1), (B) 2nd endophytic bacterial isolate (SBT2), (C) 3rd endophytic bacterial isolate (SBT3), (D) 4th endophytic bacterial isolate (SBT4), (E) 5th endophytic bacterial isolate (SBT5), (F) 6th endophytic bacterial isolate (SBT6), (G) 7th endophytic bacterial isolate (SBT7), (H) 8th endophytic bacterial isolate (SBT8).

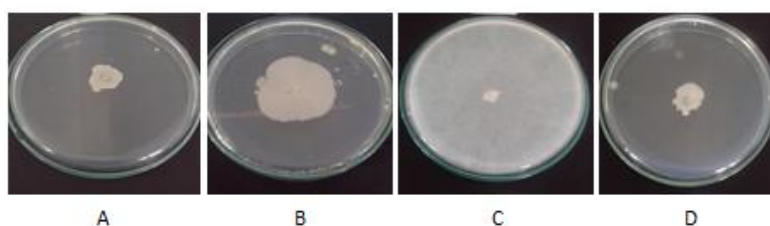


Figure 4. Macroscopic Endophytic Fungus Isolate from Tubers of Taro Glutinous (*Colocasia esculenta* L.). (A) 1st endophytic fungus isolate (SJT1), (B) 2nd endophytic fungal isolate (SJT2), (C) 3rd endophytic fungal isolate (SJT3), (D) 4th endophytic fungal isolate (SJT4).

Table 3. Macroscopic Results of Endophytic Bacterial Isolates on Glutinous Taro Bulbs

Bacteria Code	Color	Colony Shape	Edge	Elevation
ISBT ₁	White	Complex	Wavy	Flat
ISBT ₂	White	Complex	Wavy	Flat
ISBT ₃	White	Irregular and Spreading	Lobate	Flat
ISBT ₄	White	Complex	Wavy	Flat
ISBT ₅	White	Wrinkled	Smooth	Flat
ISBT ₆	White	Irregular and Spreading	Lobate	Flat
ISBT ₇	White	Concentric	Wavy	Flat
ISBT ₈	White	Complex	Wavy	Flat

* ISBT₁ : isolates of endophytic bacteria 1, ISBT₂ : isolates of endophytic bacteria 2, ISBT₃ : isolates of endophytic bacteria 3, ISBT₄ : isolates of endophytic bacteria 4, ISBT₅ : isolates of endophytic bacteria 5, ISBT₆ : isolates of endophytic bacteria 6, ISBT₇ : isolates of endophytic bacteria 7, ISBT₈ : Isolate of endophytic bacteria 8

Table 4. Macroscopic Results of Endophytic Fungus Isolates on Glutinous Taro Bulbs

Bacteria Code	Color	Colony Shape	Edge	Elevation
ISJT ₁	White	Wrinkled	Wavy	Flat
ISJT ₂	White	Wrinkled	Smooth	Flat
ISJT ₃	White	Round	Smooth	Flat
ISJT ₄	White	Wrinkled	Wavy	Flat

* ISJT₁ : Isolat jamur endofit 1, ISJT₂ : Isolat jamur endofit 2, ISJT₃ : Isolat jamur endofit 3, ISJT₄ : Isolat jamur endofit 4

4. Gram staining of endophytic bacterial isolates of glutinous taro tubers

The bacterial isolates obtained were examined microscopically in order to determine the shape of the cells/colonies of the isolates using gram staining to differentiate into gram-positive and gram-negative groups. The paints used in this painting are paint A (Crystal violet), paint B (iodine solution), paint C (ethanol) and paint D (safranin). in the following image.

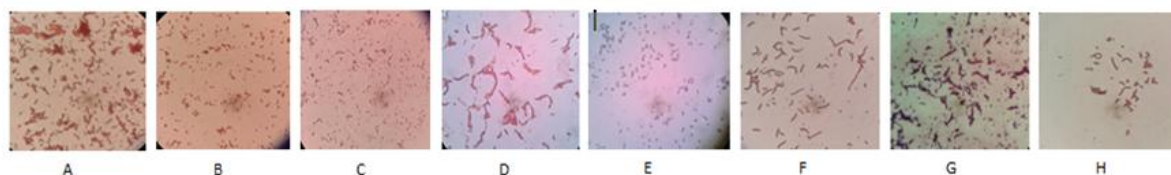


Figure 5. Microscopic isolates of endophytic bacteria from tubers of sticky rice taro (*Colocasia esculenta* L.)

The results of microscopic examination showed that the bacterial isolate was a group of Gram Negative bacteria which showed a red color with the shape of a cocci colony. Microscopic results can be seen in table 5.

Table 5. Results of Gram Painting Isolate of Endophytic Bacteria of Taro Glutinous Tubers

Bacteria Code	Color	Shape	Description
ISBT ₁	Red	Kokus	Gram negatif
ISBT ₂	Red	Kokus	Gram negatif
ISBT ₃	Red	Kokus	Gram negatif
ISBT ₄	Red	Kokus	Gram negatif
ISBT ₅	Red	Kokus	Gram negatif
ISBT ₆	Red	Kokus	Gram negatif
ISBT ₇	Red	Kokus	Gram negatif
ISBT ₈	Red	Kokus	Gram negatif

* ISBT₁ : Isolat bakteri endofit 1, ISBT₂ : Isolat bakteri endofit 2, ISBT₃ : Isolat bakteri endofit 3, ISBT₄ : Isolat bakteri endofit 4, ISBT₅ : Isolat bakteri endofit 5, ISBT₆ : Isolat bakteri endofit 6, ISBT₇ : Isolat bakteri endofit 7, ISBT₈ : Isolat bakteri endofit 8

5. Fermentation of Microbial Isolates of Glutinous Taro Tubers

The endophytic microbial isolates obtained were then fermented in Maltose Yeast Broth (MYB) medium because this medium is a source of protein needed for microbial growth, synthesis and metabolism. 200 rpm to obtain the supernatant fermentate and mycelia. To see the potential of the secondary metabolism results, antibiotic testing was carried out.

6. Testing the Antibiotic Activity of Endophytic Microbial Isolates of Glutinous Taro Tubers (*Colocasia esculenta* L.)

Testing the antibiotic activity of isolates of bacteria and fungi of glutinous taro tubers (*Colocasia esculenta* L.) with the agar diffusion method using Antimicrobial susceptibility test discs. The results of the antibiotic activity test showed that not all isolates gave activity against the test microbes, this was indicated by the inhibition zone formed. The bacterial isolate with the largest inhibition zone diameter of 18.7 mm was isolate 7 (SBT7) which was active against *Vibrio cholerae*.

Fungal isolates 1 (SJT1), 2 (SJT2) and 3 (SJT3) gave activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Salmonella typhi*, *Escherichia coli*, *Vibrio Cholerae* and *Shigella dysentery* with different diameters of inhibition zones. Meanwhile, fungal isolate 4 (SJT4) did not provide activity against the tested microbes. The fungal isolate that had the largest inhibition zone diameter of 23.4 mm was isolate 3 (SJT3) which was active against *Streptococcus mutans*. The results of activity testing can be seen in table 6.

Table 6. Results of Testing the Antibiotic Activity of Fermentate Isolates of Endophytic Bacteria on Samples of Glutinous Taro Bulbs (*Colocasia esculenta* L.)

Endophytic Bacteria Isolate	M	Barrier Zone Diameter (mm)								
		ST	SD	SM	SA	PA	VC	BS	EC	SE
SBT 1	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0

	3	0	0	0	0	0	0	0	0	0
	\bar{Y}	0	0	0	0	0	0	0	0	0
SBT 2	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	\bar{Y}	0	0	0	0	0	0	0	0	0
SBT 3	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	\bar{Y}	0	0	0	0	0	0	0	0	0
SBT 4	1	10	10	11	10	0	0	10	10	10
	2	11	10	10	9	0	0	10	10	9
	3	10	10	10	9	0	0	10	11	10
	\bar{Y}	10,4	10	10,4	7,4	0	0	10	10,4	9,7
SBT5	1	16	0	15	0	0	18	0	17	14
	2	15	0	15	0	0	15	0	16	13
	3	16	0	15	0	0	17	0	16	14
	\bar{Y}	15,6	0	15	0	0	16,7	0	16,4	13,7
SBT 6	1	14	14	15	0	0	0	0	17	0
	2	15	14	15	0	0	0	0	16	0
	3	15	13	15	0	0	0	0	17	0
	\bar{Y}	14,7	13,7	15	0	0	0	0	16,7	0
SBT 7	1	15	0	17	0	0	18	0	16	13
	2	17	0	15	0	0	19	0	15	13
	3	15	0	15	0	0	19	0	16	14
	\bar{Y}	15,7	0	15,7	0	0	18,7	0	15,7	13,4
SBT 8	1	16	15	18	0	0	0	0	19	0
	2	17	14	17	0	0	0	0	17	0
	3	17	15	18	0	0	0	0	19	0
	\bar{y}	16,7	14,7	17,7	0	0	0	0	18,4	0

* M : measurement, \bar{y} : mean, BS : *Basillus subtilis*, ST : *Salmonella thypi*, EC : *Escherichia coli*, SM : *Sterptococcus mutans*, SD : *Shigella dysentri*, SE : *Staphylococcus epidermidis*, SA : *Staphylococcus aureus*, PA : *Pseudomonas aeruginos*, VC : *Vibrio cholerae*

Table 7. Results of Testing the Antibiotic Activity of Endophytic Fungus Isolate Fermentate on Glutinous Taro Bulbs Samples (*Colocasia esculenta* L.)

Endophytic Fungus Isolate	M	Barrier Zone Diameter (mm)								
		ST	SD	SM	SA	PA	VC	BS	EC	SE
SJT 1	1	14	17	17	15	0	16	0	15	17
	2	15	17	17	15	0	15	0	15	17
	3	15	18	18	14	0	15	0	15	18
	\bar{Y}	14,7	17,4	17,4	14,7	0	15,4	0	15	17,4
SJT 2	1	12	15	15	15	0	16	0	13	12
	2	12	16	15	15	0	16	0	12	12
	3	12	16	15	15	0	16	0	13	13
	\bar{Y}	12	15,7	15	15	0	16	0	12,7	12,4
SJT 3	1	21	20	23	23	0	21	0	20	20
	2	21	21	23	24	0	22	0	20	21
	3	22	20	24	20	0	23	0	18	20
	\bar{Y}	21,4	20,4	23,4	22,4	0	22	0	19,4	20,4
SJT 4	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0

Isolation and Characterization of Antibiotic-Producing Endophytic Microbes from ..

	3	0	0	0	0	0	0	0	0	0
\hat{y}	0	0	0	0	0	0	0	0	0	0

* M : measurement, \hat{y} : mean, BS : *Basillus subtilis* ST : *Salmonella thypi*, EC : *Escherichia coli*, SM : *Sterptococcus mutans*, SD : *Shigella dysentri*, SE : *Staphylococcus epidermidis*, SA : *Staphylococcus aureus*, PA : *Pseudomonas aeruginos*, VC : *Vibrio cholerae*

The results of the antibacterial activity test showed that the diameter of the inhibition zone formed from bacterial isolates, namely bacterial and herbal isolates, was included in the parameters of the inhibition zone diameter category, namely the diameter of the inhibition zone was weak if 11 mm, the diameter of the inhibition zone was in the intermediate/medium category between 12-21 mm, and the diameter of the zone of inhibition in the strong category was 22 mm. (6,7). The results of the analysis of the antibacterial activity of bacterial isolates, namely 18.7 mm, were isolate 7 (SBT7) with an inhibition zone diameter of 18.7 mm, which was active against *Vibrio cholerae* which was a weak category and isolate 3 (SJT3) was active against *Streptococcus mutans* with an inhibition zone diameter of 23.4 mm is a strong category. These antibiotic isolates with large zones were of excellent antibiotic power (8).

IV. CONCLUSION

Based on the results of the research that has been done, it can be concluded that :

1. Isolation of antibiotic-producing endophytic microbes from glutinous taro tubers (*Colocasia esculenta* L.) resulted in 8 bacterial isolates and 4 fungal isolates.
2. From the results of antibiotic activity testing, bacterial isolate 7 (SBT7) had the largest inhibition zone diameter of 18.7 mm against *Vibrio cholerae*, while fungal isolate 3 (SJT3) had the largest inhibition zone diameter of 23.4 mm against *Streptococcus mutans*.

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