

## Antibacterial Activity Tests of Endophytic Bacteria Isolates From Tea Plant (*Camellia sinensis*) Againsts *Staphylococcus aureus* and *Staphylococcus epidermidis*

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### Abstract

*Staphylococcus* is one of the most common types of bacteria in Asia that causes local infectious diseases of the skin, nose, urethra, vagina, digestive tract, pneumonia, endocarditis, septic arthritis, and septicemia. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common types of *Staphylococcus* in Asia. Tea plants contain bioactive compounds and endophytic bacteria which are widely used as antimicrobial agents. Endophytic bacteria are bacteria that exist in plant tissues, not pathogenic, and have the ability as the host plant. The purpose of this study was to determine the antibacterial activity of endophytic bacterial isolates of tea plants (*Camellia sinensis*) against the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. The antibacterial activity test of endophytic bacteria of tea plants includes a series of processes such as sample selection, surface sterilization of samples, isolation of endophytic bacteria in agar medium, purification, screening, suspension of endophytic bacteria in 0.9 % NaCl and standardized with 0.5 McFarland, making endophytic bacterial culture in nutrient borth, making endophytic bacterial supernatant and antibacterial activity test with paper disc diffusion method. The results obtained are the antibacterial activity of the endophytic bacterial supernatant isolates B23, B14, and A2 on the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The best antibacterial activity was found in endophytic bacterial B14 isolates with inhibition zones of 7.75 mm and 12.5 mm followed by B23 isolates with 7.5 mm and 8.25 mm inhibition zones and A2 isolates with large inhibition zones of 7.42 mm and 8.16 mm. Endophytic bacteria isolates of tea plant show better antibacterial activity against the growth of *Staphylococcus aureus* than *Staphylococcus epidermidis*.

**Keywords :** Antibacterials, Endophytic bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*

### Introduction

*Staphylococcus* is a gram-positive bacterium, coccus and white<sup>16</sup>. At present the genus *Staphylococcus* has been divided into 45 species and eight sub-species<sup>2</sup>. *Staphylococcus* is commonly found in the surrounding environment, medical equipment and the human body<sup>2,1</sup>. Basically the *Staphylococcus* bacteria is one of the normal flora which is estimated to be around 30% found in the human body, especially many found in the anterior nares<sup>1</sup>. However, due to invasion, mutations and an increase in the number of *Staphylococcus* bacteria, several cases have been linked to pathogenic activities such as local infections of the skin, nose, urethra, vagina, digestive tract, and several life-threatening diseases such as pneumonia, endocarditis, septic arthritis and septicemia<sup>2, 14</sup>.

The most characterized and widely studied bacterial species of the genus *Staphylococcus* to date include two strains namely *Staphylococcus epidermidis* and *Staphylococcus aureus*<sup>2</sup>. Both are gram-positive bacteria. *Staphylococcus epidermidis* is known as *Staphylococcus albus* and includes CoNS bacteria (negative coagulation) while *Staphylococcus aureus* has a coagulase enzyme that is able to break down H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub> and called coagulate positively which is able to clot blood plasma<sup>17,28</sup>. The morphology of the *Staphylococcus aureus* bacterial colony often has a golden color when it grows on solid media, whereas CoNS group bacteria such as *Staphylococcus epidermidis* form a pale white and translucent colony<sup>9</sup>. Both of these bacteria have different levels of danger where the species of *Staphylococcus aureus* is more dangerous than *Staphylococcus epidermidis*<sup>3,4</sup>.

*Staphylococcus aureus* and *Staphylococcus epidermidis* are among the most common species in Asia<sup>7</sup>. Various ways have been done to control the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria, one of which uses synthetic antibiotics, but several cases of resistance to antibiotics such as daptomycin, vancomycin, meticylin, and penicilin cause it is necessary to look for other antibiotics that are more effective and safety<sup>11,18,19</sup>. One of them is by exploring medicinal plants as an antimicrobial agent which has the potential as an antibacterial<sup>25</sup>.

One of the medicinal plants is tea plant<sup>20</sup>. Based on 2015 global tea production data, Indonesia ranks seventh after China, India, Kenya, Sri Lanka, Turkey and Vietnam as the world's largest tea producing countries. In addition, the tea plant (*Camellia sinensis*) contains secondary metabolite compounds which have antibacterial activity including polyphenols, tannins, phenols, saponins, terpenoids, and steroids<sup>10,13</sup>. The polyphenols found in tea are mostly in the form of flavonoids. Flavonoid compounds in tea plants function as antibacterial. The main flavonoid compounds in tea are flavanol and flavonol. Potential antibacterial flavanols are catechins consisting of epicatechin (EC), epicatechin gallat (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG). The biggest ingredient in the shoots of tea leaves is catechins or epigallocatechin-3-gallate. The content (EGCG) in tea leaves ranges from 60% w/w<sup>10,31</sup>. Intake of secondary metabolites can be done through the isolation of endophytic bacteria. Endophytic bacteria are bacteria that exist in plant tissues, do not cause pathogens in the host and have the ability as a host plant<sup>6</sup>.

## Methodology

### Sampling

Sampling of the tea plant (*Camellia sinensis*) was carried out at the Medini Tea Plantation, Mount Ungaran, Kendal District, Semarang, Central Java.

### Selection and Surface Sterilization of Samples

Samples of leaves, stems, and roots of tea plants are selected which are healthy, not deformed, and not moldy, cut into  $\pm$  1cm sizes and washed with running water. Furthermore, the surface sterilization with sterile distilled water for  $\pm$  5-10 minutes, then put into 70% alcohol for 2 minutes, then sodium hypochlorite solution 5.25% for 2 minutes and soaked again with 70% alcohol for 30 seconds and finally washed again with sterile distilled water 3 times until it is clean<sup>27</sup>. The last distilled rinses are used for negative control.

### Isolation of Endophytic Bacteria

Samples of leaves, roots, stems of tea plants were planted aseptically into a petri dish containing Nutrient Agar media which had been added with nystatin (0.01% w / v) 30 mg/ml as an antifungal. Confirmation of sample sterility was carried out using 100 µl sterile distilled water in the last rinse on Nutrient Agar medium which was then incubated with isolates of tea plant samples at 37°C for 24-48 hours.

### Purification and Culture of Endophytic Bacteria

One of the endophytic bacterial isolates closest to the sample was taken and inoculated on the agar nutrient with the quadrant streak plate method. Incubated at 37°C for 24-48 hours to obtain separate bacterial colonies. Purification was repeated with an unspecified time limit to ensure the isolates obtained were truly pure.

### Preparation of Test Bacterial Suspension and Endophytic Bacteria

*Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria<sup>24</sup> hours rejuvenation results were taken one ose and suspended into a tube containing 10 ml of 0.9% sterile NaCl solution. Endophytic bacterial suspension is made aseptically by endophytic bacterial isolates on nutrient agar so that 24 hour rejuvenation results are taken one ose and suspended into a tube containing 10 ml of 0.9% sterile NaCl solution. The turbidity obtained was then compared to the 0.5 McFarland standard which is equivalent to the number of bacterial cell growth  $(1-2) \times 10^8$  CFU / ml<sup>8</sup>.

### Making Positive Control and Negative Control

Positive control used in the form of 500 IU chloramphenicol powder with a concentration of 30 ppm dissolved in 10 ml of sterile NaCl 0.9%. The negative controls used were nutrient agar medium and sterile paper disc.

### Antagonistic Test of Endophytic Bacteria Isolates

The selection of the most potential endophytic bacteria from 12 endophytic bacterial isolates was carried out through screening tests using endophytic bacterial isolates with *Staphylococcus aureus* and *Staphylococcus epidermidis* through the paper disc diffusion method on nutrient agar<sup>26</sup>. The method of endophytic bacterial isolates and indicator bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* on 24 hour rejuvenation results was taken one ose and each suspended in a tube containing 10 ml of sterile 0.9% NaCl solution. The turbidity obtained was then compared to the 0.5 McFarland standard. Next, 100 µl of indicator bacterial suspension was taken and flattened on NA media contained in different petri dishes using sterile cotton swabs. Next, 5 µl of endophytic bacterial suspension was taken and dropped on sterile paper disc. Paper disc that have been filled with endophytic bacterial suspensions are then placed and arranged in such a way on the nutrient agar that has been given a bacterial suspension test. When finished, the petri dish is sealed with plastic wrap and labeling. Then put in 37°C incubator for 24 - 48 hours.

### Making Endophytic Bacteria Culture on Medium Nutrient Broth

One ose of endophytic bacteria potential rejuvenation results in the medium nutrient agar for 24-hour, put in 5 ml nutrient broth medium for making a starter and then agitated on a rotary shaker at a speed of 120 rpm at 37°C for 24 hours. Then the starter is put in 100 ml of nutrient broth media and mixed until homogeneous. Potential endophytic bacterial culture in the nutrient broth medium

was then agitated on a rotary shaker at a speed of 120 rpm, temperature 37°C for 52 hours for sampling measurements of growth curves and the optimum time to produce secondary metabolites of potential endophytic bacteria.

### **Endophytic Bacteria Growth Curve**

Bacterial growth curves is done by measuring Optical Density (OD) using a UV-VIS spectrophotometer ( $\lambda = 620 \text{ nm}$ )<sup>12</sup>. Sampling is carried out in 52 hours every 4 hours starting at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, and 52 hours.

### **Making Endophytic Bacteria Supernatant**

A total of 10 ml of starter containing 10 ml of nutrient broth media with one ose of potential endophytic bacteria isolates that have been shaker and agitated for 24 hours is put into an erlenmeyer containing 100 ml of sterile nutrient broth media then agitated on a rotary shaker at a speed of 120 rpm at 37°C. Then as much as 10 ml of potential endophytic bacterial culture was centrifuged at 4000 rpm at 4°C for 15 minutes to obtain a separation between the supernatant at the top and debris at the bottom. The endophytic bacterial supernatant is then separated from the debris by taking part of the supernatant using a sterile microtype to be transferred to another sterile container/bottle and stored in a cooler.

### **Antibacterial Test with Endophytic Bacteria Supernatant**

Antibacterial activity test was carried out by dividing the sterile petri dish into 5 parts with an angle of 72° on the bottom outside of the petri dish. Furthermore, each corner is given the name supernatant (Sp) potential endophytic bacteria to be used. nutrient media in order to pour 20 ml of sterile petri dish. After the media solidifies, the bacteria *Staphylococcus epidermidis* or *Staphylococcus aureus* are inoculated in a petri dish using a sterile cotton swab method evenly on the surface of the media. Antibacterial test with a potential endophytic bacterial supernatant was carried out by means of each endophytic bacterial supernatant being dropped on 5 µl paper disc using a micropipet. The paper disc was transferred to the nutrient agar which had been added 100 µl of bacterial suspension test on a petri dish. Paper disc with endophytic bacterial suspension is placed on the area in accordance with the name of the supernatant (Sp) endophytic potential bacterial potential endophytic bacteria code 1, 2, or 3. One part on the agar nutrient to be given sterile paper disc as a negative control, and one paper disc containing chloramphenicol 30 µg / ml with a concentration of 5 µl was used as a positive control. All plates were incubated for 24-48 hours at 37°C. Each treatment was repeated 3 times to obtain accurate antibacterial test results. The diameter of the inhibition zone formed is measured using a calipers.

### **Measurement of Endophytic Bacterial Inhibition Zones**

Barriers are measured using calipers. The method of measurement is to take 2 lines perpendicular to each other through the center of the paper disc, while the third and fourth lines are drawn between the two lines by forming an angle of 45°. Measurements are made using 4 lines at different places namely by using the diameter of the zone resistance line AB plus the diameter of the inhibitory zone of the CD line plus the diameter of the inhibitory zone of the EF line and the diameter of the inhibitory zone of the GH line. The results of the fourth sum are then averaged and divided by four. Where as the ab, cd, ef, gh lines only indicate the size of the diameter of the paper discs used.

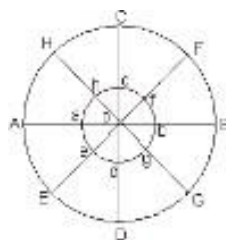


Figure 2.1 Inhibition Zone Measurement Paper Disc Diffusion Method<sup>15</sup>.

Note :

Aa, Bb, Cc, Dd, Ee, Ff : Inhibition zone formed

A, b, c, d, e, f : Paper disc diameter

o : Center of paper disc

AoE : 45° angle

### Characterization of Bacteria

Bacterial characterization was carried out macroscopically and microscopically. Macroscopic observations include the morphology of single colonies of endophytic bacterial cells. Observation of a single colony in the form of shape, edge, elevation, and color<sup>5</sup>. Microscopic observations were made through gram staining to determine the type of gram-positive or gram-negative bacteria. Gram staining of bacteria was carried out by means of isolates of potential endophytic bacteria produced by 24 hour rejuvenation made outward, fixed above the burner spiritus. Dripped violet crystal paint and allowed to stand for 45 seconds. The rest of the paint is washed with running water then dropped with iodine solution and allowed to stand for 1.5 minutes. Wash with running water and drop 70% alcohol for 30 seconds then wash with running water and dry. Then given safranin dilution solution, allowed to stand for 20 seconds, then washed with running water, dried. Observed under a microscope with low to large magnifications up to 1000X. Gram-positive bacteria show purple and Gram-negative bacteria show red in gram staining.

### Data Analysis

Quantitative data are in the form of inhibition zone diameters around the paper disc on the nutrient agar, statistically analyzed using the tabulation data stage using a completely randomized design (RAL) factorial pattern and One Way ANOVA (Analysis of Variance) at 5% level. If there are real differences, further tests are performed using the LSD (Least Significant Difference) test to determine the best effect with the SPSS 16.0 application.

### Results and Discussion

Tea plant samples (*Camellia sinensis*) were taken from the Medini Tea Plantation, Ungaran Mountain, Kendal District, Semarang, Central Java. The study was conducted at the Integrated Laboratory, Diponegoro University, Semarang.

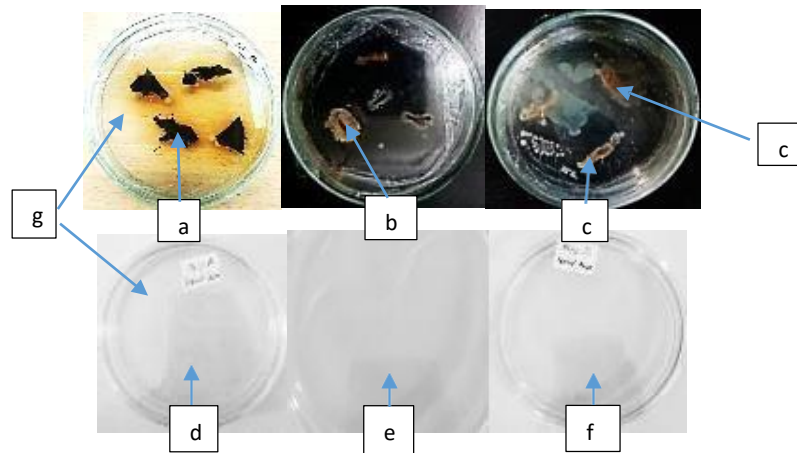


Figure 4.1 Results of Isolation of Endophytic Bacteria in Tea Plants and Control of Surface Sterilization of Samples. Note: (a) Leaves, (b) Stems, (c) Roots, (d, e, f) Control of surface sterilization of samples, (g) Nutrient agar; (h) Endophytic bacterial cell colonies.

Figure 4.1 Shows the results of the isolation of endophytic bacterium of tea plants (*Camellia sinensis*) from the three samples obtained by colonies of endophytic bacterial isolates cells around the roots, stems, and leaves of tea plants followed by the results of the surface sterilization control of each sterile sample. Cell colonies of endophytic bacterial isolates have medium size and are white to yellowish. Most bacterial cell colonies appeared in root samples, followed by stem samples and finally leaf samples. Endophytic bacteria are generally more common in the roots and stems and decreasing in number in the tubers and leaves<sup>21,22</sup>.

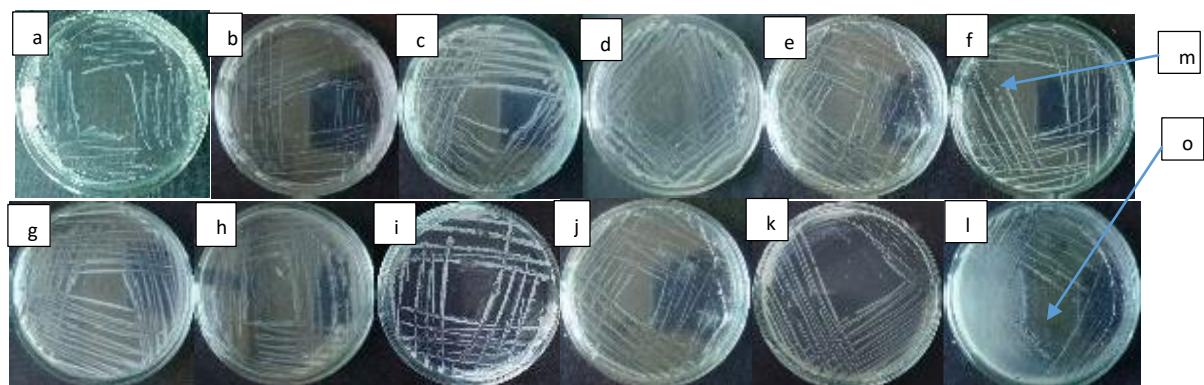


Figure 4.2 Results of Pure Isolation of Endophytic Bacteria in Tea Plants. Note: Endophytic bacterial isolates (a) D1, (b) D2, (c) B11, (d) B12, (e) B13, (f) B14, (g) B21, (h) B22, (i) B23, (j) A1, (k) A2, (l) A3, (m) Single cell endophytic bacteria; (e) Nutrient agar.

Figure 4.2 Shows the results of the purification of endophytic bacterial isolates with the results of 12 pure endophytic bacterial isolates including 2 from leaf samples (D1 and D2), 7 from stem samples (B11, B12, B13, B14, B21, B21, B22, and B23), and 3 from root samples (A1, A2, and

A3). Furthermore, macroscopically and microscopically characterize 12 endophytic bacterial isolates as the table below

Table 4.1 Macroscopic Characterization of Tea Plant Endophytic Bacteria Isolates

Isolate	Size of cell colonies	Shape of cell colonies	Elevation	Margin	Color of cell	Shape of cell	Gram Bacteria
D1	Small	Circular	Flat	Entire	White	Coccus	Positive
D2	Small	Circular	Flat	Entire	milky white	Coccus	Positive
B11	Moderate	Irregular	Flat	Lobate	milky white	Coccus	Positive
B12	Moderate	Irregular	Unbonate	Lobate	milky white	Coccus, endospora	Positive
B13	Moderate	Irregular	Flat	Entire	milky white	Coccus	Positive
B14	Moderate	Circular	Unbonate	Entire	milky white	Coccus	Positive
B21	Moderate	Irregular	Flat	Entire	milky white	Coccus	Positive
B22	Moderate	Irregular	Flat	Undulate	milky white	Coccus	Positive
B23	Moderate	Irregular	Flat	Lobate	milky white	Coccus, endospora	Positive
A1	Moderate	Irregular	Flat	Entire	milky white	Coccus	Positive
A2	Moderate	Circular	Flat	Entire	yellowish white	Coccus	Positive
A3	Moderate	filament	Flat	Filamen	milky white	Coccus	Positive

Table 4.1 Shows macroscopic characteristics of 12 endophytic bacterial isolates including the size of bacterial cell colonies with 2 different sizes namely small and moderate. The colony shape includes 3 types, namely circular, irregular, and filament. Elevation includes 2 types, namely flat and undulate. Margins include 3 types, namely entire, lobate, and filament. The microscopic characteristics of all 12 endophytic bacteria are gram positive because of the staining of gram cells of purple<sup>32</sup>. The shape of bacterial cells is coccus, the endophytic bacterial isolates B12 and B23 form endospores. Endospores are a form of bacterial defense against unfavorable environments. Endospores are thermostable and resistant to extreme environmental conditions<sup>30</sup>. Gram-positive bacteria have thick cell walls such as thick nets made of peptidoglycan (50-90% by weight of the cell envelope), a layer of cell membrane, and do not have an outer membrane, whereas gram-negative bacteria have a thin cell wall (10% by weight of the sheath cell) which is between two layers of cell membrane<sup>24</sup>.



Figure 4.3 Results of Endophytic Bacteria Screening by Paper Disk Diffusion Method. Note: Bacterial cells (a) *Staphylococcus aureus* and (b) *Staphylococcus epidermidis*, (c) Disc paper, (d) Inhibited zones (1) Endophytic bacteria isolate code B23, (2) Endophytic bacteria isolate code B14, (3) Endophytic bacteria isolate code A2, (+) positive control of chloramphenicol, and (-) negative control.

Figure 4.3 shows an overview of screening tests of 12 endophytic bacterial isolates using the disk diffusion method<sup>23</sup>. Clear zones appear to form around the paper disc that has been given a suspension of endophytic bacterial isolates B14, B23, and A2 and positive control (+). The clear zone is one indicator of inhibitory zones of endophytic bacterial isolates related to their antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The symbol (-) indicates a negative control with the result that no inhibition zone is formed around the paper disc.

Table 4.2 Average Diameter of Inhibitory Zone Test Results of Tea Plant Endophytic Bacteria against *Staphylococcus aureus*

No.	Endophytic bacteria	Inhibition Zone (mm)			Average (mm)
		I	II	III	
1.	D1	7	6,75	6	6,58
2.	D2	7,75	7,75	5,25	6,25
3.	B11	6,75	6,75	5	6,15
4.	B12	7	6,5	8	7,17
5.	B13	7,75	5	5	5,29
6.	B14	7,75	7	7,25	7,3
7.	B21	6	5,75	5,25	5,7
8.	B22	8,5	7,75	6,5	7,42
9.	B23	13,5	7,5	7,25	9,42
10.	A1	6,5	6,75	6,5	6,58
11.	A2	5,25	8,75	7	7
12.	A3	7,5	10,5	7,25	7,42

Table 4.3 Average Diameter of Inhibitory Zone Results of Tea Plant Endophytic Bacteria Screening Test on *Staphylococcus epidermidis*

No.	Endophytic bacteria	Inhibition Zone (mm)			Average (mm)
		I	II	III	
1.	D1	5,75	7	5	5,9
2.	D2	6,5	5	7	6,1
3.	B11	5	5	0	3,3
4.	B12	5	5	5	5
5.	B13	5	6	5,25	5,4
6.	B14	5,5	14,75	5,75	8,7
7.	B21	5,5	5	5	5,17
8.	B22	7,5	6,75	7,25	7,17
9.	B23	8	8,25	15,75	10,7
10.	A1	7	6	7,25	6,92
11.	A2	6	6,5	6,75	6,41
12.	A3	6,75	5,25	6,25	6,08

Table 4.2 and Table 4.3 show the average diameter of inhibition zones of 12 endophytic bacterial isolates from the screening test which have antibacterial activity against the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The largest average inhibition zone diameter was found in isolate B14 with an average inhibition zone diameter of 7, 3 mm and



8.7 mm followed by endophytic B23 isolates with inhibition zone diameter of 9.42 mm and 10.7 mm and A2 with inhibition zone diameter of 7 mm and 6.41 mm. This isolat is the most potential endophytic bacteria because these three isolates have antibacterial activity that is consistently able to inhibit the two test bacteria with large diameter inhibition zones that fall into the medium to strong category. Based on the diameter of the inhibition zone formed, the antibacterial activity is included in 3 categories, namely weak if the diameter of the inhibition zone is 1-5 mm, while the diameter of the inhibition zone is 6-10 mm, strong with the inhibition zone diameter of 11-20 mm, and very strong with inhibition zone diameters  $>20$  mm<sup>29</sup>.

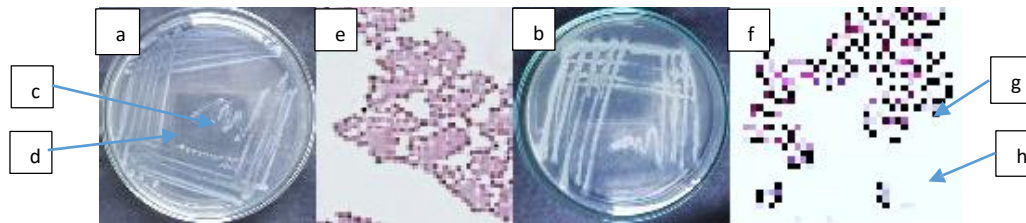


Figure 4.4 Macroscopic Characteristics of Test Bacteria. Note: (a) *Staphylococcus aureus* and (b) *Staphylococcus epidermidis*, (c) Colonized single cell colonies of test bacteria, (d) Agar nutrient. Microscopic Characteristics of Bacteria Test. Note: (e) *Staphylococcus aureus*, (f) *Staphylococcus epidermidis*, (g) Form of test bacterial coccus cells, and (h) Glass preparations.

Figure 4.4 Shows macroscopic and microscopic characteristics of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. Macroscopic characteristics of these two bacteria have small cell colonies, while the color of the colonies is white to yellowish in *Staphylococcus aureus* and pale white and shiny in *Staphylococcus epidermidis*. The microscopic characteristics show both include gram-positive bacteria in the form of *coccus* bacteria clustered like grapes<sup>2</sup>.

Table 4.4 Growth Curves of Tea Plant Endophytic Bacteria

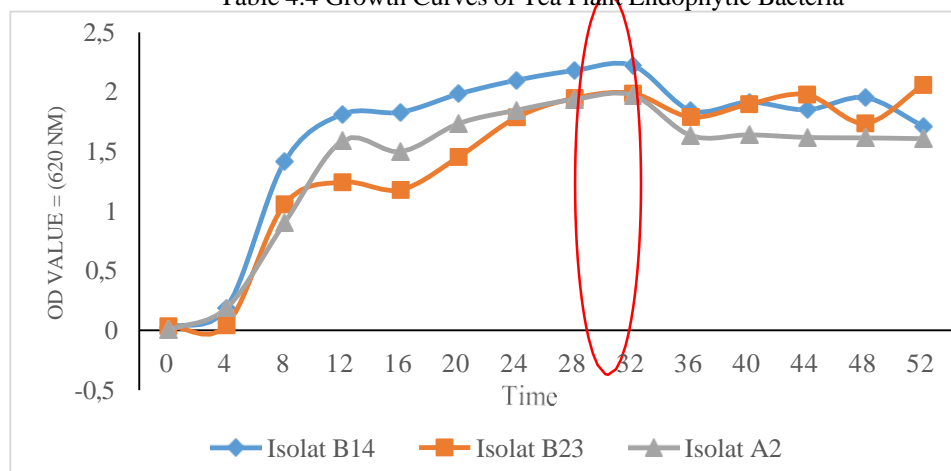


Table 4.4 shows the growth curves of endophytic bacterial isolates B14, B23 and A2. From the curve it appears that the growth of the three endophytic bacterial isolates is in a static condition because there are 4 growth phases, namely lag phase, logarithmic / exponential phase, stationary phase and death phase<sup>12</sup>. The lag phase is the phase of bacterial adaptation to the growth environment where bacterial cells grow but with very little cell division despite continued cell metabolism<sup>12</sup>. The log phase is the phase of bacteria doing cell division constantly with a very fast growth time so that the addition of bacterial cells is very high<sup>12</sup>. Stationary phase is a phase of bacterial growth that is very slow or stationary<sup>12</sup>. While the phase of death is the phase of bacteria experiencing death due to nutrition in the reduced media and the presence of toxicity in the growth media. In the phase of death many cells die, whereas those who are still alive are no longer able to divide. Depletion of nutrients and accumulation of inhibitor products such as acids are some of the factors that influence cell death<sup>12</sup>. In endophytic B14 and A2 isolates, there was no lag phase or bacterial adaptation phase to the environment. It is possible that there is an influence from the use of a starter to facilitate the adaptation of endophytic bacteria to the growth environment while in isolate B23 endophytic bacteria there is a lag phase. It is possible that this endophytic bacterial isolate requires a longer time to adapt to the growth environment. This can also be seen where the endophytic B23 bacterial isolate cells produce endospores or a spore that is used by bacteria to survive a less favorable and extreme environment. The initial stationary phase of the three isolates began at the 30th hour and the final stationer phase was obtained at the 32nd hour. The final stationary phase is the phase in which bacteria produce the most optimal secondary metabolites, one of which is antibiotics<sup>12</sup>.

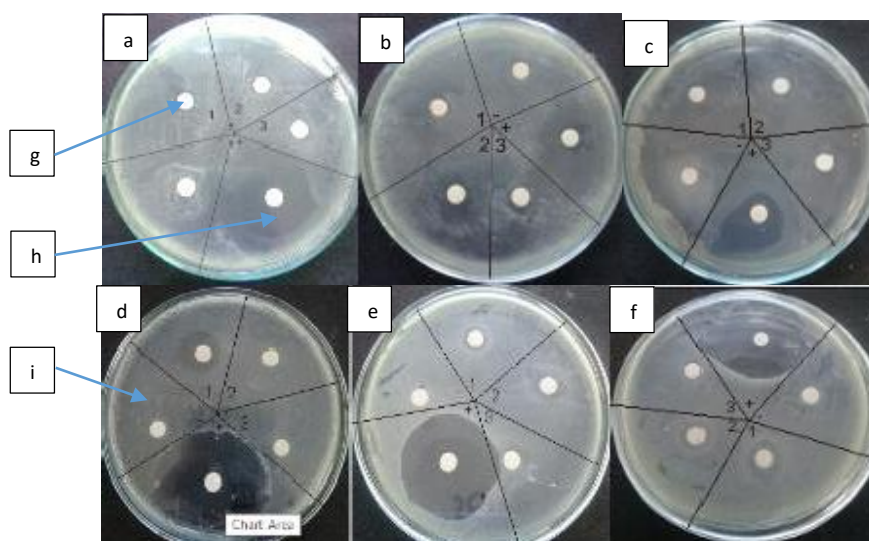


Figure 4.5 Antibacterial Activity Test Results with Endophytic Bacteria Diffusion Method of Paper Disc. Note: (a, b, and c) *Staphylococcus aureus* and (d, e, f) *Staphylococcus epidermidis*, (g) Disc paper, (h) Inhibited zones, (i) Test bacterial cells, (1) Bacterial endophytic isolates B23, (2) Bacterial endophytic isolate B14, (3) Bacterial endophytic isolate A2, (+) positive control of chloramphenicol, and (-) negative

Figure 4.5 Shows an overview of the results of antibacterial activity tests against the *Staphylococcus aureus* bacteria and *Staphylococcus epidermidis* bacteria using the paper disc diffusion method. Shown in the picture is the inhibitory zone around the paper disc that is supernatant from bacterial isolates of endophytic B14, B23, and A2 and in positive control with a suspension of antibiotic chloramphenicol. While in the negative control no inhibition zone is formed around the sterile paper disc.

Table 4.5 Average Diameter of Inhibitory Zone Test Results for Antibacterial Activity of Tea Plant Endophytic Bacteria Isolates against *Staphylococcus aureus*

Treatment	Inhibiton Zone (mm)			Average (mm)
	1	2	3	
Sp2 (B14)	8	7	8,25	7,75
Sp1 (B23)	8,25	8,25	6	7,5
Sp3 (A2)	7,75	6,25	8,25	7,42

Table 4. 5 Shows the average diameter of inhibitory zones for antibacterial activity test using supernatant isolates of endophytic bacteria B14, B23, A2 with *Staphylococcus aureus* test bacteria. The largest inhibition zone diameter was formed by isolate B14 with a large inhibition zone of 7.75 mm followed by B23 isolates with a diameter of inhibition zone of 7.5 mm and A2 with a diameter of inhibition zone of 7.42 mm. all three isolates showed antibacterial activity which was included in the medium category. Based on the diameter of the inhibition zone formed, the antibacterial activity is included in 3 categories, namely weak if the diameter of the inhibition zone is 1-5 mm, while the diameter of the inhibition zone is 6-10 mm, strong with the inhibition zone diameter of 11-20 mm, and very strong with inhibition zone diameters > 20 mm<sup>29</sup>.

Table 4.6 Average Diameter of Inhibition Zones Test Results of Antibacterial Activity of Isolate Bacteria Endophytic Bacteria of Tea Plants against *Staphylococcus epidermidis*

Treatment	Inhibitoin Zone (mm)			Average (mm)
	1	2	3	
Sp2 (B14)	14	10,25	13	12,4 <sup>a</sup>
Sp1 (B23)	10	7	7,75	8,25 <sup>b</sup>
Sp3 (A2)	8,25	7,25	9	8,16 <sup>b</sup>

Table 4. 6 Shows the diameter of inhibitory zone results of antibacterial activity test of endophytic bacterial isolates B14, B23, and A2 against *Staphylococcus epidermidis*. The biggest inhibition zone diameter was found in isolate B14 with a large inhibition zone diameter of 12.4 mm followed by isolate B23 with a large inhibition zone diameter of 8.25 mm and A2 with a large diameter of inhibition zone 8.16 mm. The antibacterial activity of B14 endophytic bacterial isolates is included in the strong category because the diameter of the inhibition zone is in the range of 11-20 mm while the endophytic bacterial isolates B23 and A2 are included in the moderate category because of the large diameter of inhibition zone in the range of 6-10 mm. Based on the diameter of the inhibition zone formed, the antibacterial activity is included

in 3 categories, namely weak if the diameter of the inhibition zone is 1-5 mm, while the diameter of the inhibition zone is 6-10 mm, strong with the inhibition zone diameter of 11-20 mm, and very strong with inhibition zone diameters  $< 20$  mm<sup>29</sup>.

#### Data analysis

The results of data analysis using the One Way ANOVA test showed that the inhibitory zone diameter data of the three isolates of endophytic bacteria B14, B23, and A2 in their antibacterial activity against the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* were significantly different at the 0.05 level.

Table 4.7. One Way ANOVA Test Result of Endophytic Bacteria (*Camellia sinensis*) against *Staphylococcus aureus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.758	3	.253	70.400	.000
Within Groups	.029	8	.004		
Total	.787	11			

Note: the data is significantly different if the significance value is less than 0.05

Table 4.8. One Way ANOVA Test Result of Endophytic Bacteria (*Camellia sinensis*) against *Staphylococcus epidermidis*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1200.542	3	400.181	14.026	.001
Within Groups	228.250	8	28.531		
Total	1428.792	11			

Note: the data is significantly different if the significance value is less than 0.05

Table 4.7 and Table 4.8 Shows the results of data analysis using the One Way ANOVA test, in which the diameter of inhibition zones of the three isolates of endophytic bacteria B14, B23, and A2 in their antibacterial activity on the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* differed significantly at the 0.05 level. This is because the significance value of the three isolates both against *Staphylococcus aureus* and *Staphylococcus epidermidis* is below 0, 05 or  $p < 0.05$ . The significance value of the three isolates of endophytic bacteria B14, B23, and A2 against *Staphylococcus aureus* test bacteria was 0,000 and against *Staphylococcus epidermidis* test bacteria was 0.001.

Table 4.9. LSD Test Results of Endophytic Bacteria (*Camellia sinensis*) against *Staphylococcus aureus*

(I)	(J)	Mean	Std. Error	Sig.	95%	Confidence
Endophytic	Endophytic	Difference			Interval	
bacteria	bacteria	(I-J)			Lower	Upper
					Bound	Bound
B23	B14	-.01786	.04892	.724	-.1307	.0949
	A2	.00314	.04892	.950	-.1097	.1159
B14	B23	.01786	.04892	.724	-.0949	.1307
	A2	.02100	.04892	.679	-.0918	.1338
A2	B23	-.00314	.04892	.950	-.1159	.1097
	B14	-.02100	.04892	.679	-.1338	.0918

Tabel L 4.10 LSD Test Results of Endophytic Bacteria (*Camellia sinensis*) against *Staphylococcus epidermidis*

(I) Endophytic bacteria	(J) Endophytic bacteria	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
B14	B23	2.08333*	4.36129	.646	-7.9738	12.1405
	A2	2.41667	4.36129	.595	-7.6405	12.4738
B23	B14	-2.08333*	4.36129	.646	-12.1405	7.9738
	A2	.33333	4.36129	.941	-9.7238	10.3905
A2	B23	-2.41667	4.36129	.595	-12.4738	7.6405
	B14	-.33333	4.36129	.941	-10.3905	9.7238

Note: differ significantly at the 0.05 level.

Tables 4.9 and 4.10 show the LSD test results there were no significant differences between groups of isolates B14, B23, and A2 against *Staphylococcus aureus*. Because the p value <0.05 Where as the effect of *Staphylococcus epidermidis* B14 was significantly different from the bacterial isolates of endophytic B23 and A2 because the P value > 0.05. While there were no differences between B23 and A2 isolates because P value < 0.05.

### Conclusion

Tea plant endophytic bacteria (*Camellia sinensis*) isolates B14, B23, and A2 have antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. The best antibacterial activity occurs in endophytic bacteria isolate B14 with moderate category of antibacterial activity on *Staphylococcus aureus* and strong on *Staphylococcus epidermidis*, followed by B23 and A2 with moderate category antibacterial activity against both of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Endophytic bacteria of tea plants (*Camellia sinensis*) isolates B14, B23, and A2 showed a better effect of antibacterial activity of *Staphylococcus epidermidis* compared with *Staphylococcus aureus* bacteria.

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