

Endophy Bacterial Phenoty of Aloe Vera (*Aloe barbadensis miller*) as the Producer of Antibacterial Compounds Towards *Eschericia coli* and *Staphylococcus aureus*

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Abstract. Aloe vera plants are known to have endophytic microbes that form colonies in the tissue from the leaves to the roots. Endophytic microbes are known to improve plant growth because of their ability to suppress the growth of pathogenic microbes by competition, producing antibiotic compounds, or inducing plant resistance. The aims of this research are to isolate endophytic bacteria in aloe vera, to determine the antibacterial ability of endophytic bacteria contained in aloe vera against the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria and to determine the characterization of endophytic bacteria found in aloe vera. This research is a laboratory experimental study with research procedures, namely sampling by purposive sampling method, isolation of endophytic bacteria carried out by strike plate, antibacterial potential test against *Staphylococcus aureus* and *Escherichia coli* using paper disc diffusion method, Morphological identification was carried out by staining bacteria gram, Phenotypic characterization of endophytic bacteria using Profile matching method. The results showed that endophytic bacteria found in aloe vera leaves were characterized by the growth of bacterial colonies on Murashige-Skoog (MS) media with different shapes and colors, the results of the antibacterial potential of endophytes against the growth of *Escherichia coli* and *Staphylococcus aureus* showed that there were 6 bacterial isolates that showed clear zones in *Escherichia coli* and *Staphylococcus aureus* bacteria and characterization results showed that isolates H2L, H9L and H10L had similar genera to *Staphylococcus* and *Bacillus* while isolates H4L, H5L and H7L had similar genera to *Pseudomonas*.

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During the current Corona virus pandemic, the government has made various efforts to prevent the spread of this virus with large-scale social restrictions policies, and adaptation of new habits by wearing masks, maintaining distance and washing hands using soaps and running water. This policy encourages some people to take various ways to prevent the spread of the virus by making various disinfectant and antiseptic liquids naturally because the supply of disinfectants and antiseptics in the market is decreasing, coupled with soaring prices.

Disinfectants and antiseptics are two materials that have different functions. Disinfectants are often used on the surface of objects such as tables, doorknobs, floors and other objects while antiseptics are used on living tissues such as human skin. Antiseptics are naturally made with various combinations of natural ingredients such as betel leaf, lime leaf, ginger and Aloe vera.

Aloe vera is a cactus-shaped shrub that has thick leaf flesh and contains a lot of water [1] Aloe vera is also known as the lily of desert. Aloe vera contains anthraquinone, glycosaminoglycans, acemannan, carbohydrates, enzymes, amino acids, vitamins A, C, E and other active ingredients that also play a role in wound healing [2]. The content of aloe vera is anti-inflammatory, antimicrobial, antiseptic, antioxidant and moisturizing. Aloe vera has always appeared in every phase of history with its appreciation in medicine [3]. Currently aloe vera is one of the natural ingredients that is often used in the manufacture of antiseptics naturally, but the antiseptic is directly distributed to the wider community without going through the laboratory testing stage to determine its ability to inhibit or kill viruses or bacteria. Laboratory trials on Aloe vera plants need to be carried out to determine the ability of antibiotics it possesses. The ability of antibiotics is a substance produced by bacteria if in low concentrations it is able to inhibit the growth or kill other microbes.

In aloe vera it is known that there are endophytic bacteria that live in plant tissues from stems to roots and colonize in the intercellular space. Plants use endophytic bacteria as growth promoters because endophytic bacteria can increase the availability of nutrients and produce growth hormone [4]. The word endophyte is generally used for fungi, but now there are many articles about endophytic bacteria that have beneficial effects but endophytic bacteria are considered neutral or cause disease. For more than 50 years, many bacterial endophytes, especially in higher plants, have shown symptoms of disease [5].

Various studies have been carried out on higher plants and have found the presence of these bacteria that live in different plant tissues. Some of the endophytic bacteria found are pathogenic, but there are several groups of endophytic bacteria that can be used to inhibit or kill other bacteria that are considered harmful [6].

Endophytic bacteria in plants have a role in producing Inole Acetid acid (IAA) which can stimulate plant growth and development. In addition, endophytic bacteria can act as biocontrol agents that can reduce the rate of bacterial infection, suppress plant diseases, inhibit fungal growth, kill nematode larvae, suppress the number of root cavities, and reduce nematode populations in large numbers. Another role of endophytic bacteria is as a pathogenic biocontrol agent that can cause disease in plants. In addition, endophytic bacteria can also produce metabolites that are toxic to pathogens [7].

Based on the role of endophytic bacteria as biocontrol agents, this study identified endophytic bacteria to prove their role as biocontrol agents in inhibiting pathogenic bacteria (disease-causing)

including *Staphylococcus aureus* bacteria which can cause ulcers and *Eschericia coli* bacteria which can cause diarrhea.

Endophytic bacteria are also able to increase plant resistance to various kinds of microbial pathogens by inducing plant resistance known as induced systemic resistance (ISR) so that they are able to withstand plant disease attacks, besides that the source of endophytic bacterial compounds can be used as medicine[8][9].

Endophytic bacteria that have been identified in plant tissue is *Bacillus polymixa* isolated from *A. annua* L, capable of producing artemisinin metabolites which have potential as anti-malarial agents [10][11]. In the isolation of endophytic bacteria, media is needed that can support the growth of endophytic bacterial host plants. Murashige-Skoog (MS) media is a medium used for almost all kinds of plants because it contains high mineral salts and N compounds in the form of NO₃ and NH₄ [12]. *Staphylococcus aureus* can hemolyze blood and coagulate plasma, also infect boils, impetigo and wound infections that cause pus [13] [14]. *Eschericia coli* can cause urinary tract infections, diarrhea, sepsis, and meningitis [15]. *S. aureus* (gram positive) and *E. coli* (gram negative) was chosen to determine the effectiveness of the antibacterial potential of the compounds produced by endophytic bacteria against gram positive and gram negative bacteria so that the spectrum of inhibition of these antibacterial compounds can be known [16].

Natural ingredients that have the potential as drugs can be used as models in the discovery of new drugs. Therefore, it is necessary to search for sources of antibiotics by exploring potential endophytic microbes in obtaining new antibiotic compounds.

2. Experimental Section

2.1 Isolation of Endophytic Bacteria by Streak Plate Method

Isolation of endophytic bacteria in aloe vera was carried out aseptically on Murashige-Shoog Media (MS) then incubated for 2-3 days. After incubation, the growth of endophytic bacteria will appear around the Aloe vera leaf flesh [17] [5]. Furthermore, the growing bacteria were separated based on their morphological characters on slanted Nutrient Agar (NA) media to obtain a pure culture collection Testing the Antibacterial Potential of Endophytic Bacteria Isolates in Aloe Vera Against *E.coli* and *S.aureus* by Diffusion Paper disc Pure cultures of endophytic bacteria obtained were tested for their antibacterial potential against *E.coli* and *S.aureus* using the Paper Disc Agar Diffusion Technique method. The endophytic bacteria were then grown in Nutrient Broth medium and shaken using a shaker incubator at 37°C at 170 rpm for 3-4 days. Separation of cell biomass with Nutrient Broth medium containing secondary metabolites of endophytic bacteria (supernatant) was carried out by centrifugation at 5000 rpm for 1 hour, the supernatant was used to screen for endophytic bacteria producing antibacterial compounds. To reduce excess water, the paper disc is dried on a sterile watch glass for approximately 1 hour. The test microbes, namely *E.coli* and *S.aureus* were grown in NA by pour plating in different petri dishes, namely by taking 1 ml suspension of the test bacteria *E.coli* and *S.aureus* then inoculated into 20 ml of Nutrient Agar which was melting at a temperature of 50°C then poured into a petri dish and shaken slowly to mix the bacterial culture with agar. After the agar solidified, the paper disc that had been inoculated with the supernatant was placed on the surface of the medium. Incubation was carried out at 37°C for 24 hours or until a clear zone was formed around the paper disc. The inhibitory potential was measured by measuring the diameter of the clear zone using a caliper[18] [9].

2.2 Characterization of Endophytic Bacteria

The characterization of endophytic bacteria was carried out by macroscopic, microscopic, biochemical and physiological observations, then the data from the characterization results were made dendograms based on similarity values in two ways, namely simple matching coefficient (Ssm) and Jaccard coefficient (S) then analyzed quantitatively with the Numerical Taxonomy System (NTSYS)

program) version 2.10. The algorithm used was average linkage, then bacterial isolates were characterized and identified based on phenotypic characters by Profile Matching method using Bergey's Manual of Determinative Bacteriology [19].

2.3 Results Analysis

The test data for the activity of endophytic bacteria were indicated by the presence of a clear zone which was formed after testing was conducted using the test bacteria *S. aureus* and *E. coli* and observed descriptively [16]. Afterward, biochemical and physiological observations were carried out to determine the phenotypic, macroscopic, microscopic characterization. The results of the phenotypic characterization obtained were then analyzed using Profile Matching to determine the suspected genus of endophytic bacterial isolates that have activity of inhibiting the growth of *S. aureus* and *E. coli* test bacteria, then the data was analyzed quantitatively with the MVSP (Multivariate Package) version 3.2 program to obtain a similarity index or similarity between isolates determined by writing (+) for positive results and (-) for negative results. The(+) result data were replaced with the number 1 and the (-) result data were replaced with the number 0 so that it will be readable in the MVSP program [11][20]

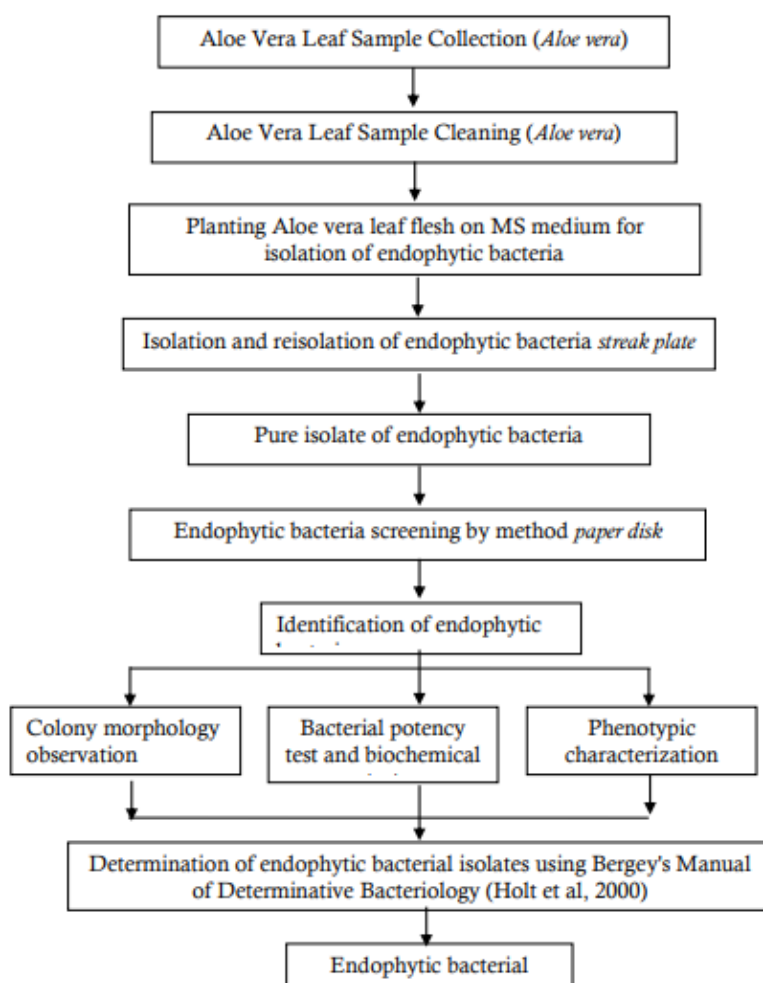


Figure 1. Method flowchart

3. Results and Discussion

3.1 Isolation of Endophytic Bacteria of Aloe vera (*Aloe barbadensis miller*)

Endophytic bacteria were successfully grown on Murashige-Shoog MS media with different bacterial colony morphology, then separated according to different shapes and colors and 10 isolates with different characteristics, shapes and colors were found; as shown in Figure 2.

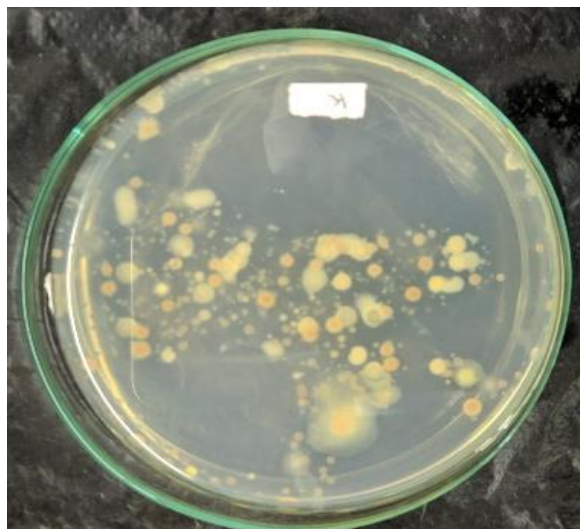


Figure 2. Isolation of endophytic bacteria from Aloe vera

Ten different bacterial isolates were then identified with different morphological characters based on shape, elevation, margin, color, cell shape, and gram as shown in Table 1 below.

Table 1. Morphological characterization of endophytic bacteria that produce antibacterial isolates from Aloe vera (*Aloe barbadensis miller*)

Isolation Code	Form	Isolate Morphological Characterization					
		Elevation	Margin	Color	Cell shape	Cell arrangement	Gram
H1L	Filamentous	Raised	Undulate	Milky white	Cocci	Unicellular	Positive
H2L	Circular	Flat	Entire	Milky white	Cocci	Multicellular	Positive
H3L	Circular	Raised	Entire	Yellow	Bacilli	Unicellular	Positive
H4L	Circular	Raised	Undulate	Yellow	Cocci	Unicellular	Negative
H5L	Circular	Raised	Entire	Yellow	Bacilli	Multicellular	Positive
H6L	Irregular	Flat	Undulate	Yellow	Cocci	Multicellular	Positive
H7L	Filamentous	Raised	Undulate	Yellow	Cocci	Unicellular	Negative
H8L	Filamentous	Raised	Undulate	Yellow	Bacilli	Unicellular	Negative
H9L	Filamentous	Raised	Entire	Whites	Bacilli	Unicellular	Positive
H10L	Circular	Raised	Entire	Yellow	Cocci	Multicellular	Positive

Information :

H : First letter of researcher's name

1-5: Bacteria isolate number

L : Sample prefix

Based on the table above, there were 10 isolates with different characteristics, namely in the shape of the colonies there were round and wrinkled colonies, the elevations of the colonies were convex and some were flat, the edges of the colonies were undulate and entire, the color of the colonies was slightly different, yellow, and some are milky white, there are cocci and bacilli cells, there are unicellular cells, and some are in pairs and some are gram positive and gram negative. The results of this study are in line with research which states that generally the shape of bacterial colonies is circular, irregular, filamentous, rhizoid, elevation in the form of raised, convex, flat, umbonate, crateriform. The edges are entire, undulate, filiform, curled and lobate [21]. Differences in the form of colonies in each species indicate characteristics for certain species [22][23].

The ten bacterial isolates found in this study were then grown in Nutrient Broth (NB) medium and shaken using a shaker at 37°C at 1,700 rpm for 1-2 days. This NB medium is used for enriched hatchery before being distributed to solid media [24][23]. Generally, this NB media is used to see the nature of bacterial growth such as cloudy, uniform, forming sandy deposits or forming caput medusa [25][20]. After the bacterial isolates were incubated for 2 days, followed by separation of cell biomass with NB medium containing secondary metabolites of endophytic bacteria (supernatant) by centrifugation at 5,000 rpm for 40 minutes, the supernatant was used to screen for endophytic bacteria producing antibacterial compounds.

3.2 Testing the Antibacterial Potential of Endophytic Bacterial Isolates Against *Staphylococcus aureus* and *Escherichia coli* by Diffusion Paper disc

The supernatant of 10 isolates of endophytic bacteria was tested for its ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. An indication of the presence of inhibition is indicated by the formation of an inhibitory zone around the paper disk. Clear zone data can be presented in table 2 and figure 2 below

Table 2. Antibacterial Potential Test of Endophytic Bacterial Isolates against *Staphylococcus aureus* and *Escherichia coli* by Diffusion Paper disc.

Isolate Code	Inhibitory zone diameter (mm)		Clear Zone Criteria	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
H1L	-	-	None	None
H2L	10	6	High	High
H3L	-	-	None	None
H4L	2	17	Medium	High
H5L	15	10	High	High
H6L	-	-	None	None
H7L	7	5	High	High
H8L	-	-	None	None
H9L	-	8	None	High
H10L	12	10	High	High

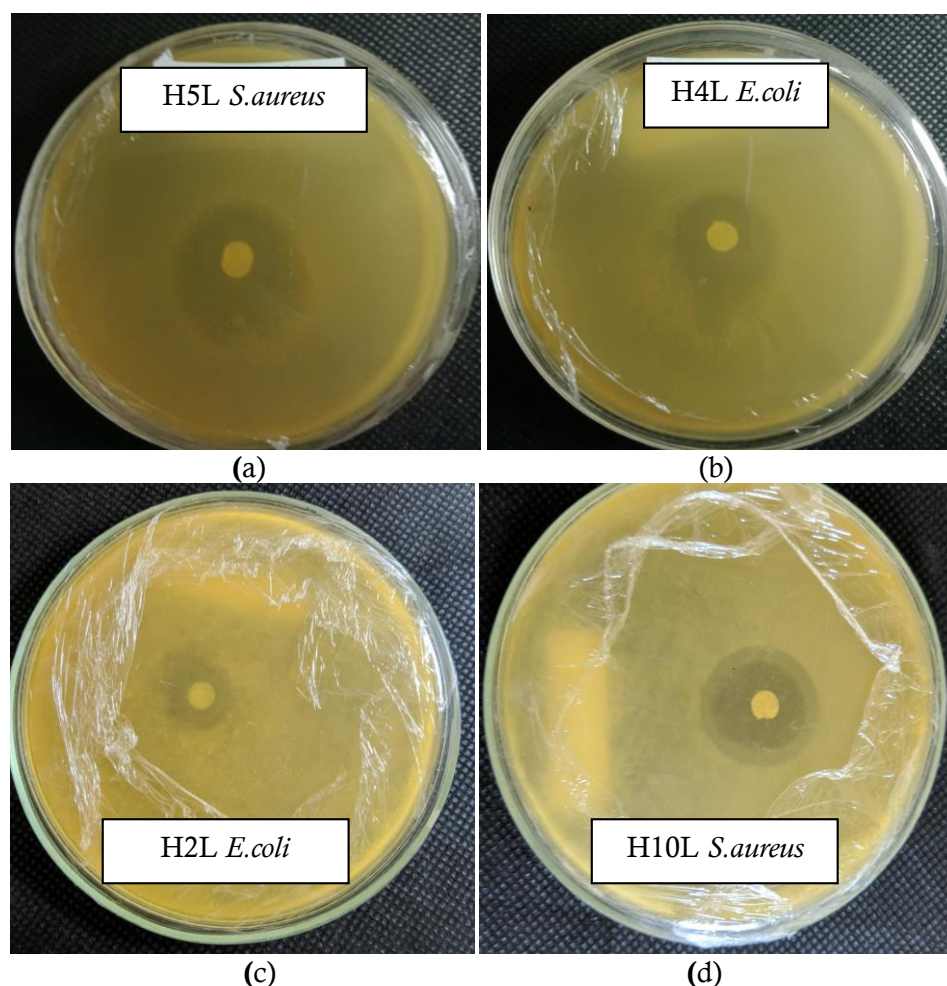


Figure 3. The clear zone shows the results of the Endophytic Bacteria Test against *Staphylococcus aureus* and *Escherichia coli*

Information:

- (a) clear zone on H5L isolate with test bacteria *Staphylococcus aureus*
- (b) clear zone on H4L isolate with test bacteria *Escherichia coli*
- (c) Clear zone on H2L isolate with test bacteria *Escherichia coli*
- (d) Clear zone on H10L isolate with test bacteria *Staphylococcus aureus*

Table 2 above shows that out of ten bacterial isolates, only six were able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. The isolates of endophytic bacteria that had the highest inhibition on the growth of *Staphylococcus aureus* were 4 isolates, namely isolates H2L, H5L, H7L and H10L, and the isolate that had moderate inhibition was only 1 isolate, namely H4L. In addition, isolates that did not have the ability to inhibit the growth of *Staphylococcus aureus* were 5 isolates, namely H1L, H3L, H6L, H8L and H9L. There were 6 isolates of endophytic bacteria that had the highest inhibition on the growth of *Escherichia coli* bacteria, namely H2L, H4L, H5L, H7L and H9L and H10L, and 4 isolates that did not have the ability to inhibit the growth of *Escherichia coli* bacteria, namely H1L, H3L, H6L, H8L. The provision of no isolate criteria indicated that when the endophytic bacterial activity was tested against the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria, the isolates did not show a clear zone on the petri dish. While the high criteria was given

because the clear zone produced on the test bacteria showed a larger size equal to 5mm, while the medium criteria was given because the clear zone produced was smaller than 5mm [26][20].

The highest clear zone diameter in endophytic bacteria isolates using *Staphylococcus aureus* and *Escherichia coli* test bacteria was produced by H4L isolates with a clear zone diameter of 17 mm for *Escherichia coli* test bacteria while the smallest diameter with moderate criteria was shown in H4L isolates for *Staphylococcus aureus* test bacteria. When compared to research [27], which obtained the highest clear zone diameter of 10.5 mm and the research [28] who obtained the highest clear zone diameter of 13.4 mm, the H4L isolate with *Escherichia coli* test bacteria had high activity in inhibiting the growth of *Escherichia coli* bacteria.

There are many microbes from the group of bacteria that have the ability to inhibit and or kill *Staphylococcus aureus* and *Escherichia coli* bacteria, however, there are some of these bacteria that have the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. Figure 2 shows the representation of isolates that have high activity in inhibiting the growth of the test bacteria, which is marked by the formation of a clear zone, this is an early indication that the bacterial isolate has the potential to inhibit the growth of the test bacteria. Therefore, isolates showing a clear zone obtained in this study were chosen to identify their phenotypic properties to determine any discovered relations between the isolates.

3.3 Phenotypic characterization of endophytic bacteria that have the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria

Six isolates of endophytic bacteria from aloe vera which have the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria were tested for phenotypic characters which include biochemical, physiological and macroscopic observations of the six isolates as presented in Table 3.

Table 3. Phenotypic characterization test results using Profile Matching endophytic bacterial isolates from aloe vera which have the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria

Characteristics	Phenotypic characters					
	H2L	H4L	H5L	H7L	H9L	H10L
Colony Morphology						
Circular	Circular	Circular	Circular	Filamentous	Filamentous	Circular
/Filamentous						
Color	White	White	Yellow	Yellow	White	Yellow
Margin	Entire	Undulate	Entire	Undulate	Entire	Entire
Elevation	Flat	Raised	Raised	Raised	Raised	Raised
Surface	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny
Morphology of cell						
Cell shape	Cocci	Cocci	Bacilli	Cocci	Bacilli	Cocci
Cell structure	Multicellular	Unicellular	Multicellular	Unicellular	Unicellular	Multicellular
Gram characteristics	+	-	-	-	+	+
Biochemical						
Catalase	+	-	-	+	+	+
Oxidase	+	+	-	-	-	-
Starch hydrolysis	-	+	+	-	+	+
Citrate hydrolysis	-	-	-	+	+	+
Acid produced from:						
Glucose	+	+	+	-	-	+
Lactose	-	+	+	+	+	-

Notes :

(+) : Productive

(-) : Unproductive

The results of the phenotypic characterization test using Profile Matching in table 3 above were followed by numerical analysis using the MVSP program to determine the similarity between isolates. The data from the similarity test of Simple Matching Coefficient (ssm) and Jaccard Coefficient (Jc) are presented in tables 4 and 5.

Table 4. Matrix of similarity (ssm) of endophytic bacterial isolates of aloe vera based on phenotypic test of 14 characters

Isolates	Simple Matching Coefficient					
	H2L	H4L	H5L	H7L	H9L	H10L
H2L	100					
H4L	42.9	100				
H5L	35.7	92.9	100			
H7L	28.6	57.1	64.3	100		
H9L	35.7	35.7	42.9	64.3	100	
H10L	64.3	50	42.9	50	57.1	100

Table 4 above shows that the H2L, H5L, and H9L isolates and the H4L isolates with H9L had the closest similarity value of 35.7%, while the H4L isolates with H5L isolates had the furthest similarity value of 92.9%. Table 5 isolates H2L with H7L had the closest similarity value of 23.1% while isolates H4L with Isolate H5L had the furthest similarity value of 87.5. The similarity values presented in table 4 and table 5 are continued by constructing a dendrogram using the Average Linkage (UPGMA) algorithm and is presented in Figure 4.

Table 5. Jaccard Coefficient (Jc) similarity matrix of aloe vera endophytic bacteria isolates based on phenotypic test of 14 characters

Isolates	Simple Matching Coefficient					
	H2L	H4L	H5L	H7L	H9L	H10L
H2L	100					
H4L	38,5	100				
H5L	30,8	87,5	100			
H7L	23,1	40	44,4	100		
H9L	30,8	25	27,3	44.4	100	
H10L	58,3	41,7	33,3	36.4	45.5	100

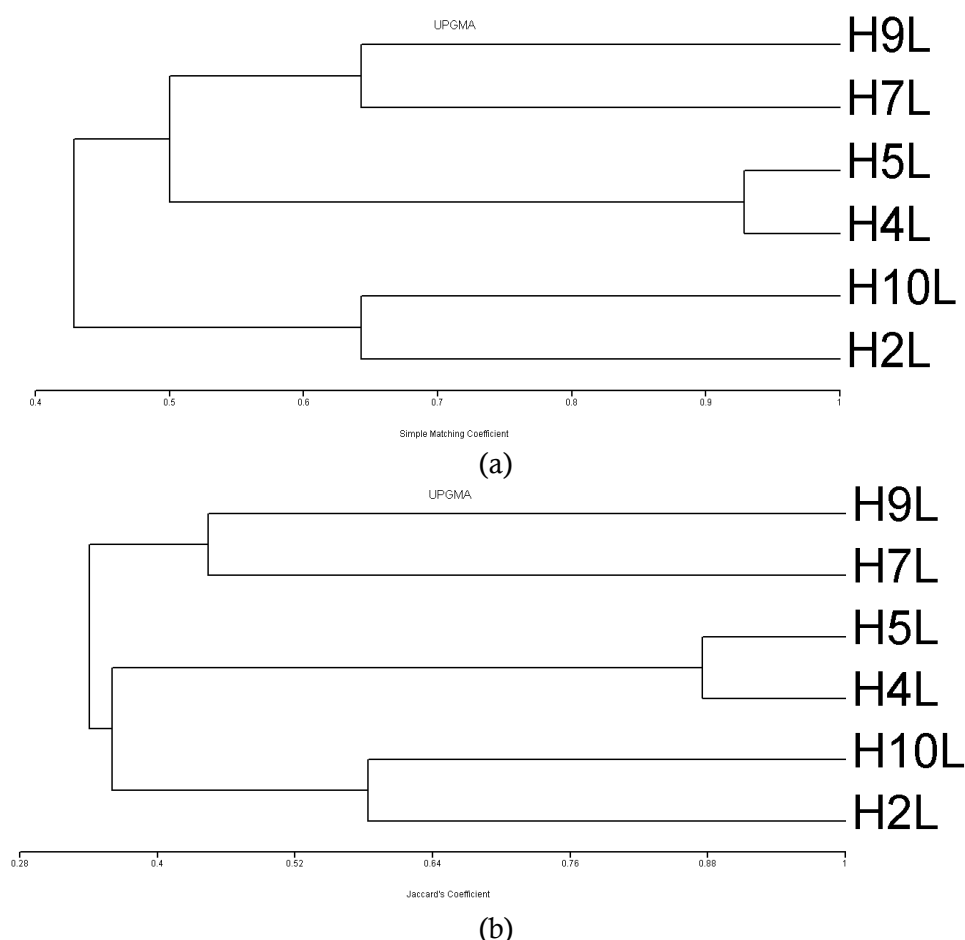


Figure 4. Dendrogram of the relationship between species of endophytic bacterial isolates based on similarity index (a). *Simple Matching Coefficient* (b). *Jaccard Coefficient*

Description: H2L, H4L, H5L, H7L, H9L H10L: isolates of endophytic bacteria from Aloe vera which have the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. The results of the dendrogram construction using UPGMA showed that several species of endophytic bacteria that had the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria were identified based on phenotypic analysis. However, based on the taxospecies concept, a microbial isolate can be grouped into one species if it has a similarity index of 70% [26]. Based on the results of similarity calculations using the Simple Matching Coefficient method, isolates H4L and H5L are the same species where the similarity value is 92.9%, while the results of similarity calculations using the Jaccard Coefficient method isolates H4L and H5L are also the same species with a similarity value of 87, 5%. The difference in similarity values is because the similarity index values of the two methods used are different [28]. The last step of this research is that six bacterial isolates that have the ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria are identified for their phenotypic characteristics using Bergey's Manual of Determinative Bacteriology. The results of this identification can be seen in table 6.

Table 6. Identification of the genus level (Generic Assignment) of the selected isolates using the Profile Matching method

Characters	Phenotypic Character								
	<i>Staphylococcus</i>	H2L	<i>Pseudomonas</i>	H4L	<i>Bacillus</i>	H5L	H7L	H9L	H10L
Cell Structure	Unicellular	Multicellular	Unicellular/ Multicellular	Unicellular	Unicellular/ Multicellular	Multicellular	Unicellular	Unicellular	Couple
Cell shape	Cocci	Coccus	Bacillus	Cocci	Bacillus	Bacillus	Cocci	Bacilli	Coccus
Gram									
Characteristics	+	+	-	-	+	-	-	+	+
Catalase	+	+	NA	-	NA	-	+	+	+
Oxidase	-	+	NA	+	NA	-	-	-	-
Starch hydrolysis	NA	-	-	+	-	+	-	+	+
Citrate hydrolysis	NA	-	+	-	+	+	+	+	+

The table above shows that based on the arrangement of the *Staphylococcus* and *Pseudomonas* cells, the cell structure was similar to the H4L, H7L and H9L isolates. While *Bacillus* and *Pseudomonas* had similarities with isolates H2L, H5L, and H10L. while based on gram characteristics, *Staphylococcus* and *Bacillus* had similarities with isolates H2L, H5L, H9L and H10L while *Pseudomonas* had similarities with H4L and H7L so it can be said that isolates H2L, H9L and H10L had similar genus with *Staphylococcus* and *Bacillus* while isolates H4L, H5L and H7L has a similar genus with *Pseudomonas*.

4. Conclusion

Based on the research above, it can be concluded that the endophytic bacteria found in Aloe vera leaves were characterized by the growth of bacterial colonies on MS media with different shapes and colors. The results of the antibacterial potential of endophytes against the growth of *Escherichia coli* and *Staphylococcus aureus* showed that there were 6 bacterial isolates that showed clear zones in *Escherichia coli* and *Staphylococcus aureus* bacteria, while the characterization of endophytic bacteria in aloe vera showed that the bacteria found had similar genera with *Staphylococcus*, *Bacillus* and *Pseudomonas*.

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