

Endophytic Bacteria Producing Antimicrobial Compounds of *Musa balbisiana* Colla

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Abstract. This study aims to obtain endophytic bacteria on Banana Hump (*Musa Balbisiana* Colla), know its ability to produce antimicrobial compounds and determine the identity of endophytic bacteria producing antimicrobial compounds. This research is quantitative descriptive research. Isolation of endophytic bacteria based on streak plate method and activity analysis antimicrobials using the Kirby Bauer method. Identification of endophytic bacteria based on morphological characteristics and molecular. The results showed that there was one type of endophytic bacteria that had the potential to have antibacterial activity hinder *Escherichia coli* and *Staphylococcus aureus* with the diameter of the inhibition zone on the test bacteria *Escherichia coli*.e. 8,10 and on *Staphylococcus aureus* 7.76mm. Bacterial isolates identified as *Enterobacter ronggenkampii* are closely related to *Enterobacter ronggenkampii* strain colony 354 chromosomes with a similarity index of 99.22%.

1 Introduction

Bananas are a member of the family Musaceae and naturally, there are two types of banana plants, namely cultivated bananas and wild bananas. One of the wild bananas found in Sulawesi was identified as *Musa balbisiana* Colla or the general public calls it Batu banana [1]. Batu banana has the characteristics of the fruit having large seeds and thick and hard skin, so it is underutilized and rarely cultivated by farmers, however, empirically some people use stone bananas as an herbal medicine. These herbal medicines are used to prevent or treat external wound infections such as wounds due to animal scratches, scrapes, and burns. This is thought to be due to the presence of bioactive compounds. Batu banana weevils contain bioactive compounds, namely glycosides, tannins, and saponins, which exhibit antimicrobial activity, i.e. can inhibit the growth of bacteria [2]. Several researchers also reported that members of the genus Musaceae produce secondary metabolites that show antimicrobial activity [3].

Metabolic activity in banana plants is supported by the presence of endophytic microbes in banana plant tissue. Endophytic microbes are microbes that live in colonies in plant tissues without causing disease symptoms in their host bodies. capable of producing secondary metabolic compounds similar to its host. It is suspected that there is genetic transfer (genetic recombination) from host plants to endophytic microbes. The role of endophytic bacteria in being able to increase the growth and development of host plants in various environmental and ecological conditions, and increase the resistance of host plants to pathogenic bacteria in the soil. Endophytic bacteria can be used as

growth-promoting agents, which associate with plant internal tissues by holding a growth stimulus that is relatively the same as PGPR (Plant Growth Promoting Rhizobacteria) [4].

Antimicrobials are metabolites produced by microorganisms that can inhibit the growth of other microorganisms. According to research [5] succeeded in isolating isolates of endophytic bacteria (66.7%) which were inoculated on cavendish banana plants. Interactions between host plants and endophytic bacteria can be neutralism (no effect on host plants), mutualism (beneficial to host plants and endophytic bacteria), or commensalism (beneficial to host plants or bacteria). endophytes) [6]. According to research [7] managed to isolate as many as 22 endophytic bacterial isolates from miana plants. Endophytic bacterial isolates were able to inhibit the growth of pathogenic bacteria. *E. Coli* and *S aureus*, and or both. Studies on endophytic bacteria on stone banana humps have not been widely studied. This research is aimed at obtaining endophytic bacteria on banana heads. Miles (*Musa Balbisiana* Colla), determines the antimicrobial properties and determine the identity of endophytic bacteria producing antimicrobial compounds

2 Material and method

2.1 Isolation and purification of endophytic bacteria

The isolation of bacteria from banana weevil was carried out using the plating method (plating). The cob of stone banana 56 was surface sterilized using 2%

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NaOCI and then cut into 1×5 cm sizes. Pieces of 57 stone banana heads were aseptically placed on the surface of the NA medium and then incubated at 37°C for 24-48 hours. 58 Endophytic bacterial colonies showing different morphological colors were purified by growing on NA 59 media using the streak pleat method. Furthermore, the separated colonies were transferred to slant agar as pure isolates or 60 culture stock Affiliations of authors should be typed in 9-point Times. They should be preceded by a numerical superscript corresponding to the same superscript after the name of the author concerned. Please ensure that affiliations are as complete as possible and include the country.

2.2 Antimicrobial activity assay

An antimicrobial activity assay was carried out based on the Kirby Bauer method. Bacteria test *S aureus* and *E. coli* Each cell were equalized by growing in 5 ml of NB medium and then incubated in an incubator shaker at 200 rpm at 37 °C for 24 hours. Furthermore, to determine the microbial density was measured using the turbidimetric method at a wavelength of 600 nm at an OD of 0.7. Each of the test bacteria was taken as much as 1 ml and grown on NA media by pour plate and then compacted. One ose of endophytic bacterial isolates was put into the Nutrient Broth (NB) medium. Then incubated in a shaker incubator at 37°C for four days (96 hours) then the medium was centrifuged at 5000 rpm for 1 hour so that the supernatant and biomass were separated. cell. Sterile paper disks are immersed in the supernatant for 30-70 minutes. Furthermore, the paper disks are dried and placed on the surface of the inoculated NA medium plate. Test bacteria *S.aureus* and *E.coli*.The same treatment was also carried out on the positive control (chloram fenicol) at a concentration of and the negative control (aquadest). Petri disks were incubated at 37°C for 24 hours. The anti-bacterial activity of endophytic bacteria isolates was indicated by the formation of an inhibition zone around the paper disk which was measured using a vernier caliper.

2.3 Identification Based on Morphological and Molecular Characteristics

Identification of endophytic bacteria based on morphological characters including shape, color, edges, and surface of 76 colonies as well as response to gram staining. Molecular identification using the 16S rRNA 77 gene sequence through the stages of DNA Extraction, 16S rRNA Gene Amplification, DNA Sequencing, and Phylogenetic Tree Reconstruction.

2.4 Extraction of genomic DNA

DNA genomics of endophytic bacteria was performed using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). ZR BashingBead™ Lysis Tubes (0.1 mm and 0.5 mm) were filled with bacterial cells weighing 50-100 mg in 200 l of PBS isotonic buffer. Add 750 µl Bashing

Bead™ Buffer to the tube. For 5 minutes, process at maximum speed in a bead beater fitted with a 2.0 mL tube holder. Centrifuge the ZR BashingBead™ Lysis Tube in a microcentrifuge at ≥10,000 x g for 1 minute. Transfer up to 400 µL supernatant to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuge at 8,000 x g for 1 minute. In the Collection Tube from Step 4, add 1,200 liters of Genomic Lysis Buffer. Transfer 800 µl of the mixture from Step 5 to a Zymo-Spin™ IICR Column 3 in a Collection Tube and centrifuge at 10,000 x g for 1 minute. Discard the flow-through from the collection tube and repeat Step 6. Add 200 µL DNA Pre-Wash Buffer to the Zymo-Spin™ IICR Column in a new Collection Tube and centrifuge at 10,000 x g for 1 minute. Add 500 µl gDNA Wash Buffer to the Zymo-Spin™ IICR Column and centrifuge at 10,000 x g for 1 minute. Transfer the Zymo-Spin™ IICR Column to a clean 1.5 ml microcentrifuge tube and add 100 µL (35 µL minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute DNA. Characterization of DNA extraction product for quality (purity) and quantity (concentration and extraction efficiency) was determined by using a spectrophotometer.

2.5 Amplification of 16S rRNA gene

Amplification PCR by using 2X MyTaq HS Red Mix (BIO-25048). PCR Master Mix 1x25µL consist of 9.5µL ddH₂O; 12.5 µL MyTaq HS Red Mix, 2x; 1 µL 10 µmol/µL 27F Primer (5' – AGAGTTTGATCMTGGCTCAG– 3'); 1 µL 10 µmol/µL 1492R Primer (5' – GGTTACCTTGTTACGACTT– 3'), and 1 µL DNA Template (Okolie et al. 2013). The forward and reverse 16S rRNA gene universal primers generate a 1.5 kb fragment. PCR Condition (35 cycles) followed an initial denaturation of 95°C for 3 min; denaturation at 95°C for 15sec; annealing at 52°C for 30 sec; extension at 72°C for 45 sec; and final extension at 72oC for 3 min. The process was held at 4°C for more than 48 hours. The PCR product was detected on agarose gel electrophoresis using a 1 Kb DNA ladder as a marker.

16S rRNA gene sequencing and phylogenetic analysis. The PCR products of endophytic bacteria were purified by using Zymoclean® Gel DNA Recovery Kit (Zymo Research) and sequenced based on the bi-directional sequencing method. All the sequences obtained from the sequencing phase were analyzed and edited by using BioEdit software [8]. Initially, all the 16S rRNA gene sequences were compared to sequences in GenBank by using the online service of Basic Local Alignment Search Tool (BLAST; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to determine the approximate phylogenetic position. Sequences were aligned using ClustalW with representative bacteria 16S rRNA sequences, and a phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) software VII. An unrooted neighbor-joining tree was constructed using the sequence of the 16S rRNA gene *Pseudomonas fluorescens* strain KU-7 AB266613.1 as an outgroup, obtained from GenBank as outgroup species.

2.6 Sequencing DNA

PCR products were then DNA sequenced at First Base Singapore. The sequencing process was carried out using the Amplification method. Then an analysis of the sequencing results was carried out by performing a BLAST of the nucleotide sequences from the sequencing results using the database that can be accessed at <https://www.ncbi.nlm.nih.gov>. Then select blasts and then nucleotide BLAST. then sequencing of each strain resulting from the blast was carried out by Lightment to find out the alignment.

2.7 Phylogenetic tree reconstruction

The method used in the reconstruction of the phylogenetic tree is by using a distance-based method, namely the Neighbor Joining Tree, and analysis using the Kimura model in MEGA X software.

2.8 Data analysis

This research is a descriptive study that describes the presence of endophytic bacteria in banana weevils. Batu identification and its potential as a producer of antimicrobial compounds and their types based on molecular characters.

3 Results and discussion

3.1 Endophytic bacteria on stone banana weevil (*Musa balbisiana* Colla)

Detection of endophytic bacteria on stone banana weevil (*Musa Balbisiana* Colla) using NA medium showed the presence of endophytic bacteria growing around the banana weevil with irregularly shaped colony morphology, smooth colony edges, and milky white colony color. The purification results of endophytic bacteria from banana weevils obtained one isolate. Subsequently named what is referred to as the banana weevil endophyte (EBP).

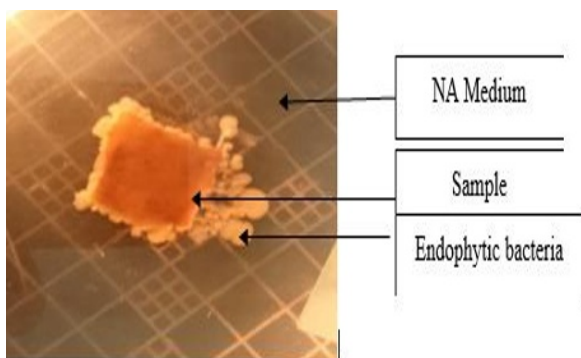


Fig. 1 Endophytic bacteria on banana weevil (*Musa balbisiana* Colla)

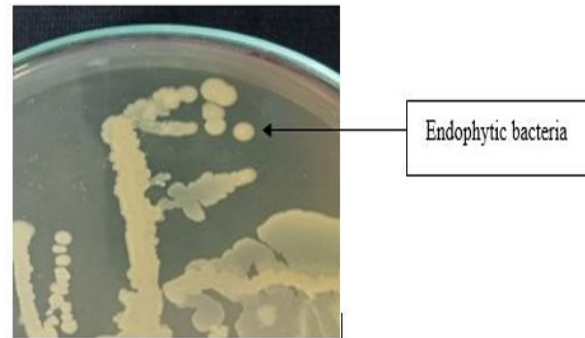


Fig. 2. Colony morphology of EBP isolates

Table 1. Macroscopic morphological characterization of bacteria

Nama Isolate	Colony characteristics			
	Form	Edge	Surface	Color
ILb01	Not order	Smooth	Flat	White milk

3.2 Antimicrobial Activity of EBP Isolate Against *Staphylococcus aureus* dan *Escherichia coli*

Showed antimicrobial activity against test bacteria *Staphylococcus aureus* and *Escherichia coli* as indicated by the formation of a clear zone/inhibition zone after incubation for 24 hours. Antimicrobial activity on the test bacteria *Staphylococcus aureus* with a diameter of inhibition zone which is 7.76 mm and for the test bacteria *Escherichia coli* with an inhibition zone diameter of 8.10 mm. The antimicrobial activity of EBP isolates was lower than that of the positive control (chloramphenicol), however, it was higher than that of the negative control (aquadest). Based on the results of the antimicrobial activity test, the diameter of the inhibition zone falls into the range of 5-10 mm which is included in the medium category (Figure 3).

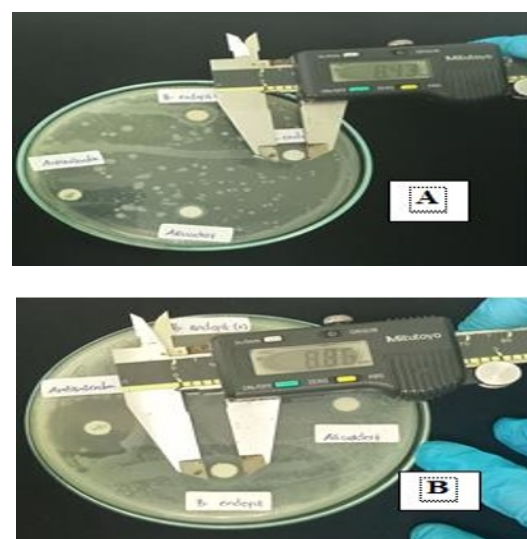


Fig. 3. Antibacterial activity of stone banana weevil isolate produced against test bacteria: A. *Staphylococcus aureus* B. *Escherichia coli*

Table 2 Diameter of Inhibition Zone of Antibacterial Compounds Produced by Endophytic Bacteria Against *Staphylococcus aureus* and *Eschericia coli* with 24-Hour Incubation Time

Sample	Diameter of Inhibition Zone (mm) Against Test Bacteria	
	<i>Staphylococcus aureus</i>	<i>Eschericia coli</i>
Endophytic bacteria	7,76	8,10
Chlorampenichol	20.05	25.07
Control negative	0	0

3.3 Molecular Identification of EBP Isolates

Endophytic bacterial isolates showing potential antibacterial activity were characterized based on molecular characteristics using the 16SrRNA gene, as a basis for identification. Identification begins with stages of identification of Genomic DNA extraction and purity is measured by nanodrop at a wavelength of 260 and 280 nm. The extracted Genomic DNA was obtained with a purity level of 1.93. This shows that the extraction of genomic DNA is feasible for further tests, namely amplification and 16SrRNA gene. 16SrRNA gene amplification using primers 27F and 1492R. The amplification results were visualized to obtain DNA bands at a size of 1,500 bp. This shows that the amplification processes correspond to the base length of the 16SrRNA gene, which is approximately 1,500 bp.

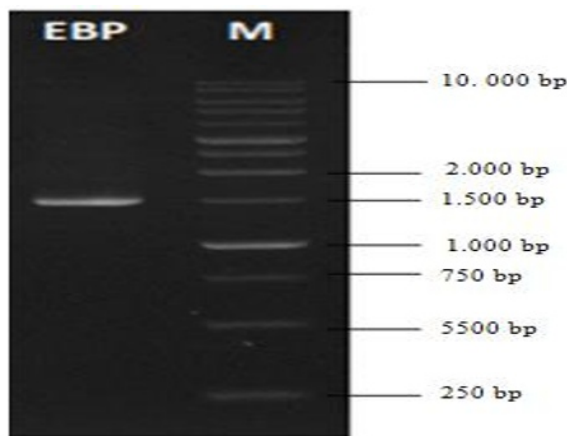


Fig. 4. Electrophoresis results of bacterial isolates with primers 27F and 1492R DNA marker 1400bp : EBP : Banana weevil endophyte M : Marker

Sequencing of 16SrRNA genes was carried out using the B-directional method. The results of sequencing the 16SrRNA genes obtained a base length of 1,415bp. The sequence of base sequence of the 16SrRNA gene was compared with the sequence or sequence of the gene bases contained in the NCBI GenBank using the blast algorithm, resulting in 10 strains with the highest similarity, namely Eterobacter roggenkampii CP080038.1, Enterobacter asburiae CP034336.1, Enterobacter chuandaensis CP080037.1, Enterobacter sichuanensis CP027986.1, Enterobacter chegduensis MZ540253.1, Enterobacter ludwigii MN641475.1,

Enterobacter cancerogenus CP045769.1, Enterobacter aerogenes EU855208.1, Enterobacter mori CP084692.1. with a presentation range of 99.22% similarity. - 99.08%. The base sequence data of the 16SrRNA gene were used as data for phylogenetic tree reconstructions using the Neighbor joining algorithm with a bootstrap of 1000 times. Results show that the EBP isolate is closely related to Enterobacter ronggenkampii with Enterobacter ronggenkampii strain colony 354 chromosomes with a similarity index of 99.22%. - 99.08%.

Table 3. Cromotogram gene 16SrRN

NO	Sample code	Sequences
1	EBP	Sequence Assembly 1415p 1 TCCAAGTGA GGGTAGCAC AGAGAGCTG CCTCGGGTG ACGAGGCGG GACGGGTGAG 81 TAATGCTGG GAAACTGCTT GATGGARGG GATAACTCT GGAAACGGTA GCTAATACG 121 CATAAGCTGG CAGACGAAA GAGGGGAGC TTGGGGCTC TTGCAATAG ATGTCCGAG 181 ATGGGATAG CTAGTAGTG GGTAAAGCC TCACCTAGC GAGCATCCT ACCTGGTGT 241 AGAGGATGAC CAGCCACACT GAACTTAGA CACGGTCAG ACCCTACGG GAGGCAGAG 301 TGGGGATAT TCACCAATGG GCGAAGCCT GATGAGCCA TCCTGCTGT ATGAGAGAG 361 CTTTGGGTT GTAAAGATTT TTAGGGGGT AGRAAGGGT TGAGGTTAT AACTGGG 421 ATTGAGCTTA CCGCGAAGG AAGCACGGC TAACCTGGT CCAGCAGCG CCGTAATAG 481 GAGGGTCAA GCGTAAATG GAATACCTG GCTGAAGCG CAGCAGGCG CAGTCCGAA 541 TGTATGTTA AATCCCGGG GTAAAGCTG GAACTGCAT CBAAACTGC AAGCTGAGT 601 CTTGTAGAG GGGGTAGAT TCCAGGTTA CGGTAAAT GCGTAGAT CCGAGGAT 661 ACCGGTGGG AAAGCGCCC CCTGCAAAA GACTACGCT CAGTCCGAA AGGTGGGA 721 GCAACAGGA TTAGATACC TGTAGTCCA GCGCTAAC GATGTGACT TGAAGTGT 781 GCGTTGAGG CCGTCTTCC GBACTAGG CCGTAACTG ACGCTGGG GAGTGGG 841 GCAAGTTAA AACTCAAAT AATCAAGG GCGCCGACA AGGCTGGAG CATGTGTT 901 AATTGATGG AACCGAAGA ACCTACTCT GCTTGACAT CCACAGACT TACCAGAGT 961 GGTGTGTC CTTGCGAGC TTGAGACAG GACTGCATG CCGTGCATG GCTGCTGTA 1021 TGAAATGTT GCTTAATCC CCGCAGGCG CCAACCTTA TCTTTGTT CCGAGGTTA 1081 GCGCGGAGC TCAAAGAGA CTCACGTTA CACTGCTAC GAAGGTGGG ATGAGCTAA 1141 GTATCATGG CCTTACGAG TAGGCTACA CAGGCTCAC AATGGCCAT ACAAGAGAA 1201 GCGACCTGC GAGAGCAAC GBACTATA AAGTATGTA TACTGGGAT TGGAGTGT 1261 AACTGACTC CATGAGTGG GAATGCTAG TAATCTAGA TCAGAACTC ACGTGAATA 1321 CCGTCCGGG CTTGTACAC ACCCGCTC ACACATGGG AOTGGTTC AAAAAAGTA 1381 GGTACCTTA CTTTGGGAG GCGCTTACC CACTT

Table 4. EBP blast result

Description	Result Links					
	Max score	Total score	Query cover	E Value	Per. ident	Accession
Enterobacter roggenkampii CP080038.1	2551	20118	100%	0,0	99,22%	CP080038.1
Enterobacter asburiae CP034336.1	2545	19919	100%	0,0	99,15%	CP034336.1
Enterobacter chuandaensis CP080037.1	2545	19895	100%	0,0	99,15%	CP080037.1
Enterobacter sichuanensis CP027986.1	2545	20207	100%	0,0	99,15%	CP027986.1
Enterobacter chegduensis MZ540253.1	2540	20218	100%	0,0	99,08%	MZ540253.1
Enterobacter ludwigii MN641475.1	2540	2540	100%	0,0	99,08%	MN641475.1
Enterobacter cancerogenus CP045769.1	2540	19853	100%	0,0	99,08%	CP045769.1
Enterobacter aerogenes EU855208.1	2540	2540	100%	0,0	99,08%	EU855208.1
Enterobacter mori CP084692.1	2540	20211	100%	0,0	99,08%	CP084692.1

Based on the results of the EBP blast by matching the sequencing results with the Gen Bank, it was found that there were 10 types of bacteria from the genus Enterobacter which has similarities with EBP as shown in Figure 4.5. The similarity can be seen based on the query cover value which shows the number 100% with percent identity 99.22

3.4 Phylogenetic Tree Reconstruction

Blast results obtained from isolates were then carried out Alignment or alignment so that phylogenetic trees will be obtained which show the relationship between the isolates obtained and the blast results. Based on phylogenetic tree reconstructions in Figure 5

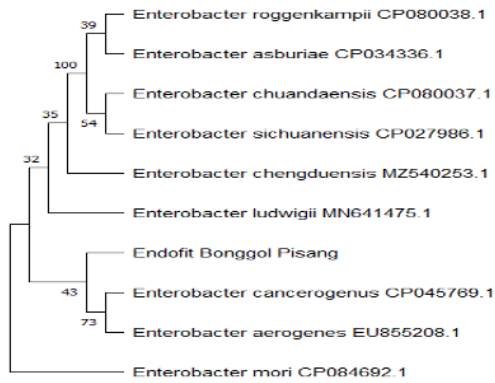


Fig. 5. Phylogenetic Tree Reconstruction

3.5 Discussion

Endophytic bacteria are bacteria associated with plants that can be found in plant parts. The results showed that there was one type of endophytic bacteria associated with the stone banana weevil (*Musa Balbisiana* Colla) located in Gorontalo. This was as reported by Rahayu [9], that there were endophytic bacteria associated with the root organs, weevils, and stalks of the Klutuk banana leaf which grew in regosol and grumusol soils. This is as reported by [10]. That the endophytic bacteria in banana weevils showed antibacterial activity against bacteria *Staphylococcus Aureus* ATCC 25923 and bacteria *Shigella Dysenteriae* ATCC 13313 [11].

The endophytic bacterial isolates that were able to associate with plant tissues varied according to the type of plant, which was determined or influenced by environmental chemical cycle conditions or the physical and chemical environmental conditions of the hosts. This is because the growth of endophytic bacteria shows a symbiotic mutualistic interaction in which the presence of endophytic bacteria will benefit the host plant, while the endophytic bacteria will get a supply of nutrients produced or as a result of plant metabolism as an energy source and carbon source [12].

The type of metabolism of each type of plant, especially in banana plants, varies depending on the type of plant. The stone banana weevil contains several secondary metabolites that show antimicrobial activity, so the endophytic bacteria associated with the banana weevil are a type of bacteria that can adapt to conditions. physical-chemical or active compounds produced by banana weevil [13].

Stone banana is a type of plant known to contain secondary metabolites which can inhibit bacterial growth including saponins, ascorbic acid, flavonoids, anthraquinones, and tannins. Among them are antibacterial compounds so that the endophytic bacteria that grow on plants can use the metabolites produced by the host plant. This is because the endophytic bacteria in plants play a role in increasing the growth and development of host plants in various environmental and ecological conditions. Endophytic bacteria are also known to increase host plant resistance to pathogens [14].

These active compounds are generally capable of producing metabolites that are almost similar to those

produced by their hosts. The results showed that endophytic bacteria could produce secondary metabolites which were able to inhibit bacterial growth *Escherichia coli* and *Staphylococcus aureus*. This is due to physiological abilities possessed by bacteria and/or due to genetic transfer from host plants so that these endophytic bacteria can produce metabolites that are almost the same as host plants [15].

Reported that based on the results of the research that had been conducted there were 3 isolates of endophytic mold on *Mussa Balbisiana*. Based on the test results of antibacterial activity against bacteria *Staphylococcus Aureus* ATCC 25923 and bacteria *Shigella Dysenteriae* ATCC 13313 [2].

The results of antimicrobial activity show that EBP isolates that can associate with banana weevils show antibacterial activity by having board spectrum properties with inhibition zones on the tested bacteria *Staphylococcus aureus* 7.76 mm and on the test bacteria *Escherichia coli*.e. 8.10mm. which means it can inhibit *Staphylococcus aureus* and *Escherichia coli* which is representative of the gram-positive and gram-negative battery groups. The ability of the EBP isolate to have antibacterial activity was in the moderate group. This was because the antimicrobial compounds present in the endophytic bacteria suppressed the growth of gram-positive and gram-negative bacteria. Based on the results of the antimicrobial activity test, the diameter of the inhibition zone falls into the range of 5-10 mm which is included in the medium category (Davis & Stout, 1978). The size of the inhibition zone of endophytic microbes against pathogenic bacteria is thought to be caused by secondary metabolites produced by endophytic microbial isolates. The higher the concentration of antibacterial compounds produced, the greater the inhibition of the growth of bacterial colonies. The diameter of the extract inhibition zone is smaller than the positive control because the extract used is not a pure compound [5].

Antibacterial ability is quite large due to the work of these secondary metabolites in carrying out their effects on intruding bacteria capable of damaging important parts of bacterial cells such as inhibiting metabolism, damaging cell walls, disrupting cell membranes, inhibiting cell protein synthesis, and inhibiting cell nucleic acid synthesis. According to Sudana [17] high or low antibacterial activity of a compound can be caused by the physical properties of the compounds, which can be seen from the length of the chain, the ability to penetrate the cell wall, the integrity of the molecules in the cell and the hydrophilic or lipophilic properties. The mechanism of action of inhibition of endophytic bacterial isolates against *S.aureus* is not yet known with certainty. However, seeing the size of the inhibition zone produced, it is possible that these bacteria can damage important parts of the cell, such as inhibiting cell metabolic processes to damage the cell wall, as a result, the growth of cells is disrupted so that the cell cannot grow [18].

In general, secondary metabolites produced by endophytic bacteria act as antibacterial compounds and can come from various groups such as alkaloids, phenolics, flavonoids, saponins, steroids, and

terpenoids. These compound groups can act as antibacterials with various mechanisms of action. Starting from inhibition by damaging the cell wall, changing the permeability of cells, changing protein molecules and nucleic acids, to coagulating protoplasm. Pathogenic bacteria can be disrupted by growth and can even die if the compound or inhibitor substance can enter the cell through the cell wall, so it can damage the cell wall, and over time it can easily damage other important parts of the cell [19].

Endophytic bacterial isolates of stone banana weevil (*Musa Balbisiana Colla*), which showed antimicrobial activity had morphological and molecular characteristics after being identified by molecular income with the Gn 16SrRNA sequence as a genetic marker, it was identified as *Enterobacter roggenkampii* because it determines the relationship between kinship strains with a colony of 354 chromosomes with a similarity index of 99.22% [11].

Jun Guo, 2020, reported that based on the results of molecular identification of endophytic bacteria originating from the roots of sugarcane plants identified by sequencing the 16SrRNA gene, namely as *Enterobacter roggenkampii* which are the most prominent strains. This is because endophytic bacterial strains interact with plants more efficiently than rhizosphere bacteria. And strains of endophytic bacteria can provide benefits to host plants, such as accelerating plant growth and tolerance to biotic and abiotic stresses in plants, and can also carry genes important for converting dinitrogen gas (N₂) into nitrogen [18].

4 Conclusion

In conclusion, *Musa paradisiaca* Colla was associated with endophytic bacteria. The endophytic bacteria potentially as producing a natural-antibacterial compound that showed antibacterial activities against *Staphylococcus aureus* dan *Escherichia coli*. The endophytic bacteria called EBP isolates showed specific characteristics including morphological dan molecular characteristics, and identify based on the sequence of 16S rRNA gene as *Enterobacter ronggenkampii* that closely related with *Enterobacter ronggenkampii* strain colony 354 chromosomes on 99.22% of similarity index.

References

1. C.W. Bacon, and D.M. Hinton. Bacterial endophytes : the endophytic niche, its occupants, and its utility. In S.S. Gnanamanickam, editor. Plant Associated Bacteria. (Netherland: Springer, 2006)
2. W.W. Davis, and T.R Stout. J. Microbiology, **4** (1971)
3. Fazhilalin Lisa. Antibacterial Activity Test of Endophytic Mold Isolates from Kepok Banana Weevil (*Musa Balbisiana* BBB) Against *Staphylococcus Aureus* ATCC 25923 and *Shigella Dysenteriae* ATCC 13313 (Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University, Jakarta, 2017)

4. Hastuti, Purnomo, I. Sumardi, and B.S. Daryono. Biodiversitas, **20**, 3 (2019)
5. H. Rizka. Ant Bacterial Activity Test of Ambon Banana Stem and Stem Extract (*Musa paradisiacvar sapientum*) Against *Staphylococcus aureus* (Scientific papers, Medical School, Diponegoro University, Indonesia,2012)
6. J. Guo, Dao, R. K. Singh, and P. S. Ingh. Microbiology, **11** (2020)
7. Fithriyah. Isolation and Identification of Endophytic Bacteria from Kebar Grass (*Biophytum* Sp.) As Producers of Antibacterial Compounds Against *Eschericia coli* and *Staphylococcus aureus* Bacteria (Department of Biology, Faculty of Science and Technology, State Islamic University of Maulana Malik Ibrahim Malam, 2015)
8. H. Marwan, M.S. Sinaga, Giyanto, and A.A. Nawangsih. J. Tropical HPT, **11**, 2 (2011)
9. D. Purnamasari, and Suhartiningsih. Journal of Cosmetology, **2**, 03 (2013)
10. M.J. Pleczar, and E.C.S. Chan. Fundamentals of Microbiology (UI Press, Jakarta, 1998)
11. R.Triastuti, Y.A. Purwestari, S. Subandiyah, and D. Widiyanto. Journal of Biology, **14**, 2 (2021)
12. I.M. Sudana. Agritop, **23** (2004)
13. A. N El-Banna; M. E. El-Mahrouk, Y. H. Dewir, M. A. Farid, D. M. A. Elyazid, and H.M. Schumacher. Horticulturae, **7**, 12 (2021)
14. M. Karthik, P. Pushpakanth, R. Krishnamoorthy, M. Senthilkumar. The Journal of Horticultural Science and Biotechnology, **92**, 6 (2017)
15. C. A. S. Junior, J. Marcon, P. A. M. Andrade, J. A. Silva, M.I.F. Faraldo, M. C. Q. Verdi, A. A. M Filho, and J. L. Azevedo. Journal of Agricultural Science (Toronto), **10**, 10 (2008)
16. A. C. Sekhar, and P. Thomas. American Journal of Plant Sciences, **6**, 7 (2015)
17. A. Souza, J.C. Cruz, N.R. Sousa, A.R.L. Procópio, and G.F. Silva. Endophytic bacteria from banana cultivars and their antifungal activity. Genetics and Molecular Research **13** (4): 8661-8670 (2014)
18. I. P. Gomes, A. D. M. Matos, S. Nietsche, A. A. Xavier, M. R. Costa, W. S. Gomes, and M. C. T. Pereira. Braz. Arch. Biol. Technol, **60** (2017)