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Molecular Characterization Of Endophytic Bacterial Isolates Of Angsana Leaves (Pterocarpus Indicus Willd) Producers Of Antibiotics Compounds

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Abstract – The increasing resistance of pathogenic bacteria to antimicrobials requires the discovery of new antibiotics. Angsana plant or Lansano (Pterocarpus indicus Willd) is one of the medicinal plants in West Sumatra. Angsana has the potential to have endophytic bacteria capable of producing antibiotic compounds like its host. The endophytic bacterial isolates of Angsana can inhibit the growth of S. aureus and E. coli bacteria. This study aims to determine the genetic characteristics of the endophytic bacteria of Angsana leaves that produce antibiotic compounds through phylogenetic tree analysis. The type of research is descriptive qualitative which was carried out from May to October 2019 at the Biotechnology Laboratory of UNP Padang. The process of amplification of the 16S rRNA gene from 3 isolates has succeeded in obtaining a single DNA band located between the 1494 bp, 1465 bp and 1163 bp DNA bands of the HindIII λ marker. The size of the PCR product for each isolate that had been amplified was approximately 1500 bp. The BLAST results showed that the bacterial isolates were identified as one genus, namely the genus Bacillus with the types Bacillus tequilensis and Bacillus subtilis. The endophytic bacterial isolate DB 1 was related to Bacillus tequilensis. Bacterial isolates DB2 and DA 3 were most closely related to Bacillus subtilis.

Keywords – Molecular Characterization; Endophytic Bacteria Isolate; Angsana or Lansano Plant (Pterocarpus indicus Willd); Antibiotic Compound

I. INTRODUCTION

To overcome the increasing cases of bacterial resistance to antibiotics, it is very necessary to source new substances that can be an alternative to further antimicrobial compounds. Currently, the potential of natural materials as candidates for producing antibacterial compounds has been widely developed, especially those from medicinal plants. According to the *World Health Organization* (WHO) the use of plants will be the best source for obtaining various drugs, so these plants must be studied to better understand the properties of plants related to their safety and efficacy [1]. About 20% of the world's plants have been tested pharmacologically or biologically and a large number of antibiotics introduced in the market are obtained from natural or semi-synthetic resources [2].

Generally antimicrobial active compounds are products of secondary metabolites of plants. These secondary metabolites can also be produced by microbes that form colonies in plant tissues, also called endophyte microbes. Each plant contains one or more

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endophytic microbes consisting of bacteria and fungi [3]. Some types of endophyte bacteria are known to be able to produce active compounds that are antibiotic, antimalarial and antifungi [4]–[6][7].

Research that is being conducted about the antimicrobial potential of endophyte bacteria in Angsana plants (*Pterocarpus indicus*) has also succeeded in isolating endophytic bacterial isolates from angsana leaves that have the potential as producers of antibiotic compounds. Based on the results of research researchers there are six isolates of Angsana leaf endophye bacteria that are successfully isolated and potentially antimicrobial. Four isolates have the ability to inhibit the growth of *Escherichia coli* and two isolates have the potential to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*.

Identification of bacteria is one of the stages that must be done in research isolation and characterization of endophyte bacteria producing antimicrobial active compounds. The determination of the right type of endophyte bacteria is necessary to facilitate in the scientific communication of scientists. With the type of endophyte bacteria well known, it is possible to develop research to more advanced stages such as the molecular engineering stage.

Identification of bacteria is not sufficient by macroscopic and microscopic means, but molecular identification is required. The PCR (Polymerase Chain Reaction) test is a more sensitive microbiological test compared to conventional methods. This test is performed by characterizing and observing the gene profile of 16S rRNA. The use of a 16S rRNA for identification and kinship studies has been widely performed. Currently there are programs available to group and find out the identity of an isolate such as blast program (Basic Local Alignment Search Tool) which is a sequence alignment program used to compare the sequence of biological information [8], [9].

Identification of bacteria based on genetic profile is considered more accurate because it is not influenced by internal factors such as growth stage or external factors such as the environment in which bacteria grow [10]. In addition, using PCR is also easier to do, reagents are easier to obtain and provide relatively faster results (depending on laboratory conditions). The success of PCR examination is influenced by several factors, including the quality and quantity of samples in the form of genetic material of the bacterial DNA [11].

Based on the description above, given the antimicrobial potential found in the endophytic bacteria of Angsana leaves, it is necessary to continue the previous research to the stage of "Molecular Characterization of Isolates of Endophytic Bacteria of Angsana Leaves (*Pterocarpus indicus* Wild) Producing Antibiotic Compounds."

II. METHOD

2.1. Molecular Identification of Bacteria

2.1.1. Molecular Test *[12]*

2.1.1.1. Genomic DNA Isolation

Isolation of the bacterial genome was carried out by boiling method. A total of 2-3 oses of bacterial colonies were put into a 1.5 mL *Ependorf* tube containing 200 µl of 1/10 TE buffer. The suspension was vortexed until homogenous. The boiling process was carried out in a water bath at a temperature of 95-100^{o}C, for 15 minutes. The suspension was then centrifuged at 12,000 rpm for 10 minutes to separate the supernatant and pellet. The supernatant was transferred to a new *Ependorf* tube, and stored at -20^{o}C until used [13].

2.1.1.2. 16S rRNA Gene Amplification of Angsana Leaf Endophytic Bacteria

The 16S rRNA gene for endophytic bacteria in Angsana leaves was amplified from the bacterial genome that had been previously isolated using universal bacterial 16S rRNA primers. The PCR reactions were carried out in 50 µl reaction, consisting of 2.5 µl 10x PCR buffer, 0.5 µl MgCl2 25 mM, 3.0 µl dNTP 10 mM, 2 µl each primer (forward and reverse) and 0.2 µl Tag polymerase. 4 µl of template DNA was added, then the reaction was added with DW until the volume was 50 µl of DNA. The temperature cycle used was initial denaturation of 95°C for 3 minutes, followed by 35 cycles consisting of denaturation of 95°C for 45 seconds, primer attachment at 55°C for 30 seconds, and elongation at 72°C for 2 minutes. The reaction was closed by final elongation at 72°C for 7 minutes.

2.1.1.3. Electrophoresis

Analysis of PCR products was carried out by electrophoresis technique using 1% agarose gel. Agarose gel was prepared by dissolving 1 g of agarose powder in 100 ml of TAE buffer. The solution is frozen in a mold that has a comb. Electrophoresis is carried out in a special tank. The PCR product to be analyzed was mixed with 6x loading buffer, then inserted into each well on the agarose gel. In addition to PCR products, the electrophoresis process also included a 100 bp DNA marker to determine the size of the DNA bands resulting from electrophoresis. Electrophoresis was carried out at 100 volts for 30 minutes. The gel from the electrophoresis was then soaked for 3-5 minutes in µg/ml Ethidium bromide and then washed with 1x TAE buffer. DNA visualization and analysis performed in UV Transilluminator.

2.1.1.4. Purification

The PCR product in the form of a single specific band was purified using the Qiaquick PCR purification (Qiagen) kit. The working procedure is carried out according to the protocol on the kit. PB buffer was added 5 times the volume of PCR product. After homogenization, the mixture was put into a QIAquick spin column and centrifuged at 12,000 rpm for 60 seconds. The membranes were washed with 750 µl Buffer PE and centrifuged at 12,000 rpm for 60 seconds. To remove residual ethanol, the column was centrifuged empty at 12,000 rpm for 60 seconds. DNA was eluted by adding 50 µl Buffer TE and centrifuged at 12,000 rpm for 1 minute.

2.1.1.5. Sequencing

The PCR results were sequenced to determine the nucleotide sequence. Sequencing was carried out at Macrogen Singapore with an automated DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystem, USA). The results of the sequencing were analyzed with BioEdi software and then assembled into one complete nucleotide sequence of the 16S rRNA gene. Furthermore, using the BLAST-N (*Basic Local Alignment Search Tool-Nucleotide*) program from the NCBI (*National Center for Biotechnology Information*) website, the sequence results were aligned with GenBank data to determine the species similarity of the isolates tested.

2.1.2. Phylogenetic analysis *[14]*

The phylogenetic tree construction was carried out using the MEGA 4.0 program, the *Neighbor Joining* (NJ) method. The phylogenetic tree was constructed to see the relationship between the endophytic bacterial isolates of Angsana leaf and similar bacteria. The sequences used in constructing the phylogenetic were downloaded from the genBank data.

2.2. Data Analysis

The data obtained from the stages of molecular testing and phylogenetic analysis were analyzed descriptively based on the type of bacteria whose molecular characteristics were identified. The data obtained is then displayed in the form of tables, graphs and images.

III. Result and Discussion

A. Amplification of the 16S rRNA Gen Gene

The first step in the molecular analysis of the endophytic bacteria of Angsana leaf (*Pterocarpus indicus* Wild.) producing antibiotic compounds was the amplification of the 16S rRNA gene. PCR reactions were carried out on 3 isolates. PCR products were analyzed using 1% agarose gel. The results of the PCR product analysis can be seen in Figure 1.

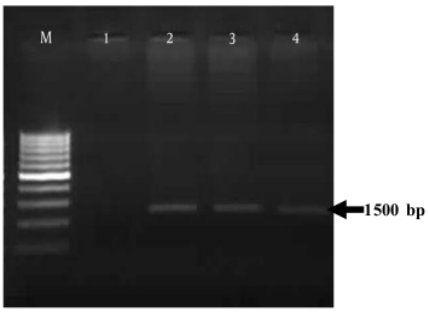

Fig 1. The results of the electrophoresis of the 16S rRNA gene amplification product. (M) Markers; (1) Negative Control; (2) DB 1; (3) DB 2; (3) DA 3

Figure 1 shows that the 16S rRNA gene amplification process of 3 isolates has successfully produced a single DNA band located between the DNA band 1494 bp and 1163 bp of the H*ind*III λ marker. The amount of PCR products of each isolate that has been successfully replicated is approximately 1500 bp.

B. Sequencing Results Analysis

The PCR product was used as template in the sequencing process. In this study, 3 isolates were sequenced, namely DB 1, DB 2, and DA 3. The total length of the 16S rRNA gene fragment, each isolate, was obtained by combining the results of the forward and forward primer sequencing. reverse (contig process). The list of bacterial isolates that have been sequenced and the length of the fragments resulting from the 16S rRNA gene contig of each bacterium can be seen in Table 1.

Table I. List Of Sequenced Bacterial Isolates

No.	Isolat Code	Sequence Length (bp)
1.	DB 1	1494
2.	DB 2	1465
3.	DA 3	1163

The average length of the 16S rRNA gene fragment contig each isolate is 1450 bp, except for a DA 3 isolate of only 1163 bp. The results are not maximal for gene fragments 16S rRNA DA 3 isolates because the sequencing process using reverse primer is not good.

C. Identify The Type of Bacteria

The contig results of the 16S rRNA gene are used to determine the type of bacteria using the BLAST program of the NCBI site. The type of bacteria is determined based on the highest similarity to NCBI data. The study analyzed 3 isolates with the BLAST program. The results of the identification of the type of bacterial isolate can be seen in Table 2. Each isolate has been uploaded to GenBank NCBI.

The BLAST results showed that the bacterial isolates were identified as one genus, namely the Bacillus genus identified as Bacillus tequilensis and Bacillus subtilis.

Table II. Results Of Identification Of 16s Rma Gene Isolated Endophytic Bacteria Using The Blast Program

BLAST Results					
Description	**Skor Maks**	**E Value**	**Similarity**	**Isolate**	**Types of Bacteria**
Bacillus tequilensis strain SJS (MK880583.1)	2760	0.0	100%	DB 1	*Bacillus tequilensis*
Bacillus subtilis strain PgBE238 (MH144308.1)	2706	0.0	100%	DB 2	*Bacillus subtilis*
Bacillus subtilis strain A3 (KY202696.1)	2021	0.0	98,02%	DA 3	*Bacillus subtilis*

Analysis of similarities between Angsana leaf endophyte bacterial isolates was done with the BioEdit program, the *sequence identity matrix* menu. The results of the analysis of similarities between bacterial isolates can be seen in Table 3. Isolates in the same genus have a partial similarity of > 95%.

Table 3. Sequence Similarities Between Angsana Leaf Endophytic Bacteria Isolates

Isolat Name	DB 1	DB 2	DA 3
DB 1	ID		
DB 2	0,97	ID	
DA 3	0,98	0,99	ID

D. Phylogenetic Analysis

The phylogenetic construction was carried out using the MEGA 10 program using the *Neighbor Joining* (NJ) method. Sequence data of each isolate was compared with several 16S rRNA gene sequence data from several bacterial species obtained from GenBank data. The results of the sequence comparison were visualized in the form of a phylogenetic tree that could show the relationship between bacterial isolates. The results of the phylogenetic construction can be seen in Figure 2.

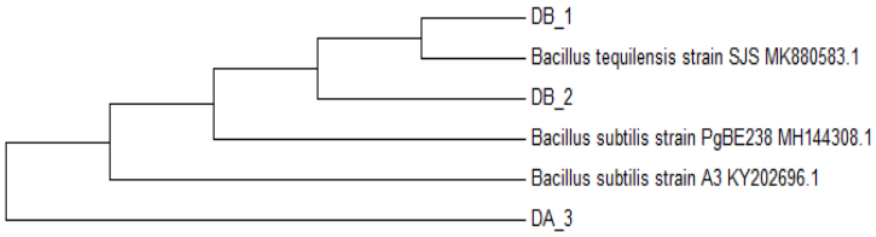

Fig 2. Phylogenetic Tree Bacteria from Angsana Leaves.

Based on the results of the phylogenetic analysis in Figure 2, it can be seen that the bacterial isolates isolated from leaf tissue are closely related. The endophytic bacterial isolate DB 1 was related to Bacillus tequilensis. Bacterial isolates DB2 and DA 3 were most closely related to Bacillus subtilis.

In this study, the sequencing process was carried out at Macrogen Singapore with an automated DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystem, USA). The results of the sequencing are issued in the form of a chromatogram of the nucleotides of the target DNA fragment. In theory, the length of the sequence that can be read in one sequencing process is around 1000 – 1500 bp, but in practice usually the average nucleotide length that is read in one sequencing process is only around 400-700 bp [15]. With the limitations of these sequencing results, it is necessary to do the sequencing process twice for each isolate using forward and reverse primers to obtain the complete sequence of the 16S rRNA gene (±1500 bp). Several factors that can affect the reading of the sequencing results are the purity of the printed DNA, the quality of the enzymes used, the accuracy of the reaction, the accuracy of the primers and others [15].

Analysis of the sequencing results was carried out with the help of BioEdit software to obtain the desired DNA fragments. Whole 16S rRNA gene sequences were analyzed using the BLAST program to look for homologous or similar reference sequences from GenBank data so that the type of bacterial isolate could be determined. Based on the BLAST results for all isolates, homologous results were obtained with reference sequences ranging from 98-100%. The BLAST results for each isolate can be seen in Table 4.2. GenBank sequences are said to be similar if they have a similarity percentage of 98%. The percentage of similarity <98% is considered unrecognized so it is recommended that further research be carried out to ensure that the isolate is a rumor [16].

Several hundred different strains of *B. subtilis* have been collected, studied and tested for antibacterial proven *B. subtillis* strains have the potential to produce antibiotic compounds with various amazing structures (Moszer *et al*., 2002). This was also proven in isolates DB2 and DA 3 in the inhibitory test to inhibit the growth of *S. aureus and E. coli*. bacteria *B. subtillis* was the most productive species of the genus *Bacillus*. 4-5% of the genome synthesizes 66 types of antibiotic substances [17]. *B. subtilis* has been widely used for genetic and biochemical assays and for decades, this bacterium and has been described as an endospore bacterium is classified as Gram-positive.

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REFERENCES

[1] G. G. F. Nascimento, J. Locatelli, P. C. Freitas, and G. L. Silva, "Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria," *Brazilian J. Microbiol.*, vol. 31, pp. 247–256, 2000.

[2] R. A. A. Mothana and U. Lindequist, "Antimicrobial activity of some medicinal plants of the island Soqotra," *J. Ethnopharmacol.*, vol. 96, no. 1–2, pp. 177–181, 2005.

[3] G. Strobel and B. Daisy, "Bioprospecting for microbial endophytes and their natural products," *Microbiol. Mol. Biol. Rev.*, vol. 67, no. 4, pp. 491–502, 2003.

[4] S. Yandila, D. H. Putri, and M. Fifendy, "Endophytic Bacteria Colonization on Root Andaleh Plant (Morus macroura Miq .)," *Bio-Site*, vol. 04, no. 2, pp. 1–7, 2018.

[5] N. Afifah, I. Irdawati, and D. H. Putri, "Isolation and Identification of Endophytic Bacteria from the Andalas Plant Stem (Morus macroura Miq.)," *Bioscience*, vol. 2, no. 1, pp. 72–75, 2018.

[6] M. F. Putri, M. Fifendy, and D. H. Putri, "Diversitas Bakteri Endofit Pada Daun Muda dan Tua Tumbuhan Andaleh (Morus macroura miq.)," *EKSAKTA*, vol. 19, no. 1, pp. 125–130, 2018.

[7] U. M. Safira, F. H. Pasaribu, and M. Bintang, "Isolasi bakteri endofit dari tanaman sirih hijau (Piper betle L.) dan potensinya sebagai penghasil senyawa antibakteri," *Curr. Biochem.*, vol. 1, no. 1, pp. 51–57, 2014.

[8] D. H. Putri, M. Anika, and W. Wahyuni, "Bioinformatics Study Genes Encoding Enzymes Involved in the Biosynthesis of Carotenoids Line Cassava (Manihot esculenta)," *EKSAKTA Berk. Ilm. Bid. MIPA*, vol. 20, no. 1, pp. 10–16, 2019.

[9] Y. P. Cita and D. H. Putri, "Analisis Mutasi pada Kodon 531 Pada Gen Rpob Mycobacterium tuberculosis Penyebab Resistensi Rifampisin," *J. ILMU KEFARMASIAN Indones.*, vol. 15, no. 2, p. 140, 2017.

[10] D. Dwiyitno, "Molecular-Based Identification for Fish and Seafood Pathogenic Bacteria," *Squalen Bull. Mar. Fish. Postharvest Biotechnol.*, vol. 5, no. 2, pp. 67–77, 2010.

[11] S. Sunarno, K. Sariadji, and H. A. Wibowo, "Potensi gen dtx dan dtxR sebagai marker untuk deteksi dan pemeriksaan toksigenisitas Corynebacterium diphtheriae."

[12] A. Kusumawati and F. Fatimah, "Kombinasi One-step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) dan Nucleic Acid Lateral Flow (NALF) sebagai Metode Deteksi Gen env-tm Virus Jembrana Strain Tabanan 1987," *J. Sain Vet.*, vol. 36, no. 2, pp. 137–143.

[13] J. Sambrook, D. W. Russell, R. Szewzyk, et al, "DNA Isolation Methods," *Genomic DNA Purif.*, vol. 13, no. 2, pp. 1–8, 2014.

[14] V. Singh, D. K. Chaudhary, and I. Mani, "Molecular characterization and modeling of secondary structure of 16S rRNA from Aeromonas veronii," *Int J Appl Biol Pharm Technol*, vol. 3, no. 1, pp. 253–260, 2012.

[15] I. M. Tasma, "PEMANFAATAN TEKNOLOGI SEKUENSING GENOM UNTUK MEMPERCEPAT PROGRAM PEMULIAAN TANAMAN," *J. Penelit. dan Pengemb. Pertan.*, vol. 34, no. 4, 2015.

[16] J.-G. Oh, W. H. Lee, and H. Kim, "The inhibition of electrochemical carbon corrosion in polymer electrolyte membrane fuel cells using iridium nanodendrites," *Int. J. Hydrogen Energy*, vol. 37, no. 3, pp. 2455–2461, 2012.

[17] T. Stein, "Bacillus subtilis antibiotics: structures, syntheses and specific functions," *Mol. Microbiol.*, vol. 56, no. 4, pp. 845–857, 2005.

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