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Antibacterial Activities of Endophytic Bacteria Isolate AKEBG28 from Yellow Root Plant (*Arcangelisia flava* (L.) Merr) Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT
Yellow root plant (*Arcangelisia flava* (L.) Merr) is one of the plants widely used as a medicinal plant. Yellow root plant stems have secondary metabolites of the Alkaloid groups that have the potential as antibacterial. Antibacterials can be produced by microorganisms such as endophyte bacteria. So far, there is no research has been reported on the antibacterial activities of yellow root plant endophyte bacterial isolates. This study aims to determine the antibacterial activities produced by yellow root plant endophyte bacterial isolates against *Escherchia coli* and *Staphylococcus aureus*. Endophyte bacterial isolates were collected from the microbiology laboratory, Biology Department, University of Bengkulu. The isolate was repurified on Nutrient Agar (NA) media for further steps. Isolate is further molecularly identified using 16S rRNA genes. The antagonistic results of antibacterial activities using culture, pellets, supernatants showed that isolate AKEBG28 had antibacterial activities against Escherichia coli and Staphylococcus aureus. Molecular identification based on 16S rRNA genes showed that the AKEBG28 isolate was genetically close with genus Bacillus cerreus Strain MS54.

Keywords: *antibacterial, endophytic bacteria, yellow root plant*

1. INTRODUCTION

Indonesia is one of the tropical countries that have high biodiversity, one of the areas that contribute to the potential of biodiversity in Bengkulu Province, especially Enggano Island. The plants found in the Enggano sub-district are among those with high biodiversity. Based on the results of the Enggano Island Biosources Expedition reported that there are 545 types of plants found on Enggano Island, and 545 of them are yellow root plants [1].

Yellow root plants are liana plants, up to 20 m long, living in lowlands, up to 800 m above sea level. The leaves are thick and strong as the skin, oval-shaped, growing not sharp, the width of the leaves 7 cm to 20 cm, the top surface shiny and the stalks long. The flowers are two houses with small sizes arranged in a series in the form of glaborous 20 cm to 50 cm, the title is greenish-white or yellowish-white [2]. The wood is yellow, the stem stew can be used to treat jaundice, digestion, worming, strong medicine/tunikum, fever, menstrual decay, and thrush [3].

Yellow root plants contain many active compounds that make this plant has many benefits as a medicinal plant. The stem of yellow root plants contained protoberberine alkaloid compounds, reportedly active as antibiotics against gram-positive

and gram-negative bacteria [4]. In general, bioactive compounds contained in certain plants can be obtained by directly using these parts of the plant, if this method continues to be used will cause its availability in nature to decrease, so it is necessary to use a more efficient way to obtain bioactive compounds from certain plants i.e. using endophytic bacteria [5].

Endophyte bacteria are bacteria that live in the host tissue without treating the symptoms of the disease [6], endophyte bacteria can be found in various plant tissues, including seeds, ovula, fruit, stems, roots, tubers, and leaves [7]. Some types of endophyte bacteria are known to produce active compounds that are antibiotics. Some experts have isolated and examined endophyte bacteria from various plants including, medicinal plants [9], plantation plants [10], forest plants [11].

Endophyte bacteria isolated from a medicinal plant can produce secondary metabolites similar to their host plant or even in higher quantities, so there is no need to use the original plant in large quantities for simplisia to take [12]. Therefore, it is necessary to study the potential of yellow root plant endophyte bacteria (*Arcangelisia flava* (L.) Merr) as a producer of compounds that serve as anti-bacterial against *E. coli dan S. aureus.*

2. METHODS

2.1. Tools and Material

The tools used in this study were autoclave, laminar air flow, incubator, oven, Petri cup, Polymerase Chain Reaction (PCR), eppendorf tube, spritus lamp, erlenmeyer, analytical scales, matches, digital calipers, micropipet, microtype, inoculating loop, electrophoresis, and centrifuges.

The materials used in this study are, Isolate bacteria endophyte yellow root plant from Enggano (collection laboratory of microbiology, biology major, University of Bengkulu), Nutrient Agar (NA), Tryptic Soy Agar (TSA), Nutrient Broth (NB), Tryptic Soy Broth (TSB), Primer 63f and 1387r, PCR kit, GoTag Green Mastermix, ethidium bromide dye (EtBr), 70% alcohol, 96% alcohol, aquades, spritus, disc paper (Whatman 6 mm), pathogenic bacteria (*Staphylococcus aureus*, dan *Escherichia coli*).

2.2. Reculture of Endophityc Bacterial Isolates

Endophyte bacterial isolates that have been in the previous collection, repurification in nutrient agar (NA) media, by scratch method, then in incubation at 30 ^{o}C during 2x24 hours [13].

2.3. Antibacterial Activity Test Using Culture

Qualitative testing of antibacterial compound activity [14]. One pathogenic bacterial loop is cultured into 50 mL of Tryptic Soy Broth (TSB) media and incubation on shaker for 24 hours. A total of 1 mL of pathogenic bacterial culture is added to the TSA's 100 mL temperature of ± 40oC, homogenized, then ed into a Petri dish of ± 15 mL, waiting until the media is solid. Endophyte bacterial isolates are inoculated into media that already contain pathogens using the loop, incubation at 30 ^{o}C for 2x24 hours. Positive results are characterized by the formation of clear zones around endophyte bacterial isolates. The clear zone that is formed is measured using digital calipers.

2.4. Antibacterial Activity Test Using Pellets

As many as one loop isolates the endophyte bacteria that will be used for testing antibacterial activity not cultured on NB media and incubation on the shaker at a speed of 100 rpm for 24 hours. 1.5 mL of culture is centrifuged at a speed of 10,000 rpm for 5 minutes inside the eppendorf tube, then the pellets are separated for use in the activity test process against pathogenic bacteria. The pellets are dissolved using a supernatant ± 150 µL, then a vortex pellet. Activity tests are carried out using sterile disc paper placed on media that already contains test pathogens. The vortex pellets are dredged onto disc paper by 20 µL, then incubated at 30 ^{o}C for 2x24 hours. Positive results are indicated by the absence of a clear zone around the disk paper [15].

2.5. Antibacterial Activity Test Using Supernatant

Supernatants resulting from centrifugation are separated from pellets for use in activity tests against test pathogens. Activity tests are carried out using sterile disc paper placed on media that already contains test pathogens. Supernatants are dripped onto disc paper by 20 µL, then inkubation at 30 ^{0}C for 2x24 hours. Positive results are shown by the presence of clear zones around disc paper [16].

2.6. Molecular Identification

Isolates with the best potential are then molecularly identified, bacterial genome DNA isolation is performed using the PrestoTM Mini gDNA Bacteria (Geneaid) kit protocol. DNA insulation results are then amplified using a PCR (Polymerase Chain Reaction) machine with a primary forward of 63F (5'-CAGGCCTAACACAT GCAAGTC-3') and a primary reverse of 1387R (5'-GGGCGGGTGTGTACAAGGC-3') with a target fragment ± 1300bp. Pre-denaturated PCR conditions (94 ºC, 5 minutes), denaturation (94 ºC, 45 seconds), primary slinging (55 ºC, 1 minute), lengthening (72 ºC, 1 minute 10 seconds), post-elongation (72 ºC, 7 minutes), and cooling (15 ºC, 15 minutes). PCR reaction is carried out as much as 30 cycles, then PCR amplification results are then migrated to agarose gel at 1% with electrophoresis technique at 50 Volt voltage for 40 minutes. The electrophoresis results are then visualized using Axygen's Gel Document System after being soaked using ethidium bromide (EtBr) dye for 15 minutes. The amplification-resulting 16S rRNA gene will be seen with the formation of a ± 1300 pb. The amplification results are then carried out sequencing through PT. Genetika Sains Indonesia [17].

3. RESULT AND DISCUSSION

Endophyte bacterial isolates from yellow root plants to be rejuvenated are a collection from microbiological laboratories, there are as many as 29 isolates of endophyte bacteria to rejuvenate. All endophyte bacterial isolates are then rejuvenated for initial testing of the activity of antibacterial compounds from endophyte bacterial isolates against pathogens. Colonies of bacteria are rejuvenated using scratch methods and in incubation in incubators for 2 x 24 hours.

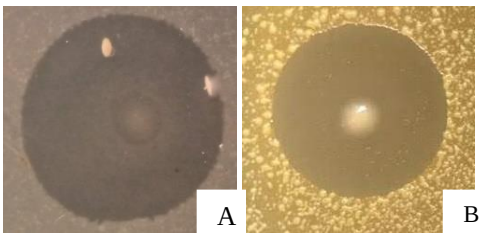

Figure 1. activity test results using endophyte bacterial isolates, (A) AKEBG28 against *E. coli* dan (B) AKEBG28 against *S.aureus.*

Initial testing of the activity of antibacterial compounds from endophyte bacterial isolates against *E. coli* and *S. aureus* test pathogens obtained the result that AKEBG28 isolation was able to inhibit the growth of both pathogenic bacteria. this is characterized by the formation of a clear zone around the tested endophytic bacterial isolate (Figure 1)

Inhibition activity in the form of clear zones formed is measured by the term of the digital shove to know the size of the inhibition zone formed (Table 1). Based on the diameter of the inhibitor zone, the positive test results were compared to 4 categories of inhibition of antimicrobial compounds, diameter > 20 mm belongs to the category of very strong inhibition, 10-20 mm including strong category, 5-10 mm including medium category, and > 5 mm including weak category [18]. Endophyte bacterial isolate AKEBG28 inhibits the growth of *E. coli* by 15.8 mm and inhibits the growth of *S.aureus* by 16.7 mm. Previous research has successfully isolated 38 endophyte bacterial isolates from life-saving medicinal plants, of which there are eight of the best isolates that have antimicrobial activity against pathogens *P. aeruginosa*, *E. coli*, and *C. albicans* [19].

Testing using breeding showed the endophyte bacterial isolate AKEBG28 was able to inhibit the growth of test pathogens, so that continued by testing using pellets and supernatant, for testing using pellets and supernatant isolates endophyte bacteria AKEBG28 first cultured for 24 hours before centrifugation and testing with disc paper. The results of the test using pellets and supernatant suggest that growth inhibition activity is characterized by the formation of clear zones around disc paper (Figure 2).

Table 1. Antibacterial Activity Measuring Results Endophyte Bacteria Isolate AKEBG28

No	Isolate code	Pathogenic Bacteria	The Diameter of the clear Zone Activity (mm) ±SD	Clear Zone Activity
1	AKEBG28	*E. coli*	15,8±1,7	strong
		S. aureus	16,7±0,1	Strong

Test measurements using pellets showed the greatest activity was generated at 3.1 mm of *E. coli* inhibition and 0.7 mm of *S.aureus*. In tests using supernatant also the largest activity occurred in the inhibition of E. coli which is 2.4 mm and inhibits *S. aureus* by 0.5 mm. previous research has reported results that supernatant isolates endophyte B2d bacteria isolated from yacon plant stems (*Smallanthus sonchifolius* (Poepp. & Endl.) H. Rob.)

Table 2. AKEBG28 endophyte isolates activity measurement results using pellets and supernatant

No	Isolate code	Testing methods	Pathogenic bacteria	Clear zone activity diameter (mm) ±SD	Clear zone activity
1	AKEBG28	Pellets	*E. coli*	3,1±0,07	Weak
			S. aureus	0,7±0,28	Weak
		Supernatant	*E. coli*	2,4±0,14	Weak
			S. aureus	0,5±0,28	Weak

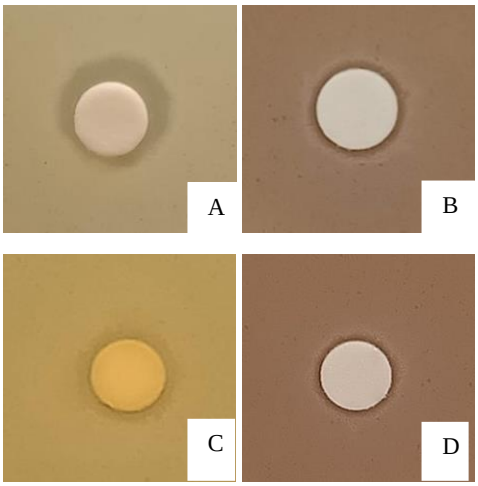

Figure 2. Test results of antibacterial activity of endophyte bacterial isolate AKEBG28 using pellet (A) against *E.coil*, (B) against *S.aureus* and supernatant (C) against *E.coli*, (D) against *S.aureus.*

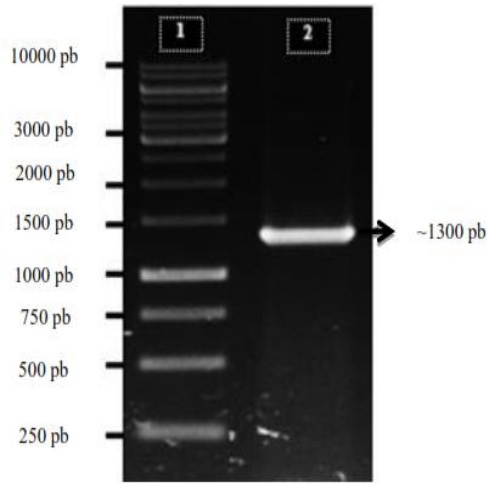

Figure 3. Electrophoresis results from gene 16S rRNA isolate AKEBG28 with primary 63f and 1387r on agarose 1%; 1= marker, 2= isolate AKEBG28

have antibacterial activity against *S. aureus* and *Salmonella thypimurium* [20]. Tests using pellets and supernatants showed that the resulting antibacterial compounds were extracellular and intracellular.

The results of the study using pellets and supernatant were then measured to determine the diameter of the resulting slave zone. The measurement results are displayed in Table 2.

AKEBG28 potential isolates are then molecularly identified using the 16S rRNA gene. Molecular identification of 16S rRNA is used because gene 16S has a conservative nucleotide sequence. The conservative nucleotide part is used as the primary attached place so that it can be amplified using a polymerase chain reaction (PCR) [21]. The amplification of the 16S rRNA gene will produce a DNA fragment band with a ±1300 pb (Figure 3).

The results of sequencing isolate AKEBG28 obtained in the form of DNA fragments, the results of sequencing DNA fragments that have been obtained then analyzed using Basic Local Alignment Search Tools nucleotide (BLASTn), from the analysis will be obtained bacterial isolates that have a resemblance to isolates AKEBG28.

Molecular identification of the 16S rRNA genes showed that the AKEBG28 isolate was genetically close with genes *Bacillus cereus* strain MS54 with similarity value of 99%. Bacterial isolates that have more than 97% resemblance in the 16S rRNA gene homology sequence indicate they are the same species [22].

4. CONCLUSION

Based on the results of the study can be concluded that endophyte bacteria isolate AKEBG28 from yellow root plants (*Arcangelisia flava* (L.) Merr) has antibacterial activity that is able to inhibit the growth of *E. coli* and *S.aureus* both testing using culture, pellets, and supernatant. The best antibacterial activity is shown in the inhibition of *E. coli* growth using pellet and supernatant testing of 3.1 mm and 2.4 mm. Molecular identification of the 16S rRNA genes showed that the AKEBG28 isolate was genetically close with genes Bacillus cereus strain MS54.

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