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Article

Antibacterial Potential of Actinomycetes Isolates from Imperata (*Imperata cylindrica L.*) Rhizosfer Against *Eschericia coli* and *Staphylococcus aureus*

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Abstract. Research on the Antibacterial Potential of Actinomycetes Isolates from the Rhizosphere of imperata (*Imperata cylindrica L.*) Against *Eschericia coli* and *Staphylococcus aureus* has been conducted at the Biota Laboratory of Sumatera, Andalas University from July to September 2020. This study aims to obtain actinomycetes isolates from plant rhizosphere reeds (*Imperata cylindrica L.*) which has the potential as antibacterial against *Escherichia coli* and *Staphylococcus aureus*. This study used a survey method. The results showed that 4 isolate actinomycetes were found, all of which have antibacterial potential, which could inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*.

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1. Introduction

Antibiotic resistance currently has the greatest impact on human health. World health leaders describe antibiotic resistance as "nightmare bacteria" which threatens the lives of people in every part of the world. The increase in infections and deaths caused by antibiotic resistance is a challenge that must be resolved [1]. It is estimated that there will be 10 million deaths in 2050 due to antimicrobial resistance, and 4.7 million of them will be Asians. The big impact of antibiotic

resistance is that the morbidity (illness rate) and mortality (death rate) is increasing due to the risk of spreading infection due to resistant bacteria and the higher cost of treatment [2].

Multidrug-resistant (MDR) is a condition in which bacteria are resistant to at least one type of antibiotic from 3 antibiotic groups [3]. Increased ability of pathogens to inhibit the effect of drugs can lead to the emergence of resistance. Several pathogenic bacteria in humans have been reported to have developed resistance to more than one class of antibiotics [4]. Some of these microorganisms include *Escherichia coli* and *Staphylococcus aureus*.

The emergence of various infectious diseases caused by bacteria and the occurrence of antibiotic resistance by pathogenic bacteria, encourage further research to be able to produce new antibiotics and have optimal efficacy to treat infectious diseases. One of the antibiotic-producing microorganisms is actinomycetes. Actinomycetes are bacteria that resemble fungi and are classified as Gram positive bacteria [5]. Actinomycetes produce many bioactive compounds which tend to produce antibiotic compounds to treat symptoms of infection [6].

Streptomycetes actinomycetes members can be found in the rhizosphere and non-rhizosphere of plants [7]. Some isolates were found to be able to produce antibiotics against several test bacteria. The types of antibacterial compounds found were Erythromycin, Tetracycline, Rimfampicyn, Polymyxin and Chloramphenicol. Many microorganisms are attracted to the nutrients produced by exudates from the root system of a plant [8]. So that the population of microorganisms in the rhizosphere is much higher than other parts of the soil [9].

Several studies have shown that actinomycetes isolated from plant rhizosphere have the potential to produce antibiotics. Isolated actinomycetes from the rhizosphere of pangola grass (*Digitaria decumbens*), the isolates obtained had potential as antibiotics which were proven to be able to inhibit multi-resistant Escherichia coli bacteria. The population of actinomycetes in the grass rhizosphere is about 40% of the total soil microflora [10]. In the rhizosphere soil of the Poaceae family of plants, namely bitter grass (*Axonopus compressus*) and jampang grass (*Elusine indica*), streptomyces isolates can be obtained which have the potential as antibiotics [11].

Imperata (*Imperata cylindrica L.*) is a species of grass family or Poaceae. Imperata has large and many roots. Root characteristics can affect the nutrients needed by microorganisms in the soil [10]. In the rhizosphere of imperata plants which is located on the Limau Manis campus, it is hoped that actinomycetes have the potential to produce antibiotics as an alternative in the search for antibacterials to overcome several pathogenic bacteria that have been resistant to several antibiotics. which is expected to have antibacterial potential, especially against *Escherichia coli* and *Staphylococcus aureus*.

2. Experimental Section

2.1 Preparation Stage

This researched was carried out for 3 months starting in July – September 2020 in Biota Sumatera Laboratory, Universitas Andalas. The tools used in this study were petri dish, spatula, oven, micropipette, blue tips, analytical balance, 50 mL beaker glass, plastic, pH meter, test tube, vortex mixer, erlenmeyer, autoclave, sterile loop, object glass, stopwatch, gas lighter, tissue, microscope , camera, 6 mm disc paper, centrifuge, plastic wrap, rubber bands, markers, label paper, reject paper, 37°C incubator and 1.5 ml eppendorf. The materials used in this study were SCA (Strach Casein Agar), Nutrient Agar, aquadest, Imperata rhizosphere soil sample, 70% alcohol, safranin, lugol, crystal violet, and nystatin.

2.2 Procedures

Sampling of this study was carried out at around campus Universitas Andalas then continued with the antagonist test of actinomycetes isolates and test of actinomycetes isolates as a producer of antibiotics. Soil samples were taken randomly from the rhizosphere of the Imperata cylindrica plants at 2 points around the Universitas Andalas campus, namely point 1 behind the Universitas Andalas

Campground and point 2 around the Universitas Andalas FMIPA basketball court. then the pH is measured.

All equipment that has been sterilized by autoclaving at 121˚C at a pressure of 15 lbs. 1 ml of soil solution was taken and then diluted with 9 ml of sterile distilled water (10-1) and homogenized with a vortex. Take 1 ml of the dilution of 10-1 put into 9 ml of sterile aquadest (10-2) homogenized with a vortex, the dilution is carried out to 10-4. Put in a Petri dish and then pour 10-15 ml of Starch Casein Agar (SCA) medium with soil extract added to the petri dish. Then incubated at 37°C in an inverted position for 3-5 days.

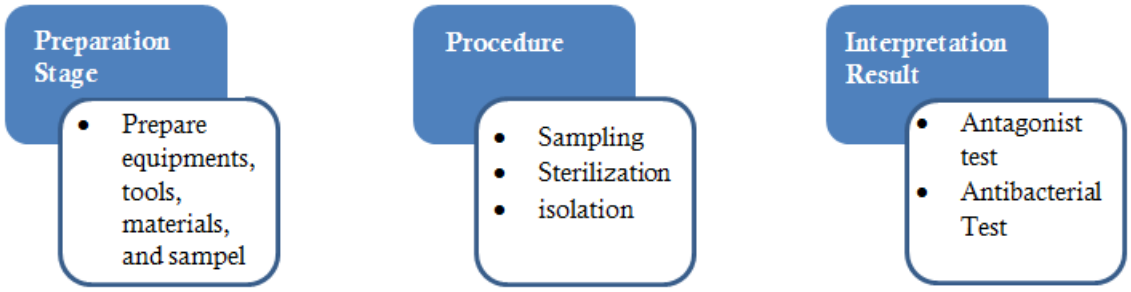

Figure 1. Time and locations of research

2.3 Interpretation Result

Antagonism test was carried out by taking the purified actinomycetes isolate and then scratching it into a petri dish that already contained SCA medium. Then scratched with actinomycetes isolate on the right side and then on the left side the test bacteria were scratched. Then incubated for 5-7 days and then calculated the increase in size and diameter between the test bacteria and isolates. then the actinomycetes isolate was fermented, by taking the actinomycetes in a petri dish and growing it in starch casein broth and then putting it in an incubator at 37°C for 3-5 days [12]. Then 1.5 ml of actinomycetes isolate was taken in starch casein broth medium and centrifuged for 10 minutes at 5000 rpm.

The purified actinomycetes isolates were tested on and Staphylococcus aureus. The test bacteria were taken and then put into sterile aquadest and then incubated for 24 hours. After that, 0.1 ml of the liquid isolate of the test bacteria was taken and poured into a petri dish containing solid Nutrient agar medium and then flattened with a sterile cotton bud until smooth, left for 15 minutes at room temperature. Then the actinomycetes isolate supernatant was taken which had been centrifuged and immersed in 6 mm paper discs and then placed in a petri dish containing the test bacteria that had been smeared. Disc paper dipped in chloramphenicol as a control. The next step, all cultures were incubated at 37°C for 24 hours. Then observed the growth of bacteria and the formation of inhibition areas by measuring the diameter of the inhibition (areas that are not overgrown with bacteria). So that the resistance can be determined, >21 mm is very strong, 11-20 mm is strong, 6-10 mm is moderate and <5 mm is weak [13].

3. Results and Discussion

3.1. Isolation of Actinomycetes From The Rhizosphere of Imperata

Based on the isolation of actinomycetes from the rhizosphere of imperata plants obtained results as presented in Table 1 and Figure 1

Table. 1. Actinomycetes Isolates From The Rhizosphere of Imperata Plants

No	dilution	colonies	Isolates	Isolate code
1.	10^{-1}	93	4	ARA 1
				ARA 2
				ARA 3
				ARA 4

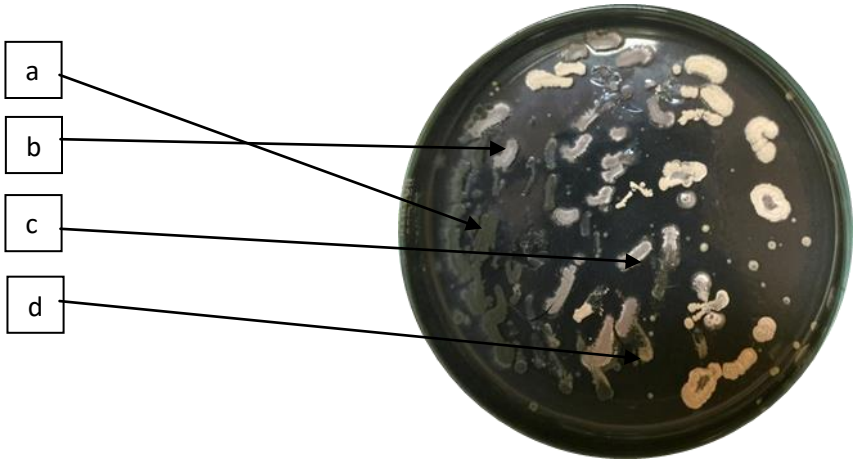

Figure 2. Actinomycetes Colonies in SCA Medium
a. ARA 1, b. ARA 2, c. ARA 3, d. ARA 4

Based on Table 1 and Figure 1 shows that the isolation results obtained 4 isolates of actinomycetes from the rhizosphere of Imperata plant. The number of actinomycetes in the soil is influenced by humidity, temperature, and pH. The normal pH for the growth of Actinomycetes ranges from pH 7.0-8.0 [6]. Thirty eight isolates of actynomycetes were obtained from the rhizosphere of rice plants (*Oryza sativa L.*) with a soil pH of 7.48 [14]. Forty isolates of actinomycetes from the rhizosphere of bone grass (*Eleusine indica L.*), soil pH 7.78 [15]. The size of the pH can affect the number of actinomycetes. The more acidic the content of actinomycetes, especially streptomyces, will decrease [16]. At pH < 5 there are only <1% [5]. In this study, the soil pH obtained was 6.96.

3.2. Characteristics of Actinomycetes Isolates From Rhizosphere Imperata

The partial characterization of the 4 actynomycetes isolates is presented in Table 2. It shows that the colony shape of actinomycetes is circular, the edges are undulate and entire while the elevation is convex. The colony colors of the 4 isolates found were different, dark green, white, and beige. The color that appears in actinomycetes colonies is due to pigmentation, resulting in different colony colors according to the type of actinomycetes obtained [17]. Colony observation takes 2 weeks [18] because actinomycetes are slow-growing and aerobic bacteria that require oxygen to grow [5].

Table 2. Characteristics of Actinomycetes Isolates From Rhizosphere Imperata

Isolate Code	Macroscopic					Microscopic
	Color	Shape	Margin	Elevation	Diameter (mm)	Gram Stain
ARA 1	Dark green	Circular	Undulate	Convex	2,5	Positif
ARA 2	White	Circular	Undulate	Convex	1,5	Positif
ARA 3	Beige	Circular	Entire	Convex	2,6	Positif
ARA 4	Beige	Circular	Entire	Convex	3,5	Positif

3.3. Antagonist Test of Actinomycetes Rhizosphere of Imperata

Based on the results of the antagonist test of actinomycetes isolates from the rhizosphere Imperata against *Staphylococcus aureus* and *Escherichia coli*, it was found that all actynomycetes isolates found were able to inhibited the growth of the 2 test bacteria as shown in Table 3 and Figure 2.

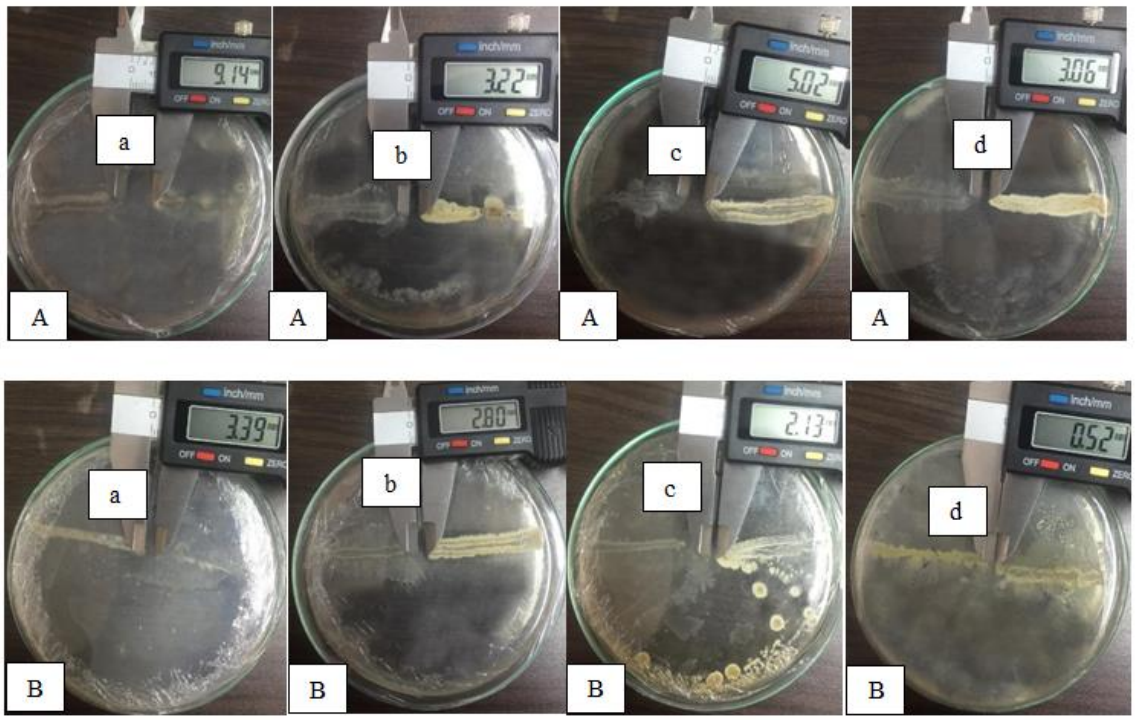

Figure 3. Antagonist Test of Actinomycetes Rhizosphere of Imperata *Staphylococcus aureus,* B. *Echerichia* coli, a. ARA 1, b.ARA 2, c. ARA 3, d. ARA 4

Table 3. Antagonist Test of Actinomycetes Rhizosphere of Imperata Plants Against *Staphylococcus aureus* and *Escherichia coli*

No.	Isolate Code	Clear Zone (mm)

		Staphylococcus aureus	*Escherichia coli*
1.	ARA1	9,15	3,39
2.	ARA2	3,22	2,80
3.	ARA3	5,02	2,13
4.	ARA4	3,06	0,52

Based on Table 3 and Figure 2, it is shown that ARA 1 isolate has the greatest diameter of inhibition against Staphylococcus aureus 9.15 mm and Escherichia coli 3.39 mm. The smallest diameter of inhibition was found in ARA 4 isolate where for Staphylococcus aureus 3.06 mm and Escherichia coli 0.52 mm. The difference in the diameter of the inhibition of actinomycetes isolates was due to the different actinomycetes isolates tested. Each actinomycetes isolate has different characteristics and different inhibitory activity abilities. This is in accordance with the that the difference in inhibitory power is caused by different antagonist isolates, so that the strength of the inhibitory activity of different isolates will result in different inhibition and activity of metabolite components [19].

3.4. Antibacterial Test of Actinomycetes Rhizosphere Isolates of Imperata

Based on the antibacterial test results, all the actinomycetes isolates obtained were able to inhibit the growth of Staphylococcus aureus and Escherichia coli test bacteria as presented in Table 4 and Figure 3

Table 4. Antibacterial Test of Actinomycetes Rhizosphere Isolates of Imperata against *Staphylococcus aureus* and *Escherichia coli*

Isolate Code	Clear zone (mm)	
	Staphylococcus aureus	*Escherichia coli*
ARA 1	35,89	34,60
ARA 2	34,24	29,54
ARA 3	31,23	28,60
ARA 4	23,67	8,91
Control +	42,13	48,83
Control -	0	0

Based on Table 4 and Figure 3, it shows that all actinomycetes isolates that were found had the potential to produce antibacterial because they could inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* by forming an inhibition zone. The diameter of the inhibition zone produced by ARA 1 isolates was 35.89 mm against *Staphylococcus aureus* and 34.60 mm against *Escherichia coli*. ARA 2 isolate resulted in inhibition zone diameter of 34.24 mm against *Staphylococcus aureus* and 29.54 mm against *Escherichia coli*.

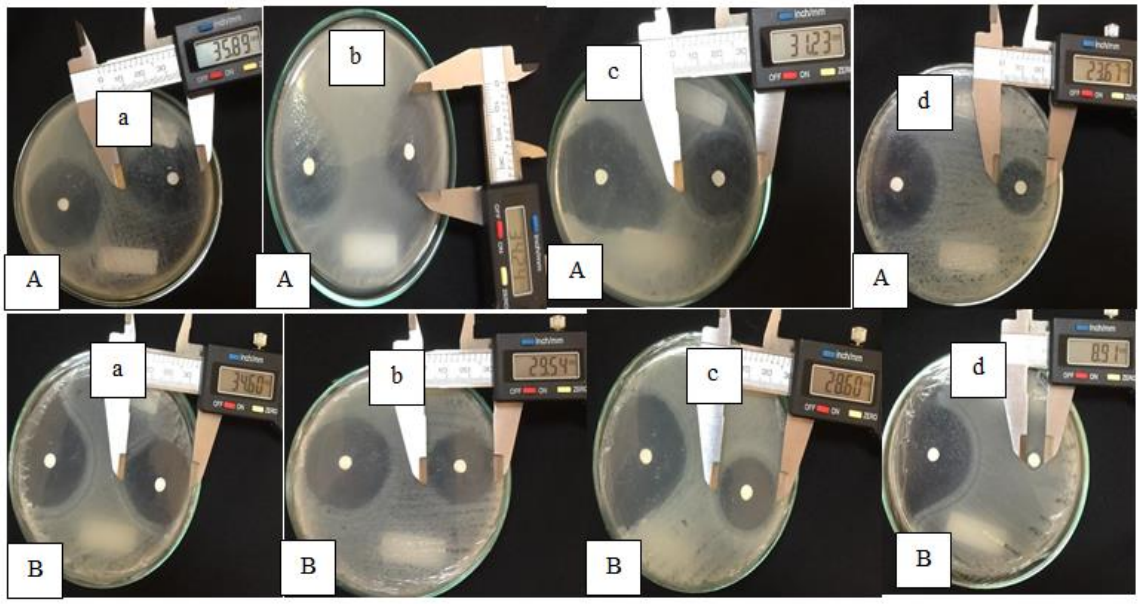

Figure 4. Antibacterial Test of Actinomycetes Isolate. A. *Staphylococcus aureus,* B. *Echerichia coli*, a. ARA 1, b.ARA 2, c. ARA 3, d. ARA 4

The inhibition zones produced by ARA 3 isolate were 31.23 mm in *Staphylococcus aureus* and 28.60 mm in *Escherichia coli*. While the inhibition zone produced by ARA 4 isolates was 23.67 mm in Staphylococcus aureus and 8.91 mm in *Escherichia coli*. The zone of inhibition produced by isolates ARA 1, ARA 2 and ARA 3 was categorized as very strong on both test bacteria, while the zone of inhibition produced by isolates ARA 4 was categorized as very strong against *Staphylococcus aureus* and moderate category for *Escherichia coli*. This is in accordance with the statement that the strength of the inhibition zone is categorized as >21 mm very strong, 11-20 mm strong, 6-10 mm moderate <5 mm weak [13]. In this study, the positive control used was chloramphenicol while the negative control was distilled water.

4. Conclusion

Four isolates of actinomycetes were obtained from the rhizosphere of *Imperata cylindrica L.*, ARA 1, ARA 2, ARA 3 and ARA 4. All actinomycetes isolates found from the rhizosphere of Imperata cylindrica L. had potential as antibacterial against *Escherichia coli* and *Staphylococcus aureus*. ARA 1, ARA 2, ARA 3 isolates had an inhibitory zone categorized as very strong on both test bacteria, while ARA 4 isolates had an inhibition zone categorized as very strong for *Staphylococcus aureus* and moderate categories for *Escherichia coli*.

5. Acknowledgement

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