ISOLATION OF Actinomycetes FROM MANGROVE SOIL IN THE VILLAGE OF SUNGAI RAWA, SUNGAI APIT SUB-DISTRICT, SIAK REGENCY, RIAU PROVINCE AND ANTIMICROBIAL TEST AGAINST *Escherichia coli* AND *Staphylococcus aureus*

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ABSTRACT

The purpose of this study was to isolate *Actinomycetes* bacteria from mangrove soil and antimicrobial test against *Escherichia coli* and *Staphylococcus aureus*. Soil sampling was conducted using zigzag. *Actinomycetes* isolation was carried out with surface plate while the potency test isolates was done with diffusion method. A total of 2 isolates of *Actinomycetes* were isolated, namely Isolate RR-1 with the color of white colonies and Isolate RR-2 with colony color of gray. Two isolates of the RR-1 were able to inhibit the growth of bacteria *E. coli* and *S. aureus*, while the RR-2 was only able to inhibit the bacteria *E. coli.*

Keywords: Actinomycetes, Mangrove, Antimicrobial.

INTRODUCTION

Health problems that often arise and develop at this time are infectious diseases. One way to minimize infectious diseases is by using antibiotics. Antibiotics in Indonesia are mostly imported from China and India. This is very contradictory to the fact that antibiotic raw materials in Indonesia are very abundant. Antibiotics can come from various sources, for example from bioactive substances from microorganisms, animals and plants. The emergence of various new infectious diseases that require antibiotics on the one hand and the existence of germ resistance to existing antibiotics on the other hand encourage researchers to continue conducting research to produce new types of antibiotics that are more effective to kill germs (Suwandi, 1993).

At present many studies are focused on Actinomycetes which are indicated as bacteria that can produce the most antibiotics. The presence of Actinomycetes in the soil has been widely studied by researchers. Oskay *et al.* (2004) found 50 different Actinomycetes strains from agricultural field samples taken from the Manisa region in Turkey where 34% of all isolates had the potential as antibiotics, and 7 isolates produced new antibiotics.

One source of Actinomycetes is the mangrove ecosystem. The condition of mangrove areas in general is an environment that is rich in organic matter and is a habitat that supports microorganism growth.

Some literature also supports that mangrove areas are very potential for microbial isolation, especially Actinomycetes which have activities to produce useful compounds (Solingen *et al*., 2001).

Hong *et al.* (2009) concluded that mangrove habitat is a very potential environment to exploit Actinomycetes which is capable of producing secondary metabolites with anti-infective, anti-tumor and agents for treatment of neurodegenerative diseases and diabetes.

Compounds for the treatment of diabetes are produced especially by 2 genera Actinomycetes namely *Micromonospora* and *Streptomyces*. Sweetline *et al.* (2012) also succeeded in isolating Actinomycetes from mangroves in India, where 17 isolates from 38 isolates that were isolated were able to produce antibacterial substances, 8 isolates were able to inhibit *Staphylococcus* sp. and 3 isolates could inhibit *Salmonella* sp bacteria. Therefore, with the abundance of organic materials in the mangrove area, it is still necessary to do research on the isolation of Actinomycetes in mangrove soils to obtain isolates that have potential as a new antimicrobial producer.

METHODOLOGY

Measurement of Physical Factors and Chemical Environment

Measurement of physical and chemical factors at the sampling location includes measurement of temperature, soil moisture and acidity or pH of the soil.

Collecting Soil Sample

Soil samples were taken from mangrove areas in Sungai Rawa Village, Sungai Apit Sub-District, Siak Regency, Riau Province by using zig zag method (Saraswati *et al.*, 2007) where the area was divided proportionally into 5 points for sampling. Soil samples were taken at a depth of ± 15 cm from the soil surface as much as 5 g. Then the soil sample was put into the ziplock and the sample obtained was composite into one to get an unbiased estimation of various microbes in a relatively homogeneous area or plot of land. After that, soil samples were analyzed in the laboratory.

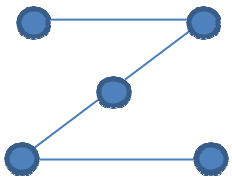


Figure 1. Sampling Method

Isolation and Purification of Actinomycetes

The soil samples that had been obtained were dried by aerating for 4 to 6 days, then were crushed and filtered to separate large-sized dirt and make a homogeneous sample. After that, as much as 1 g of filtered soil was suspended into 9 ml of distilled water and homogenized for 5 minutes (10^{-1} dilution). Then the suspension of the sample was placed in hot water (50º C) for 10 minutes to prevent the growth of other bacteria. Next, a series of 10^{-2} to 10^{-5} dilution was carried out, where 1 ml of a suspension of 10^{-1} was taken and

added to a test tube containing 9 ml of aquades, then homogenized (10^{-2} dilution). In the same way, a suspension with a level of 10^{-3}, 10^{-4} and 110^{-5} was made. Suspension of each dilution level was taken as much as 0.1 ml and inoculated on a surface plate in starch-casein agar (ScA) media with the addition of Nistatin 0.05 mg / ml as an anti-fungus. The inoculated media was then incubated at 25º C for 2 weeks. Growing colonies on the media were observed. Each colony that had a different colony color was purified on the new ScA media to obtain pure isolates. Isolates were incubated at 28º C for 2 weeks. Furthermore, observing the color and shape of the colony was done (Sembiring *et al*., 2000).

Production of Antimicrobial Compounds from Actinomycetes Isolates

Actinomycetes isolates that had been obtained were then produced in Starch-casein Broth media, where the isolates were inoculated into 9 ml Starch-casein Broth media and incubated at 28º C for 7 to 14 days. Bacterial growth was characterized by the formation of small or large granules, and changes in color and media became thick.

Isolate Potential Test as Antimicrobial Producer

Antimicrobial activity test was carried out using the diffusion method (Susilowati *et al.*, 2007). Nutrient Agar media was rubbed evenly with the test bacteria suspension which had been equated with the Mc Farland standard. Then filter paper 8 mm in diameter was sterilized and dipped in suspension of Actinomycetes isolates, using sterile tweezers. The piece of filter paper was placed on Nutrient Agar media which had been rubbed with the test bacteria. Next, all the dishes were incubated at 28ºC for 24 hours - 48 hours. After that, the inhibition zones around the filter paper were observed which showed no microbial growth. The diameter of the obstacle area was calculated and determined the level of resistance in accordance with the categorization of Lathifah (2008), namely the clear zone with a diameter of <5 mm was categorized as weak, 5-10 mm was categorized as moderate, 10-20 mm was categorized as strong, while the clear zone with a diameter of> 20 mm was categorized very strong.

Data Analysis

Data from the research results were tabulated in the table and then were analyzed descriptively to describe the physical condition of the environment, the number of Actinomycetes isolates, and inhibition zone measurements as well as categorization of inhibitory zones produced by Actinomycetes isolates towards test bacteria.

RESULTS AND DISCUSSION

Results of Physical Condition and Chemical Environment Measurement

The results of the measurement of Physical Condition and Chemical Environment in the mangrove area can be seen in Table 1.

Table 1. The results of the measurement of Physical Condition and Chemical Environment

No	Physical and Chemical Parameters	Value
1	Temperature (ºC)	30º C
2	Soil moisture (%)	60 %
3	Soil pH	5

Table. 1 shows that the temperature in the mangrove area of Sungai Rawa Village, Sungai Apit Sub-District, Siak Regency, is 30º C. The temperature is the optimum temperature for the growth of Actinomycetes. According to Rollin & Joseph (2000), the optimum temperature for the growth of Actinomycetes is 25º C-30º C. Furthermore, the soil moisture is 60% or 0.60, while the pH of the soil is 5 which are included acidic. Moisture conditions and soil pH can affect the amount of Actinomycetes obtained. This is in accordance with the opinion of Zenova et al. (2007) where Actinomycetes populations vary in fertile soils with humidity ranging from 0.67 to 0.89. Furthermore, Rao (2001) states that generally Actinomycetes are not acid tolerant and the amount decreases at pH 5.0. Actinomycetes are suitable for growth in the pH range of 6.5–8.0.

Results of Isolation and Purification

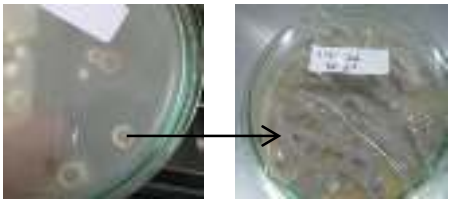
The results of isolation and purification of Actinomycetes from the soil in the mangrove forest area using Starch-casein Agar (ScA) media can be seen in Table 2.

Table 2. Results of Isolation and Purification

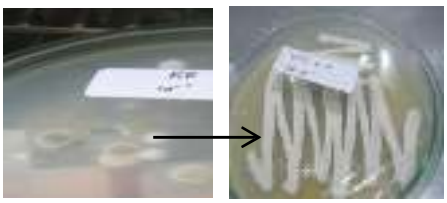
Isolate Code	Colony Color	Colony Shape
RR-1	White	Round
RR-2	Grey	Round

Based on the results of isolation and purification (Table 2.) two different isolates were obtained. Both isolates showed differences in colony morphology which was marked by differences in colony color. Morphology of RR-1 isolates was characterized by white colonies, while RR-2 isolates were marked with gray colonies. Actinomycetes have different colony colors due to the different pigment content of each isolate. Actinomycetes colonies are characterized by hard colonies, such as powdered, attached to agar media and have a characteristic smell of soil (Figure 1). Rao (1994) stated that Actinomycetes colonies are very easy to distinguish from other bacteria. Bacterial colonies grow rapidly and slimy, while Actinomycetes colonies appear slowly and powdered as well as stick to agar media. Actinomycetes colonies are usually hard, rough, and grow high above the surface of the media.

The results of isolation and purification of Actinomycetes obtained were not much different from Rosida (2014) who had isolated 4 isolates of Actinomycetes from mangrove soil in Teluk Lecah Village, Rupat Sub-District, Bengkalis Regency, Riau Province. Ravikumar *et al.* (2011) successfully isolated 17 Actinomycetes from the Karangkadu mangrove ecosystem in India and 10 isolates showed antibacterial activity.



(a)



(b)

Figure 1. Results of Actinomycetes Isolation and Purification. a. Isolate Morphology RR-1, b. Isolate Morphology RR-2.

Isolate Potential Test Results as Antimicrobial Producers

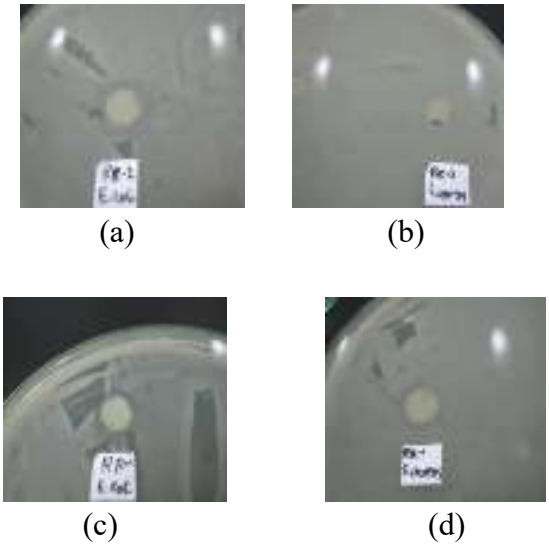
Test results of the potential of Actinomycetes isolates towards E. coli and S. aureus bacteria using Nutrient Agar (NA) media can be seen in Table 3.

Table 3. Isolate Potential Test Results as Antimicrobial Producers

Isolate code	Clear Zone Diameter (mm)	
	E. coli	*S. aureus*
RR-1	4	3
RR-2	4	-

From table 3, it can be seen that the isolates that had been isolated were then tested for potential as a producer of antimicrobial compounds using E. coli and S. aureus test bacteria. Isolate RR-1 was able to inhibit E. coli with a diameter of 4 mm (weak) and S. aureus with a diameter of 3 mm (weak) while isolate RR-2 was only able to inhibit bacteria E. coli with a diameter of 4 mm (weak) (Figure 2). The formation of clear zones due to pathogenic bacteria around the isolate was unable to grow. It was suspected that there were antimicrobial compounds capable of being secreted into Nutrient Agar (NA) media. The more antimicrobial compounds secreted into the media, the larger the clear zone was formed. White isolates (RR-1) were able to produce clear zones in both test bacteria. These isolates have the potential to produce antimicrobial compounds against E. coli and S. aureus. Gray isolates (RR-2) were only able to inhibit bacteria E. coli and unable to inhibit S. aureus bacteria. It was suspected that metabolites produced by RR-2 isolates have the potential to produce antibiotics in inhibiting Gram negative bacteria. Susilowati et al. (2007) stated that the inhibition of test bacteria by Actinomycetes isolates was caused by the presence of antimicrobial compounds capable of being secreted into the media, the more antimicrobial compounds secreted in the media the

greater the inhibitory zone formed. Anugrahwati (2011) also stated that the variation of the diameter of the inhibitory zone formed was due to differences in antagonism power and antimicrobial compounds produced by Actinomycetes isolates in inhibiting the growth of pathogenic bacteria.



(a) (b)

(c) (d)

Figure 2. Isolate Potential Test Results as Antimicrobial Producers. a. RR-1 isolate was able to inhibit E. coli bacteria; b. Isolate RR-1 is able to inhibit S. aureus bacteria; c. RR-2 isolate was able to inhibit *E. coli* bacteria; d. RR-2 isolates were not able to inhibit *S. aureus* bacteria.

The test results of potential isolates as antimicrobial producers are able to produce inhibitory zones with weak inhibitory power. Rosida (2015) also found inhibitory zones with weak inhibitory power against E. coli and inhibitory zones with moderate inhibitory power against S. aureus, then Nedialkova and Naidenova (2005) successfully found 40 strains of Actinomycetes isolated from Antarctica. After being tested on 7 species of pathogenic bacteria, the results showed that 60 % of strains have the potential to produce antibiotics, and 10 strains have a wide spectrum of inhibitory power.

CLOSING

Conclusion

Based on the research that has been done, it can be concluded that: Actinomycetes which were successfully isolated from mangrove soil in Sungai Rawa Village, Sungai Apit Sub-district, Siak Regency, Riau Province as many as 2 different isolates, namely isolate RR-1 (White) and isolate RR-2 (Gray). Isolate RR-1 (White) was able to inhibit E. coli bacteria with a 4 mm (weak) inhibitory zone diameter, inhibiting S. aureus bacteria with a 3 mm (weak) inhibition zone diameter. Isolate RR-2 (Gray) was only able to inhibit E. coli bacteria with a 4 mm (weak) inhibition zone diameter.

Suggestion

Further research is needed to identify Actinomycetes isolates that have been obtained and characterize growing media and optimum growth conditions (temperature and pH) for these isolates.

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