ANACTINOMYCETES (ISOLATE T34) AS ANTIBIOTIC PRODUCER AGAINTS *Staphylococcus aureus* AND BIOAUTOGRAPHY ANALYSIS

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Abstract

Background. *Actinomycetes is a group of microorganisms producing many kinds of active compounds. One of them is antibiotic.*

Objective. *The purpose of this study is to determine the potency of Actinomycetes (isolate T34)as antibioticproducer against Staphylococcus aureus and to know the spot of thin layer chromatography showing activity as antibiotic based on bioautography test.*

Methods. *The Actinomycetes (isolate T34) was taken from rhizosphere of Tin (Ficuscarica L.) plant which is grown on Starch Nitrate Agarmedium.The broth culture was prepared using 25 ml of Starch Nitrate Brothmedium having been givena quarter plate of the Actinomycetes isolate and then shaken for 5days.After that, the25 µl of actinomycetesbroth culture was put on the well of plate that had been planted with S. aureus and incubated at 37 °C for 24 hours to measure the inhibition zone diameter.The active broth culture was extracted using ethyl acetate solvent. Components in the extract were analyzed by thin layer chromatography and followed by bioautography process on Mueller Hinton medium having been cultivated with S. aureus for 30 min and incubated for 24 hours.*

Outcome measured. *Inhibition zone diameter against S.aureusgrowth.*

Results. *The results shows that the Actinomycetes (isolate T 34) can inhibit S. aureus with 24,2 mm of inhibition zone diameter. The result of the thin layer chromatography using silica gel GF254 as stationary phase and n-hexane : ethyl acetate (2:1) as mobile phase shows that one spot appears by UV 254 detection and two spots appear by UV 366. One of spots with Rf of 0.56 shows activity as antibiotic.*

Conclusion. *Actinomycetes (isolate T 34)produces antibiotic against S. aureus.*

Keyword : *Actinomycetes, bioautography, Staphylococcus aureus*

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INTRODUCTION

Infectious disease is one of the problems in the health sector that continues to grow. Infection can be transmitted from one person to another, from animals to humans. Some microorganisms that cause infections such as bacterial, viral, rickettsial, fungal, and protozoal (Gibson, 1996). One of them is *Staphylococcus aureus. S. aureus* can cause infections in humans both intissues and organs and cause typical signs such asinflammation, necrosis and abscess formation. Infection can bemild to the skinfurunclestobe afatalpiemia (Nelnick, 1994). This bacterium was resistant to most antibiotics. MRSA (methicillin resistant *S. aureus*) were obtained from the hospital environment and occurs due to the exposure of a semisynthetic penicillin and methicillin (Enright, 2003). Sulistyani *et al.* (2009) have isolated bacteria from hospital sewage and found a lot of resistance occurs in *S. aureus* primarily on Betalaktam and erythromycin derivatives.

With increasing misuses of antibiotics, the serious problems of antibiotic resistance are developing at an alarming rate. Hence, intensive search for new antibiotics has become imperative worldwide (Haque*et al.,* 1995; Oskay*et al.,* 2004; Parungao*et al.,* 2007) especially from actinomyceteswhich is known as the greatest source of antibiotics (Ogumnwonyi, 2010).Actinomycetes is best known for their ability to produce antibiotics and Gram-positive bacteria consists of a group of unicellular microorganisms branched. They produce mycelium branches consisting of two types: the substrate mycelium and aerial mycelium (Sivakumar, 2010).

The purpose of this study was to find out whether actinomycetes (isolate T34) were isolated from the rhizosphere of plants Tin (*Ficuscarica* L.) has potential as an antibiotic against *S. aureus*. In addition, to determine which patches of thin-layer chromatography shows potential as an antibiotic against *S. aureus*.

MATERIALS AND METHOD

A. Materials

Materials used in this study are sterile distilled water, the bacteria *S. aureus*, Nitrate Starch Agar (SNA) medium, Starch Nitrate Broth (SNB), Mueller Hinton agar (MH), Brain Heart Infusion, Standard McFarland, nystatin 100 mg / mL, glycerol 20% v / v, n-hexane, ethyl acetate, silica gel GF254.

B. Procedure

1. Turification of the Actinomycetes (isolate T34).

   Actinomycetes isolates cultured from the rhizosphere samples of tin plant (*F.carica* L.) was done by streaking on SNA plate (Rante, 2010), then incubated at 28 ° C for ± 10 days. Observation of morphological characteristics of Actinomycetes (isolate T34) was carried out toward the pigment, aerial mycelium and vegetative mycelium.

2. Preparation of *S.aureus* culture

   Several colonies of bacteria growing on agar taken 24 hours,were suspended in 1 mL of BHI broth and incubated 4-8 hours at 37 ° C. The suspension was added with sterile distilled water up to a certain turbidity in accordance with the standard concentration of 10^8 CFU/mLof bacteria.

3. The activity test of antibiotic-producing Actinomycetes with the wells diffusion method a quarter plate of isolates included in 25 mL of Starch Nitrate Broth (SNB) medium, incubated at room temperature for 5 days with the shaking. To obtain the cell-free supernatant, the culture broth was centrifuged at 8000 rpm for 10 min. The culture of *S.aureus* with concentration of 10^8 CFU/mL was spread using sterile cotton on Mueller Hinton Agar (MH), and then made three wells with diameter of 5 mm and each well wasloaded with 25 µL of the clear

supernatant. The dishes were preincubated at 4.0 °C for 2 hours to allow uniform diffusion into the agar, then followed by incubation for 24 hours at 37 º C, then measured the diameter of inhibition zone (Oskay, 2009).

4. Extraction of secondary metabolites the broth cultures were centrifuged, then the supernatant was extracted with ethyl acetate using a ratio of 1:1 (Sulistiyani, 2006). The extraction was conducted twice (Rante, 2010), strongly shaken and then left in place to form ethyl acetate phase and liquid phase. Phases were separated and the ethyl acetate phase was evaporated in a hood.

5. Thin-layer chromatography and bioautography

   Bioautography test was conducted to determine spotting potentially active compounds as antibiotics by using thin layer chromatography. Bioautography was done with spotting of extract on silica gel GF254 plate, then developed with the appropriate mobile phase for the separation of compounds contained in the fraction. The mobile used phase in this study is n-hexane: ethyl acetate (2:1). Chromatogram plate were placed on a agar surface that has been spread with *S. aureus* suspension, chromatograms were left clinging on agar for 30 minutes so that the active compound diffuses into the agar medium, then carefully removed and the dish was incubated for 24 hours. Furthermore, it can be seen patches that provide clear zone (zone of inhibition) that suggest the potential as antibiotics.

RESULTS AND DISCUSSION

1. Isolation and Purification Actinomycetes

The actinomycetes (isolate T34) was isolated from the rhizosphere of Tin plant (*Ficuscarica* L.) were obtained from Gergunung (North Klaten). The rhizosphere taken was located 5-10 cm below the soil surface. In the process of isolation, the rhizosphere samples were dried at room temperature until completely dry or no water content again, evidenced by the absence of water absorbed by the paper after tested with put on paper. Then the dried soil samples was carried propagules sediment extraction by making serial dilutions 10^{-1}-10^{-5}, after that, each dilution was inoculated on media SNA to do selective isolation and purification Actinomycetes.

Actinomycetes are soil bacteria that have a slower growth rate when compared with other soil bacteria that isolation requires a technique that allows maximum bacteria isolated Actinomycetes. Some methods used are: pre-treatment by heating the sample at a temperature of 50 º C for 10 minutes to prevent the growth of other bacteria, the addition of nystatin 100 mg/mL in order to prevent the growth of fungus and selective use of media that can eliminate the growth of other bacteria. Selective media were used to grow Actinomycetes in this research that Starch Nitrate Agar (SNA), this medium can be used by microorganisms including Actinomycetes as a source of nitrogen is a nutrient for growing Actinomycetes. Thus, the purification of Actinomycetes is done by planting the alleged Actinomycetes isolates on solid medium SNA.

In the process of isolation of Actinomycetes from tin plant rhizosphere (*F. carica* L.), Actinomycetes colonies grow slowly, showing the consistency of powdered, firmly attached to the surface of the agar and have a different appearance from each other (Rao, 1994), including a variety of different colors both the vegetative mycelium and aerial mycelium (Holt *et al*., 1994).

2. The morphology of colonies of Actinomycetes (isolate T34)

Gram staining is one way for microscopic identification of Actinomycetes. Actinomycetesisa group of Gram-positive bacteria. Gram staining was conducted to determine whether the classification of

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Gram-positive microorganisms (results are purple) or Gram negative (red result). The Gram staining result showed that the Actinomycetes (isolate T34) has the characteristics of class members Actinomycetes that have branched mycelium and purple. Picture of the Gram staining result of Actinomycetes (isolate T34) is presented in Figure 1.

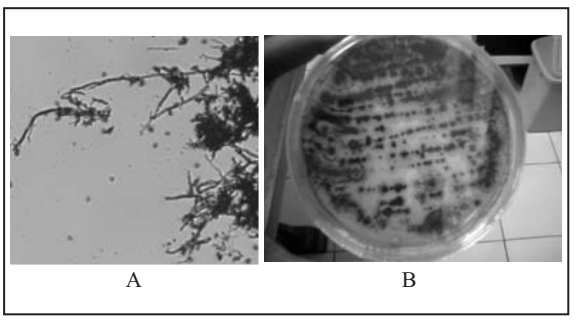

Figure 1. The morphology of Actinomycetes (Isolate T34), microscopic (A) and the colony (B)

Figure 1 shows there sult of painting is colored purple orare Gram positive, so that it can be concluded that these isolates have characteristics of Actinomycetes asgram-positive bacteria. The observation of the morphological characteristics of Actinomycetes (isolate T34) suggests that it has the characteristics of class members of Actinomycetes that their colonies are initially relativelys mooth surface but then form a kind of wovenaerial my celium which can manifest granularnya like powder, velvet, produces pigments that cause colors inaerial my celium and vegetative my celium. This Actinomycetes isolate has moss green color aerial my celium and brownish green color vegetative my celium.

3. The Potency of Actinomycetes (Isolate T34) as Producer Antibiotics against *S. aureus*

In this study, the activity test of Actinomycetes as antibiotics producers use *S. aureus* as test bacteria. The method used to test the activity of antibiotic-producing Actinomycetes is wells diffusion method. The wells diffusion method is done by making a hole in the solid medium that had been inoculated with bacteria. The number and location of holes were adapted to the purpose of the study, then the hole is filled with the sample to be tested. After the incubation, bacterial growth was observed to determine the presence or absence of inhibition areas around wells. The advantage of this method is much easier to measure the diameter of inhibition zone formed by the activity of the compounds in the test sample that is not only on the surface of the nutrient agar but also get to the bottom (Kusmayati and Agustini, 2007). The figure of potential test results of Actinomycetes (isolate T34) as producers of antibiotics against *S. aureus* is presented in Figure 2.

Figure 2.The Activityof Actinomycetes (Isolate T34) against *S. aureus*

The strength of antibiotics to inhibit the growth of bacteria is classified by Davis Stout as very strong (inhibitory area 20 mm or more), strong (inhibitory region 10-20 mm), medium (5-10 mm inhibitory region) and weak (local inhibitory 5 mm or less) (Sulistiyani, 2006). At the test potential of Actinomycetes (isolate T34) activity as a producer of antibiotics, it is done three planting replication in a liquid culture plate. The first major inhibitory diameter was 23.7 mm, the diameter of the second inhibitory diameter is 24.5 and the third inhibitory diameter was 24.5 mm. Based on this measurement, it is known that the Actinomycetes (isolate T34) has the potential to produce antibiotic categorized very strong inhibitory activity in inhibiting the growth of *S.aureus* (average diameter of 24.2 mm barrier region (not including the 5 mm

diameter wells)) with SD values of 0.46 and 1.9% CV values.

4. Thin Layer Chromatography (TLC) and Bioautography

Before doing Bioautography-TLC, it is previously carried out the extraction of metabolite from the culture broth of Actinomycetes (isolate T34) using ethyl acetate.The metabolite extractyield results are 0.102% w/v. Antibacterial activity of this extract then is tested by bioautography, the method previously performed TLC to separate the good patches for gettinge specially active spot as an antibacterial. Bioautography-TLC testinthis studyaims to determine the spots of TLC which shows activity as antibiotics against *S.aureus*.The TLC mobile phase used in this study are n-hexane: ethyl acetate in the ratio of 2:1 and the stationary phase is silica gel GF254. The TLC results shows the presence of one spot that appears on the detection using a 254 nm UV light and two spot on observations with the 366 nm UV light. The bioautography results indicate a potential spot as the antibiotic on the spot with Rf of 0.56. The figure of bioautography chromatogram and test results are presented in Figure 3.

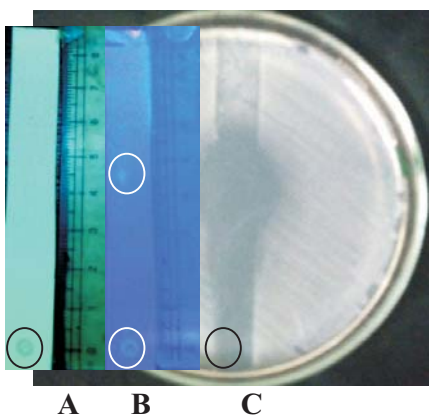

Figure 3. The chromatogram of ethyl acetate extractof Actinomycetes (isolate T34) culture brothon detection with 254 nm UV light (A), UV 366 nm (B) and the results of the TLC bioautography against *S. aureus* (C)

Bioautography method is done bychromatography plate is placed on the surface of the media, so that the compounds that have been separated into spots on the chromatogram will be able to diffuse into the agar medium. Chromatography plate affixed for about 30minutes on solid medium previously spread *S.aureus*.During attachment, the content of the compounds contained in the chromatogram spots diffuses into the agar medium. If the sepatches have potential as antibiotics,it will form a clear zone, which is an inhibitory zone of the chromatogram spot of metabolites extract of Actinomycetes(isolate T34) culture broth against *S.aureus*.

CONCLUSION

Based on theresults ofthe studyit can be concludedthat:

1. The Actinomycetes (isolate T34) produce antibiotics that inhibitsthe growth of *Staphylococcu saureus.*
2. The TLC with mobile phase of n-hexane: ethyl acetate in the ratio of 2:1 and the stationary phase of silicagel GF254 shows that the TLC patch of ethyl acetate extract of Actinomycetes (isolate T34) culture broth containing antibiotics against *S.aureus* has Rf of 0.56.

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