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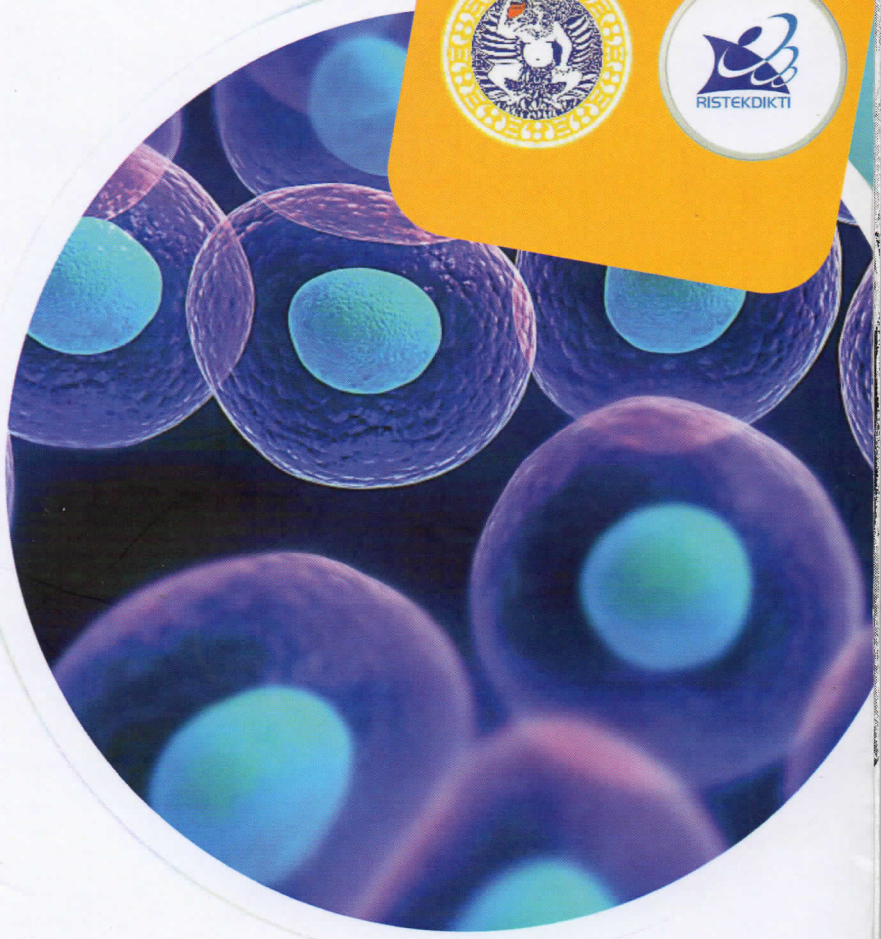

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CONTENTS:

ISOLASI DAN IDENTIFIKASI GEN 16S rRNA *Streptomyces* sp. ISOLAT TANAH GUNUNG SEMERU

ISOLATION AND IDENTIFICATION OF 16S rRNA GENE *Streptomyces* sp. SOIL ISOLATE SEMERU MOUNTAIN

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ABSTRACT

Streptomyces sp has been isolated from the antibiotic-producing slopes of Mount Semeru. Soil taken from an altitude of 2000 meters to 3500 meters above sea level which is impassable lava flow from Mount Semeru. Then isolation of *Streptomyces* sp. Special media for growth of *Streptomyces* sp. is an ISP-4. Based on the research method that has been done, the results obtained from this study are: the optimization of PCR only produce a tape that shows a length of about 1350 bp. It has been found five species of *Streptomyces* sp isolates. of land around Ranu Pane named Streptomyces sm-2, *Streptomyces* sm-5 and *Streptomyces* sm-6, while those found on the ground around Ranu Regulo named *Streptomyces* sm-7 and *Streptomyces* sm-9. The conclusion from this study is that there are different types of colonies found in Ranu Pane and Ranu Regulo on the slopes of Mount Semeru and have found 5 strain of *Streptomyces* sp., Which later was named *Streptomyces* s sm-2, *Streptomyces* sm-5, *Streptomyces* sm-6, *Streptomyces* 7 and *Streptomyces* sm-sm-9.

Key words: Isolation and Identification, the 16S rRNA gene of Streptomyces sp. Isolate the land of Mount Semeru,

PRELIMINARY

One way businesses discovery of new antibiotics is the isolation and identification of soil bacteria are thought to have an important role in the field of biotechnology, especially capable of producing the active ingredient in the form of antibiotics. One such bacterium is Streptomyces sp. About 3,000 kinds of antibiotics known, approximately 70% is produced by actinomycetes, especially the genus Streptomyces (Hoopwood, 1999).

Streptomyces can live on land or mountain plateau (Korn and Jurgen, 2002; Madigan, et al., 2002) as the land around the area of Mount Semeru. The big difference in the texture of the soil at each location is expected to be found *Streptomyces* different, specific, survive and are able to produce antibiotics.

Based on the important role of *Streptomyces* sp. found in the mountainous land habitats in the area of Mount Semeru, and is expected Streptomyces sp. The later can be developed as an alternative treatment for the infection, this study is to find isolates *Streptomyces* sp. The new, then identify and view the characteristics of the 16S rRNA gene of *Streptomyces* sp.

Based on this background, the problem can be formulated as follows: How do the characteristics of the 16S rRNA gene *Streptomyces* sp. isolates the soil found in the area Ranu Pane and Ranu Regulo in Mount Semeru?

RESEARCH METHODS

Soil samples were taken from the Ranu Pane and Ranu Regulo Mount Semeru with the criteria of the area once passed the lava flow.

The tools used in this study is a metal cylindrical, microscopy, homogenizer, vortex, analytical balance, petri disk, test tubes, Bunsen burner, ose, micropipette, mikrotube, shaker, spectophotometer, autoclaves, UV light, electroelusi, ultra centrifuges, cling wrap, aluminum foil; 1.5 ml Eppendorf 2000; Disposable gloves; Mini Eppy storage tubes; tissue; Pippetes tips 1 ml to 1000; Pippetes tips 0.2 ml 1000; Pippetes tips 10 µl 1000; Pippetes tips 2 µl 1000; Plastic cling wrap; 100 Syringe 2.5 ml, 1.0 ml Syringe 100; Millipore membrane 20, sekunser ABI Prism 310.

Materials used in this study is a soil sample from the slopes of Mount Semeru, pH7 phosphate buffer, primer foward, distilled water, pA-AGAGTTTGATCCTGGCTCAG 5'-3 'and reverse 5'-AAGGAGGTGATCCAGCCGCA pH-3', crystal violet, safranin, the iodine solution , alcohol, acetone, oil emersi, ISP-4, NA,, 6 kinds of carbohydrates, Nucleospin Tissue kit Cat # 740952.50, TE buffer, ethanol absolute, pH7,5 Tris buffer, EDTA, master mix, destilated water, agarose, TBE, ehidium bromide, loading buffer, DNA marker, ET-OH 96%, buffer PB and EB buffer.

Research procedure

This study will be divided into two stages as follows:

1. Isolation and identification of Streptomyces sp. Mount Semeru

Weigh as much as 10 grams of soil, then put in 90 ml of phosphate buffer pH 7 and homogenized. 1 ml suspension was diluted by adding 9 ml phosphate buffer pH 7 (10-1) into a test tube, then made dilution with up to 10-3. Each dilution is taken 1 ml using a micropipette and put in a sterile petri dish plus 10 ml of medium ISP-4 To be thawed at 45oC, then incubated at 28°C for 2-4 days.

Planted colonies grown on solid media ISP-4 on a petri dish and incubated for 4 days at a temperature of 28°C. After Streptomyces sp. grow, then transferred to the ISP-4 media for oblique. Examined macroscopic, microscopic, Gram staining and Test carbohydrates.

2. Isolation of genomic DNA, PCR amplification of 16S rRNA, electrophoresis of PCR products Streptomyces sp.

Streptomyces DNA isolation using a Qiagen kit Cat Cat # 51304. and done according to procedure. Primer used was Sigma Proligo with the sequence: 5'-AGAGTTTGATCCTGGCTCAG foward pA-3 'and reverse 5'-AAGGAGGTGATCCAGCCGCA pH-3' (Davelos, A.L., et al., 2004)

Amplification was carried out with a mixture of reagents as follows: 25 µL master mix kit, 2 µL forward primer, reverse primer 2 µL, destilated water 11 µL, DNA 10 µL so we get a total of 50 µL reaction. Initial denaturation 95 ° C for 5 minutes. Doing amplification for 35 cycles: denaturation 95 ° C for 1 min, annealing 600C for 1 min, 72 ° C for 1 minute elongation. In the 35th round of post elongation of 72 ° C for 10 minutes.

Electrophoresis was performed using 2% agarose gel run at 100 V for 20 minutes and the results of electrophoresis seen under UV light. PCR products were purified with the QIA quick PCR Purification Kit.

RESULTS AND DISCUSSION

1. Isolation and identification of Streptomyces sp. Mount Semeru

Soil taken from the highlands in East Java, the area Ranu Regulo and Ranu Pane on the slopes of Mount Semeru in general have the characteristics and texture is almost the same in the form of a mixture of ground sand, clay and organic matter and tend to have a reddish color. Mount Semeru with a height of ± 3,676 meters above sea level (masl), is the highest mountain in Java and includes one of the active volcanoes in East Java. Selection of the plateau on the slopes of Mount Semeru especially around Ranu Pane (2000 masl) and Ranu Regulo (2200 masl) as a test site for sampling in the form of land, because the area once passed volcanic lava which may lead to fewer and fewer types of microbes that can survive life, so that only the species that survive against the extreme environment to live.

Selection of Streptomyces sp. in this study because the genus potential as a producer of mainly antibacterial antibiotics. Of the ten diverse group of antibiotics, it can all be produced by genus *Streptomyces* sp. The *Streptomyces* sp. commonly found in soil include soil of the plateau among others Semeru.

Types of *Streptomyces* sp. which is found in both regions Ranu Pane and Ranu Regulo generally similar when viewed macroscopically (Figure 1). Isolated *Streptomyces* sp. on ISP-4 medium after storage at a temperature of 28oC for 2 days is characterized, among others, have a distinctive smell like soil, a small colony, colony forming mycelium surface.

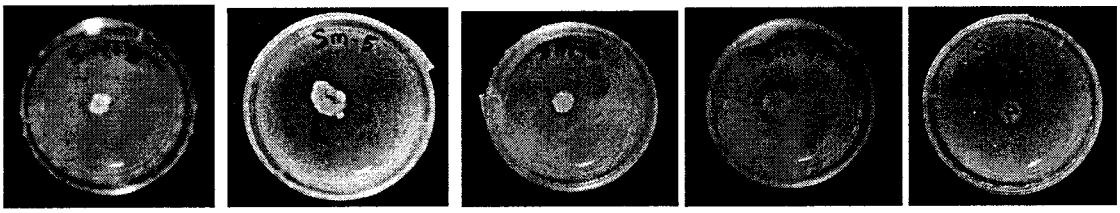

Figure 1. Colonies of Streptomyces sp. of the area Ranu Pane (SM-2, SM-5 and SM-6) and Ranu Regulo (Sm-7 and Sm-9) in cultured agar plate with media ISP-4

Morphological characteristics of isolates of *Streptomyces* sp. antibiotic-producing macroscopically on ISP medium-4 To be skewed showed that isolates *Streptomyces* sp. derived from Ranu Pane (Sm-2, Sm-5 and Sm-6) has the form colonies circular, convex surface, white, opaque with spores growing thick and fast. While isolates *Streptomyces* sp. derived from Ranu Regulo (Sm-7 and Sm-9) has the form colonies circular, convex surface, greenish-white colored, opaque with spores growing thick and fast.

The observation of morphological characteristics of the isolates of *Streptomyces* sp. antibiotic-producing microscopically performed under a microscope Olympus U-SRE2 with 1000x magnification by adding oil emersi generally reveals characteristics which is almost the same look that isolates *Streptomyces* sp. from the two regions Ranu Regulo and Ranu Pane has a slender hyphae, mycelium air when the adult form a chain with three to many spores. The observation of the effect of carbohydrates on the growth of *Streptomyces* sp. showed that *Streptomyces* sp. can grow on some media. Observations by Gram staining showed that all isolates both from Ranu Regulo and Ranu Pane shows of the same color is blue-purple, which means *Streptomyces* sp. is a Gram-positive bacteria.

2. Isolation of genomic DNA, PCR amplification of 16S rRNA, electrophoresis products *Streptomyces* sp.

PCR DNA Isolation of *Streptomyces* sp. using PCR method, which aims to multiply the target DNA strand segments quickly. For such purposes and to obtain good results it is necessary to a certain annealing time and temperature for optimal PCR conditions that do not cause non-specific band or a low amount of product. At this stage of PCR have been isolated *Streptomyces* sp. having a nucleotide up to 1500 bp, because the primers used in this study has a target to 12-34 bp nucleotide is 5'-AGAGTTTGATCCTGGCTCAG-3 'and nucleotides to 1508 to 1529 bp, namely 5'-AAGGAGGTGATCCAGCCGCA-3' (Davelos *et al.* , 2004). This primary election based on universal primers that can be used for all bacteria, which corresponds with the primers used for the 16S rRNA gene of *Streptomyces* sp. the research that has been done by Ueda *et al.*, 1999 and Zhang *et al.*, 2003. Selected primer sequence with the target 12-34 bp and 1508-1528 bp for primer sequences can be reached sequences of 16S rRNA to 1500 bp, it is appropriate with the opinion of Davelos *et al.*, 2004 and Zhang *et al.*, 2003 that the target area 12-34 bp and 1508-1528 bp is a conservative area genus *Streptomyces* thus obtained sequences necessary for identification of the species *Streptomyces* sp.

Results PCR isolates of *Streptomyces* sp. derived from Ranu Pane and Ranu Regulo with formulations that PCR mix PCR kit 25mL, aquabidest 10 mL, pA (forward) 10 pmol 5 mL, pH (reverse) 10 pmol 5 mL, DNA 5 mL and optimization PCR with an initial denaturation 95 ° C for 5 minutes, amplification for 35 cycles: denaturation 95 ° C for 1 min, annealing 60 ° C for 1 min, elongation 72 ° C for 1 minute and on lap 35 post elongation of 72 ° C for 10 minutes, followed by electrophoresis of PCR products can be seen in Figure 2.

Based on Figure 2, it appears that the five isolates of *Streptomyces* sp. by using a primer which has target of 1500 bp, it can be seen that *Streptomyces* sm-2 and *Streptomyces* sm-6, producing a thick ribbon but smear at about 1250 bp, *Streptomyces* sm-5 produced a thick ribbon but smear around 400 bp, while *Streptomyces* sm-7 and *Streptomyces* sm-9 produces a thick band around 1250 bp. Resulting ribbon smear, it is likely due to the levels of DNA low or annealing does not correspond to the 5 'end so

that the primer could not stick optimally cause the results of electrophoresis thinner, while the results of the tape thickness, indicating there is a match between the sequences of 16S rRNA Streptomyces sp. the area occupied by the primary sticking optimally.

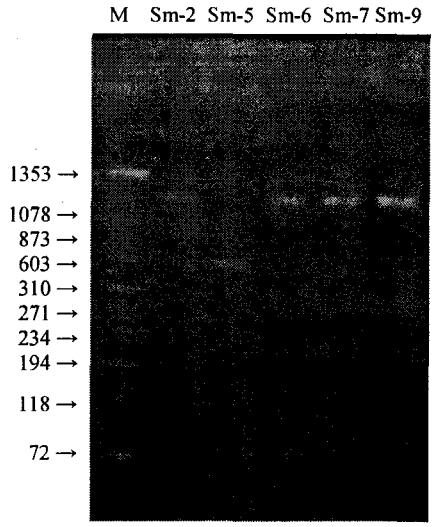

Figure 2 The results of the PCR product isolates of *Streptomyces* sp.

Information :
M : Marker
Sm-2 : *Streptomyces* sm-2
Sm-5 : *Streptomyces* sm-5
Sm-6 : *Streptomyces* sm-6
Sm-7 : *Streptomyces* sm-7
Sm-9 : *Streptomyces* sm-9

PCR amplification process to obtain the results of the optimization to occur primer template DNA 16S rRNA gene of *Streptomyces* sp. Electrophoresis is the standard method used to separate, identify and degrade DNA fragments, where the process is using gel electrophoresis Agarose. Agarose concentration elections are very important in the separation efficiency of various sizes of DNA (Sambrook, 1989). The presence of DNA bands was evident on electrophoresis, amplification can be said that the successful and on target. Purity DNA amplification product will greatly determine the outcome of the nucleotide sequence.

Failure detection of DNA in a DNA examination with the techniques of PCR can be caused by: 1) too little DNA template, 2) does not occur cDNA synthesis, 3) the kontaminase DNA, 4) the target DNA has been degraded, 5) reverse transcriptase enzyme inactivated, 6) DNA polymerase enzyme less, 7) cycles of PCR were insufficient, 8) extention time is too short, 9) annealing temperature is too high, 10) primer unsuitable and 11) the presence of PCR inhibitors (Suwarno, 2010).

CONCLUSION

Characteristics of the 16S rRNA gene of *Streptomyces* sp. soil isolates found in Ranu Pane and Ranu Regulo in Mount Semeru is *Streptomyces* sm-2 nucleotides in length = 1152 bp; *Streptomyces* sm-5 nucleotides in length = 395 bp; *Streptomyces* sm-6 nucleotides in length = 1253 bp; *Streptomyces* sm-7 nucleotides in length = 1247 bp; and *Streptomyces* sm-9 nucleotides in length = 1248 bp.

SUGGESTION

Continuing its next research plan antibacterial test, test and test thin layer chromatography bioautografi generated antibacterial *Streptomyces* sp. which compared to a variety of antibiotics.

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