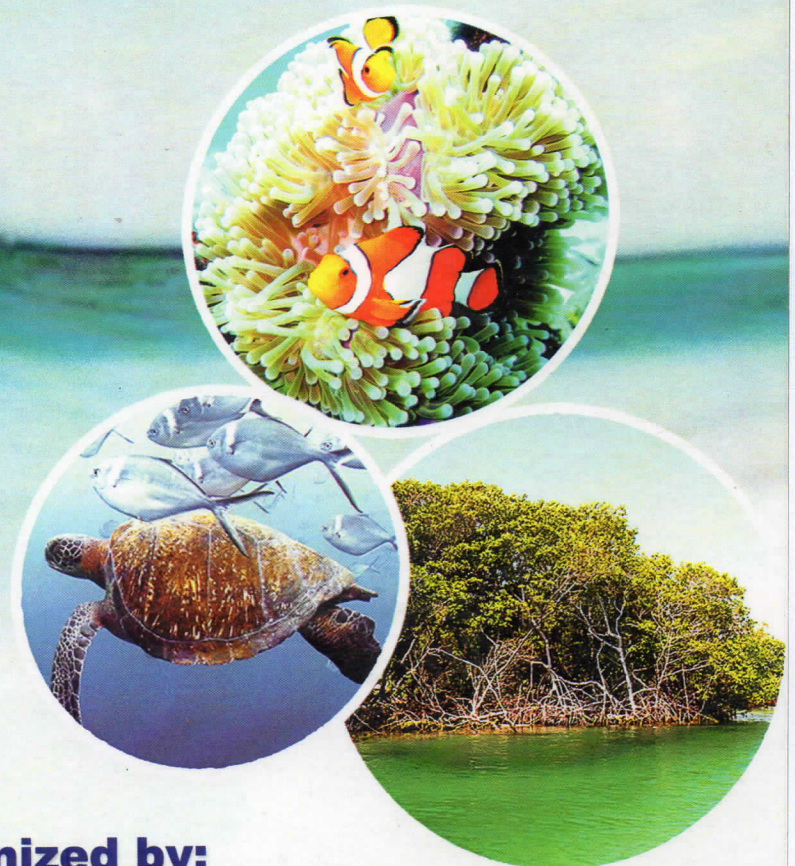


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*“From Ocean for Food Security,
Energy, and Sustainable Resources
and Environment”*

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**at Airlangga University
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PREFACE

In the name of Allaah the Most Gracious and Merciful. We would like to express our deep gratitude to our God, we could finish Proceedings of International Seminar. This proceedings was set of articles or papers that has been presented at International Seminar on *From Ocean for Food Security, Energy, and Sustainable Resources and Environment*. This seminar is organized by cooperation between Fisheries and Marine Faculty, Airlangga University, Surabaya and Research Center for Marine and Fisheries Socio Economics, Agency for Marine and Fisheries Research, Ministry of Marine Affairs and Fisheries, Jakarta, and Agrotechnology and Food Science Faculty, Universiti Malaysia Terengganu, Malaysia. The seminar is attended by researchers, lecturers, students of undergraduate, master and doctoral degrees, and also by government official. The papers cover broad topics about food production technology, product value improvement, resources and environment biophysics, alternative energy and environment biophysics, and socio-economic.

We would like to express our sincere thanks to Rector and Vice Rector of Airlangga University, Vice Counsellor and Dean of Agrotechnology and Food Science Faculty of Universiti Malaysia Terengganu, Head of Research Center for Marine and Fisheries Socio Economics, Dean of Fisheries and Marine Faculty, Airlangga University, keynote speakers: Prof. Dr. Gunawan Sumodiningrat from Gadjah Mada University, Prof. Dr. Sakri Ibrahim from Universiti Malaysia Terengganu, and Prof. Hassan Hj. Mohd Daud, DVM., Ph.D. from Universiti Putra Malaysia, moderators, presenters, participants, and colleague for supporting and kind help in the seminar. We also wish to thank to all sponsorships: Vice President of PT. CP Prima, Director of PT. Sufie Bahari Lines, Head of Fisheries and Marine Office, Regency of Tuban, Head of Fisheries and Marine Office, Regency of Pasuruan, General Manager of PT. Sanbe Farma, Director of PT. Petrokimia Gresik, Director of CV. Antika, Coordinator of Education Fish Pond, Fisheries and Marine Faculty, Airlangga University, and Director of PT. SIER for good contributions and partnership in the seminar. Finally, we would like to express our sincere thanks to the Steering Committee and Organizing Committee either staff and students from Faculty of Fisheries and Marine, University of Airlangga or staff from Research Center for Marine and Fisheries Socio Economics, Agency for Marine and Fisheries Research, Ministry of Marine Affairs and Fisheries, Reviewer: Prof. Ir. Sukoso, M.Sc., Ph.D from Fisheries and Marine Science Faculty, Brawijaya University, Ir. Murwantoko, M.Sc., Ph.D. and Ir. Triyanto, M.Si., Ph.D. from Department of Fisheries, Agriculture Faculty, Gadjah Mada University, Ir. Agung Sudaryanto, M.Sc., Ph.D. from Fisheries and Marine Science Faculty, Diponegoro University, Mohammad Yunus, DVM., M.Kes., Ph.D. from Department of Parasitology, Veterinary Medicine Faculty, Airlangga University, Ir. H. M. Pujoyuwono, M.Sc. from Research Center for Marine and Fisheries Socio Economics, Agency for Marine and Fisheries Research, Ministry of Marine Affairs and Fisheries, and Prof. Sayed Mohd Zain Hasan, Ph.D. from Agrotechnology and Food Science Faculty, Universiti Malaysia Terengganu.

Surabaya, 1 December 2009

Editors

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THE ISOLATION OF ANTIBIOTICS PRODUCED *Streptomyces* sp. FROM SOIL AT EAST JAVA

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ABSTRACT

The research was done isolation of antibiotic produced *Streptomyces* sp. from soil of the highlands of East Java of the area of the slopes of Semeru Mountain. Land was taken from a height of 2,000 m above sea level and altitude of 2,390 m above sea level that had traveled lava flow of Semeru Mountain. Isolation breeds done by *Streptomyces* sp. Germination medium specialized in *Streptomyces* sp. the ISP-4. Then, performed by macroscopic and microscopic observations. The observations of *Streptomyces* sp. are macroscopically showed some characteristics: a small colony with a diameter of 2-3 mm, white, fluffy, like the skin and form hard, spores at the end of smell miselia and provide land. By microscopic observations, Gram staining, isolates showed a purplish blue color, which means *Streptomyces* sp. was a Gram-positive bacteria, in addition to the examination with a microscope with 1,000x magnification it appears that *Streptomyces* sp. has slender hifa, mycelium forms chains with spores and 3-5 show the activity test of carbohydrates by showing a yellow color in the media candy. In this research can be concluded that the soil with altitude 2,000 and 2,390 m above sea level there is still a *Streptomyces* sp. who survive, the expected antibiotic produced also have greater potential as an antibacterial.

Keywords: *Streptomyces* sp., upland soil

INTRODUCTION

Treatments of infectious diseases in general use of antibiotics among other drugs from groups such Aminoglycosides Streptomycin (Katzung and Trevor's, 2005). Along with the increase of infectious diseases as well as resistance and toxicity of existing antibiotics, the development of antibiotics is still going on rapidly both through search and discovery of new antibiotics are more potent or modification of the molecular structure of antibiotics are semi-synthetic long time to get a more powerful antibiotics to (Jhoni, 1991).

In a new antibiotic discovery efforts, one way is to do the isolation and identification of soil microbes that allegedly has an important role in biotechnology in particular are able to produce several bioactive secondary metabolites of antibiotics. One such microbe is *Streptomyces* sp.

Based on the important role of *Streptomyces* sp. that can later be developed as an alternative treatment of infection, the study was conducted to find new isolate *Streptomyces* sp. heavily on a variety of habitats, such as upland soil and expected *Streptomyces* sp. found at that location is the species that survive in area.

Based on these background problems can be formulated as follows: is there a difference *Streptomyces* sp. isolate of the grounds in the area of Semeru Mountain? Hypotheses can be proposed in this study is: there is no difference *Streptomyces* sp. isolate of the grounds in the area of Semeru Mountain.

MATERIALS AND METHODS

This research was conducted at the Laboratory of Microbiology, Faculty of Veterinary Medicine, Airlangga University, and Laboratory of Microbiology, Faculty of Sains and Technology, Airlangga University, and ITD Airlangga University. This research was conducted from July until November 2009. This

research is divided into several stages as: preparation and bacterial culture, making inoculum *Streptomyces* sp., and identification of isolate *Streptomyces* sp.

Preparation and Bacterial Culture

Soil sampling carried out according to the method of Alexander and Strete (2001) is a sterile cylindrical metal device (5.5 cm long, 5 cm diameter) placed on the ground and pressed to a depth of 5-10 cm. Soil sampling conducted in the highlands of East Java Province of Ranu Pane and Ranu Regulo located on the slopes of Semeru Mountain.

Isolation of soil *Streptomyces* sp. performed according to the method of Alexander and Strete (2001), by weighing as much as 10 g of soil, then placed in 90 mL of phosphate buffer pH 7 and homogenizer. Obtained suspension was taken using 1 mL micropipet, then made dilutions by adding 9 mL of phosphate buffer solution pH 7 (10^{-1}) into the test tube, and then made dilutions with phosphate buffer pH 7 to 10^{-3} . Each dilution was taken using 1 mL micropipet and inserted into a sterile petri dish 10 mL of medium plus ISP-4. In order that has been thawed at a temperature of 45°C, and then incubated at a temperature of 28°C for 2-4 d.

Macroscopic observations made by determining the colony of *Streptomyces* sp. with features: a small colony with a diameter of 2-3 mm, finely hairy, like the skin and form hard, miseria spores at the end of the air, grain-shaped colonies of powders, granules or velvet, forming kinds of pigments, and provide the smell of the soil

Making Inoculum *Streptomyces* sp.

According to the method of Alexander and Strete (2001), from the various colonies that grew in isolation media captured the colony 1 Ose *Streptomyces* sp. character. Then grown in medium ISP-4 solid and incubated to petri dishes for 4 d at 28°C. After *Streptomyces* sp. grow, then transferred to the ISP-4 medium to side. For further processing,

according to Davelos *et al.* (2004) that the isolate *Streptomyces* sp should be stored on ISP-4 medium containing liquid glycerol 20% with a temperature-80°C to isolate no change in morphology and physiology.

Identification of Isolate *Streptomyces* sp. by Microscopic

Microscopic observations performed with the colony took 1 Ose *Streptomyces* sp. and placed on glass objects containing sterile water with 1000x magnification. For the Gram staining is done by taking colonies of *Streptomyces* sp. and placed on the glass object and then colored with crystal violet for 30 s. Crystal violet solution is removed with a solution of water and then added to increase the affinity jodium crystal violet. The solution is removed by flowing water, then add alcohol for 10-20 s and washed with running water until the blue colour disappeared. Added safranin for 30 s, then removed by flowing water and the water remains clean it with filter paper. Preparations examined with 100-400x magnification. To test used six different carbohydrates are carbohydrates arabinose, lactose, xilosa, mannitol, sakarosa, and starch. This test done to prove that able *Streptomyces* sp. isolates using sugar for growth.

RESULTS AND DISCUSSION

Soil taken from the highlands of East Java Province of Ranu Regulo and Ranu Pane on the slopes of Semeru Mountain in general have characteristics almost the same and the texture of the soil mixture of sand, clay and organic matter and tend to have a blackish color.

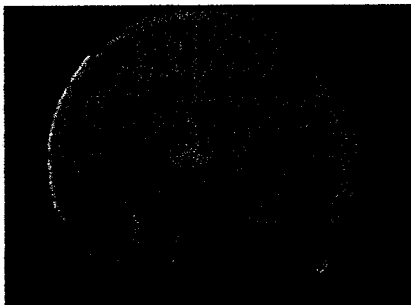


Figure 1. Colonies of *Streptomyces* sp. from region Ranu Regulo the culture medium to the plate with ISP-4



Figure 2. Colonies of *Streptomyces* sp. from region Ranu Pane the culture medium to the plate with ISP-4

The types of *Streptomyces* sp. found in both regions Ranu Regulo and Ranu Pane generally about the same when viewed in a macroscopic (Figures 1 and 2). Results isolates *Streptomyces* sp. on ISP-4 medium after storage at a temperature of 28°C for 2 d were seen distinctive odor, such as land, a small colony, the colony forming mycelium.

Morphological characteristics of *Streptomyces* sp. isolates antibiotic-producing the macroscopic on ISP-4 medium side order presented in Figure 3. In the picture looks isolate *Streptomyces* sp. from Ranu Regulo (C1 and C2) have a circular colony shape, convex surface, white, opaque with a thick growth of spores and fast.. While isolate *Streptomyces* sp. from Ranu Pane (D1 and D2) has a circular colony shape, convex surface, greenish white, opaque with a thick growth of spores and fast.

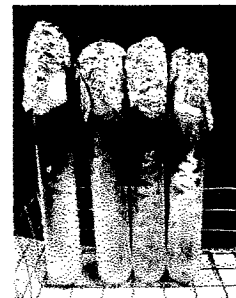


Figure 3. Isolate *Streptomyces* sp. producing antibiotics from Ranu Regulo region (C1 and C2) and Ranu Pane of (D1 and D2) in the culture media in order to side with ISP-4

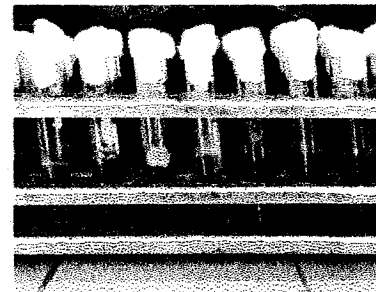


Figure 4. *Streptomyces* sp. with test



Figure 5. *Streptomyces* sp. Semeru Mountain isolates by Gram staining

Morphological characteristics of the observations isolate *Streptomyces* sp. antibiotics in producing microscopic carried out under the microscope Olympus U-SRE2 with 1000x magnification with oil adding generally emersi characteristics. Isolate *Streptomyces* sp. derived from both regions Ranu

Regulo and Ranu Pane have hifa slender, air mycelium when grown to form a chain with three or more spores.

Observations carbohydrate source influence on the growth of *Streptomyces* sp. can be seen in Figure 4. In Figure 4, it appears that *Streptomyces* sp. can grow on some media carbohydrates include mannitol, lactose and sucrosa.

CONCLUSIONS

Based on research results as can be concluded that in the highlands of Semeru Mountain showed no difference *Streptomyces* sp. isolate the ground between the regions of Mount Semeru

SUGGESTIONS

Suggestions need to be addressed in this study are: continuing sensitivity test antibiotic produced by *Streptomyces* sp. upland soil isolates at Semeru Mountain and continuing different DNA profile of 16S rRNA sequences *Streptomyces* sp. observations isolates the ground so that the species is found in expected new species.

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