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Screening of antimicrobial producing strains isolated from the soil of grassland rhizosphere in Pocut Meurah Intan Forest Park, Seulawah, Aceh Besar

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ABSTRACT

Fitri L, Bustam BM (2010) Screening of antimicrobial producing strains isolated from the soil of grassland rhizosphere in Pocut Meurah Intan Forest Park, Seulawah, Aceh Besar. Biodiversitas 11: 129-132. This research was a part of some works that was conducted to find antibiotics from soil microbes. The aim of this research was to screen isolates of antibiotics-producing microbes. Soil samples were collected from grassland rhizosphere in Pocut Meurah Intan Natural Reserved Forest Seulawah, Aceh Besar. This research was conducted at the microbiology laboratory Department of Biology , Faculty of Mathematics and Natural Sciences, Syiah Kuala University. This research covers six steps i.e. collecting soil samples, isolation of microbes, making colony library, purifying colony library, antagonism test and disk method test. Eleven isolates of microbes were selected, and purified for colony, library. However, only six isolates were assumed to have an ability to produce antibiotics, as confirmed by antagonism test. Those isolates have greater ability to inhibit the growth of *Staphylococcus aureus* than that of *Escherichia coli*. 13.25 mm was The average of clear zones formed for *Staphylococcus aureus* and *Escherichia coli* were 13.25 and 11.33 mm, respectively.

Key words: grassland rhizosphere, antibiotic, microbes.

INTRODUCTION

Indonesia, like some others tropical countries, is the site of easily spreading diseases caused by microbes. This is because of tropical areas provide good environment for growth of pathogens or useful ones. At present, in Indonesia, some diseases caused by microbial infection are still at the top list. Using antibiotics for curing the diseases is always the option. As a result, Indonesia has been spending quite large amount of money to provide antibiotics (Akmal et al. 1993). Improper uses of antibiotics, however, are leading to microbial resistance. Microbes are able to produce enzymes that can destroy antibiotics (Sudarmono 1994). Soeripto (2002) added that resistance of bacteria can be transferable to other bacteria that make those bacteria also resistant.

Antibiotics are compounds produced by microorganisms that are able to inhibit the growth of other microorganisms (Lay 1994). Antibiotics are spread in the world, as key role in organizing soils, water, and compos microbes' population (Chatim and Suharto 1994). Antibiotics also have enormous economic values in health because these can be used to cure many infection diseases. Generally, antibiotics are used to cure the infections caused by bacteria, virus, fungi and parasites. Typically, antibiotics have selective toxicity. It means those antibiotics are dangerous for parasites only but not for the host (Jawetz et al. 1989).

There have been some studies conducted in order to find useful microorganism, particularly microorganisms that are able to produce antibiotics. Although microbes can be found everywhere, soil is the popular site in conducting that kind of research (Reinhold et al. 1986; Grayston et al. 1998; Handelsman et al. 1998; Burgess et al. 1999; Miya and Firestone 2000; Fang et al. 2001; Hamilton and Frank 2001; Jensen et al. 2001; Marschner et al. 2001; Porazinska et al. 2003; Reynolds et al. 2003; Schlüener et al. 2003; Krutz et al. 2005; Voget et al. 2005; Pesaro and Widmer, 2006; Chung et al. 2008). Moreover, rhizosphere soil has a more diverse and active microbial communities compared to non vegetated soils (Krutz et al. 2005). One kind of rhizosphere soil is soil from grasslands. Even though grassland rhizosphere is a promising place to get soil microbial-rich samples, merely few studies have been performed in Aceh to address this issue particularly in Pocut Meurah Intan Natural Reserved Forest Seulawah, Aceh Besar. Pocut Meurah Intan Natural Reserved Forest Seulawah, Aceh Besar is a forest conservation which is approximately 6.622 km^2 large area, about 21% is grassland area (Department of Forestry 2003). To address the issue that grassland is important microbial resources then the study has been conducted at Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. Soil samples were taken from Pocut Meurah Intan Natural Reserved Forest Seulawah, Aceh Besar.

The objectives of this study were: (i) finding antibiotics-producing microbes, (ii) measuring the ability of isolates to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Making media

Nutrient Agar (NA) disc and Aslant Agar. Nutrient agar (NA) was used as growing and purifying medium. Nutrient agar media was made by weighing out 23 grams of nutrient agar powder then dissolving it into 1 liter of pure water (aquadest) until fully dissolved. Bring it to boil. Afterwards, the media was sterilized in an autoclave for 15 minutes at 121^0C. To make a disc agar, approximately 20 ml of the sterile media was poured in a petridisc aseptically. Let it dry. To make an aslant agar, approximately 5 ml of the sterile media was put in a test tube. Then, put it sideways.

Nutrient Broth (NB). To make nutrient broth media, 8 grams of nutrient broth powder was weighed. Then, the media powder was dissolved in 1 liter of aquadest. Bring it into boil. Afterwards, 5 ml of NB media was put in a test tube. The media was placed in an autoclave for 15 minutes at 121^0C.

Microbes isolation

Soil samples were taken from 10 randomly grassland rhizosphere areas in Pocut Meurah Intan Natural Reserved Forest Seulawah, Aceh Besar. One gram of soil sample was diluted in 9 ml of sterilized aquadest. Next, the sample was vortexed to homogenize the solution. Afterwards, one mL of the soil was diluted into 9 ml of sterilized aquadest to make 10^{-1} soil dilution. The processes were repeated until we have 10^{-5} soil dilution. 0.1 ml of soil dilution was spreaded into the nutrient agar disc media. Then, it was incubated upside down at room temperature for 3x24 hours. Microbes' isolations were done in every 24 hours.

Making colony library

Maximal 3 colonies that are morphologically similar were inoculated from every soil dilution. Afterwards, the inoculations were put in a library. The library then was incubated for 24 hours at room temperature.

Purifying isolates of colony library

Purifying isolates were done from colony libraries. Purification process was conducted through quadrant scratching in purification media. The media was incubated upside down. Purification processes were done at least twice to get colonies which are similar in size and morphology.

Antagonism test

Antagonism test was conducted to select the isolates that are potentially as antimicrobials. Scratching method was used to distinguish the potential antimicrobials isolates. *S. aureus* (representative of Gram positive bacteria) and *E. coli* (representative of gram negative bacteria) were used as test organisms. *S. aureus* and *E. coli* were grown in separate petridisc. Then, the bacteria in petridiscs were incubated for 24 hour at the temperature of 30^0 C. After that, the purified isolates were scratched to the petri discs that consist of *S. aureus* and *E. coli.* Positive potential antimicrobials isolates can be distinguished if the isolates are able to inhibit the growth of *S. aureus* and *E. coli*. As confirmation test, disc paper method was used. Only the positive potential antimicrobials isolates will be used in the test.

Disc paper method

Positive potential antimicrobials isolates were grown in nutrient broth media for 4 days. After 4 days, to homogenize isolates and media, the isolates were vortexed for about three minutes. Twenty µL of homogenized isolates, were then placed in a 5 mm paper disc. The isolates filled paper discs were put in petridiscs that have previously filled with *S. aureus* and *E. coli.* Positive isolates were distinguished from the ability to inhibit the growth of either *S. aureus* or *E. coli* or both of them with the appearance of clear zone (inhibited zone) surrounding the paper discs.

RESULTS AND DISCUSSION

There were 11 isolates retrieved from the soil samples (Figure 1), namely A1, A2, A3, A4, A5, A6, A7, A8, A9, A10 and A 11. All isolates are white. In order to separate other organism from isolates, all isolates were sampled as colony library before purification. This research used twice purification to make sure having pure isolates (Figure 2). Having pure isolate is an important thing in doing microbiological research. This is supported by Sofa (2008) that in an attempt to get better result, microbiological processes require purification of organism. There were six isolates (A1-A6), however, that are assumed contain antibiotics because they are able to cut lines of *S. aureus* or *E. coli* (Figure 2).

Five other isolates (A7-A11) are assumed having no antibiotics because there have no cutting lines of bacteria test in the media. It means that those isolates have no ability to inhibit the growth of bacteria test. This is supported by Lay (1994) that stated about antagonism test. According to him, antagonism test is a test that involve two kind of organism (bacteria), first organism (bacteria) is produced something that has ability in inhibiting the second organism (bacteria). Moreover, Hidayat et al. (2006) stated that producing antibiotics are the way of microorganisms to protect them from endangered habitat. This mechanism is happened because of metabolism processes. Results of metabolism can be grouped as acid or any other compounds that are able to kill other microorganisms. Morphological characters of the six colony isolates that are assumed to produce antibiotics can be seen in Table 1.

Table 1. Isolates code and they morphological characters

Isolate code	Colony tipe	Colony surface	Colony edge
A1	Round	Dome-shaped	Wavy
A2	Un-arrangement	Curve	Wavy
A3	Dots	Flat emerge	Unimpaired
A4	Coil	Curve	Serrated
A5	Round	Flat	Serrated
A6	Round	Flat	Unimpaired

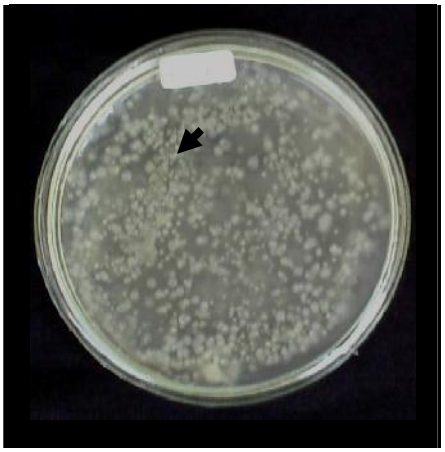

Figure 1. Isolates of microbes, the arrow shows the colony of bacteria

Figure 2. Purification of colony library, the arrow shows the colony of bacteria

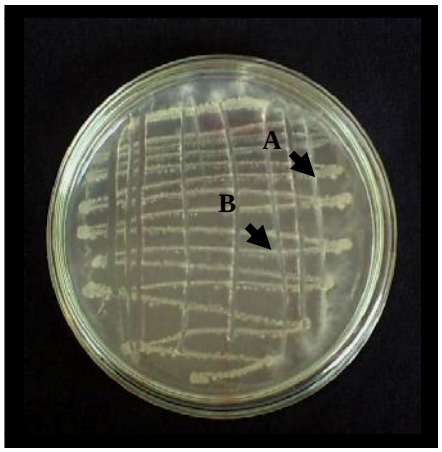

Figure 3. Antagonism test. A = Bacteria test (*E. coli*), B = Isolate that is assumed to contain antibiotics

As the way to confirm the antagonism test, the disc paper method test was conducted. The disc paper method was used to measure the ability of microbes isolates in inhibiting the growth of *S. aureus* and *E. coli.* This method was supported by Hatmanti et al. (2009). She and her colleagues stated that the disc paper test can be used to measure the inhibiting ability of microbes on the growth of pathogenic bacteria. Table 2 shows the clear zone average that was formed from the disc paper method.

Table 2. Clear zones from disc paper method

Isolate Code	Clear zones to *E. coli* (mm)	Cear zones to *S. aureus* (mm)
A1	11.0	12.0
A2	12.5	13.0
A3	13.0	16.0
A4	10.5	13.5
A5	10.5	14.0
A6	10.5	11.0
Average	**11.33**	**13.25**

According to Table 2, the wider clear zones were produced by the A3 isolate. The A3 isolate was able to inhibit the growth of *E. coli* and S. aureus as wide as 13 mm and 16 mm in diameter, respectively. Other isolates are able to produce clear zones in the range of 10.5-14 mm. The differences in the ability to produce the clear zone were presumably dependent on the secondary metabolites that were produced by test isolates. This assumption was supported by Dharmawan et al. (2009) that stated the variation of clear zone diameter happen because every isolate produces different types of secondary metabolites. Different types of secondary metabolites have different chemical structure, compounds and also different in chemical concentration. To measure the inhibiting response of clear zones can be classified as follow in Table 3.

Table 3. Classification of clear zones response

Diameter of clear zones	Inhibiting respon
20 mm > …….	Very strong
10-20 mm	Strong
5-10 mm	Medium
>5 mm	No response

Source: Davis and Stout (1971)

As stated above that the average clear zones were produced in both bacteria test, were 11.33 mm and 13.25 mm for *E. coli* and *S. Aureus*, respectively. Those ranges of clear zones are classified as having strong inhibiting response. However, the average of clear zones of *S. aureus* as representative of gram positive are wider than the average of clear zones of *E. coli* as representative of gram negative. It shows that isolates have more ability to inhibit the growth of *S. aureus* than to inhibit the growth of *E. coli*. This is because gram negative bacteria usually have better protection to other antimicrobial compound rather than positive bacteria because both kinds of bacteria have different cell wall components. Cell wall of gram positive bacteria contains peptidoglican while cell wall of gram negative bacteria contains peptidoglican and lipopolysaccharide. The statement was supported by Zuhud et al. (2001) and Ajizah et al. (2007) stated that cell walls of gram positive bacteria contain very thick peptidoglican to protect the bacteria. Campbell et al. (1996) added that cell walls of gram negative bacteria, besides peptidoglican, they also contain lipopolysaccharide to protect the bacteria from antibiotics. Jawetz et al. (1989) added that the death of bacteria caused by antibacterial compounds happened because the antibacterial produce chemical components that are able to inhibit the synthesis of cell wall, inhibit function of cell membrane, inhibit the protein synthesis and or inhibit the nucleate acid synthesis.

CONCLUSION

There are eleven isolates found from grassland rhizosphere area in Pocut Meurah Intan Natural Reserved Forest Seulawah, Aceh Besar. Six isolates are assumed to be able to produce antibiotics. Average of clear zone to inhibit the growth of *S. aureus* is 13.25 mm whereas 11.33 mm is the average of clear zone to inhibit the growth of *E. coli*. The ability of isolates in inhibiting the growth of *S. aureus* is higher than they ability to inhibit the growth of *E. coli*.

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