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ISOLATION AND SCREENING OF ANTIMICROBIAL-PRODUCING ACTINOMYCETES FROM MARINE SEDIMENT OF GALESONG COAST, INDONESIA

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Key words : *Actinomycetes, Marine Sediment, Antimicrobial,* Streptomyces sp.

Abstract–Isolation and screening of antimicrobial-producing marine actinomycetes has been conducted on isolates taken from marine sediment samples which were collected from various depths of Galesong Coast. Isolation was carried out by using heat shock treatment (heat at 50° C for 10 minutes) and isolates were grown in Starch Nitrate Agar medium added with Nistatin as antifungal. The results obtained 2 isolates of actinomycetes. The screening revealed that isolate GLS-01 has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* which was then fermented in Starch Nitrate Broth for 11 days. In the test of antimicrobial activity of ethyl acetate extract of fermented isolate GLS-01 with concentrations of 3%, 1.5%, 0.375%, and 0.1875% against *Staphylococcus aureus* resulted the diameters of inhibition of 14.5 mm, 12.51 mm, 12.31 mm, and 12.26 mm, respectively, and against *Escherichia coli* resulted the diameters of inhibition of 10.88 mm, 9.65 mm, 9.03 mm, and 7.88 mm, respectively. Based on macroscopic and microscopic features, isolate GLS-01 was suspected to be *Streptomyces sp*.

INTRODUCTION

Infection diseases has become the second leading cause of death worldwide after heart disease (Anonymous, 2013), while the cases of antibiotic-resistant microbes lately has been emerged rapidly along with the increasing use of antibiotics as a medicine to treat the infectious diseases. This situation encourages the growing importance of efforts to get the new antibiotic compounds which were easily to be cultured with less cost and continously available in large quantities (Sunaryanto *et al*., 2009). The efforts to find the new antibiotic has been made within the fields of chemical synthesis and engineered biosynthesis, but compared to them, nature still remains the richest and the most versatile source to find new antibiotic (Baskaran *et al*., 2010).

Microbes are one of the antibiotic compound sources found in nature which are easier to be cultured and is continuously available in large quantities compared to plants and animals. Among the various kinds of microbes, actinomycetes had known to be the most antibiotic-producing microbes, compared to fungi and eubacteria (Makut and Owolewa, 2011).

Actinomycetes is generally isolated from terrestrial environment, but lately much attention has been focused on marine actinomycetes since the discovery of new bioactive compounds derived from terrestrial actinomycetes has been declined (Valli *et al*., 2011). Marine environment provided high biodiversity of organisms which also influenced the variety of secondary metabolites they produced (Sunaryanto *et al*., 2011).

New strain of actinomycetes with potential secondary metabolites as bioactive compounds have been found from marine sediments since several decades, among others, such as *Salinispora*, *Marinispora*, and *Thermomonospora* (William *et al*., 2005, Ward, and Naganami, 2006, Hasegawa *et al*., 1986). One of antibiotics which is derived from marine actinomycetes is Madumycin-I which has strong activity against *Bacillus subtilis* with minimum inhibitory concentration of 120,86 µg/mL (Sunaryanto *et al*., 2009).

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Indonesia is a tropical and maritime country with a vast expanse of ocean that is approximately 3.1 million km² or nearly twice its land area (Sunaryanto *et al.*, 2009), and this is potentially to be explored for discovering new potential antibiotics from marine actinomycetes. Galesong Coast which is located in South Sulawesi, is one of the coasts in Indonesia with potential as the source of iron sand and has not been explored yet in efforts to find the new antibiotic compounds from marine actinomycetes. Thus, the aim of this research is to isolate the actinomycetes from marine sediment of Galesong Coast, and screening for its antimicrobial activity against selected pathogenic microbes (*Staphylococcus aureus, Escherichia coli*, and *Candida albicans*).

MATERIALS AND METHODS

Samples collection

Samples of marine sediment were collected from various depths (20 cm, 50 cm, and 1 m from sea level) of Galesong Coast. Samples were collected in vials which were previously washed with 70% alcohol. The samples contained vials then put in cool box along the way from field to the laboratory.

Isolation of actinomycetes from marine sediment samples

The method of isolation was carried out according to (Sunaryanto *et al.* 2009), with some modifications. One gram of each solid sample were diluted in 4 mL sterilized sea water then were stirred for 10 minutes. Then 1 mL of diluted sample were diluted again in 4 mL sterilized sea water and continued to be heated at 50⁰ C in 10 minutes for pre-treatment in order to minimize the presence of other bacterial growth. Five folds of serial dilutions (10^{-1} up to 10^{-5}) of the diluted samples which have been heated were made using sterilized sea water. One milliliter of the serially diluted samples then poured into the plates and added with Starch Nitrate Agar medium (which has been added with 25 µg/mL nistatin as antifungal after the medium has been sterilized) g/L: Soluble starch 20 g, KNO_3 1 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.5 g, NaCl 0.5 g, $FeSO_4$ 0.01 g, agar 15 g, sea water and pH 7.0 ± 2. The Petri plates were incubated at 28 ± 2⁰ C for 7 to 21 days.

All the morphologically different actinomycetes colonies were sub-cultured on SNA medium by streak plate technique. After growth appeared, the actinomycetes colonies were maintained in SNA slants for further investigation.

Screening for antimicrobial activity (antagonist test)

The method of antagonist test was carried out following (Herlina *et al.*, 2010. All pure culture of actinomycetes isolates were cultured on SNA medium by streak plate technique and were incubated at 28 ± 2⁰ C for 7 days. Each of 7 days incubated actinomycetes isolates were cut using stainless steel cylinder with diameter of 6 mm. Each of disk-shaped isolate's surfaces stacked on the seed agar surface which has been prepared previously. The Petri plates were incubated at 37⁰ C for 24 h for antibacterial test, and for antifungal test, the Petri plates were incubated at 25⁰ C for 72 h. The diameter of the inhibition zones were observed for qualitative screening after the incubated time. The seeded agar prepared by cultured the selected pathogenic microbes (*Staphylococcus aureus, Escherichia coli*, and *Candida albicans*) in broth media (NB for the bacterial and were incubated at 37⁰ C for 24 h; and PDB for the yeast and were incubated at 25⁰ C for 72 h) and then were swabbed on the NA medium (for cultured bacterial) and PDA medium (for cultured yeast) surface. The active isolates according to the qualitative observation were then identified by their macroscopic and microscopic features.

Preparation of ethyl acetate extract of fermented isolate GLS-01

The active isolate (GLS-01) was cultured on SNA slants at 28 ± 2⁰ C for 2 weeks. The mature spores were inoculated in 100 mL SNB in 500 mL Erlenmeyer flask and incubated at 28 ± 2⁰ C on rotary shaker at 150 rpm for 11 days. After 11 days, 100 mL ethyl acetate was added into the Erlenmeyer flask containing fermented isolate GLS-01 and the mixture was kept overnight to ensure that the actinomycetes cells died. The mixture was then moved to a 250 mL separation funnel to separate the ethyl acetate phase. The extraction was repeated twice and all of ethyl acetate phase were collected and were evaporated in desiccators. This method was carried out following (Herlina *et al.*, 2010).

Antimicrobial test of ethyl acetate extract of fermented isolate GLS-01

The antimicrobial test of ethyl acetate extract of fermented isolate GLS-01 was done by using agar disk-diffution method as described in (Mounyr *et*

al., 2015). The ethyl acetate extract were prepared in various concentrations (3%, 1.5%, 0.375%, and 0.1875%) using ethyl acetate as the solvent. The paper disks with 6 mm of diameter were prepared in the empty Petri plates and were added with 25 µL of each concentrations of extract and after about 45 minutes (the solvent have been evaporated) the paper disks were then put on seeded agar surface which have been prepared previously. The Petri plates were then incubated at 37^0 C for 24 h for antibacterial test, and for antifungal test, the Petri plates were incubated at 25^0 C for 72 h. The diameter of the inhibition zones were measured after the incubated time. The seeded agar prepared by cultured the selected pathogenic microbes (*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*) in broth media (NB for the bacterial and were incubated at 37^0 C for 24 h; and PDB for the yeast and were incubated at 25^0 C for 72 h) and then were swabbed on the NA medium (for cultured bacterial) and PDA medium (for cultured yeast) surface.

RESULTS AND DISCUSSION

Isolation of Actinomycetes from Samples

Two isolates of marine actinomycetes were isolated from marine sediments of Galesong Coast. Isolations of the marine actinomycetes were performed based on observation of the color, form, and surface of the isolate colonies on agar plates. The two isolates, named GLS-01 and GLS-02, have different macroscopic features as shown in Table 1

Table 1. Macroscopic colony features of isolates GLS-01 and GLS-02

Isolates code	Color, form, and surface of the isolates
GLS-01	White, round, powdered surface
GLS-02	Yellow, round, smooth surface

Fig. 1. Isolates GLS-01 and GLS-02

and Figure 1. One actinomycetes isolates (named GLS-01) was found in sample at depth of 1 m from the sea level, one actinomycetes isolates (named GLS-02) was found in sample at depth of 50 cm from the sea level, and no actinomycetes isolates was found in sample at depth of 20 cm from the sea level. Sea waves may be the cause of the less amounts of actinomycetes isolates obtained at a depth of 20 cm to 1 m from sea level.

Antagonist Test of Isolates

The qualitative screening of antimicrobial activity of the two isolates done by antagonist test and the result revealed that isolate GLS-01 has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* by the presence of inhibition zone and it did not show antifungal activity against *Candida albicans*. While, isolate GLS-02 did not show any inhibition zone for the three selected pathogenic microbes.

Macroscopic and Microscopic Features of Isolates GLS-01

Isolates GLS-01 was identified for its macroscopic and microscopic features. The colony characteristics of isolates GLS-01 on agar plates is white, powdered surface, raised, and adhering to medium and its microscopic features show long and limited branched hyphae and some of them show a perfect spiral form of hyphae (Fig. 3). By these features, Isolates GLS-01 is suspected to be *Streptomyces sp.* according to Waksman and Lechevalier, 1953.

Fermentation and Extraction of Isolate GLS-01

Isolates GLS-01 was fermented to obtain its secondary metabolites which then were extracted with ethyl acetate. The flakes of mycelium were shown starting in days 3 of fermentation. Bright blue pigment was seen in ethyl acetate phase when the broth fermented of isolates GLS-01 was extracted but the pigment wasn't seen in SNB medium along the fermentation days. The isolates GLS-01 produced the bright blue pigment which was soluble in organic solvent such as ethyl acetate. The ethyl acetate extract obtained was 30 mg.

Antimicrobial Test of Ethyl Acetate Extract

The results of antimicrobial test of ethyl acetate extract are shown in Table 2. The isolates GLS-01 has antibacterial activity which inhibited both of *Staphylococcus aureus* and *Escherichia coli* but doesn't have antifungal activity against *Candida albicans*. The diameters of inhibition shown were increased along

Table 2. Antibacterial activity of isolates GLS-01

Concentration (% w/v)	Diameter of inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
3	14.5	10.88
1.5	12.51	9.65
0.375	12.31	9.03
0.1875	12.26	7.88

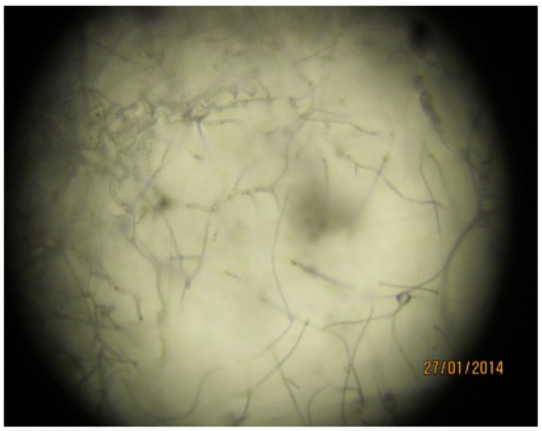

Fig. 2. Microscopic features of isolates GLS-01

with the increasing concentration of extract.

CONCLUSION

Two isolates of marine actinomycetes were isolated from Galesong Coast named GLS-01 and GLS-02. The screening revealed that isolate GLS-01 has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Based on macroscopic and microscopic features, isolate GLS-01 was suspected to be *Streptomyces sp*.

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