INTRODUCTION

Actinomycetes are soil-borne microbes capable of producing antibacterial compounds. They are Gram-positive, filamentous, and saprophytic bacteria (Oskay et al., 2004). An example of these microbes is Streptomyces (Thakur, 2011). Antibacterial compounds produced by Streptomyces are streptomycin, aureomycin, chloromycetin, tramycin, erythromycin, and magnamycin. These compounds have varied potentials and specification (Holt et al., 2000).

Explorations of Streptomyces have been widely done, due to the high demand for antibacterial compounds, and triggered by the emergence of resistance problems of pathogenic microbes toward antibiotics. The exploration has been done in various environments, even in a unique habitat.

Mangrove forest is a possible environment for the source of Streptomyces. It has rich organic substances that very likely to be the source of antibacterial producing actinomycetes (Alanis, 2005). Mangrove forest of Segara Anakan, Cilacap, is an intertidal zone influenced by the sea tidal waves. According to Suryono (2006), various mangrove species grew according to zonation, the distance from where they grew to the shore.

Avicennia marina was a species that grow in the closest distance to sea waters with higher environment salinity compared to other mangrove vegetation (Sulmarani et al., 2008). The explorations of Streptomyces in mangrove area is crucial because this bacteria has a superior ability to survive the intertidal zone, thus considered to have some specific potential related to primary and secondary metabolite productions (Sathya and Ushadevi, 2009).

METHODOLOGY

The assay of antibacterial activity could be done with diffusion and dilution method towards Gram-negative bacteria, Escherichia coli, and Gram-positive bacteria, Staphylococcus aureus. The species have different cell wall structure located on the peptidoglycan layer. Thus, different responses toward antibacterial compounds were expected. The mechanisms of microorganism growth inhibition included cell wall synthesis inhibition, a disturbance of cell membrane permeability, and blockage of replication, transcription, and translation of bacterial genetic materials (Oskay, 2004). The advance assay of antibacterial compounds is bioautographic test towards chromatography results, to test the antibacterial ability of antibacterial compounds (Oedijono et al., 1993; Patil et al., 2013).

Based on this review, the objectives of this research were to obtain antibacterial producing Streptomyces isolate from the Avicennia marina rhizosphere, and to examine the antibacterial ability and antibacterial compounds characters produced.
The antibacterial substance was produced on liquid SCN mediums with 7, 14, 21, and 28 days incubation at room temperature. The evaluated parameters were inhibition zone diameter of rough extract, mycelium biomass (X), filtrates pH, RF and MIC values. Filtrates were extracted with ethyl acetate and methanol solution (4:1). The extracts were diluted in various concentrations (1%, 2.5%, 5%, 10%, 20%, 40%, 80%, 100%), and then the antibacterial ability was tested with diffusion method. After 24 hours incubation at 37°C, inhibition zone diameters were measured. MIC assay was done by mixing 0.8 mL NB medium, 0.1 mL S. aureus and E. coli cultures, and 0.1 mL solution of antibacterial compound extracts in various concentrations. These cultures were incubated for 24–48 hours at 37°C. The observation was focused on the turbidity of the cultures. MIC value was the lowest concentration to inhibit bacterial growth which was indicated by the dear culture broth.

The characters of antibacterial compounds were observed with TLC using eluant acetic acid, butanol, and distilled water (3:1:1). Antibacterial compounds were placed (15 µL), sprayed with ninhydrin 1% diluted in NaOH 1% solvent, to obtain the compound spots. TLC plate containing spots was used for the bioautography test. It was placed in a sterile petri dish, added with agar medium, and incubated for 24-28 hours at room temperature. The evaluated parameters were inhibition zone diameter of rough extract, mycelium biomass (X), and biomass increase, but inhibition zone became wider than previously.

Culture filtrate of Streptomyces isolates E404 had the greatest inhibitory activity towards E. coli and S. aureus with an inhibition zone of 12.5–13.0 mm in diameter. This isolate also had the highest growth rate (X = 119.2 mg) (Table 1). The biomass production corresponded to enzyme and secondary metabolite productions in which isolate with the greatest inhibitory value produced the most biomass (Waites et al., 2001).

**Results and Discussion**

There was 16 Streptomyces isolated A. marina rhizosphere, 12 from site E40 and four from site E46. They have diverse morphological and biochemical characters. The differences between isolates were the presence of aerial mycelium, substrate mycelium (color), and melanoid pigment. According to Holt et al. (2000), the isolates produced a hard colony, powdery surface, wrinkle and lichenoid mycelium, with 2–3 mm of colony diameter. Aerial mycelium had straight and bent or spiral shape to support spores arrangement. Its colors were red, purple, brown, white, yellow and gray. The colony secreted black exudates and fused pigment with black color. Thakur et al. (2011) reported that almost all Streptomyces isolates found in mangrove rhizosphere had substrate mycelium and aerial mycelium. Substrate mycelium served to strengthen bacteria to survive and absorb nutrition, and aerial mycelium functioned for sporangium reproduction. Amal et al. (2011) stated that there was the various color of melanoid pigment and that mycelium produced by Streptomyces colony were blue, purple, red, yellow, green, brown, and black. The pigment which was undiffused in the medium appeared in its substrate and aerial mycelium.

The results of the biochemical test showed that almost all isolates were able to use sugar, as tested in the sole carbon source. IMVIC test responded positively to all isolates, except for VP test. Holt et al. (2000) described 150 isolates of Streptomyces; and showed several key characters as references for genus and species determination. The characters included mycelium shape, positive response to casein (as N source) test, carbon source tests (monosaccharides, disaccharides, and polysaccharides), reduce nitrates test, positive catalase test, and gelatin suspension survival.

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**Table 1.** The average diameter of inhibition zone (IZ) of bacteria by culture filtrates and Streptomyces isolates biomass (X)

| No | Isolate Code | S. a | E. c | X (mg) |
|----|--------------|------|------|--------|
| 1  | E461         | 10.0 | 12.0 | 101.5  |
| 2  | E401         | 9.0  | 7.0  | 30.9   |
| 3  | E402         | 7.0  | 8.0  | 91.9   |
| 4  | E403         | 9.0  | 8.0  | 83.3   |
| 5  | E404         | 8.0  | 9.0  | 63.7   |
| 6  | E405         | 13.0 | 12.5 | 119.2  |
| 7  | E406         | 8.0  | 7.0  | 63.6   |
| 8  | E407         | 9.0  | 8.0  | 106.3  |
| 9  | E408         | 12.5 | 7.0  | 102.3  |
| 10 | E409         | 10.5 | 10.0 | 84.4   |
| 11 | E4010        | 9.0  | 8.0  | 102.1  |
| 12 | E4011        | 11.5 | 7.0  | 86.8   |
| 13 | E4012        | 10.0 | 7.0  | 106.3  |
| 14 | E4013        | 11.0 | 8.0  | 75.6   |
| 15 | E4014        | 8.0  | 8.0  | 63.7   |
| 16 | E4015        | 1.0  | 8.0  | 103.5  |

Culture filtrates inhibited the pathogenic bacteria growth (Table 2). The longer the incubation period, the wider the diameter of the inhibition zone because of a larger amount of antibacterial compounds produced by isolate E404.

**Table 2.** The average diameter of inhibition zone of isolate E404 culture filtrate towards E. coli and S. aureus by different incubation periods.

| No | Incubation Period (Day) | ZH (mm) | S. a | E. c | pH | X (mg) |
|----|--------------------------|---------|------|------|----|--------|
| 1  | 0                        | 0.0     | 7.19 | 4.5  |
| 2  | 7                        | 9.0     | 6.80 | 40.4 |
| 3  | 14                       | 17.0    | 6.73 | 25.5 |
| 4  | 21                       | 20.0    | 6.90 | 13.6 |
| 5  | 28                       | 25.0    | 8.23 | 12.0 |

The cultures were in the exponential phase during seven days of incubation, then idiophase, the phase of secondary metabolites production. Thus, during the incubation period, high X value but little ZH was obtained (40.4 mg). Secondary metabolites antibacterial compounds accumulated during 28 days incubation period. During this period, there was no biomass increase, but inhibition zone became wider than previously.
Inhibitory activity of the antibacterial rough extract compounds of isolate 404 towards *E. coli* and *S. aureus* was unstable (known as bacteriostatic). The high concentration of the extract showed low inhibitory effect. Cultures produced inhibition zone (clear zone) of >16-20 mm were characterized as the moderate class (Nedialkova & Naidenova 2004). Polarity and saturation of antibiotic after dilution caused the diffusion inhibition in the medium (Fauziah, 2010). Preparations in obtaining rough extracts of antibiotic affected the ability of antibiotic compounds. Evaporation and heating steps might decrease the antibacterial compound ability which might not be the case in the screening step (Andrews, 2000).

**Table 3.** The results of antibacterial rough extract tests for *Streptomyces* 404 with dilution method

| Extract Concentration | *S. aureus* 24 hours | *S. aureus* 48 hours | *E. coli* 24 hours | *E. coli* 48 hours |
|------------------------|----------------------|----------------------|-------------------|-------------------|
| 1 %                    | -                    | -                    | -                 | -                 |
| 2.5 %                  | -                    | -                    | -                 | -                 |
| 5 %                    | -                    | -                    | -                 | -                 |
| 10 %                   | +                    | -                    | -                 | -                 |
| 20 %                   | +                    | +                    | +                 | -                 |
| 40 %                   | +                    | +                    | +                 | +                 |
| 80%                    | +                    | +                    | +                 | +                 |
| 100%                   | +                    | +                    | +                 | +                 |

The assay of antibacterial activity with dilution method showed that the highest concentration of 20% towards both *E. coli* and *S. aureus*. (Table 3). Some factors, such as antibiotic group, work mechanism, and pathogenic bacterial resistance influenced inhibitory activity.

The thin layer chromatography test separated compounds of the extract (Figure 1). There were three spots, indicating fractions with different distances to origin spot. In TLC, the solvent used, types of movement and static phase determined the test results (Lade et al., 2014). Maatoui et al., (2014) reported that *Streptomyces* could produce more than one active compounds that corresponded to the fractions formed in chromatography.

Bioautography showed the compounds that inhibited *E. coli* and *S. aureus* the growth had different Rf value. This difference explained that *Streptomyces* isolate 404 produced two types of antibacterial compounds (Figure 2). Bioautography is a test for activity of antimicrobial compounds in situ along with TLC test. Compounds on the TLC plate diffused with agar containing microbes. Thus the inhibition effect was seen on the microbial growth (Oedjijono et al., 1993; Patin et al., 2013). Active compound with Rf value of 0.47 inhibited *E. coli*, while that with Rf value 0.72 inhibit *S. aureus*. *Streptomyces* could produce more than one active compound in every life phase (Kishore, 2011).

**C O N C L U S I O N  A N D  S U G G E S T I O N**

There were 16 isolates of *Streptomyces* obtained from *A. marina* rhizosphere. The *Streptomyces* isolate E404 showed antibacterial activity towards *E. coli* and *S. aureus* indicated by the bioautography test. The Rf characterized *Streptomyces* secondary metabolites at the values of 0.47 and 0.72.

**R E F E R E N C E S**

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