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RESEARCH ARTICLE

STUDIES ON BIOSURFACTANT PRODUCED USING *Exiguobacterium profundum*

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ABSTRACT

Background: The manufacture of pharmaceutical preparations generally adds surfactants. Microbial biosurfactants can be an alternative because biodegradable and have antibacterial properties.

Objective: This study aimed to examine the biosurfactant activity of *Exiguobacterium profundum*.

Methods: Hemolysis and spreading oil tests were performed as an initial screening. Biosurfactant production was carried out by growing bacteria on oil-enriched media with shaker system for 7 days. Biosurfactant activity can be seen from the emulsification index, while the characterization of biosurfactant were used thin layer chromatography and antibacterial qualitative testing.

Results: *Exiguobacterium profundum* could spread the oil layer and form micelles. The emulsification index on days 0, 1, 3, 5, and 7 showed percentage in sequence 44.83%, 48.28%, 48.28%, 40%, and 43.75%. The result of TLC showed lipopeptide group which is marked with red stain with ninhydrin appearance. Antibacterial testing using *Escherichia coli* showed the formation of clear zones around the disk paper.

Conclusion: The biosurfactant produced by *Exigoubacterium profundum* can be classified into lipopeptide group which has antibacterial activity against gram-negative.

Keywords : Antibacterial, Biosurfactant, Emulsification, *Exiguobacterium profundum*, Lipopeptide

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INTRODUCTION

Surfactant is an amphiphilic chemical compound in which hydrophilic and hydrophobic properties exist in one molecule so that it has the ability to reduce the surface tension of a fluid.[1] Surfactants are widely used in industry as an emulsifier, corrosion inhibition, foaming, detergent, dan hair conditioning. The use of synthetic surfactants is considered less profitable because it cannot be degraded naturally (nonbiodegradable) and are of high toxicity and inhibit the degradation process by microorganisms.[2] To reduce these effects of surfactants need to be developed the alternate compound that can be degraded naturally (biodegradable), one of them by using biosurfactants.

Biosurfactants are amphiphilic compounds produced by microbes on cell surface and synthesized extracellular, which can reduce the surface tension between two phases.[3] Biosurfactants from microorganisms have stable chemical properties, renewable, environment-friendly, and low toxicity so that it can be applied in the pharmaceutical industry.[4] In the pharmaceutical field, biosurfactants are used as emulsifiers, foaming agents, wetting agents, and moisturizers.[3] Because of many benefits of biosurfactants, it needs to explore the potency of biosurfactant-producing microorganisms. Preliminary tests and biosurfactant activity tests were carried out on the bacterium *Exiguobacterium profundum*, marine bacterium that has a wide temperature and pH range so that it can adapt to extreme environments. *Exiguobacterium* is a potential genus that is widely used in industry and agriculture. Several studies have shown that the bacteria can be applied in bioremediation and toxic degradation.[5] However, there is no exploration related to its use as a biosurfactant.

MATERIAL AND METHODS

Rejuvenation of *Exiguobacterium profundum*

Exiguobacterium profundum is a bacterial isolate collection in the Microbiology Laboratory Sekolah Tinggi Farmasi Indonesia. Working culture was made by rejuvenating the bacteria into some Nutrient Agar slant by streak method. Then, inoculums were incubated at 37°C for 24 hours.

Screening of biosurfactant-producing bacterial isolate

Biosurfactant screening of *Exiguobacterium profundum* was carried out through hemolysis tests on blood agar and oil spreading techniques. Sterile fresh horse blood (1.5 ml) was added to the Erlenmeyer flask containing 100 ml of sterile Blood Base media. After that, the medium was poured into a sterile petri dish until it solidified. Bacterial isolate was inoculated by streak method on blood agar and incubated at 37°C for 48-72 hours.

The oil spreading technique was conducted by pouring 30 mL of distilled water into a petri dish, coconut oil (1 mL) was pipetted and dropped in the middle. Next step, 20µL of bacterial culture was added in the middle of the oil layer. Emulsion and clear zone formation were observed.

Biosurfactant production

Exigoubacterium profundum bacterial suspension (1 mL) was inoculated into 100 mL of Nutrient Broth media containing 3.3 mL of coconut oil. Next, it was incubated at 25^{o}C for 7 days with a shaker system. Observations were

made at T0, T1, T3, T5, and T7.[6] Separation of biosurfactants and bacterial cells were carried out by centrifugation at 3600 rpm for 20 minutes and the supernatant was taken.

Emulsification test

Exiguobacterium profundum suspension (2 mL) and 2 mL of coconut oil were added into test tube, then vortex for 2 minutes and allowed to stand for 24 hours.[7] Emulsification index was calculated by the equation :

$$\text{Emulsification index (E24)} = \frac{\text{height of emulsified layer}}{\text{height of the liquid column}} \times 100\%$$

Characterization of biosurfactant

The biosurfactant characterization was done through thin layer chromatography and antibacterial activity. Cell-free supernatant was spotted on TLC and eluted using chloroform, methanol, and water in a ratio (65: 25: 4 of volume). The results formed were visualized with UV 254nm, 366nm, and sprayed with ninhydrin.

Antibacterial activity was carried out using the paper disc method. Suspension of Staphylococcus aureus and Escherichia coli were inoculated using the pour method on MHA (Mueller Hinton Agar) media, homogenized, and allowed to solidify. Paper disc containing 20 µl biosurfactant were placed on top of the media layer, incubated at 37º C for 24 hours, and the formation of inhibition zones around the disc paper was observed. The positive control used chloramphenicol 250 µg/50µL[8], while the negative control used sterile distilled water.

RESULTS

Screening of biosurfactant-producing bacterial isolate

Screening of biosurfactant-producing through hemolysis and oil spreading test with the results in Figure 1 was done. The results of hemolysis tests on blood agar were negative because there were no clear zones arranged based on inoculum streak, while the oil spreading technique test showed positive results with the formation of emulsions and clear zones from oil drops of bacterial culture.

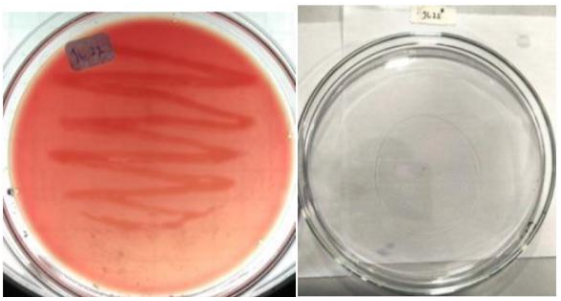

Figure 1. Hemolysis and oil spreading test of *Exiguobacterium profundum*

Emulsification test

There were five observation points for measuring the emulsification index with the biggest results on Day 1 and Day 3 of 48.28% according to Figure 2. The emulsification index decreased on day 5 and increased again on day 7.

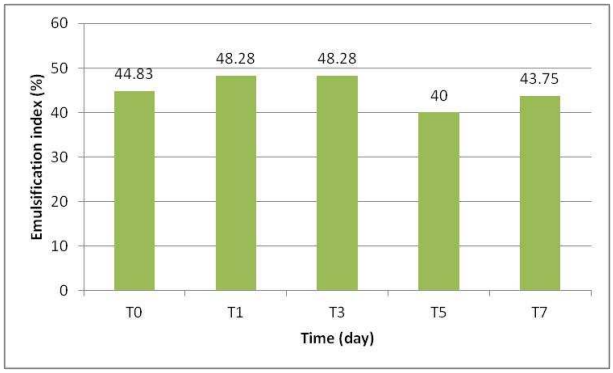

Figure 2. Emulsification test results

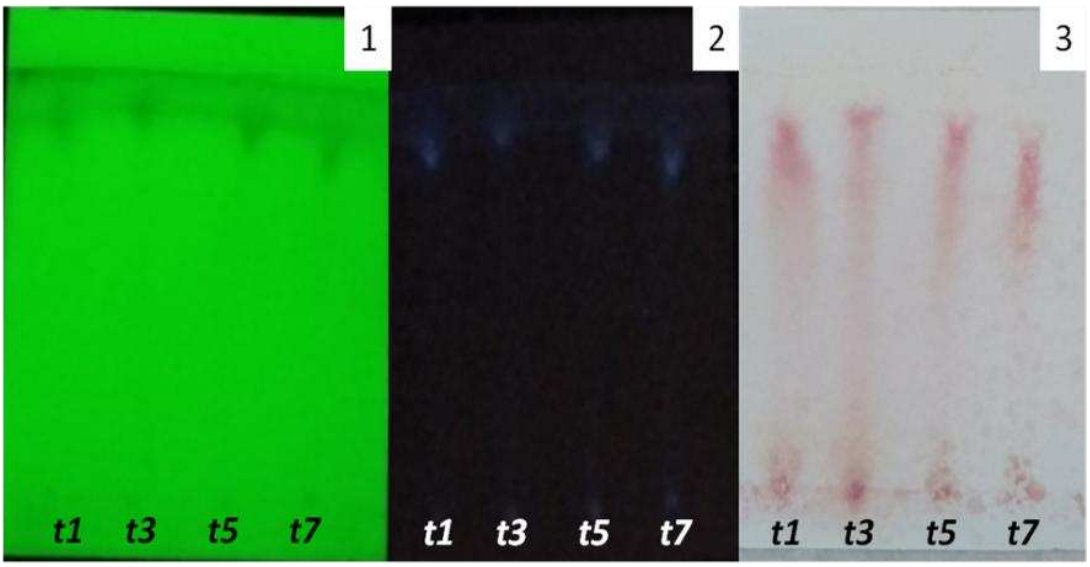

Figure 3. TLC results of the *Exiguobacterium profundum* supernatant using the mobile phase of chloroform: methanol: water (65: 25: 4 of volume) under UV 254nm (1) 366 nm (2) and the appearance of Ninhydrin spots (3).

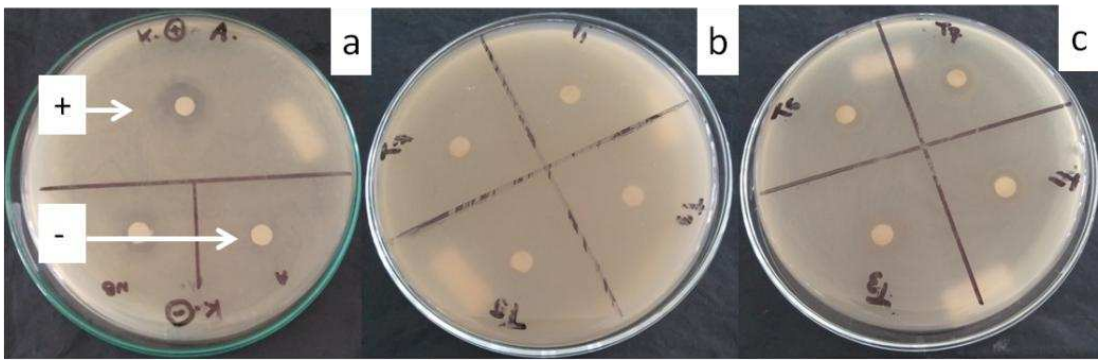

Figure 4. Antibacterial activity test of crude surfactant from *Exiguobacterium profundum* on four observation points (Day 1, Day 3, Day 5, and Day7) a) positive and negative control (b) *Staphylococcus aureus* c) *Escherichia coli*

Characterization of biosurfactant

The results of thin layer chromatography in Figure 3 showed the formation of red spot after being sprayed with the ninhydrin reagent. The antibacterial test in Figure 4 showed that biosurfactant could not inhibit the growth of *Staphylococcus aureus* (Gram-positive) because there was no clear zone around the disc paper, but it could inhibit the growth of *Escherichia coli* (Gram-negative) as indicated by the formation of a clear zone around the disc paper.

DISCUSSION

The manufacture of pharmaceutical preparations generally adds surfactants. Microbial biosurfactants can be an alternative because biodegradable, non-toxic and have antibacterial properties. Based on result, *Exiguobacterium profundum* has the potential to be a source

of biosurfactants. This was proven by oil spreading technique test and emulsification index. Oil spreading technique as initial screening tests showed the oil layer was emulsified and forms micelles because the hydrophobic and hydrophilic parts of the supernatant coalesce thereby reducing surface tension.[9]

Emulsification index test is one method to determine the character of biosurfactant production. *Exiguobacterium profundum* produced an emulsification index of 48.28% which was classified as a good biosurfactant. The higher emulsification index value has better surfactant properties.[10] The ability of bacterial emulsification was influenced by the structure and components possessed by biosurfactants.[11] This ability facilitates the uptake of hydrophobic substrates by cells.

Biosurfactants produced by microorganisms categorized into high and low molecular mass. The main classes of biosurfactants are glycolipids, phospholipids, lipopeptides, and polymeric biosurfactants.[12,13] Characterization using thin-layer chromatography showed that *Exiguobacterium profundum* produced lipopeptide biosurfactants. This indicated by the appearance of red spots after being sprayed with the ninhydrin reagent.[14] Lipopeptide is a low molecular mass biosurfactant component that has broad-spectrum antimicrobial activity and is currently applied in industry, cosmetics, and pharmaceutical.[13] It is not only decreases surface tension but has bioactivity as an antibiotic. Biosurfactants of this group can inhibit the attachment of pathogenic microorganisms to the surface at the site of infection and destroy bacterial colonization.[15]

Further testing of antimicrobial activity showed the biosurfactant produced by *Exiguobacterium profundum* only has antibacterial activity against *Escherichia coli*, gram-negative bacteria. The chemical composition and microbial origin affect biosurfactant activity.

CONCLUSION

Based on the results of research that has been done it can be concluded that the *Exiguobacterium profundum* can produce biosurfactants which belong to the lipopeptide group and have antibacterial activity against gram negative bacteria. Further research needs to be done on optimizing the biosurfactant production of *Exiguobacterium profundum* on various factors such as carbon source, pH, temperature, aeration, and inoculum concentration.

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