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The biosurfactant activity of supernatant fermentation broth isolates bacterial origin of Surabaya's Wonorejo mangrove sediment and its potential as an antifungal against *Candida albicans* ATCC 10231

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ABSTRACT

The purpose of this research was to know genus, biosurfactant activity and antifungal activity potency of bacterial isolate from mangrove sediments of Wonorejo Surabaya. Mangrove Wonorejo Surabaya is known as contaminated of heavy metals and hydrocarbon. Heavy metals and hydrocarbon contamination could induce microorganism to produce biosurfactants. Biosurfactant activity test used oil spreading, drops collapse, and parafilm test and antifungal activity test used agar well diffusion method. The result of this research showed that Genus of bacterial isolate belong to *Bacillus* because the bacterial isolate was Gram positive, bacil shape, aerob, and having endospore with ellipse shape which was in center of the cell. The result of biosurfactant activity with oil spreading method showed by clear zone. The average clear zone was 48.88 mm, the average droplets of parafilm test is 6.41 mm, and the result drops collapse test is positive showed by spread and drowned cell free supernatant of bacterial isolate. Cell free supernatant of bacterial isolate could inhibit growth of *Candida albicans*. The average inhibitory diameter is 31.25 mm. The conclusion of this research proved that bacterial isolate belong to Genus *Bacillus* which have biosurfactant and antifungal activity of *Candida albicans*.

Key words: *Biosurfactant activity, Supernatant fermentation, Bacterial origin, Candida albicans*

Introduction

Candidiasis is a fungal disease caused by yeast infections of the genus Candida. This disease often occurs in broilers, layers, ducks, pigeons, quails, and ornamental birds. *Candida albicans* species are the most frequently found in candidiasis cases (Liu, 2018).

Candida albicans infections have increased significantly in the last two decades so that synthetic antifungal drugs as agents for treating fungal infections are widely developed (Lo, 2015). However, the use of antifungal drugs made from chemicals such as amphotericin, nystatin, ketoconazole, and griseofulvin have disadvantages such as serious side effects, toxicity, potential for resistance, and high prices. The weakness of antifungal drugs made from these chemicals encourages efforts to find alternative antifungal agents that have minimal side effects, low toxicity, are environmentally friendly and inexpensive (Rintiswati, 2004).

Biosurfactants are potential compounds as antifungal alternatives (Singh, 2004). Biosurfactants have the advantage of being safe because of lower

toxicity, high biodegradability, good environmental compatibility, special activities under extreme conditions (temperature, pH, and salinity), as well as inexpensive because they are synthesized from renewable raw materials and substrates can be taken from waste or the environment (Cameotra, 2010).

Biosurfactants are produced by microorganisms in the fermentation process of production media (Mukherjee, 2006). Media production which is commonly used is Mineral Salt Medium (MSM). Production media fermented by microorganisms is often also called fermentation broth (Sunaryanto, 2015). The fermentation broth must be centrifuged and filtered using a bacterial filter to obtain the supernatant of the fermented broth if you want to utilize its biosurfactivity.

Bacterial isolates were isolated from Wonorejo mangrove sediments in Surabaya's East Coast (Sugihartuti, 2018). Biosurfactants are mostly produced by microorganisms in polluted ecosystems (Secato, 2016). Mangrove ecosystems in the Wonorejo River estuary are quite often found in heavy metal and hydrocarbon contamination (Harnani, 2017). Heavy metal pollution accompanied by pressure, temperature, increased salinity and limited nutrition will create stress conditions for microorganisms and encourage microorganisms to produce secondary metabolites as a defense mechanism (Nofiani, 2008). Hydrocarbon pollution encourages microorganisms to produce biosurfactants to reduce surface tension so that the carbon substrate needed by microorganisms is more easily absorbed and metabolized (Santos, 2016). Fermented broth supernatant isolates have the potential to have antifungal activity because they have the potential to contain biosurfactants which are known to have antifungal properties.

Materials and Methods

The materials used in this study were bacterial isolates from Mangrove Wonorejo sediment Surabaya, nurient agar, nutrient broth, saboroud dextrose agar, mineral salt medium, NaCl 0.9%, *Candida albican* ATCC 10231 isolate, violet crystal, safranin, malachite green, lactophenol cotton blue, acetone alcohol, lugol, oil, parafilm, paraffin liquid, aquades, 70% alcohol, bacterial filter of pore diameter 0.22 µm (Millex®), and emersion oil.

Sterilization of tools made of glass and materials using an autoclave with a temperature of 121 ºC with a pressure of 2 atm for 15 minutes. Ose is sterilized using a bunsen combustion fire, while equipment that cannot stand the heat is sterilized using 70% alcohol (Adji, 2007).

Rejuvenation of bacterial isolates is done by transferring one ose to nutrient media so that it is tilted and incubated for 24 hours at 37 ºC (Fernandes, 2007).

Rejuvenation of *Candida albicans* by transferring 1 ose to oblique SDA media and incubated for 7 days at 37 ºC (Pharmacopoeia Indonesia Edition V, 2014).

Macroscopic and microscopic identification of bacterial isolates by Gram staining, spore staining (Handijatno, 2016). Confirmation of *Candida albicans* ATCC 10231 macroscopically and microscopically with Lactophenol cotton blue staining (Sulivan, 1995).

Bacterial isolates that had been rejuvenated on NA skewed media were then inoculated on a 10 mL liquid NB medium then incubated using a shaker incubator at a speed of 150 rpm for 24 hours at 37 ºC. The incubation results of 1 mL were transferred using a pipette to the second liquid NB media containing 9 mL then incubated using a shaker incubator at a speed of 150 rpm for 24 hours at 37 ºC. The next step is to measure optical density (OD) to have a turbidity of 1.5 OD using a spectrophotometer (Fernandes, 2007).

Transferring the results of inoculation of 2 mL liquid NB media isolates into 18 mL MSM solution then fermented using a shaker incubator at 37 ºC at a speed of 150 rpm for 72 hours. The fermentation broth is transferred to the centrifugation tube, then the fermentation broth is centrifuged. The centrifugation results were filtered using a bacterial filter with a pore diameter of 0.22 µm (Millex®) to obtain cell free supernatants.

Drop collapse test is carried out in small test tubes. Then the test tube is filled with 1 mL of liquid paraffin. After that a supernatant of 20 microliters was dropped on the surface of liquid paraffin (Thavasi, 2011).

Parafilm test was carried out by dripping the supernatant on the surface of the hydrophobic parafilm then measuring the droplet diameter and compared with the MSM droplet diameter as a negative control.

Oil spreading test is done by pouring 50 mL of distilled water in a petri dish. Next 80 microliters of motor oil are dripped in the middle of the cup which has been spiked with distilled water until the

surface of the distilled water is covered by motor oil. Then drop 10 microliters of the supernatant in the middle of a petri dish (Thavasi, 2011).

Candida albicans ATCC 10231 rejuvenation results for 5 days at 37 °C in a test tube containing a skewed media Sabouraud Dextrose Agar (SDA) filled with 0.9% NaCl solution to half the volume of the tube then divortexed to homogeneous and measured fungal spore suspension with spectrophotometer λ 580 nm of 108 spores/mL which is equivalent to 25% transmittance.

A total of 10 mL of sterile SDA media is poured into a sterile petri dish as a base layer and wait for it to solidify. A total of 3 µL of *Candida albicans* mushroom suspension was taken using a micropipette then inserted into a test tube containing 10 mL of sterile SDA before solidifying and divortex. A total of 10 mL SDA with a suspension of the mushroom that has been divortex inserted into a petri dish that already contains the base layer. SDA second layer media on a petri dish if it has been solidified then made a hole in the media so that there are 3 replications and in 1 petri dish there are 3 holes in the well. 1 well as negative control of 100 µL MSM solution, 1 well as positive control with Ketoconazole 0.005% 100 µL and the third well containing supernatant of bacterial fermentation broth as much as 100 µL.

Results and Discussion

Based on Bergey's Manual of determinative genus of bacterial isolates is *Bacillus* because Gram is positive, rod-shaped, has ellipse-shaped endospores in the central part, and is aerobic. The morphology of the milky white bacterial isolate (Figure 1a), on microscope observation of 40x magnification of the colony appeared irregularly shaped with undulate edges (Figure 1b).

Gram staining of bacterial isolates showed Gram-positive and rod-shaped (Figure 2a). Bacterial isolates have ellipse-shaped spores and are located in the central part (Figure 2b). Morphological confirmation of the isolate *Candida albicans* ATCC 10231. Macroscopic observation on Sabouroud Dextrose media to make it look circular, creamy-white, shiny, smooth slippery texture and convex elevation (Figure 3). The results of microscopic confirmation using a microscope with 1000x magnification showed that the *Candida albicans* ATCC 10231 cells formed blastospores and pseudohifa (Figure 4).

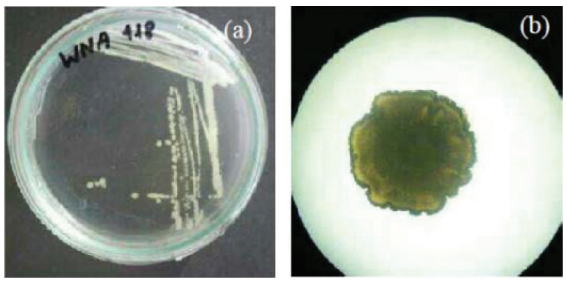

Fig. 1. Identification results of bacterial isolates colonies

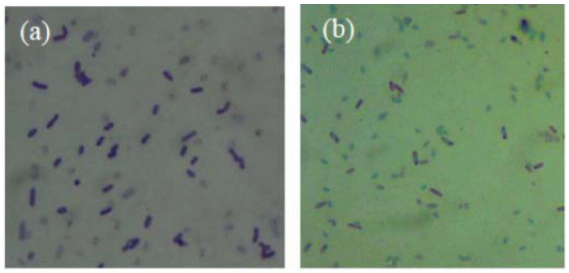

Fig. 2. The results of the morphology identification of 1000x magnification cells

Fig. 3. Confirmation of the morphology of the ATCC 10231 *Candida albicans* colony

Biosurfactant activity of bacterial isolates. The drop collapse test showed positive results (+) marked by the diameter of the supernatant droplets of the fermentation broth of bacterial isolates that were larger and could fall to the bottom of the tube compared to negative controls (MSM) whose diameter was smaller and could not fall to the bottom of the tube (Figure 5).

Test results on bacterial isolate supernatant gave positive results in this test, namely the diameter of supernatant droplets of fermentation of bacterial isolates was greater and could fall to the bottom of the tube compared to negative controls (MSM)

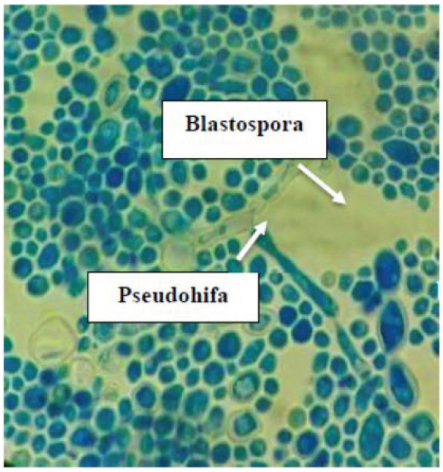

Fig. 4. Confirmation of *Candida albicans* ATCC 10231 cell morphology

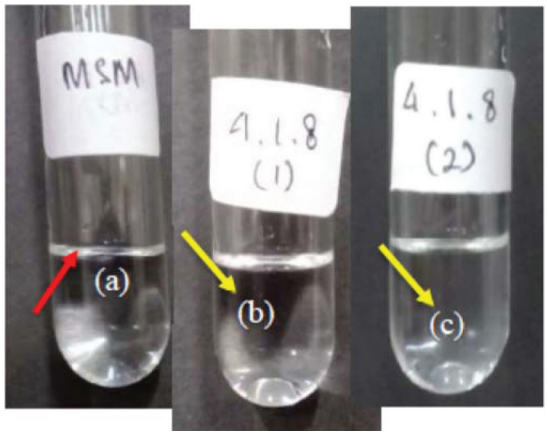

Fig. 5. Drop Test Collapse Bacterial Isolate Fermentation Broth Supernatant Broth. (a) Negative control (MSM); (b) The first test sample; (c) Second replication sample. Yellow arrows are droplets of supernatant fermentation broth for bacterial isolates and red arrows are MSM droplets.

whose diameter was smaller and could not fall to the bottom of the test tube. This happens because the biosurfactant of the bacterial isolate supernatant decreases the interface voltage between the supernatant fluid and the hydrophobic surface of the paraffin so that the droplet diameter becomes wider or larger.

The parafilm test for bacterial isolate broth supernatant droplets showed positive results because the first and second replication sample droplets were seen to spread (larger diameter) and flatter than the negative control droplets (MSM) which appeared more convex and smaller in diameter (Figure 6). The sample diameter of the first test was 6.38 mm, the diameter of the second test was 6.44 mm, and the diameter of the negative control was 4.72 mm.

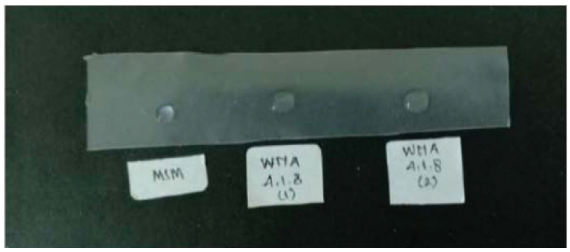

Fig. 6. Bacterial Isolate Fermentation Supernatant Broth Parafilm Test

Oil spreading test is done by duplicate. Test results show positive results. Positive results were marked by the formation of clear zones in the first and second replication samples (Figure 7). The clear zone diameter in the first sample was 50.34 mm and the second sample was 47.26 mm, while the diameter of the negative control (MSM) was 0 mm. Figure 8 shows that supernatant fermentation of bacterial isolates (with the letter "P") indicates an inhibitory zone for the growth of the fungus Candida albicans ATCC 10231 so it is known that the fermentation broth has antifungal properties.

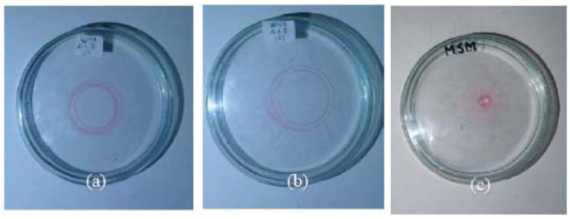

Fig. 7. Bacterial isolate fermentation supernatant broth oil spreading test.
Note: (a) first test sample (b) second test sample; (c) negative control (msm). The arrows indicate the clear zone.

Measurement of the diameter of the resistance is done using a calipers. The first sample of inhibition zone supernatant fermentation broth of bacterial isolates was 31.57 mm and positive control was 25.66 mm. The second sample obtained the results of the inhibition zone of the supernatant broth of bacterial fermentation isolate of 31.05 mm and positive control of 24.06 mm. The third sample obtained the results of inhibition zone of the supernatant broth of bacterial fermentation isolate of 31.13 mm and positive control of 24.33 mm. The negative con-

Table 2. Diameter of inhibition zones.

Repetition	Treatment	
	Control positive of ketokonazol 0.005%	Fermented broth supernatant isolates
Repetition 1	25.66 mm	31.57 mm
Repetition 2	24.06 mm	31.05 mm
Repetition 3	24.33 mm	31.13 mm
Average	24.68 mm	31.25 mm
Standard deviasi	0.85	0.28

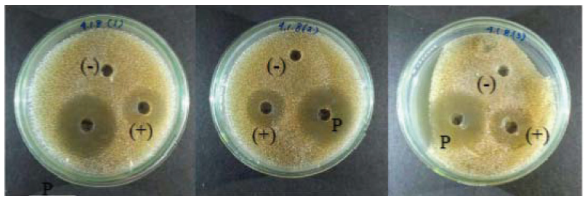

Fig. 8. Test results of bacterial isolate antifungal activities against *Candida albicans* atcc 10231.
Note: (a) first sample; (b) second sample; (c) the third sample. The sign (p) is a supernatant; (+) ketoconazole 0.005%; (-) msm.

trol of the three samples did not produce any resistance zone at all. The results of measurement of the diameter of the obstacles are presented in Table 2.

Conclusion

WNA isolates 4.1.8 origin of the Wonorejo mangrove sediment from the East Coast of Surabaya belong to the genus *Bacillus*. Fermentation broth supernatant WNA 4.1.8 origin of Wonorejo mangrove sediments from Surabaya East Coast has the potential to contain biosurfactants. Fermentation broth supernatant WNA 4.1.8 origin of Wonorejo mangrove sediment from East Coast Surabaya has antifungal activity against *Candida albicans* ATCC 10231.

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