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Antifugal potency againts *Candida albicans* (ATCC 10231) and its activity as biosurfactant of WNA 4.1.13 fermented growth of sediment from mangrove Wonorejo Surabaya Indonesia

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ABSTRACT

The purpose of this study was to identify the genus of bacteria that produces biosurfactants and its potency as antifungal to *Candida albicans* (ATCC 10231). WNA bacteria 4.1.13 were isolated from Wonorejo mangrove sediments in Surabaya. The bacteria were identified by examining the morphology and and cell colony.Cell morphology identification was carried out by Gram staining and spores identification. Biosurfactant activity was carried out by oil spreading test, drop collapse test and parafilm test. Antifungal activity was performed by agar diffusion method. The results showed that WNA 4.1.13 isolates from Surabaya Wonorejo mangrove sediments were rod shaped, including Gram positive and had endospores located at the center of bacterial cells. Based on the morphological character of the colony and genus cell bacterial isolates WNA 4.1.13 including *Bacillus* sp. The result showed that in the broth collapse test, the droplets were flat, forming a clean zone in the oil spreading test and widened diameter in the parafilm test. Antifungal activity test showed inhibition zones formed by fermentation broth isolates of WNA 4.1.13. In conclusion, the fermentation of broth of WNA 4.1.13 isolates from the Wonorejo Surabaya have biosurfactant activity and antifungal potency against *Candida albicans* (ATCC 10231).

Key words *: Biosurfactant, Antifungal, Mangrove, Candida albicans Fermented broth*

Introduction

Biosurfactants are surface active compounds produced by microorganisms. Biosurfactants have amphilic properties that have function to reduce surface tension and biological components. Biosurfactants can be applied to several biotechnology products (Secato *et al.,* 2016). The last period of biosurfactant is widely used in the pharmaceutical and medical fields as antifungal, antiviral, antibacterial, immunomodulatory, anti-adhesion, antioxidant, and anticancer drugs (Donio *et al.,* 2013). According to Banat *et al.* (2010), biosurfactants have high biodegradable properties, good emulsifying agents, low toxicity, environmentally friendly, effective in conditions of salinity, pH, and extreme temperatures (Secato *et al.,* 2016).

An appropriate environment for biosurfactant-producing bacteria is a polluted environment (Secatp *et al.,* 2016). According to Ranjan (2008), polluted ecosystems are generally found in mangrove sediments (Ranjan *et al.,* 2008). Widjajanti *et al.* (2013) reported having obtained 29 types of biosurfactant-producing bacteria from mangrove

areas contaminated with petroleum, namely the genus *Alcaligenes, Bacillus, Pseudomonas, Enterobacter, Flavobacterium* and *Pseudomonas* (Widjajanti *et al.,* 2013). Biosurfactants have diverse structures, for example glycolipids, phospholipids, lipopeptides and others.

Based on research conducted by Desai and Banat (1997) biosurfactants have potential as antimicrobials, namely antibacterial and antifungal (Desai and Banat, 1997). Research conducted by Abruzzo *et al.,* (2018) that biosurfactants produced by Lactobacillus gasseri are proven to have antifungal activity against *Candida albicans* (Abruzzo *et al.,* 2018).

Candida spp is a dimorphic fungus that normally exists in the digestive tract, upper respiratory tract and mucosal genity in mammals, but if the population increases it will cause a problem. *Candida albicans* is the main cause of candidiasis. This disease is opportunistic, so it is largely determined by the animal's immunity, water conditions, environment and feed quality (Jordan *et al.,* 2009).

Antifungal drugs commonly used by people is ketoconazole. The weakness of antifungal drugs currently circulating in the community is the interaction between antifungal drugs and other drugs when the host immune system is weak, and can cause serious problems such as liver and kidney damage (Hassanshahian, 2014). According to Apsari and Adiguna (2013) antifungal resistance can become a serious problem in the future due to the widespread of fungal infections and the lack of treatment options available (Apsari *et al.,* 2013). Fungus can originally resistance to antifungal drugs (primary resistance) or sometimes the resistance can occur in response from exposure to antifungal drugs during treatment (secondary resistance). Data from the Ministry of Industry (Kemenperin) in 2015 reported that the drug industry imported 90% of drug raw materials. Resistance and complications due to the use of antifungals and the dependence of the drug industry on drug raw materials from abroad are a serious health problem (Ministry of Agriculture of Indonesia, 2014)

One alternative to overcome the above problem is finding for new compounds that have potency as antifungals. The search for new biosurfactant-based compounds that have potency to be antifungal begins with screening of biosurfactant activity using fermentation broths of WNA isolates 4.1.13. Fermentation broth of WNA isolates 4.1.13 is the result of fermentation of biosurfactant-producing bacteria in Mineral Salt Medium (MSM) (Gozan *et al.,* 2014). Sugihartuti *et al.* (2018) have isolated the 4.1.26 foreigners from the Wonorejo mangrove in Surabaya. If it is proven to be potential as an antifungal, it can be developed into a new compound as an alternative or alternative antifungal in the future (Sugihartuti *et al.,* 2018).

Materials and Methods

Tools sterilization and materials

Sterilization of tools made of glass and materials using autoclave with a temperature of 121 ºC with a pressure of 2 atm for 15 minutes. Ose was sterilized by incandescent in a unsen-burning flame, while equipment that cannot withstand heat were sterilized using 70% alcohol (Sari and Apridamayanti, 2014). Sterilization of the media was carried out by taking as much material as needed and then mixed with distilled water. The material is heated and stirred until homogeneous and then autoclaved at 121 ºC at a pressure of 2 atm for 15 minutes (Anggraini *et al.,* 2016).

Isolate Rejuvenation

WNA 4.1.13 rejuvenation was carried out by transferring one ose to nutrient media so that it was tilted and incubated for 24 hours at 37 °C (Fernandes *et al.,* 2007).

Rejuvenation of *Candida albicans* ATCC 10231 was performed by transferring one ose to oblique SDA media and incubated for 3-5 days at 23 ºC.

Identification of WNA Isolates 4.1.13 and Confirmation of *Candida albicans* ATCC 10231

Identification of WNA 4.1.13 isolates was performed macroscopically and microscopically with Gram staining and spore staining. *Candida albicans* ATCC 10231 was confirmed macroscopically and microscopically with Lactophenol cotton blue staining (Handijatno *et al.,* 2016).

Fermentation of WNA isolates 4.1.13 on MSM media

Collection of 4.1.13 WNA bacteria that had been rejuvenated on NA skewed media was then inoculated on 10 Ml NB media then incubated for 24 hours at 37 ºC using a shaker incubator at a speed of 150 rpm. The incubation result of 1 ML was transferred using a pipette to 9 ML of NB media then in-

cubated at a speed of 150 rpm. The next step was to measure optical density (OD) to have a turbidity of 1.5 OD using a spectrophotometer (Fernandes, 2007).

Transfer the results of 2 ML WNA inoculation as much as 2 ML into 18 ML of MSM solution then fermented using a shaker incubator at 37ºC at a speed of 150 rpm for 72 hours. The fermentation broth was transferred to the centrifugation tube. Next, the fermentation broth was centrifuged at a speed of 5,000 rpm at 4 ºC for 30 minutes. The centrifugation result was filtered using a sterile membrane.

Identification of WNA isolates 4.1.13

WNA isolate 4.1.13 was identified by observing the colony and cell morphology. Observation of the colony were performed to identify the form of shape, color, surface, edge and elevation. Cell observation was carried out microscopically by Gram and spore staining.

Test of biosurfactant activity of WNA fermentation broth isolates 4.1.13

Drop collapse test

Removing paraffin liquid in the test tube as much as 1 Ml and 10 µL of fermentation broth fermentation of WNA 4.1.13 was dripped in the middle of the paraffin liquid. Positive results are shown by forming flat droplets.

Oil spreading test

Transfer 40 ML of sterile distilled water to a petri dish and 80 µL of oil was transferred to sterile distilled water and placed in the center. 10 µL of fermented broth was placed above the central oil. Positive results are shown by the formation of clean zones (Hassanshahian, 2014).

Parafilm test

25 µL of fermentation broth isolate WNA 4.1.13 was placed above parafilm. Droplet diameter was measured after one minute (Tugrul, 2005).

Antifungal activity test of fermentation broth isolates of WNA 4.1.13 against *Candida albicans* ATCC 10231.

Mushroom suspension

A sterile physiological NaCl was transferred into a test tube containing a rejuvenated *Candida albicans* ATCC 10231 culture. The suspention was vortexed and measured by fungal spore suspension with a spectrophotometer λ 580 nm at 25% T equivalent to 108 cells per mL.

Test media

3 µL suspension of the *Candida albicans* ATCC 10231 was dropped on SDA media, then in the vortex, and was poured into a petri dish containing 10 ML of agar which has solidified and leveled. The final mediawas incubated in room temperature for 15 minutes.

There were 3 replications and in 1 petri dish there were 3 wells. The first well was negative control 100 Ìl MSM solution, the second well was positive control with 100 µL 0.005% Ketoconazole and the third well was containing 100 µL WNA fermentation broth 4.1.13.

Data analysis

Data analysis was qualitative in the drops collapse and quantitative tests in the oil spreading test, parafilm test and antifungal activity test processed using SPSS paired sample test.

Results and Discussions

Based on Bergey's Manual of determinative, the genus of WNA 4.1.13 isolates was *Bacillus* because it was Gram positive, rod-shaped, has a central endospora, and aerobic growth.

The morphology of the isolation colonies of WNA 4.1.13 was yellowish white, round in shape and smooth, non-slimy (Figure 1a), at 40x magnification the microscope observed a slightly wavy edge (Figure 1b).

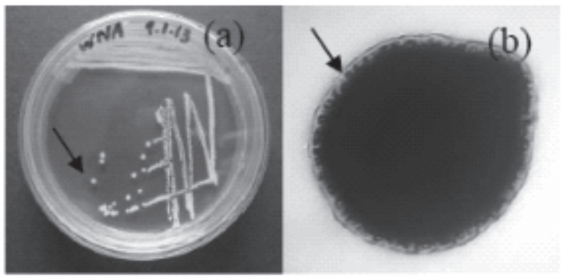

Fig. 1. Results of identification of WNA isolates colonies 4.1.13. (a) The colony was yellowish white, round in shape and smooth. (b) observation under the microscope with 40x magnification

Gram staining of WNA 4.1.13 isolates shows Gram-positive and rod-shaped (Figure 2a). the staining of WNA isolates 4.1.13 has spores located in the central section (Figure 2b).

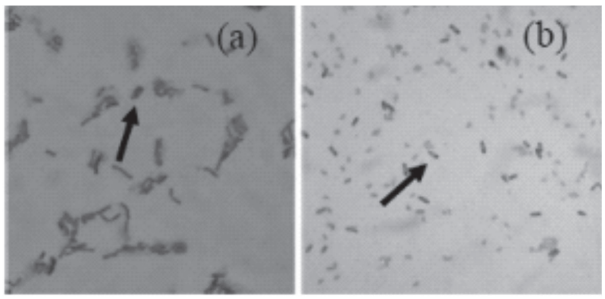

Fig. 2. Results of identification of 1000x magnification cell morphology. (a) Gram staining was identified as Gram-positive. (b) spore was located in the central section.

Morphological confirmation of *Candida albicans* ATCC 10231 isolates

Macroscopic observations on Sabouroud Dextrose media So that the colonies look milky white in color (Figure 3a).

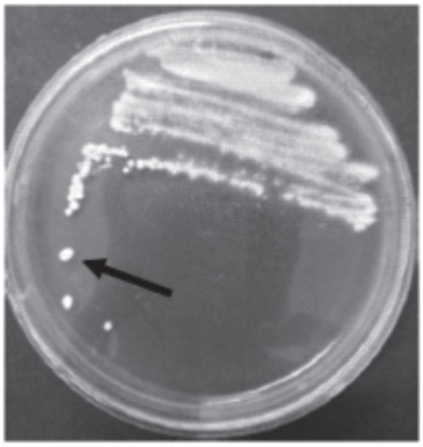

Fig. 3. Confirmation of morphological results of ATCC 10231 *Candida albicans* colony

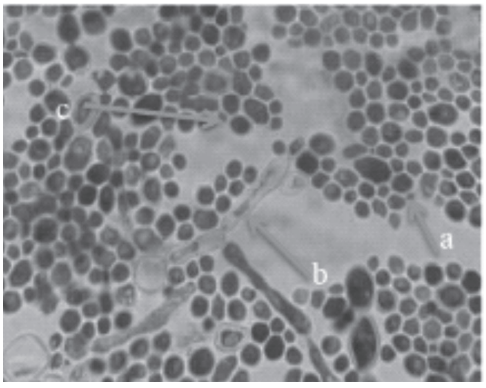

Fig. 4. Confirmation of *Candida albicans* ATCC 10231 cell morphology.

Biosurfactant activity of WNA fermentation broth isolates 4.1.13

The drop collapse test showed positive results as evidenced by the fall and widening of the WNA fermentation broth 4.1.13 (Figures 5b and 5c). The results of the drop collapse test on the negative control of the MSM solution show the negative results shown by the floating MSM droplets (Figure 5a).

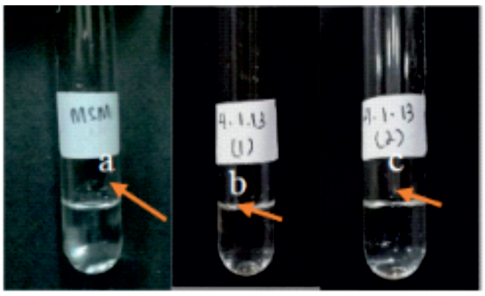

Fig. 5. Results of drop collapse test for WNA isolates 4.1.13.

The droplets were flat because the voltage between the samples' superficial and oil decreases. The diameter of the droplets formed depends on the biosurfactant concentration contained in the sample.

The MSM oil spreading test as a negative control was not formed a clean zone (Figure 5a) and in the fermentation broth isolates WNA 4.1.13 formed a clean zone (Figure 5b.). The net zone diameters of WNA 4.1.13 isolates were 53.75 mm, 52.89 mm, respectively.

The diameter of the drop WNA isolates 4.1.13 in

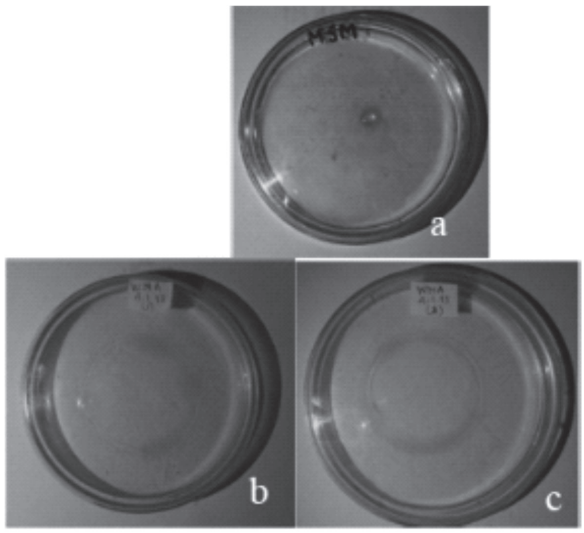

Fig. 6. Oil spreading test results on WNA isolates 4.1.13

the parafilm test appeared to be greater than the negative control of the MSM solution (Figure 6). Diameter of droplet isolates of WNA 4.1.13 were 8.02 mm and 7.9 mm, respectively. The diameter of the MSM solution as a control was 6.12 mm.

The measurement results of the diameter of inhibition zone of the WNA fermentation broth 4.1.13 against *Candida albicans* ATCC 10231 were 38.03 mm, 35.74 mm and 37.58 mm respectively, while the diameter of the positive control ketoconazole 0.005% inhibitory zone were 23.36 mm, 24.27 mm, 23.16 mm respectively. and negative control of MSM solution did not have inhibitory zones. The mean diameter of the ketoconazole inhibitory zone was 23.60 mm and the fermentation broth of WNA isolates 4.1.13 was 37.12 mm. The standard deviation of ketoconazole is 0.59 and the standard deviation of the supernatant deviation of the WNA fermentation broth 4.1.13 is 1.21 (Figure 8). The data can be seen in Table 1.

Fig. 7. Droplets diameter of the isolate WNA isolate 4.1.13*3.3 Antifungal potency of fermented broth of WNA isolate 4.1.13*

The clear zone diameter of WNA fermented broth 4.1.13 with OD 1.5 is greater than 0.005% ketoconazole, but it cannot be said to be better as an antifungal compound. Ketoconazole 0.005% is an active ingredient that functions as an antifungal, where the concentration that should be used is 2%. In addition, the fermentation broth is still in the form of a mixture of compounds that do not all function as antifungals, so that further research should be done on pure active compounds contained in the fermentation broth of WNA isolates 4.1.13.

Conclusion

WNA 4.1.13 isolates from Wonorejo Surabaya mangrove sediments were clasified in the genus Bacillus. Fermented broth of WNA isolates 4.1.13 from the origin of Surabaya's Wonorejo Mangrove sediment had biosurfactant activity. Fermented broth isolates of WNA 4.1.13 from Wonorejo Surabaya mangrove sediments had potency as antifungal againts *Candida albicans* (ATCC 10231).

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