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The Activity Of Lactic Acid Bacteria From Ale-Ale (Fermented Clams) And Cincalok (Fermented Shrimp) As Antioxidant And Antimicrobial

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Abstract

Lactic acid bacteria (LAB) have been isolated from fermented clams (Meretrix sp) called ale-ale and fermented shrimp called cincalok using the selective method of Man Rogosa Sharpe (MRS). The characteristics of LAB were gram-positive bacteria, non-spore-forming, non-motile, and facultative anaerobes. Lactic acid is produced from the anaerobic carbohydrate fermentation process. LAB activity was evaluated using the agar well diffusion method in nutrient agar (NA) media, showing that the 6-10 mm inhibition zone was categorized as moderate. The LAB inhibitory activities against Staphylococcus aureus, from ale-ale and cincalok, were 5.42 mm and 6.34 mm, respectively. Meanwhile, the LAB inhibitory activities of both fermented food against Salmonella thypi, were 6.96 mm and 7.48 mm, respectively. The antioxidant activities were relatively weak, with IC50 values of 4,061.331 ppm in ale-ale and 1,782.075 ppm in cincalok.

Keywords: Ale-ale, Cincalok, Lactic Acid Bacteria (LAB), Antibacterial, and Antioxidant

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INTRODUCTION

Fermented food processed products that are quite popular among the people of West Kalimantan are ale-ale and cincalok. Both are prepared through a fermentation process that involves microbes in the processing. The microbes that help the processing of these products are Lactic Acid Bacteria (LAB), which are a group of gram-positive bacteria, not spores, round or rods, facultative anaerobes, and non-motile (Desniar et al., 2012). LAB has an important role in providing food preservation effects by inhibiting spoilage microbes (pathogens); this makes LAB classified as a food-grade microorganism category.

LAB isolates from cincalok were able to inhibit the growth of *Vibrio parahaemolyticus* and *Listeria monocytogenes* (Samboja et al., 2019). The inhibitory activity contributes to lactic acid produced by LAB, H_2O_2, and bacteriocin (Desniar et al., 2012). Also, LAB can inhibit free radicals. The antioxidant is an inhibitor of free radicals, as molecules containing unpaired electrons in their outer orbits. Free radicals are unstable electron acceptors because they have unpaired electrons and look for electron pairs in biological macromolecules (Mishra et al., 2015). The antioxidants available in the body are not proportional to the number of free radicals that may enter the body (Mishra et al., 2015). Therefore, additional antioxidants from outside the body are needed to scavenge free radicals.

In the preservation of food, which aims to extend the shelf life, the LAB has an important role because it can inhibit the growth of rotting microbes (pathogens). LAB's availability on the market is still very limited and expensive, so efforts are needed to explore LAB from materials sourced from marine products.

Materials of the sea may have positive impacts on health. Several observations on food materials from the sea have been carried out. Fresh and fermented clams may contribute as an antioxidant (Minsas et al., 2020) and hepatoprotector agent (Fadly et al., 2020a). *Euchema spinosum* seaweed was also found to have free radical inhibition capability by their phytochemicals compounds (Sofiana et al., 2020) and *Niphate sp.* Sponge extract also indicates antioxidant and antimicrobial activities (Warsidah et al., 2020b).

In this study, the LAB was isolated from fermented small clams (*meretrix sp*) known as ale-ale and fermented fine shrimp (cincalok), which are seafood from West Kalimantan. This study aims to determine the type of LAB obtained from ale-ale and cincalok. LAB was isolated using the agar de Man Rogosa Sharpe (MRS) medium method, cell morphology observations, Gram stain, and test for bacterial growth inhibition and antioxidant activity.

METHODOLOGY

Materials

Materials needed in this study were cincalok and ale-ale, test bacteria (*S. thypi* and *S. aureus*), sterile distilled water, ethanol, Man Ragosa Sharpe Broth (MRSB) and Man Ragosa Sharpe Agar (MRSA), Nutrient Agar (NA), Nutrient Broth (NB), ascorbic acid, ethyl acetate, tetracyclin and 1,1 Diphenyl-2-Phicrylhydrazyl (DPPH).

Sampling

The fermented products included ale-ale and cincalok were gathered from the traditional market, West Kalimantan, Indonesia. Preparation of the sample was carried out at the Laboratory of Marine Science, Faculty of Mathematics and Natural Sciences, Tanjungpura University.

Sample Preparation

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traditional market, West Kalimantan, Indonesia. Preparation of the sample was carried out at the Laboratory of Marine Science, Faculty of Mathematics and Natural Sciences, Tanjungpura University. Sample preparation was executed following the procedure of (Sari et al., 2013).

Isolation of LAB from ale-ale and cincalok

Five grams of each sample (ale-ale and cincalok) were transferred to 45 mL of MRSB media, then homogenizing it so that a 10^{-1} dilution was obtained, then incubated at 37 °C for 24 hours. After incubation, gradual dilution was carried out by taking 1 mL of the sample into a test tube containing 9 mL of peptone water, graded dilution until a 10^{-7} dilution was obtained. Furthermore, the culture was grown on MRSA media with the pour plate method (incubation temperature 37 °C for 48 hours). The single LAB colonies grew purified again by inoculating the colony into MRSA media by scratch, and this was used as LAB culture (incubation temperature 37 °C for 48 hours) (Sari et al., 2013).

Antibacterial Test

30 µl of tested bacterial culture was put into a petri dish, and 20 mL of NA medium was added. After the NA media hardened, a 6 mm diameter well was made using a dropper tip. Then, as much as 20 µl of LAB culture (which had been grown in MRSB) was also carried out on the antibiotic with the same treatment put into the well and then incubated for 24 hours at 37 °C. The clear zone that is formed is then measured using a caliper (Khotimah and Kusnadi, 2014). The calculation of the clear zone or zone of inhibition is as follows (Warbung, 2014) :

$$\text{Inhibition zone} = \frac{d1 + d2}{2} - X$$

Where is:
d1 = vertical diameter of the clear zone on the media
d2 = horizontal diameter of the clear zone on the media
X = pit

Antioxidant Determination

One ose of LAB isolate was inoculated in 100 mL NB medium then shaken for 72 hours to obtain LAB suspension (Kumala et al., 2006). The suspension, which has been shaken for 72 hours, is then separated using a centrifuge. The supernatant was then extracted by maceration method using ethyl acetate as an organic solvent. The extract was concentrated using a vacuum rotary evaporator to remove the solvent until a dry extract was obtained (Kumala et al., 2006). The antioxidant activity test was carried out using the DPPH method dissolved in ethanol p.a (Fadly et al., 2020b). Then, the maximum wavelength was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm. In this study, a comparison was used, namely ascorbic acid. The stock solution of DPPH was prepared by dissolving 10 mg of the sample in 10 mL of ethanol p.a to obtain a concentration of 1000 ppm. The ascorbic acid concentration varied at 2, 4, 6, 8, and 10 ppm. At the same time, the sample variations of LAB secondary metabolite extract were 50, 100, 200, 400, and 800 ppm. A total of 1 mL of sample solution of various concentrations was added with 2 mL of p.a ethanol, then added with the 1 mL DPPH solution 100 ppm and then homogenized. The test tubed incubated in a dark place at room temperature for 30 minutes (Blois, 1958).

The absorbance of each sample variation is measured at a predetermined maximum wavelength. The ability of the DPPH radical inhibitor is determined from the absorbance value. The concentration of the test solution extract to reduce 50% free radical activity is determined by the IC_{50} value calculated by the percentage of absorbance inhibition of the extract solution using the equation obtained from the linear regression curve.

$$\%\text{Inhibisi} = \frac{\text{Abs. DPPH} - \text{Abs. Sample}}{\text{Abs. DPPH}} \text{ x } 100\%$$

The sample concentration and percent inhibition were processed using SPSS to obtain a linear regression equation. This equation is used to determine IC_{50}.

RESULTS AND DISCUSSION

Antibacterial Activity

The inhibition zone of the two tested bacteria (Table 1) was classified as moderate because the inhibition zone obtained was 6-10 mm on average. The inhibition zone obtained from the Gram-negative *Salmonnella thypi* test bacteria was 6.96 mm from LAB from ale-ale (BAL.AL) and 7.48 mm from BAL from cincalok (BAL.CN). Meanwhile, the inhibition zone obtained from the Gram-positive test bacteria *Staphylococcus aureus* was smaller, namely 5.42 mm from the LAB from ale-ale (B.AL) and 6.34 mm from the LAB from cincalok (B.CN).

Based on the inhibition zone's diameter, it can be concluded that a more significant inhibition was obtained in the Gram-negative *Salmonella thypi* test bacteria isolate compared to the inhibition zone obtained in the Gram-positive *Staphylococcus aureus* test bacteria isolate. This shows that *Salmonella thypi* is more sensitive to the antibacterial activity obtained from LAB than *Staphylococcus aureus.* The Gram-negative cell wall is thinner so that LAB will more easily enter the cell membrane to damage the cell walls of lactic acid bacteria. Gram-positive bacteria have thicker cell walls so that antibacterials will find it more difficult to penetrate lactic acid bacteria (Yulinery et al., 2009).

The diameter of the inhibition zone that is formed is the diameter of the clear zone around the well, which indicates bactericidal properties. The inhibition zone obtained from positive control on each of the tested bacteria is 11.28 mm from *Staphylococcus aureus* and 12.68 mm from *Salmonella thypi.* The microbial inhibitory activities may influenced by their bioactive compounds, involving phytochemicals (Dewi et al., 2020a; Warsidah et al., 2020a).

Antioxidant Activity

Based on (Table 2.), the higher concentration of the sample solution, the higher the percentage inhibition in inhibiting free radicals. The antioxidant activity of the sample to scavenge free radicals from the DPPH method is expressed by the IC_{50} (inhibition concentration) value, which is the concentration that can reduce 50% of free radicals from DPPH. The smaller the IC_{50} value, the greater the antioxidant activity in a sample. Compounds have very strong antioxidant activity if the IC_{50} value is less than 50 ppm, strong 50-100 ppm, intermediate 101-150 ppm, and weak if the IC_{50} value is> 150 ppm (Batubara, 2018).

Table 1. Results of Antibacterial Activity Test for LAB Isolated from Ale-ale and Cincalok

No	Test Bacteria	The diameter of the Inhibition Zone (mm)			
		Tetracycline	Aquades	BAL. Ale-ale	BAL.Cincalok
1	*S. aures*	11,28	-	5,42	6,34
2	*S. thypi*	12,68	-	6.96	7,48

(-) no clear zone formed; Very strong (> 20 mm); strong (11-20 mm); Moderate (6-10 mm); weak (< 5 mm) (Susanto, et.al., 2012).

Table 2. Results of Antioxidant Activity

No	Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50} (ppm)
1	Ascorbic acid	2	0.415	36,442	
		4	0.394	39,653	
		6	0.397	39,194	11.43
		8	0.415	36,493	
		10	0.256	60,754	
2	BAL. Ale ale	50	0.424	35,066	
		100	0.404	38,532	
		200	0.392	40,010	4,061.33
		400	0.386	40,978	
		800	0.357	45,361	
3	BAL. Cincalok	50	0.364	44,240	
		100	0.414	36,646	
		200	0.389	40,519	1,782.08
		400	0.356	45,463	
		800	0.325	50,254	

Non-linear logarithmic regression equation, the relationship between sample concentration and % inhibition of each y = 22.38 + 3.324 (ln X) secondary metabolite LAB from ale-ale, y = 27.491 + 3.007 (ln x) secondary metabolite LAB from cincalok, and y = 26,761 + 9,540 (ln x) and ascorbic acid as comparison. From the non-linear logarithmic regression equation results, the IC_{50} value can be determined, namely ascorbic acid 11.426 ppm, secondary metabolite LAB ale-ale 4,061.33 ppm, and cincalok secondary metabolite 1,782.08 ppm. Ascorbic acid was used as a comparison because ascorbic acid is an antioxidant compound. LAB from ale-ale and cincalok has a relatively weak antioxidant activity value with an IC_{50} value in both samples of more than 150 ppm. The capability of substances to affect the oxidation is influenced by their bioactive compounds (Masriani et al., 2020). The DPPH free radicals will be neutralized by the donated H^+ from antioxidant (Dewi et al., 2020b).

CONCLUSION

The antibacterial activity of LAB in this study had an inhibition zone of 6-10 mm, classified as moderate. The antioxidant activity produced from LAB from ale-ale and cincalok is classified as weak; the IC_{50} value of ale-ale was 4,061.33 ppm, and cincalok was 1,782.08 ppm.

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