SCREENING AND PARTIAL CHARACTERIZATION OF BACTERIOCIN FROM LACTID ACID BACTERIA ISOLATED FROM FAN PALM SUGAR (*Borassus flabellifer L*)

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Abstract

Bacteriocin is a protein compound that has bactericidal action against microorganism. Bacteriocins from lactid acid bacteria are very potensial as natural food biopreservatives. The aim of this present study is to obtain the isolate of lactic acid bacteria which has a potential to produce bacteriocin from fan palm sugar, to attain the bacteriocin characterization such as its stability against heat and proteolytic enzyme. Other goal is to observed the inhibitory activity of bacteriocin against Gram positive and Gram negative bacteria. This research found that 2 isolates LB.9 and LB.30 have a potency to produce bacteriocin. LB.9 sensitive to protease enzyme and heat labile whereas isolate LB.30 sensitive to protease enzyme and heat stable. The bacteriocin are able to inhibit the growth of Gram positive and Gram negative bacteria.

Keywords*: Isolation, Characterization, LAB, Bacteriocin, Fan Palm Sugar*

Introduction

Preservation by natural ingredients have an antimicrobe characteristic and have been consumed by humans for a long term without adverse effects on its health. The compound is a biological component called biopreservatif agent. One of the biopreservatif compounds is bacteriocins (Anonymous, 2007). Bacteriocin is a protein compounds released by bacteria that are inhibiting the growth of other bacteria that have a particularly strong kinship with producing bacteria. Some of the BAL have been known to produce bacteriocins that have activity inhibiting the growth of spoilage bacteria and pathogens so as to enhance food safety and the shelf life of food (Tahara et al., 1996). Bacteriocins from lactic acid bacteria used as biopreservatif has several advantages such as bacteriocins rather than toxic materials and is readily biodegradable because it is a protein compound. Its usage does not harm the intestinal microflora because it is easily digested by enzymes in the digestive tract. The use of bacteriocins can reduce use of chemicals which have been used as a preservative and can be used in superior bacterial culture capable of producing antimicrobial compounds against bacterial pathogens and can be used in the form of a purified antimicrobial compounds (Sudirman 1996, in nurliana 1997).

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Bacteriocin is classified into 4 groups: group I: bacteriocins with small peptide molecule (molecular weight <5 kDa) contains an unusual amino acid, lanthionin. A heat-resistant peptide, group II: the heat stable peptide (molecular weight <10 kDa). Group III: bacteriocin to heat labile, large molecule peptides (molecular weight> 30 kDa). Group IV: complex bacteriocin, which is a complex protein whose activity is associated with carbohydrate or lipid (Rajaram, et al., 2010).

The purpose of this study is a bacteriocin-producing isolates BAL from palm sap, knowing the inhibitory activity of isolates BAL bacteriocins against positive Gram and negative Gram and to know the stability of the bacteriocin to heat and enzymes.

Methodology

Composition

The raw materials that used in this research were from Tuban palm sap that has been fermented for 12 hours. The chemicals which used in this research are MRSB and a media for growing lactic acid bacteria (BAL), NB for growing the bacteria indicators (S.aureus and E.coli) and NaOH to neutralize the pH.

The bacteria indicators which used to test bacteriocin activity was S.aureus (Gram positive) and E. coli (Gram negative) that obtained from the food microbiology laboratory, agricultural technology department, Brawijaya University.

The Isolation of Bacteriocin Producing Lactic Acid Bacteria

The isolation method conducted by previous pour plate method done dilution to 10-8 and captured four dilution series last then diplating into the medium was added to a 1% CaCO3 that serves to clear zone formed in plain sight, then performed at a temperature of 30ºC inkubasi, 48 hours to appear BAL colonies under the surface of the agar

Bacteriocin-producing BAL initial screening

Bacteriocin-producing BAL initial screening is done as follows: after the agar media that has inoculated (*S.aureus*) condenses then it is put into the refrigerator for 30 minutes, and then the isolate BAL was scratched on the media and incubated at 37ºC for 24 hours. The isolate that has the ability to inhibit the indicator bacteria will produce a clear zone around the scratches.

Potential Test of Bacteriocin Activity

The method used to test the potential of bacteriocin activity was agar diffusion method, the way is as follows: 20 ml agar medium was poured into a petri/dish that has been filled with bacteria indicator at age 20 hours and then mixed with the pour plate method and allowed to solidify. After changing into a solid, a well was made and it was filled with the supernatant, then it was incubated at 4ºC for 24 hours, and then it was incubated again at 37ºC for 24 hours.

Supernatant obtained from centrifugation of liquid culture ages 24hours (6000rpm, 15 minutes) to separate the cells and the supernatant was neutralized to pH ±6.

The Bacteriocin Activity Resistance Against The Enzyme Protease

This test is done by adding the supernatant neutral with a crude extract of protease enzymes which were incubated at 370C for 3 hours. Then, the bacteriocin activity of this supernatant was tested by agar diffusion method. The result is positive if there is no clear zone

Then Resistance of Bacteriocin Activity against the Heat.

This test was done by heating the neutral supernatant at 100ºC for 5 minutes. Then, the bacteriocin activity of this supernatant was tested by agar diffusion method.

Spectrum Bacteriocin

To find the spectrum bacteriocin inhibitor, it was used *S.aureus* and *E.coli* indicator bacteria by the agar diffusion method.

Result and Discussion

The Isolation of Bacteriocin Producing Lactic Acid Bacteria

Isolation of microbes intended to have a single culture of lactic acid bacteria with properties that we expect. Isolation method performed by pour plate method that dilution were previously performed and taking the 4 last series of dilution, then diplating into the jelly growth medium for lactic acid bacteria which previously added by CaCO3 1% so the clear area is clearly seen. According Rodroguesz et al. (2000) insavadogo et al. (2006) Lactic acid bacteria found naturally in some raw materials. Insulation is a phase of

separation of microbial microbial mixture so as to obtain the pure microbial (Hadioetomo,1990).

The results obtain 30 isolates of isolation and to see the antibacterial activity of the bacteriocin then the initial screening is performed. Screening is done by scraping the suspected producing bacteriocins isolates onto solid media has been inoculated by bacteria *S.aureus*. Initial screening results 15 isolates were thought to have potential as a producer of bacteriocin. The next test is to use the supernatant which is neutralized and was only a few isolates have the potential to produce bacteriocins.

Bacteriocin Activity Resistance Against Enzymes Protease

The Presence of protease enzyme causes constituent protein of bacteriocin will be degraded so that the bacteriocin does not contain an inhibitory activity. This test is done in a way that the supernatant produces clear zone sinks around the added protease enzyme. Based on the observation of two isolates the isolates LB.9 and LB.30 that does not produce clear zones around the wells (Table 1). By Tahara et al. (1996) bacteriocins are protein compounds that are released by bacteria that inhibit the growth of other bacteria that have a particularly strong kinship with the producing bacteria. These compounds are easily degraded by proteolytic enzymes in the digestive tract of humans and animals.

Table 1. Result Bacteriocin Activity resistance against Enzymes Protease

Isolat	Clear Zone
LB.1	+
LB.7	+
LB.9	-
LB.10	+
LB.11	+
LB.25	+
LB.30	-

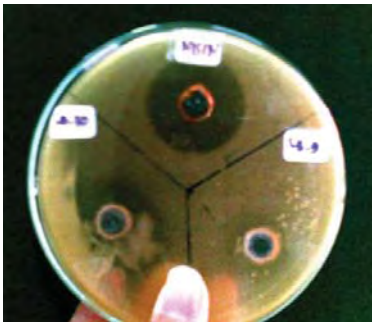
Note: + produces clear zone, - not produce clear zones

Bacteriocin Activity Resistance Against Heat

The observation shows that isolates LB.30 produce clear zone around the wells, which showed that the isolates are heat stable, whereas the isolates LB.9 did not produce clear zone around the wells.The absence of clear zone showed that the isolates LB.9 is unstable to heat. The stability of the bacteriocin to heat associated with the molecular bacteriocins weight. Some bacteriocins from BAL which have relative small molecular

size are usually stable to heat (Jimenez-Diaz et al., 1993). Meanwhile, according to Ray (1992) that short-chain peptide bacteriocins are stable to heat. Another suggestion that the cysteine amino acid is able to maintain the structure of bacteriocin structure of the heating process. According to Tahara (1996), high molecular weight bacteriocins can not stand the heat. This is related to the cohesiveness of its constituent molecules.

Klaenhammer (1988) in Rahayu, et al (2004) describes the general properties found in bacteria bacteriocins produced by lactic acid bacteria that is sensitive to protease, stable to heat, and bactericidal with a narrow spectrum is inhibitors



Picture 1. Bacteriocin activity resistance against heat

Note: LB.9 (Not clear zone)

LB.30(Produce clear zone)

Bacteriocin Inhibitors Spectrum

To determine the inhibitory spectrum of bacteriocin, S.aureus bacteria is used to represent positive Gram and E. coli to represent negative Gram. Inhibitory activity against isolates microbial indicators from palm sap indicated the inhibition zone diameter is formed. The greater the inhibition zone which is formed, the greater the inhibitory activity of microbial indicators by the isolates. The results showed the two isolates (LB.9 and LB.30) can inhibit the growth of bacteria *S.aureus* and *E.coli* (Table2).

Negative Gram bacteria generally more resistant to the bacteriocin.This is caused by the outer membrane of negative Gram acts as a protective agent of bacterial cells, especially in the presence of the molecule lipopolysaccharide (LPS) on the outer membrane leading to cell resistance from various substances Alakomi et al (2006). However, in this study, showed that the Gram negative can be inhibited by the bacteriocin.

Table 2. Resistance to indicator bacterium

Sample	Clear Zona (cm)	
	S.aureus	*E.coli*
LB.9	0,7	0,5
LB.30	0,8	0,6

Conclusion

The conclusion of this research is the bacteriocin produced by isolates LB.9 is sensitive to protease enzymes and unstable to heat, while the bacteriocin produced by isolates LB.30 is sensitive to protease enzymes and heat stable, The bacteriocins that produced by isolates LB.9 and LB.30 have inhibitory against the Gram positive and Gram negative bacteria.

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