



Antagonistic activity of *Pediococcus* isolated from bakasang againts *Pseudomonas fluorescens* (producing-histamine bacteria)

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Abstract

Pediococcus as a member of Lactic acid bacteria (LAB) was known as potential to improving quality and safety of food through natural inhibition against harmful flora that are producing-histamine and serves as a food preservative. In the process of fermentation bakasang, *Pediococcus* will produce lactic acid and through the process of carbohydrate metabolism that can inhibit the growth other bacteria. In addition to lactic acid, *Pediococcus* is able to produce antimicrobial components such as hydrogen peroxide, diacetyl and bacteriocin. The objective of the research are isolated *Pediococcus* on bakasang (fermented Cakalang fish) and test the antagonistic activity against *Pseudomonas fluorescens* (producing-histamine bacteria) by using well-diffusion method. The indicator bacteria used was *Pseudomonas fluorescens* FNCC 0070.

Result of isolation on bakasang obtained 15 of which 9 isolate were considered to be LAB as determined by culture on MRS agar, gram stain appearance, catalase test, spore-forming, motility and gas production from glucose. The LAB isolates were characterized further to the genus level and the results showed that 9 isolates LAB were classified into the genus *Pediococcus*. In general, *Pediococcus* isolates having antagonistic activity against *Pseudomonas fluorescens* (producing-histamine bacteria). Isolate B5.1 has highest diameter of inhibition zones (15 mm).

Keywords: Bakasang, *Pediococcus*, antagonistic activity, LAB

Introduction

In Indonesia, there are many types of traditional fermented food made of various food product such as fruits and vegetables (Trias et al., 2008), milk, meat and fish (Tanasupawat, 2009). Bakasang is fermented fish products traditionally made from the guts of big fish (*Katsuwonus pelamis* L.), small fish and fish eggs which is the typical food of North Sulawesi (Manado). In fermented food fish, it turns out Lactic Acid Bacteria (LAB) is a bacterium which has an important role in the process of fermentation. One role of lactic acid bacteria that produce antimicrobial components that can inhibit the growth of pathogenic bacteria and spoilage bacteria. Components that are antagonistic in the form of an organic acid, hydrogen peroxide, and

bacteriocin diacetyl (Daeschel, 1989; Danil, 1995; De Vuyst, L. dan E. J. Vandamme; Park et al., 2005; Savadogo et al., 2004). Natural antimicrobial produced by microbes especially lactic acid bacteria have been widely used as chemotherapeutic agents that can control the growth of pathogens mikorbia (Ogunbanwo, 2005). LAB including microorganisms safe when added to food because its not toxic and does not produce toxin, so-called food-grade microorganisms and are designated as "Generally Recognized as Safe" (GRAS). These microorganism is not at risk to health, even some types of bacteria are useful for health. In Indonesia, has been widely reported research results that reveal the potential of

lactic acid bacteria as producers of antimicrobial substances from fermented foods (De Vuyst and Vandamme, 1994; Rahayu, 2000; Rahatu and Eka, 1999). Lactic acid bacteria have 12 genera namely *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Axelsson, 2004).

Pediococcus is the dominant bacteria during fermentation process of Bakasang (Lawalata, 2015; Ijong and Ohta, 1996). The objectives of the research are isolated *Pediococcus* on bakasang (fermented Cakalang fish) and test the antagonistic activity against *Pseudomonas fluorescens* (producing-histamine bacteria) by using well-diffusion method.

Materials and Methods

Indicator Strains and Growth Conditions

Pseudomonas fluorescens FNCC 0070. These strain was grown in NA (Nutrient Agar) at 37°C.

Pediococcus acidilactici PAF 11 (positive control) was grown in De Man Rogosa Sharpe (MRS) at 37° C.

Isolation of Lactic Acid Bacteria

Guts of Big fish and egg fish were collected from market in Manado city, these samples were transported to the laboratory using cool box (4°C). They were cut into small pieces and mashed. Salt was added and rise was also added and mix thoroughly. The mixture was packed into bottles, corked and then incubated at 37°C for 7 days. LAB were isolated from sample bakasang. 10 g samples were taken aseptically and homogenized in 90 ml of NaCl solution. Serial dilutions up to 10⁻⁷ were prepared and appropriate dilutions were plated onto deMan Rogosa and Sharpe Agar supplemented with CaCO₃1%, Na Azida and Syclo-hexamide. All plates were incubated at 37°C for 48 hours. Only lactic acid producing bacterial colonies were selected. This can be observed from clear zones around the colonies which indicated the dissolving of CaCO₃ by an acid. Colonies with different morphology were counted, picked up and purified by restreaking on the same medium.

Cell morphology, Gram staining and catalase test, motility, non-spore forming were performed as a preliminary screening for lactic acid bacteria.

The selected lactic acid bacteria were maintained as stock cultures at -80 °C in 10% skim milk and 20% glycerol.

Identification of *Pediococcus*

The isolated LAB strains showing antimicrobial activity were identified based on profile matching method by cell shape coccus, cell arrangement tetrad, production gas from glucose, spore formation, catalase and motility.

Screening of *Pediococcus* isolate for Antimicrobial Activity

The antimicrobial activity of *Pediococcus* (Culture) against *P. fluorescens* FNCC 0070 was performed by the well diffusion assay. *Pediococcus* culture was grown in MRS broth at 37 °C for 24 hours. Indicator bacteria was grown in Nutrient Broth at 37° C for 24 hours. 10 ml of Nutrient soft agar inoculated with 50 µl broth culture of producing-histamin bacteria (*P. fluorescens* FNCC 0070). MRS hard agar poured on petri dish and allow to solidify, then overlaid with nutrient broth were prepared previously and then in place at a temperature of 4 °C for 1 hour. Wells were made and filled with 50 µl *Pediococcus* culture. Incubation petri dish at 37° C for 24 hours. LAB isolates which gave clear zones are isolates that have antimicrobial activity against indicator bacteria. The diameter of the inhibition zone was measured. The antimicrobial activity was determined by measuring the clear zone around the wells.

Results and Discussion

Isolation of Lactic Acid Bacteria

Sample of bakasang were used for isolation of lactic acid bacteria. 15 isolates of LAB in which production clear zone around their colonies were obtained from bakasang. The clear zone appearance is due to the dissolution of CaCO₃ on MRS medium by acid agent. Among the 15 isolates were rearrange and confirmed as LAB in amount of 9 isolates. All these isolates were gram positive, cocci, appeared tetrad. Cell were non motile and non sporing, they gave negative reaction for catalase. These strains were then classified into genus level using profile matching method. Based on the profile matching method that 9 isolates were represented as cocci (tetrad) homofermentative which were identified as genus *Pediococcus* (Table 1).

Table 1. Identification genera level (Generic Assignment) BAL isoletes BAL from Bakasang by profile matching metode

Characteristics	<i>Lactobacillus</i>	<i>Pediococcus</i>	<i>Leuconostoc</i>	<i>Enterococcus/ Streptococcus</i>	I ^a
Number of Isolates					9
Gram stain	+	+	+	+	+
Shape of cell :					
Rod	+	-	-	-	-
Coccus	-	+	+	+	+
Cell arrangement (tetrad)	-	+	-	-	+
Production gas from glucose	+/-	-	+	-	-
Catalase	-	-	-	-	-
Spore formation	-	-	-	-	-
Motility	-	-	-	-	-

*Key characters description of genus *Lactobacillus*, *Enterococcus/Streptococcus*, *Leuconostoc* dan *Pediococcus* by Bergey's manual Systematics of Bacteriology (Sneath et al., 1987).^a*Pediococcus*

Screening of *Pediococcus* for Anti-microbial Activity

The antimicrobial activity of *Pediococcus* isolates (culture) were tested against producing-histamine bacteria (*P.fluorescens*) are summarized in (Table 2) by using agar well diffusion assay, illustrate the zones of inhibition against pathogenic bacteria and spoilage bacteria under study. The diameters of the inhibition zones were varied it ranged between 3.0 to 15.0 mm. In general, LAB have inhibitory activity against producing-histamine bacteria (*P.fluorescens*). Isolate B5.1 has the highest diameter of inhibition zones (15

mm). This revealed that the LAB (*Pediococcus*) inhibited all the producing-histamine bacteria tested according to (Santoso et al., 1999) who mentioned that inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger. Similar study was carried out in Morocco by Kalalou who studied the activity of LAB on some gram positive and negative pathogenic bacteria such as *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus cereus* and the inhibition zones were in the range of 1.4 to 2.8 cm (Kalalou et al., 2004).

Tabel 2. Antimicrobial Activity of *Pediococcus* Culture by Wells Diffusion Assay

No	Isolate Code	Indicator Bacteria (50µl)	
		<i>P.fluorescens</i> FNCC 0070 (mm)	
		K	SA
1	B1.0*	14,0	11,0
2	B2.0	9,0	9,0
3	B3.0	10,0	11,0
4	B3.1	5,5	3,0
5	B4.1	6,5	4,0
6	B5.1*	15,0	13,0
7	B2.3	-	-
8	B3.3	7,0	5,0
9	B4.3*	12,0	11,0

Many studies were carried out in Nigeria, Adeskan in 2008 using poultry meat to isolate LAB and study its antimicrobial activity against several microorganisms. The results showed that LAB inhibited *Staph. aureus*, *E. coli*, *Pseudomonas aeruginosa* with the exception of *Candida albicans* and *Proteus vulgaris* (Adeskan et al., 2008).

Selection of *Pediococcus* isolates based on inhibitory on growth of producing-histamine bacteria (*P. fluorescens* FNCC 0070) showed that overall isolates of *Pediococcus* can inhibit the growth of test bacteria but there is one isolate of *Pediococcus* (B2.3) that are unable to inhibit the growth of *P. fluorescens* FNCC 0070. The inability of *Pediococcus* isolates (B2.3) inhibiting the growth of test bacteria because each test bacterium has sensitivity and resistance to antimicrobial compounds. This is in accordance with research conducted by Tadesse et al. (2005) indicating that the bacteria *St. aureus* is sensitive to antimicrobial compounds produced by *Pediococcus* isolates while *E. coli* is less sensitive to antimicrobial compounds produced by isolates belonging to the genus *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus*.

Generally the antimicrobial components produced by LAB can inhibit the growth of gram-positive bacteria and gram-negative (De Vuyst and Vandamme, 1994) and the same was stated by (Rahayu and Ekasari, 1999). The activity of inhibition variety of bacteria by LAB due to a combination of many factors produced by LAB e.g. production of lactic acid which reduce pH of bakasang and also other inhibitory substances such as bacteriocins which are responsible for the most antimicrobial activity (Ogunbanwo, 2005). Produced organic acids give the antimicrobial effect directly by lowering the pH of the environment so it is unfavorable for the growth of other bacteria, and besides it can also affect the permeability of the bacterial cell membrane so that the substrate transport system becomes disturbed (Yang, 2000). However, other antimicrobial compounds other than organic acids may also play a role in the antimicrobial effects that occur because the antimicrobial compounds produced by *Pediococcus* work together synergistically in giving antimicrobial effects even though their mechanism of action is not yet known (Ouwehand, 1998).

Conclusion

The results were obtained 9 isolates of lactic acid bacteria which can be grouped into the *Pediococcus*. In general, isolates of lactic acid bacteria (*Pediococcus*) have inhibitory activity against the growth of producing-histamine bacteria. Isolate B5.1 has the highest antimicrobial activity.

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