Antimicrobial activity of lactic acid bacteria isolated from bekasam against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella* sp.

Melia Sari, Dwi Suryanto*, Yurnaliza

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sumatera Utara, Jl. Bioteknologi No. 1, Kampus USU, Medan 20155

*Email: dwisuryanto@usu.ac.id

Abstract. Bekasam is an Indonesian fermented food made of fish. As a fermented food, this food may contain some beneficial bacteria like lactic acid bacteria (LAB), which usually have antimicrobial properties such as organic acid, hydrogen peroxide, and a bacteriocin. A study on antimicrobial activity of LAB isolated from bekasam against some pathogenic bacteria has been conducted. The purpose of this study was to know the ability of crude bacteriocin produced LAB of bekasam against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella* sp. Bekasam sample was taken from South Sumatera. LAB isolation was done using de Man Rogosa and Sharpe agar. A bacterial colony with clear zone was selected and purified to get a single colony. The antagonistic assay of the LAB was conducted in Muller-Hinton agar. Selected isolates with higher clearing zone were assayed for antibacterial effect of their crude bacteriocin of different culture incubation time of 6, 9, and 12 hours. The results showed that the crude extract bacteriocin of isolate MS2 of 9 hours culture incubation time inhibited more in *Staphylococcus aureus* ATCC 25923 with inhibition zone of 13.1 mm, whereas isolate MS9 of 9 hours culture incubation time inhibited more in *Escherichia coli* ATCC 25922 and *Salmonella* sp. with inhibition zone of 12.7 and 7.3 mm, respectively.

Keywords: crude extract bacteriocin, inhibition zone

1. Introduction

“Bekasam” (traditional food) is an Indonesian fermented food made of fish. Bekasam is widely known in the areas of Southern Sumatera and Kalimantan, especially Central Kalimantan. Bekasam is produced by mixing freshwater fish mixed with salt, rice, or fermented tape as a source of carbohydrate. This usually takes 4-10 fermentation days [1]. It has been known that fermented food is often associated with special benefit fermenting bacteria such as a LAB. LAB produced antibacterial substances which is important in the utilization LAB as probiotics agent and food preservative. It is related to the production of metabolites such as organic acids, hydrogen peroxide, diacetyl, and bacteriocin [2]. Bacteriocin is one of the
antimicrobial metabolites produced by the LAB.

Bacteriocin can be safely consumed and be applied as food preservative. It can be degraded by proteolytic enzyme in human digestion, so no harm to human health. It was known that bacteriocin has antagonistic properties against some pathogenic bacteria such as *Listeria*, *Clostridium*, *Staphylococcus*, *Bacillus* spp., *Brochotrix*, *Aeromonas*, and *Vibrio* spp. [3]. Crude extract bacteriocin inhibited the *E. coli* and *S. aureus* [4].

Many bacteria produce bacteriocin. Gram-negative bacteria produce a relatively narrow inhibiting bacteriocin, while Gram-positive bacteria produce relatively broad inhibiting bacteriocin [5]. Many bacteriocins showed stable activity to the temperature of -20 to 100ºC, while it might be sensitive to pH. Bacteria may produce bacteriocin in synthetic media such as de Man Rogosa and Sharpe broth (MRS broth), Tryptone Glucose Extract Yeast (TGE), or other synthetic media. However, MRS broth is a common one [6].

Bacteriocin is potentially used in the control of contaminant bacteria in the food industry, but the availability of bacteriocin is very limited and the price is very high, therefore to find LAB bacteriocin producers from traditional fermented food like bekasam is still necessary.

2. Methods
2.1. Isolation and characterization of LAB from bekasam
Isolation of LAB of bekasam from South Sumatra was conducted in de Man Rogosa and Sharpe (MRS) agar added with 1% CaCO₃. The culture was incubated at 37°C for 24-48 hours. Different colonies growing on agar were purified. The colony was characterized by its shape and color. Selected isolates after assay antibacterial of crude extract bacteriocin were characterized for gram staining, motility test, catalase, and sugar fermentation on triple sugar iron agar.

2.2. Antagonistic assay of LAB to three pathogenic bacteria
Antagonistic assay was done on Muller Hinton agar. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Salmonella* sp. was sub-cultured in nutrient agar. All bacterial cultures were diluted with 0.9% NaCl to 0.5 MacFarland. A bacterial suspension of pathogenic bacteria was swab on Mueller-Hinton agar (MHA) using sterile cotton bud. A-10 µl suspension of the LAB was dropped on the blank disc. The blank disc was placed on the bacterial lawn on MHA. The culture was incubated at 37°C for 24 hours. The diameter of inhibition zone was measured using calipers.

2.3. Assay antibacterial of crude extract bacteriocin
Selected LAB isolates were sub-cultured in MRS broth at 37°C for 6, 9, and 12 hours. The culture was adjusted to pH 6.0 to inactivate antimicrobial activity of organic acids. The culture was spin at 10.000 rpm for 15 minutes and subjected to pH re-adjusted to 4.0 by using HCl 1N to release bacteriocin from the cell prior kept into the refrigerator at 4°C for 24 hours [7]. The cool suspension was filtered using millipore 0.20 µm [4]. Assay antibacterial of crude extract bacteriocin was conducted as previously described in the antagonistic assay of a LAB to some pathogenic bacteria but using filtered supernatant.

3. Results and Discussion
3.1. Isolation and characterization of LAB
Eleven isolates were obtained in MRS agar with different colony characteristics. Clear zone produced by the colony indicated that it was LAB colony [8,9,10]. LAB produces lactic acid and reacts with CaCO₃ to produce soluble lactate calcium, characterized by a clear zone around the growing bacterial colonies [11,12]. Two selected isolates MS2 and MS9 were further characterized for their cell morphology and biochemistry (Table 1).
Table 1. Colony and cell characterization of LAB isolates

| Isolates | Colony shape | Colony edge | Colony elevation | Colony color | Gram | Catalase | Motility | Oxidase | TSIA | Ferment | Gam | Slant | Butt | Gas | H2S | TSIA |
|----------|--------------|-------------|------------------|-------------|------|----------|----------|---------|------|---------|------|-------|------|-----|-----|-------|
| MS1      | circular     | Flat        | convex            | white       |      |          |          |         |      |         |      |       |      |     |     |       |
| MS2      | circular     | Wavy        | convex            | white       | +    | -        | -        | -       | yellow | yellow | -    | -     |      |     |     | Hetero|
| MS3      | circular     | Irregular   | flat              | white       |      |          |          |         |       |         |      |       |      |     |     |       |
| MS4      | circular     | Flat        | convex            | white       |      |          |          |         |       |         |      |       |      |     |     |       |
| MS5      | circular     | Wavy        | flat              | white       |      |          |          |         |       |         |      |       |      |     |     |       |
| MS6      | circular     | Flat        | convex            | white       |      |          |          |         |       |         |      |       |      |     |     |       |
| MS7      | circular     | Flat        | flat              | white       |      |          |          |         |       |         |      |       |      |     |     |       |
| MS8      | circular     | Flat        | convex            | white       |      |          |          |         |       |         |      |       |      |     |     |       |
| MS9      | circular     | Flat        | convex            | creamy      | +    | -        | -        | -       | yellow | yellow | -    | -     |      |     |     | Hetero|
| MS10     | circular     | Flat        | convex            | white       |      |          |          |         |       |         |      |       |      |     |     |       |
| MS11     | circular     | Wavy        | convex            | white       |      |          |          |         |       |         |      |       |      |     |     |       |

3.2. Antagonistic assay of LAB to three pathogenic bacteria

Assay on antimicrobial activity of LAB against three pathogenic bacteria showed that the LAB isolates were capable to inhibiting the pathogen to some extent (Figure 1). This indicated that LABs produced antibacterial metabolites such as organic acids and bacteriocin during growth [13]. Isolates MS2, MS4, MS8, and MS9 showed to inhibit more (Figure 2). All four isolates showed relatively high inhibition zone [14].

Figure 1. MS9 showed to inhibit (a). *S. aureus*, (b). *E. coli*, and (c). *Salmonella* sp.

MS9 showed to inhibit more to *S. aureus*, *E. coli*, and *Salmonella* sp. with an inhibition zone of 18.1, 9.6, and 7.5 mm, respectively which was higher compared to that of Desnjar et al.(2012) and Indriati et al.(2006) studies [15][12]. However, it was lower in inhibiting *E. coli* compared to that of Nigam et al. [16] study.
3.3. Assay antibacterial of crude extract bacteriocin
In general, bacteriocin is produced optimally by a LAB in the logarithmic until the stationary phase [17]. In this study, there was a variation of incubation time of LAB culture. This was to determine the optimum producing time of bacteriocin of our LAB isolates. It was shown that supernatant of potential LAB culture inhibited all pathogenic bacteria of all incubated time of LAB, especially of 9 hours incubation time (Figure 3.). MS2 and MS9 supernatant inhibited more to three pathogenic bacteria compared to other two isolates of MS4 and MS8. Unlike others, MS9 inhibited more to *E. coli*. This indicated that bacteriocin produced by the isolates might different because bacteriocin has a specific binding site to the target bacteria, types of bacteriocin and bacteriocin concentrations used [18].

**Figure 2.** Diameter of inhibition zone of LAB to three pathogenic bacteria
Figure 3. The diameter of inhibition zone of crude extract of bacteriocin of different incubation time to three pathogenic bacteria

Bacteriocin attacks cytoplasmic membrane of pathogenic bacteria through the formation of cytoplasmic membrane pores [19,20], penetrating cell membrane thus increasing the permeability of the cytoplasmic membrane or inhibiting septal formation. The formation of pores in the cell membrane stimulates membrane permeability and disrupts intracellular ADP/ATP balance due to inorganic phosphate leakage, in turn reduce the power of the proton [21] and the number of bivalent cation (Mg$^{2+}$ or Ca$^{2+}$) causing neutralization of phospholipid negative charges [22], increases permeation of the ion (K$^+$ and Mg$^{2+}$), amino acid (glutamate and lysine acid), and ATP. The failure of proton motive force mechanism causes cell death through the cessation of all reactions that require energy [23].

4. Conclusions

Eleven LAB isolates were isolated from bekasam. Of that four isolates MS2, MS4, MS8, and MS9 showed to inhibit more to three pathogenic bacteria $S. aureus$, $E. coli$, and $Salmonella$ sp. However, crude bacteriocin of isolates MS2 and MS9 inhibited more to the pathogens, especially in 9 hours incubation time. The bacteriocin produced by our LAB isolates might differ since it showed to inhibit differently to pathogenic bacteria species.

Acknowledgement

Special thanks to my parents for providing a scholarship and research fund, and Yenni Veronika for providing bekasam sample.
References

[1] Desniar R, Iman A, Suwanto A, Mubarik NR (2011) Aktivitas bakteriosin dari bakteri asam laktat asal bekasam. Jurnal Pengolahan Hasil Perikanan Indonesia. XIV(2): 124-133.

[2] Gálves A, H Abriouel R, L López, and NB Omar (2007) Bacteriocins-based strategies for food biopreservation. Int.J.Food Microbio. 120: 5

[3] Brillet A, Pilet MF, Prevost H, Cardinal M, Leroi F (2005) Effect of inoculation of Carnobacterium divergens V41, a biopreservative strain against Listeria monocytogenes risk, on the microbiological, and sensory quality of cold-smoked salmon. Int.J.Food Microbial. 104:309-324.

[4] Abubakar and Arpah M (2015) Pengaruh suhu produksi terhadap aktivitas ekstrak kasar bakteriosin dari berbagai galur Lactobacillus sp. dalam menghambat Escherichia coli dan Staphylococcus aureus. Buletin Peternakan. 39(3): 189-198.

[5] De Vuyst L, and F Leroy (2007) Bacteriocins from lactic acid bacteria: production, purification, and food applications. J. Molecular Microbiol. Biotechnol. 13:194-199.

[6] Todorov SD, and LMT Dicks (2004) Influence of growth conditions on the production of a bacteriocin by Lactobacillus lactis subp. Lactis ST34BR, a strain isolated from barley beer. J.Basic Microbial. 44: 305-316.

[7] Usmiati S dan WP Rahayu (2011) Aktivitas hambat bubuk ekstrak bakteriosin dari Lactobacillus sp. galur SCG 1223. Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner, Puslitbang.

[8] Chen YS, Wu HC, Yanagida F (2010) Isolation and Characteristics of Lactic Acid Bacteria Isolated from Ripe Mulberrien In Taiwan. Brazilian Journal of Microbiology. 41: 916-921.

[9] Djie MN, dan Sartini (2008) Isolasi, Identifikasi Bakteri Asam Laktat dari Kol Brassica oleracea L. dan Potensinya sebagai Antagonis Vibrio harveyi in vitro. Torani. 18(3): 211-216.

[10] Lawalata HJ, and Satiman U (2015) Identification of Lactic Acid Bacteria Proteolytic Isolated from An Indonesia Traditional Fermented Fish Sauce Bakasang by Amplified Ribosomal DNA Restriction Analysis (ARDRA). International Journal of ChemTech Research. 8(12) : 630-636.

[11] Indriati N, Setiawan, IP, dan Yulneriwarni (2006) Potensi antibacterial bakteri asam laktat dari peda, jambal roti, dan bekasam. Jurnal Perikanan VIII (2): 153-159.

[12] Romadhon, Subagiyto, Margino S (2012) Isolasi dan Karakterisasi Bakteri Asam Laktat dari Usus Udang Penghasil Bakteriosin sebagai Agen Antibakteria pada Produk-produk Hasil Perikanan. Jurnal Saintek Perikanan. 8 (1): 59-64.

[13] Theron MM, and Lues JFR (2011) Organic Acids and Food Preservation. United State: CRC Press. Hlm: 273.

[14] Pan J, Magoulès F, and Biannic YL (2009) Executing multiple group by query in a mapreduce approach. In ICDB and ICCSNA. Hongkong.

[15] Desniar, Rusmana I, Suwanto A, and Mubarik NR (2012) Senyawa antimikroba yang dihasilkan oleh bakteri asam laktat asal bekasam. Jurnal Akuatika. III No.2.

[16] Pan J, Magoulès F, and Biannic YL (2009) Executing multiple group by query in a mapreduce approach. In ICDB and ICCSNA. Hongkong.

[17] Khoiriyah, H, Ardiningsih P, Jayuska A (2014) Penentuan Waktu Inkubasi Optimum Terhadap Aktivitas Bakteriosin Lactobacillus sp. RED4. JKK. 3(1): 7-11.

[18] Parada JS, Caron CR, Medeiros ABP, and Soccol CR (2007) Bacteriocins from Lactic Acid Bacteria: Purification, Properties and use as Biopreservatives. Brazilian Archives of Biology and Technology. 50(3): 521-542.

[19] Chen H and DG Hoover (2003) Bacteriocins and their food application. Comprehensive reviews in Food Science and Food Safety. 2: 82-100.
[20] Sablon E, B Contreras, and E Vandamme (2000) Antimicrobial peptides of lactic acid bacteria: Mode of action, genetics and biosynthesis. Adv. Biochem Eng Biothenol. 68: 21-60.

[21] Eijsink VGH, L Axelsson, DB Diep, LS Havarstein, H Holo, and IF Nes (2002) Production of class II bacteriocins by lactic acid bacteria; an example of biological welfare and communication. Antonie van Leeuwenhoek. 81: 639-654.

[22] Cleveland J, TJ Montville, IF Nes, ML Chikindas (2001) Bacteriocins: safe, natural antimicrobials for food preservation. Intern. J. Food Microbial. 71: 1-20.

[23] Gajić O (2003) Relationships between MDR proteins, bacteriocin production and proteolysis in Lactococcus lactis. Dissertation University of Groningen. Netherlands.