BIODIVERSITAS
Volume 24, Number 5, May 2023
Pages: 2830-2835

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240536

Bacteriocin-producing *Pediococcus acidilactici* BAMA 15 isolated from "*naniura*" traditional foods in North Sumatra, Indonesia

SUBANG AINI NASUTION[1,2,♥], EDY FACHRIAL[2], CHRISMIS NOVALINDA GINTING[2], LIENA[2,♥♥], FINNA PISKA[3]

[1]Doctoral Program of Medicine, Faculty of Medicine, Universitas Prima Indonesia. Jl. Sampul No. 4, Kampus 5, Medan 20118, North Sumatra, Indonesia. Tel./Fax.: +6261-457-8890, ♥email: subang_4aini@yahoo.com

[2]Faculty of Medicine, Universitas Prima Indonesia. Jl. Sampul No. 4, Kampus 5, Medan 20118, North Sumatra, Indonesia. Tel./Fax.: +62-61-457-8890, ♥♥email: liena@gmail.com

[3]Department of Pharmacy, Universitas Prima Indonesia. Jl. Sampul No. 4, Kampus 5, Medan 20118, North Sumatra, Indonesia

Manuscript received: 13 February 2023. Revision accepted: 23 May 2023.

Abstract. *Nasution SA, Fachrial E, Ginting CN, Liena, Piska F. 2023. Bacteriocin-producing* Pediococcus acidilactici *BAMA 15 isolated from "*naniura*" traditional foods in North Sumatra, Indonesia. Biodiversitas 24: 2830-2835.* Lactic Acid Bacteria (LAB) have beneficial properties for their hosts, potentially promoting health and balancing the normal gut flora. Meanwhile, bacteriocin is a bacteriostatic polypeptide used as a food preservative. LAB are generally produced from fermented products, such as dengke *naniura*, a typical Toba Batak food. Therefore, it is estimated that the LAB present in this food has the potential as a probiotic and produces bacteriocin, an antimicrobial polypeptide compound. The purpose of this study was to identify the LAB species found in dengke *naniura* and to characterize the bacteriocin generated by them. The first study stages included the isolation and biochemical and morphological characterization of LAB. Nine LAB isolates (BAMA 10-11, BAMA 15-20, and BAMA 25) were isolated from the samples. The best strain for use as a probiotic was *Pediococcus acidilactici* BAMA 15 because its antimicrobial activity against *E. coli* and *S. aureus* was 6.44 and 7.53 mm, respectively. The bacteriocin produced has a molecular weight estimated to be ~12 kDa.

Keywords: Acid, bacteriocins, bile salt, catalase, LAB, molecular

INTRODUCTION

Lactic Acid Bacteria (LAB) are found in various food products, specifically those based on fermentation. These bacteria are also the microbiota in the intestines of humans and animals. LAB are classified as safe microorganisms to be added to food because they are not toxic and do not produce toxins (Lawalata et al. 2015). LAB, naturally occurring in meats, dairy products, and vegetables, are well known for their role in food fermentation and are also often bacteriocin producers (Ünlü et al. 2015). Antibacterial efficacy of lactic acid bacteria and bacteriocin were isolated from Dadih's against *Staphylococcus aureus* (Pato et al. 2020).

LAB, with antimicrobial properties, has been commonly associated with food. Therefore, using LAB strains as probiotics and bioprotective culture in fermented products has also been widely studied. According to the Food and Agriculture Organization and the World Health Organization (FAO/WHO) definition, probiotics are living microorganisms that confer a health benefit on the host when administered in adequate amounts. The preservative effect of these bacteria is due to the production of one or more active metabolites, such as organic acids (lactic, acetic, formic, propionic and butyric acids), that intensify their action by reducing the pH of the media, and other substances, such as ethanol, fatty acids, antifungal compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate, cyclic dipeptides and 3-hydroxy fatty acids), hydrogen peroxide, diacetyl, acetoin, bacteriocins (nisin, reuterin, reutericyclin, pediocin, lacticin, enterocin and others) and Bacteriocin-Like Inhibitory Substances (BLIS) (Davani-Davari et al. 2019; Dinoto et al. 2020).

The probiotic LAB could be present in the spontaneous fermentation of different food, and this group is generally recognized as safe. LAB with *Pediococcus* strain has been isolated by Gutiérrez-Cortés et al. (2018) from Minas cheese and Pato et al. (2020) from *dadih*. One of the researchers' interest in LAB is due to the ability of LAB to produce bacteriocins as antimicrobials. Exploration of new and effective antimicrobial compounds is increasing over time, so an alternative is to utilize bacteriocins isolated from LAB. Dina et al. (2013) have investigated the ability of *P. acidilactici* to produce bacteriocins in cheese. The ability of *Pediococcus* strains to produce bacteriocins has been investigated by Gutiérrez-Cortés et al. (2018) in Mina's cheese media against *Listeria monocytogenes*. LAB isolated from fermented products such as yogurt, curd, cheese, *dangke*, and *dadih* can produce bacteriocins (Mohammed and Ijah 2013; Pei et al. 2018; Jatmiko et al. 2019; Pato et al. 2020). The benefits of bacteriocins have been studied by Simha et al. (2012), namely as a milk biopreservative.

Bacteriocins are extracellular protein compounds secreted by LAB and have bactericidal activity against different pathogens (Fatimah et al. 2020). Bacteriocins can be produced from Gram-positive and Gram-negative bacteria (Abbasiliasi et al. 2017). Bacteriocins produced from LAB isolated from various sources have increased recently, especially their applications in health science and

the food industry. Bacteriocins produced by LAB can be antibacterial against resistant bacterial strains such as Multi-Drug Resistant (MDR) *Staphylococcus aureus*, which provides great inhibition with an inhibition zone of 8.0 mm (Francois et al. 2013). *Pediococcus acidilactici* PFC69 inhibited the growth of *Bacillus aureus* and *S. aureus* during the fermentation process and was found to be stable under high temperature and low pH conditions (Kaya and Şimşek 2020). LAB, as probiotics, can grow in the human gut and stomach to protect from pathogens that can cause disease. Probiotics are categorized as microorganisms that can benefit human health (Manik et al. 2020). The applications of bacteriocin from LAB in dairy products have demonstrated effectiveness against pathogenic bacteria such as *S. aureus*, *Escherichia coli*, and *Salmonella* spp. (Reis et al. 2012). The inhibitory activity of crude bacteriocin-produced lactic acid bacteria isolated from *dadih* against Listeria monocytogenes has been studied (Pato et al. 2020).

Some fermented foods are made into traditional foods, including *naniura*, which also has the effect of a probiotic or LAB. *Naniura* is a local fermented food typical of the Batak tribe in North Sumatra. *Naniura* is formed from carp fish (*Cyprinus carpio*) cooked and fermented using citrus fruit extract (*Citrus jambhiri*). This is the first study to report that probiotics isolated from *naniura* produce bacteriocin, an antimicrobial peptide. This study aimed to isolate and identify LAB from *naniura* that have potential as probiotics and determine the molecular weight of bacteriocins produced. Next, to test the ability of the isolates, their cells will be grown in acid and bile salt media; after that, molecular identification of isolates that have more potential as lactic acid bacteria and determination of the molecular weight of bacteriocins.

MATERIALS AND METHODS

Isolation of lactic acid bacteria

Naniura was obtained from a local restaurant in Medan, North Sumatra. First, 1 g of the sample was inoculated into 9 mL of sterile MRS broth in a test tube, incubated at 37°C for 24 h, and then continued with serial dilution up to 10^{-5}. Next, 0.1 mL aliquots from the last dilution were spread onto the surface of MRS agar in a petri plate and incubated in an incubator at 37°C for 48 h. Bacterial growth was observed and continued with purification by re-streaking the isolate onto new MRS agar media. The purified colonies were stored in 25% (v/v) glycerol at -20°C.

Characterization of lactic acid bacteria

Characterizations were performed on all of the strains grown for 24 h. First, gram staining with the fixation method due to LAB strains was observed with a microscope with a 100x lens. The assay used a violet solution for 1 min before being rinsed with distilled water. Next, an iodine solution was added for 1 min, then rinsed with distilled water. Next, the slide was rinsed with 96% alcohol until no more solution flowed, then with distilled water. Finally, after 45 sec, safranin solution was added and rinsed with distilled water. The glass object was dried and then observed under a microscope. Next, all strains grown for 24 h were subjected to catalase assay, and the LAB strains were prepared and transferred to a glass object and dripped with H_2O_2 solution. Next, observing air bubbles formed on the objects determined the assay's results; the test is positive once air bubbles are formed, and no air bubbles signify a negative.

Antibacterial assay of the strains

LAB strains were incubated in MRS broth at 37°C for 24 h. The method used was disk diffusion for this assay (Driscoll et al. 2012) with some modifications. LAB strains were incubated in MRS broth for 24 h, while pathogens *E. coli* and *S. aureus* were incubated in nutrient broth (Merck, Germany) for 24 h and adjusted to OD_{600nm} approximately 0.1. The cultured pathogens were spread on a nutrient agar (Merck, Germany) plate with a sterile cotton swab. Each disk paper containing inoculated LAB strains was placed on the pathogen-inoculated plate and incubated at 37°C for 24 h. Chloramphenicol (30 µg/mL) was used as the positive control, and MRS broth was the negative control. Antibacterial activity was evaluated with the formula of the Kirby and Bauer method.

Acid and bile tolerance assay

Therefore, to mimic the gut and intestines, the identified isolates' acid and bile salt survivability was assessed based on the method described by Li et al. (2020). First, the tolerance capacity under the acidic pH condition was evaluated by MRS broth was adjusted to pH 3 with HCl 1 M. Next, the tolerance capacity under bile salt was prepared in bile salt solution was designed by adding 0.3% bile acid factors (Jarrow Formula, Los Angeles) into MRS broth. The strains' cells were harvested at an exponential growth phase (18 h), inoculated in MRS broth as a regular media, acid, and bile solution, and incubated at 37°C for 4 h. The wavelength was adjusted to 600 nm, and the percentage of absorbance was calculated as follows:

Abs. of cell growth in acid/Abs. of cell growth in MRS broth x 100% (1)

The cells that survived in the bile solution were calculated by % cell growth (Li et al. 2020):

Abs. of cell growth in bile solution/Abs. of cell growth in MRS broth x 100% (2)

Molecular characterization

DNA of potential LAB isolates was isolated using the Quick-DNA Bacterial Miniprep kit with procedures based on the manufacturer's instructions. The strain was added directly to a ZR BashingBead™ Lysis Tube (0.1 and 0.5 mm) and rapidly and efficiently lysed by bead beating without using organic denaturants or proteinases. The DNA is then isolated and purified using Zymo-Spin™ Technology, making it ideal for downstream molecular-based applications such as PCR and array. Next, 0.5% (v/v) beta-mercaptoethanol was added to a final dilution of genomic lysis buffer. Bacterial cells of about 50-100 mg resuspended in up to 200 µL of water or isotonic buffer (PBS) were added to a ZR Bashing Bead™ lysis tube (0.1 and 0.5 mm). BashingBead™ buffer was added 750 µL to the tube. The ZR BashingBead™

lysis tube (0.1 and 0.5 mm) was centrifuged in a microcentrifuge at 10,000 x*g* for 1 min, and the supernatant was transferred to a 400 µL Zymo-Spin™ III-F filter.

The previous step added a genomic lysis buffer of about 1,200 µL to the filtrate in the collection tube. Next, 800 µL of the mixture was transferred to a Zymo-Spin™ IICR Column in a collection tube and centrifuged at 10,000x g for 1 min. Subsequently, the flow through was discarded from the collection tube, and the precious step was repeated. In a new collection tube, 200 µL of DNA pre-wash buffer was added to the Zymo-SpinTM IICR Column and centrifuged at 10,000x g for 1 min. Next, the Zymo-SpinTM IICR Column was loaded with 500 µL of g-DNA wash buffer and centrifuged at 10,000 x g for 1 min. Afterward, the solutions on Zymo-Spin™ was transferred to a clean 1.5 mL microcentrifuge tube, and 100 µL DNA elution buffer was added directly to the column matrix. The mixture was centrifuged at 10,000x g for 30 sec to elute the DNA. The result of one strain was mainly identified as LAB in its 16S rRNA with 27F/1492R PCR primer. PCR amplification was performed using the (2x) My Taq HS Red Mix (Bioline, BIO-25048). Its products were assessed by electrophoresis with 0.8% TBE agarose with Non-Template Control (NTC). The sequences with Bi-directional sequencing were compared with those in the NCBI Genbank database (http://www.ncbi.nlm.nih.gov/BLAST/) using BLAST (NCBI, Bethesda, MD, USA).

Isolation of bacteriocin of the strain

BAMA, which is code strain was taken by this research. BAMAstrain with high potency as LAB was inoculated in MRS medium at 37°C at 24 h. The strain was inoculated in MRS Broth for 24 h, 150 rpm, producing the bacteriocin. Lei et al. (2020) method was used to purify bacteriocin with modification. The culture was centrifuged (10,000 x g rpm, 1 min) and compared with the ammonium sulfate precipitation method for crude bacteriocin extraction. Meanwhile, the aliquot of fermentation supernatant was precipitated with ammonium sulfate at ±4°C to 40% - 80% saturation levels at ±4°C. The crude extract was collected by centrifuging the mixture (10,000 x g, 30 min) and resuspended in phosphate buffer (pH 7). This precipitate was subjected to purification through a corning to remove the salt. The extract mainly contained protein and was stored at 4°C, and its molecular weight was determined by SDS-PAGE electrophoresis. This method used 15% separating gel and 6% stacking gel by protocol (Gowans 2018) because the buffer Tris-glycerin was used for running the gel, and the coomassies blue for staining the gel. In addition, the coomassie blue gel was destained using 0.5 M NaCl for 4-5 h.

RESULTS AND DISCUSSION

Isolation and characterization of LAB

In this study, nine strains of LAB (BAMA 10-11, BAMA 15-20, and BAMA 25) were isolated from the *Naniura* traditional food of Medan, North Sumatra, Indonesia. The strains had white colonies, rounded edges, convex, small size, and soft surfaces; several assays, such as gram staining and catalase, tested all. Based on observation, it was revealed that some strains were bacilli and cocci cell shapes, pink and purple.

Antibacterial activity of strains

The ability of nine strains to produce antibacterial compounds was tested in this study by the diffusion method against pathogens *E. coli* and *S. aureus*. Disk paper was used in the petri plate for each LAB strain. The data on the antibacterial test results are shown in Table 1. The diameter values in the following table are the diameter values that were formed as a clear zone, including the diameter size of the disk paper used. Based on the antibacterial activity test results against *E. coli* and *S. aureus* from the nine strains, the BAMA 15 strain has the greatest potential to inhibit the growth of both organisms. BAMA 15 had a more stable inhibitory effect than others, which was also the result of the repeated assay.

Survival cells in acid and bile of strains

The nine LAB strains were also tested for their ability to grow in acid and bile solutions. The percentage of survival cells of BAMA 10-25 strains show in Figure 1. Among the strains with a positive gram, BAMA 15 strain had the optimal percentage of survival cells, with 17.9% in acid and 69.5% in bile.

Table 1. The average diameter of the inhibition zone was used to calculate the antibacterial activity assay results for isolates with codes BAMA 10-BAMA 25

Isolate codes	Inhibition zone against *E. coli* (mm)	Inhibition zone against *S. aureus* (mm)
BAMA 10	6.37	8.37
BAMA 11	6.91	6.99
BAMA 15	6.44	7.53
BAMA 16	6.82	7.25
BAMA 17	7.13	6.98
BAMA 18	6.98	7.08
BAMA 19	7.41	8.09
BAMA 20	6.97	8.38
BAMA 25	6.82	9.12
Chloramphenicol	26.57	12.35
MRS broth	0	0

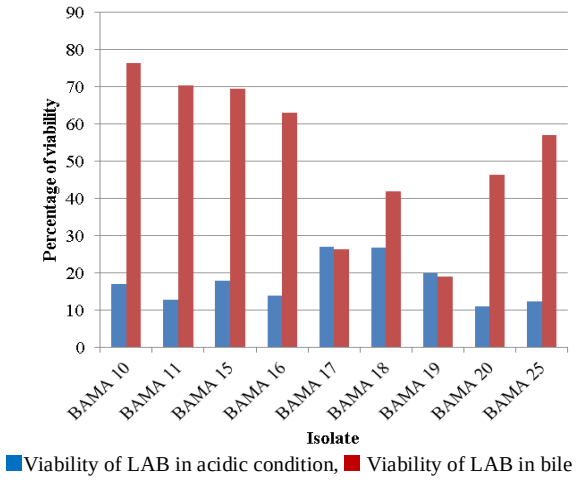

Figure 1. The percentage viability of LAB in MRS broth contains a pH of 3, and MRS broth contains bile salt of 3%

Molecular identification of BAMA 15 strain

The results of gel electrophoresis DNA (Figure 2) and the BLAST results of BAMA 15 against the NCBI database showed that the isolate belongs to *P. acidilactici*, with query cover reaching 100%. Furthermore, a percentage identity of 100% was confirmed by phylogenetic analysis, which indicates that the isolate was a bacterium with the genus *Pediococcus* (Figure 3).

Isolation of bacteriocin of the strain

Bacteriocin from *P. acidilactici* BAMA 15 strain was isolated, and the weight of its molecules was calculated by vertical electrophoresis using the SDS-PAGE gel. The molecular weight value was estimated as ~12 kDa (Figure 4).

Discussion

In this study, the LAB was isolated from *naniura*, a traditional food from North Sumatra, and yielded nine strains with probiotic-like properties. The most optimal probiotic was BAMA 15 strain. Based on the results, the survival cell growth percentage above showed that BAMA 15 strain growth is better than the others. BAMA 15 strain had a cell growth percentage in the acid of 17.9%, lower than BAMA 17, 18, and 19 strains. However, compared to the cell growth percentage in bile, BAMA 15 strain was 69.5%, which was higher than BAMA 17, 18, and 19 strains but lower than BAMA 10 and 11 strains. From the characterization and antibacterial assay results, BAMA 15 strain has more potential as LAB. It produces antibacterial compounds and growth more optimally in acid and bile, gut, and intestinal-like environments.

LAB must be resistant and able to live in acidic conditions, which is one of the probiotics characteristics. Most of the LAB grow more slowly at low pH condition and causes decreased cell growth; however, it depends on bacterial strains. Resistant to living in bile salts condition is important for LAB because it affects the LAB activity in the upper intestinal tract. The lipolytic enzymes in the pancreas react with fatty acids in the bacterial cytoplasmic membrane. Therefore, it causes permeability properties and membrane structure changes to affect resistance to bile salts. The Bile Salt Hydrolase (BSH) enzyme was related to the resistance of LAB towards bile salts reducing the toxic effects on cells (Aritonang et al. 2022).

BAMA 15 isolates were molecularly identified as *P. acidilactici*, with a similarity percentage of 99.73% from the NCBI database (Figure 5). The identity percentage value is above 97.5%, and according to Rossi-Tamisier et al. (2015), a value above 95% is at the genus level similarity. Bacteriocin produced from *P. acidilactici* BAMA 15 has a molecular weight of ~12 kDa.

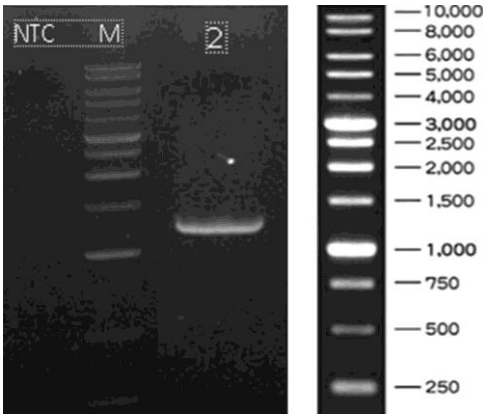

Figure 2. DNA isolated from *P. acidilactici* BAMA 15 1455 bp separated by gel electrophoresis with 0.8% TBE agarose

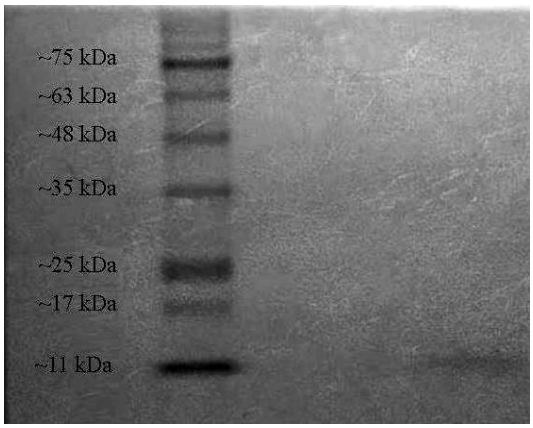

Figure 4. Vertical electrophoresis SDS-PAGE gel photo result of bacteriocin from *P. acidilactici* BAMA 15 strain

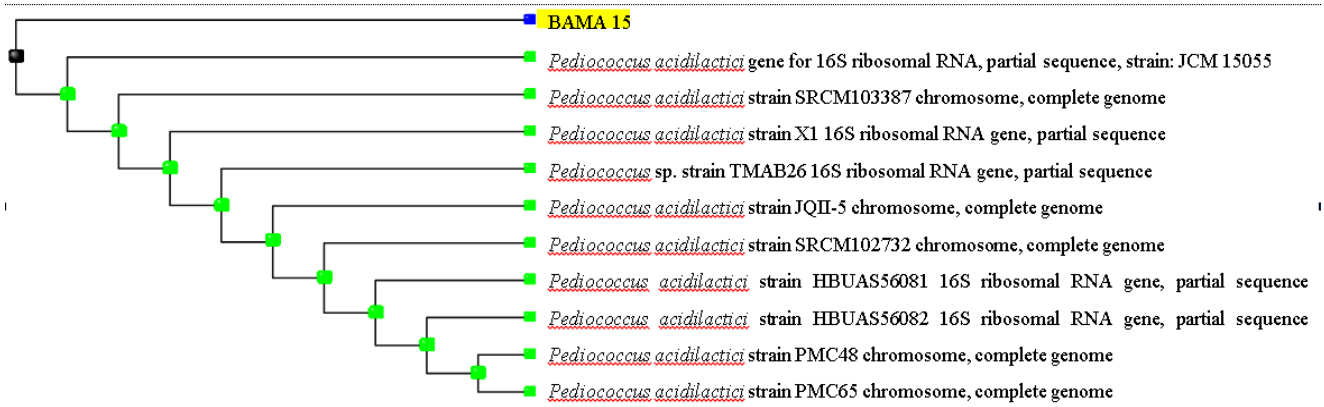

Figure 3. Phylogenetic tree analysis of the 16S rRNA gene sequence isolated from *P. acidilactici* BAMA 15 strain

Pediococcus acidilactici strains are found in many foods. Ahn et al. (2017) isolated LAB from malts. They obtained *P. acidilactici*, which produced bacteriocin with a molecular weight of 6 kDa; 6 - 12 kDa is a relatively large molecular weight because bacteriocins produced from gram-positive bacteria are often smaller than 6 kDa. Bacteriocins from LAB are peptides (small proteins) with specific physical and chemical characteristics that influence their specific interactions with targeted cells (Todorov et al. 2022). For example, Simha et al. (2012) showed bacteriocin-producing by *P. pentosaceous* NCDC 273 that purification by RP-HPLC resulted in a protein with a molecular mass of 4.6 kDa. Bacteriocin of LAB is a metabolite synthesized by ribosomes as a bacteriostatic protein (Wang et al. 2018), which is the reason for the name. The mechanism action of bacteriocin is based on their ability to target the cell membrane, DNA, or protein metabolism of the microbial strain, whose function is distinct from those (Ibrahim et al. 2021), usually acts on the bacterial cytoplasmic membrane, their genetic determinants are usually plasmid-encoded, facilitating genetic manipulation, there is no cross-resistance to antibiotics (Reis et al. 2012). Bacteriocin from *P. acidilactici* K10 mixed with succinic acid, lactic acid, and acetic acid was synergistically effective against target cells of *E. coli* O157:H7 in vivo and in vitro. Moreover, the same bacteriocin from *P. acidilactici* K10 mixed with 0.35% lactic acid and 0.25% acetic acid decreased the population of *E. coli* O157:H7, inoculated in a ground beef sample (Ibrahim et al. 2021).

The effect of bacteriocin from *P. acidilactici* strains was also proven by Qiao et al. (2022). Bacteriocin can increase the immune system and the number of flora in the intestines of normal mice. Peptides from bacteriocin can be signal peptides to regulate the host immune system. LAB has been suggested to have several modes of action that eliminate pathogenic bacteria, including competition for physical attachment sites, competition for nutrients, a synergistic interaction of two or more of the above activities, enhancement of host immune system activity, and production of antimicrobial compounds (e.g., volatile fatty acids) (Capita and Alonso-Calleja 2013). The toxicity metabolites of pathogenic bacteria can play an irreplaceable role in the antibiotic that inhibits it (Cheng et al. 2014).

Bacteriocins can kill pathogens without causing detrimental imbalances to the host microbiota and are often very specific (O'Connor et al. 2020). Inhibition was scored positive once the inhibition zone's width around the producer strain's colonies was 0.5 mm or larger (Bungenstock et al. 2020). The antibacterial activity of *P. acidilactici* BAMA 15 against *E. coli* was 6.44 mm, and *S. aureus* was 7.53 mm in diameter in both disk papers. The activity against *S. aureus* was higher in both inhibition zones. Also, the resistance level of gram-negative bacteria was higher than that of gram-positive bacteria. The zone of inhibition is divided into four classifications are following: >20 mm (very strong), 10-20 mm (strong), 5-10 mm (moderate), and <5 mm (no response). Zone of inhibition obtained different results because bacterial viability differs in producing metabolite compounds and their responses to pathogenic bacteria (Nasri et al. 2021).

Bacteriocin is mainly studied in the following aspects: bacteriocin-producing screening by LAB and evaluation of antibacterial ability; bacteriocin is used in food preservation and disease treatment to antibiotics as an alternative; bacteriocin as a bioengineering (Qiao et al. 2022). Bacteriocin production by LAB usually follows primary metabolite growth-associated kinetics, which occurs during the exponential growth phase and once the stationary phase is reached (Abbasiliasi et al. 2017). Bacteriocins produced by Gram-negative bacteria are grouped into colicins and microcins. The bacteriocins were coded by genes coding can be present either on the plasmid or chromosome as a subset of three genes, bacteriocin encoding gene, immunity protein genes, and lysis genes involved in releasing bacteriocins from cells. Colicins are classified into two classes based on translocation and three classes based on their action modes. Microcins are grouped into classes: I and II. Class II is further subdivided into two subclasses IIa and IIb. Pediocin PA-1 bacteriocin expressing gene PedA from *P. acidilactici* (Juturu and Wu 2018). The microcins are divided into Class I, which contains ribosomally synthesized and post-translationally modified peptides (RiPPs) and Class II, which contains predominantly unmodified peptides (Cotter et al. 2013).

In conclusion, nine LAB isolates were isolated from *naniura* fish in Medan, North Sumatra, with cell forms in the form of cocci and bacilli, gram-negative and Gram-positive, with BAMA codes 10, 11, 15, 16, 17, 18, 19, 20, 25. One among the nine isolates, namely isolate BAMA 15, was optimal in inhibiting the growth of *E. coli* and *S. aureus*, with inhibition of 6.44 and 7.53 mm, respectively. Meanwhile, the viability percentage of BAMA 15 isolates in acid and bile salt media was 17.9% and 69.5%, respectively. Furthermore, the molecular weight of the bacteriocin was ~12 kDa. Therefore, the results obtained from BAMA 15 isolate can be a LAB candidate with good potential in producing antibacterials. Therefore, BAMA 15 isolate can be used in fermented foods and beverages that benefit health.

ACKNOWLEDGEMENTS

This study was financed by the Directorate of Research, Technology, and Community Service (DRTPM) of Research, Technology, and Higher Education of Indonesia 2022 under contract number: 63/LL1/LT/K/2022, scheme Research of Doctoral Dissertation (PDD) awarded to Chrismis Novalinda Ginting as principal investigator.

REFERENCES

Abbasiliasi S, Tan JS, Ibrahim TAT, Bashokouh F, Ramakrishnan NR, Mustafa S, Ariff AB. 2017. Fermentation factors influencing the production of bacteriocins by lactic acid bacteria: A review. RSC Adv 7: 29395-29420. DOI: 10.1039/c6ra24579j.

Ahn H, Kim J, Kim WJ. 2017. Isolation and characterization of bacteriocin-producing *Pediococcus acidilactici* HW01 from malt and

its potential to control beer spoilage lactic acid bacteria. Food Control 80: 59-66. DOI: 10.1016/j.foodcont.2017.04.022.
Aritonang SN, Roza E, Yetmaneli, Sandra A, Rizqan. 2022. Characterization of lactic acid bacteria from buffalo dairy product (*dadiah*) as potential probiotics. Biodiversitas 23 (9): 4418-4423. DOI: 10.13057/biodiv/d230906.
Bungenstock L, Abdulmawjood A, Reich F. 2020. Evaluation of antibacterial properties of lactic acid bacteria from traditionally and industrially produced fermented sausages from Germany. PLoS One 15 (3): e0230345. DOI: 10.1371/journal.pone.0230345.
Capita R, Alonso-Calleja C. 2013. Antibiotic-resistant bacteria: A challenge for the food industry. Crit Rev Food Sci 53 (1): 11-48. DOI: 10.1080/10408398.2010.519837.
Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L, Yuan Z. 2014. Antibiotic alternatives: The substitution of antibiotics in animal husbandry?. Front Microbiol 5: 217. DOI: 10.3389/fmicb.2014.00217.
Cotter PD, Ross RP, Hill C. 2013. Bacteriocins - A viable alternative to antibiotics?. Nat Rev Microbiol 11: 95-105. DOI: 10.1038/nrmicro2937.
Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, Berenjian A, Ghasemi Y. 2019. Prebiotics: Definition, types, sources, mechanisms, and clinical applications. Foods 8 (3): 92. DOI: 10.3390/foods8030092.
Dina AM, Ekbal MI, Adham MA, Hamdi AM 2013. Effect of *Pediococcus acidilactici* and its bacteriocin on soft cheese quality and validity. Benha Vet Med J 25 (1): 64-76.
Dinoto A, Rosyidah A, Susilo ARPS, Julistiono H. 2020. Isolation, identification and antimicrobial activities of Lactic Acid Bacteria from fruits of wild plants in Tambrauw Forest, West Papua, Indonesia. Biodiversitas 21 (7): 3391-3397. DOI: 10.13057/biodiv/d210764.
Driscoll AJ, Bhat N, Karron RA, O'Brien KL, Murdoch DR. 2012. Disk diffusion bioassays for the detection of antibiotic activity in body fluids: Applications for the *Pneumonia* etiology research for child health project. Clin Infect Dis 54 (2): 159-164. DOI: 10.1093/cid/cir1061.
Fatimah MP, Imam M, Trianing TKA. 2020. Kajian pustaka: Pemanfaatan bakteriosin dari produk fermentasi sebagai antibakteri terhadap *Staphylococcus aureus*. Indones Med Veterinus 9 (5): 835-848. DOI: 10.19087/imv.2020.9.5.835. [Indonesian]
Francois ZN, Marie KP, Noëlle TAH, Emeric GWR. 2013. Antimicrobial activity of a bacteriocin produced by *Lactobacillus plantarum* 29V and strain's viability in Palm kernel oil. Intl J Nutr Food Sci 2 (3): 102-108. DOI: 10.11648/j.ijnfs.20130203.12.
Gutiérrez-Cortés C, Suarez H, Buitrago G, Nero LA, Todorov SD. 2018. Characterization of bacteriocins produced by strains of *Pediococcus pentosaceus* isolated from Minas cheese. Ann Microbiol 68: 383-398. DOI: 10.1007/s13213-018-1345-z.
Gowans GJ. 2018. SDS-PAGE gel recipes. In: Bioprotocol Excange. https://bio-protocol.org/
Ibrahim SA, Ayivi RD, Zimmerman T, Siddiqui SA, Altemimi AB, Fidan H, Esatbeyoglu T, Bakhshayesh RV 2021. Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. Foods 10 (12): 3131. DOI: 10.3390/foods10123131.
Jatmiko YD, Howarth GS, Barton MD. 2019. Naturally fermented milk and its therapeutic potential in the treatment of inflammatory intestinal disorders. AIP Conf Proc 2019: 060009. DOI: 10.1063/1.5061918.
Juturu V, Wu JC. 2018. Microbial production of bacteriocins: Latest research development and applications. Biotechnol Adv 36 (8): 2187-2200. DOI: 10.1016/j.biotechadv.2018.10.007.
Kaya HI, Şimşek Ö. 2020. Characterization of *Pediococcus acidilactici* PFC69 and *Lactococcus lactis* PFC77 bacteriocins and their antimicrobial activities in tarhana fermentation. Microorganisms 8 (7): 1083. DOI: 10.3390/microorganisms8071083.
Lawalata HJ, Sembiring L, Rahayu ES. 2015. Molecular identification of lactic acid bacteria producing antimicrobial agents from Bakasang, an Indonesian traditional fermented fish product. Indones J Biotechnol 16 (2): 93-99. DOI: 10.22146/ijbiotech.16368.
Lei S, Zhao R, Sun J, Ran J, Ruan X, Zhu Y. 2020. Partial purification and characterization of a broad-spectrum bacteriocin produced by a *Lactobacillus plantarum* zrx03 isolated from infant's feces. Food Sci Nutr 8 (5): 2214-2222. DOI: 10.1002/fsn3.1428.
Li M, Wang Y, Cui H, Li Y, Sun Y, Qiu HJ. 2020. Characterization of lactic acid bacteria isolated from the gastrointestinal tract of wild boar as potential probiotics. Front Vet Sci 7: 49. DOI: 10.3389/fvets.2020.00049.
Manik M, Kaban J, Silalahi J, Ginting M. 2020. Lactic Acid Bacteria (LAB) with probiotic potential from dengke *naniura*. Baghdad Sci J 18 (1): 35-40. DOI: 10.21123/bsj.2021.18.1.0035.
Mohammed SSD, Ijah UJJ. 2013. Isolation and screening of lactic acid bacteria from fermented milk products for bacteriocin production. Ann Food Sci Technol 14 (1): 122-128.
Nasri, Harahap U, Silalahi J, Satria D. 2021. Antibacterial activity of lactic acid bacteria isolated from Dengke *Naniura* of Carp (*Cyprinus carpio*) against diarrhea-causing pathogenic bacteria. Biodiversitas 22 (8): 3098-3104. DOI: 10.13057/biodiv/d220802.
O'Connor PM, Kuniyoshi TM, Oliveira RPS, Hill C, Ross RP, Cotter PD 2020. Antimicrobials for food and feed; a bacteriocin perspective. Curr Opin Biotechnol 61: 160-167. DOI: 10.1016/j.copbio.2019.12.023.
Pato U, Yusuf Y, Fitriani S, Jonnadi NN, Wahyuni MS, Feruni JA, Jaswir I. 2020. Inhibitory activity of crude bacteriocin produced by lactic acid bacteria isolated from dadih against *Listeria monocytogenes*. Biodiversitas 21 (4): 1295-1302. DOI: 10.13057/biodiv/d210404.
Pei J, Li X, Han H, Tao Y. 2018. Purification and characterization of plantaricin SLG1, a novel bacteriocin produced by *Lb. plantarum* isolated from yak cheese. Food Control 84: 111-117. DOI: 10.1016/j.foodcont.2017.07.034.
Qiao Y, Qiu Z, Tian F, Yu L, Zhao J, Zhang H, Zhai Q, Chen W. 2022. Effect of bacteriocin-producing *Pediococcus acidilactici* strains on the immune system and intestinal flora of normal mice. Food Sci Hum Wellness 11 (2): 238-246. DOI: 10.1016/j.fshw.2021.11.008.
Reis JA, Paula AT, Casarotti SN, Penna ALB. 2012. Lactic acid bacteria antimicrobial compounds: Characteristics and applications. Food Eng Rev 4: 124-140. DOI 10.1007/s12393-012-9051-2.
Rossi-Tamisier M, Benamar S, Raoult D, Fournier PE. 2015. Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. Intl J Syst Evol Microbiol 65: 1929-1934. DOI: 10.1099/ijs.0.000161.
Simha BV, Sood SK, Kumariya R, Garsa AK. 2012. Simple and rapid purification of pediocin PA-1 from *Pediococcus pentosaceous* NCDC 273 suitable for industrial application. Microbiol Res 167 (9): 544-549. DOI: 10.1016/j.micres.2012.01.001.
Todorov SD, Popov I, Weeks R, Chikindas ML. 2022. Use of bacteriocins and bacteriocinogenic beneficial organisms in food products: Benefits, challenges, concerns. Foods 11 (19): 3145. DOI: 10.3390/foods11193145.
Ünlü G, Nielsen B, Ionita C. 2015. Production of antilisterial bacteriocins from lactic acid bacteria in dairy-based media: A comparative study. Probiotics Antimicrob Proteins 7 (4): 259-274. DOI: 10.1007/s12602-015-9200-z.
Wang Y, Qin Y, Xie Q, Zhang Y, Hu J, Li P. 2018. Purification and characterization of plantaricin LPL-1, a novel class IIa bacteriocin produced by *Lactobacillus plantarum* LPL-1 isolated from fermented fish. Front Microbiol 9: 2276. DOI: 10.3389/fmicb.2018.02276.