BIODIVERSITAS
Volume 21, Number 9, September 2020
Pages: 4192-4198

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d210933

Characterization of a bacteriocin as biopreservative synthesized by indigenous lactic acid bacteria from dadih soya traditional product used in West Sumatra, Indonesia

ENDAH RETNANINGRUM[1,♥], TANIA YOSSI[1], RINI NUR'AZIZAH[1], FADILLA SAPALINA[2], PERISKILA DINA KALI KULLA[2]

[1]Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Sleman 55281, Yogyakarta, Indonesia. Tel./fax. +62-274-580839, Fax.: +62-274-6492355, ♥email: endahr@ugm.ac.id

[2]Graduate Program, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Sleman 55281, Yogyakarta, Indonesia

Manuscript received: 9 June 2020. Revision accepted: 20 August 2020.

Abstract. *Retnaningrum E, Yossi T, Nur'azizah R, Sapalina F, Kulla PDK. 2020. Characterization of a bacteriocin as biopreservative synthesized by indigenous lactic acid bacteria from dadih soya traditional product used in West Sumatra, Indonesia. Biodiversitas 21: 4192-4198.* A total of 4 isolates of lactic acid bacteria (strain BDL08, BDL11, BDL12, and BDL13) isolated from dadih soya were investigated for the ability to produce bacteriocin based on their antibacterial activities against *Listeria monocytogenes* ATCC 7644. Based on comparative 16S rDNA sequencing analysis, isolates BDL 11, BDL12, and BDL13 were identified as *Lactobacillus plantarum* while the isolates BDL 08 was identified as *Leuconostoc mesenteroides*. All crude bacteriocins producing strains revealed broad antibacterial spectrum against 7 different indicator bacteria, including Gram-positive and Gram-negative bacteria. *L. plantarum* BDL11 displayed the highest bacteriocin activity relative to others. The bacteriocin produced by the strain was not affected by pH, heating, and NaCl concentration but was sensitive to proteolytic enzymes. This research indicated that the bacteriocin in the food industry has the potential to be used as a biopreservative.

Keywords: 16S rDNA, antibacterial activities**,** food industry, *Lactobacillus plantarum*, proteolytic enzyme

INTRODUCTION

Dadih soya is an Indonesian traditional milk fermentation product that replaced soya milk as a substrate. This product is mainly consumed by people living in West Sumatera, Indonesia. During fermentation process, soya milk is poured into a bamboo tube capped with banana leaves. It is then fermented spontaneously at room temperature for 48 hours by indigenous lactic acid bacteria derived from both of soya milk and bamboo tube. Generally, two species of bamboo, Gombong bamboo (*Gigantochloa verticilata*) and Ampel bamboo (*Bambusa vulgaris*) are used in this fermentation process.

Lactic acid bacteria contain group of bacteria which play role in the dadih soya fermentation. During this fermentation process, the bacteria can reduce pH value by producing lactic acid. This lactic acid can inhibit undesirable contamination of microorganisms in the product. In addition, these bacteria may produce metabolites such as diacetyl, hydrogen peroxide, some enzymes, antibiotics, reuterin, and bacteriocins (Mazzoli et al. 2014; Fernández-Cruz et al. 2016; Yépez et al. 2017). This bacteria group is characterized as Gram-positive, non-spore-forming cocci, coccobacilli or rods, low G + C content, and non-motile. These bacteria are also characterized by their capability to ferment sugar into lactic acid, catalase-negative, and oxidase-negative (Nguyen et al. 2013; Gänzle et al. 2015; Renschler et al. 2020).

Bacteriocins have been the subject of particular interest among the metabolites developed by LAB because of their potential advantages in applying them as natural food biopreservatives (Field 2018; Juturu et al. 2018; Kumariya et al. 2019). These bacteriocins are antimicrobials that are ribosomally synthesized, releasing bioactive peptides or peptide complexes with bactericidal or bacteriostatic effects (Singh et al. 2015; Zou et al. 2018).

The advantages of bacteriocins produced by LAB in food preservation have been reported by several researchers. Their properties, including nontoxic to eukaryotic cells, have little influence on the gut microbiota and pH, heat tolerant, have a wide antimicrobial spectrum against many pathogenic food-borne spoilage bacteria and are capable of being bactericidal has attracted number of researchers to investigate this group of bacteria (Yi et al. 2016; Barman et al. 2018). This leads a way to carry out the research to identify LAB producing bacteriocin isolated from dadih soya-based on 16S rDNA gene as molecular marker and to characterize the bacteriocins which would be used as biopreservatives in food products.

MATERIALS AND METHODS

Sampling and isolation of LAB

Samples (dadih soya fermentation) were collected from traditional market in West Sumatera, Indonesia. A 25 g of each sample was added to 225 mL of sterile buffered

peptone water, homogenized in a stomacher for 2 min and serial dilutions were plated onto De Man, Rogosa Sharpe (MRS) Agar (Merck). Plates were incubated under microaerophilic conditions at 30˚C for 72 h. Isolates were purified by repeated streaking onto the respective growth media. All isolates were tested for morphological and biochemical properties such as Gram-reaction, spore-formation, motility, catalase, and oxidase production. The Gram-positive, catalase-negative, and oxidase-negative isolates were selected as lactic acid bacteria and further analyzed.

Screening of bacteriocin producing LAB strains

The strains were screened for bacteriocin production by analyzing antimicrobial activity against indicator pathogenic bacteria (*Listeria monocytogenes* ATCC 7644) using agar well diffusion assay (Pasteris et al. 2014; An et al. 2015). The isolates bacteria which showed an inhibition zone diameter against *Listeria monocytogenes* ATCC 7644 were selected for further investigation.

Genotypic identification by 16S rDNA gene sequencing

All isolates producing bacteriocins were identified using 16S rDNA gene sequencing. The genomic DNA of each strain was directly isolated from overnight liquid culture using DNA extraction kit (MOBIO protocol) and used as template for PCR reaction. The DNA was then amplified by polymerase chain reaction (PCR) using primers designed to amplify 1500 bp fragment of the 16S rDNA region. The primer used was 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CCGTCAATTCMTTTRAGTTT-3'). The amplification was performed using Eppendorf Gradient thermocycler with initial denaturation 95˚C (2 min), 35 cycles at denaturation 95˚C (1 min), annealing 55˚C (1 min), extension 72˚C (1 min). Finally with extension of 72˚C (10 min). Sequencing was performed using enzymatic chain terminator technique, developed by (Sanger et al. 1977), by using a PRISM BigDye Terminator v3.1 cycle sequencing kit and then analyzed by ABI Prism 3730XL DNA Analyser (Applied Biosystems, CA, USA). The sequences were BLAST in the GenBank database (www.ncbi.nhm.nih.gov) for species assignment. The phylogenetic tree was constructed by the Neighbor-Joining method (Saitou and Nei 1987), based on the Kimura 2-parameter model (Kimura, 1980) with bootstrap analysis (1000 replications) using the software MEGA (version 7) (Kumar et al. 2016).

Antimicrobial spectrum of bacteriocin producing isolates

The selected LAB isolates were tested against pathogenic microorganisms listed in Table 1. Crude bacteriocins were prepared and analyzed by the agar well diffusion assay. Previously the cell-free supernatants (CFS) as crude bacteriocin was prepared and obtained by centrifugation of culture at 10,000 g, 20 min, and at 4˚C. These supernatant collections were then neutralized to pH 7.0 with 3 M NaOH for the complete inactivation of organic acids, which might be produced by the strains. In addition, inhibitory activity of hydrogen peroxide might also be produced by the strains that were eliminated by the addition of catalase of 1 mg/mL catalase (Sigma, St. Louis, USA). Therefore, the CFS samples were not related to the production of either organic acids or hydrogen peroxide, confirmed as crude bacteriocin. The crude bacteriocin obtained then subjected to filter sterilization (0.22 µm).

All samples were examined using the agar well diffusion assay for their antimicrobial activity. One hundred µL of each pathogenic bacteria indicator (10^6 CFU/mL) listed in Table 1. was spread on the nutrient agar plates. Previously, all pathogens were grown with nutrient broth (NB) medium under aerobic conditions at 37 ºC. Equidistant wells (4 mm diameter) were formed by boring the nutrient agar plate with corer borer. Those wells then were filled with 80 mL of crude bacteriocin and incubated at 37ºC for 24 h. The antimicrobial activity of those bacteriocins was measured and expressed as AU (mm^2/ mL), which is the unit area of inhibition zone per unit volume. Therefore, the antimicrobial activity was calculated using the following equation.

Antimicrobial activity (AU) = Lz-Ls/V

Where: LZ is clear zone area (mm^2), LS is well area (mm^2), and V is volume of sample (mL) (Abbasiliasi et al. 2012).

Characterization of the crude bacteriocin stability to pH, temperature, NaCl concentrations, and proteolytic enzymes

The sensitivity of crude bacteriocins produced by selected isolate to pH was investigated by adjusting pH of crude bacteriocins from pH 2 to 10 and incubated at 37 ˚C for 4 h. To analyze effects of temperature, NaCl concentrations, and proteolytic enzymes, the crude bacteriocins were adjusted previously to pH 7.0 by 3 M HCl or NaOH. Effects of temperature were investigated at 60˚C, 80 ˚C, and 100 ˚C for 30 min. Effect of of NaCl on crude bacteriocins were also observed at NaCl concentrations of 1.0%, 2.0%, 3.0%, 4.0% and 5.0%. Proteolytic enzymes that used to analyze of bacteriocins stabilities were trypsin (1000 - 2000 units/mg solid, Sigma-Aldrich, St. Louis, MO, USA), a-chymotrypsin (≥ 40 units/mg; Sigma-Aldrich, St. Louis, MO, USA) and proteinase K (20 mg/mL; TaKaRa Bio Inc., Otsu, Shiga, Japan). Then, all samples were assayed against the indicator strain *Listeria monocytogenes* ATCC 7644 using the agar well diffusion assay as described above.

Table 1. Pathogenic microorganisms used in the antimicrobial spectrum test for selected LAB producing bacteriocin

Strains	Origin	Growth conditions
Streptococcus mutans	ATCC 25175	Nutrient broth, 37 ˚C
Staphylococcus aureus	ATCC 29213	Nutrient broth, 37 ˚C
Bacillus cereus	ATCC 11778	Nutrient broth, 37 ˚C
Pseudomonas aeruginosa	ATCC 27853	Nutrient broth, 37 ˚C
Escherichia coli	ATCC 25922	Nutrient broth, 37 ˚C
Salmonella typhi	ATCC 6539	Nutrient broth, 37 ˚C

Statistical analysis

The experiments were done in triplicates. All data are shown as means ± standard deviation. Mean data of treatments were compared by the analysis of variance (one-way ANOVA) followed by Duncan Multiple Range Test (DMRT) approach for the measurement of mean differences. Differences at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Lactic acid bacteria producing bacteriocin isolated from dadih soya

Total of 55 bacteria cultures was obtained in the MRSA medium as a result of isolation in this research. Those isolates of lactic acid bacteria (LAB) group were selected based on morphological and biochemical properties. Ten strains (BDL01, BDL05, BDL08, BDL09, BDL11, BDL12, BDL13, BDL15, BDL18, BDL20 strain) clearly showed characteristics as Gram-positive, rods shape, non-spore-forming, non-motile, catalase, and oxidative negative. Therefore, 10 LAB isolates were further screened for their producing bacteriocin ability.

Previously, researches reported that bacteriocins could inhibit foodborne pathogenic bacteria, *L. monocytogenes*. This pathogen showed high virulence and resistance even though grew at stressful condition, such as high salt levels, freezing, drying, pH levels and heat processes (Changcheng et al. 2017; Huang et al. 2019). Therefore, it is difficult to control in food products. Its species has been found to contaminate several food products, such as smoked salmon, dairy product, cheese, pasta, fruit and vegetable (Vitas et al. 2014; Fei et al. 2016; de Cesare et al. 2018; Hamidiyan et al. 2018; Haubert et al. 2018; Hiko et al. 2019; Huang et al. 2019). In addition, *L. monocytogenes* were used successfully as indicator strains to screen bacteriocin-producing LAB isolates (Tan et al. 2014; Md Sidek et al. 2016; Wong et al. 2017).

Therefore, for screening the ability of LABs producing bacteriocin, ten strains originating from Indonesian fermented food "dadih soya" were assayed for their antimicrobial activity and potential bacteriocin production against *L. monocytogenes* ATCC 19115 using well diffusion method. Table 2 demonstrated that among 10 strains, 4 isolates (40%) exhibited inhibitory activities against this indicator bacteria. These isolates displaying an inhibition zone greater than 3 mm. The Inhibition zone diameters observed at strain BDL 8, BDL 11, BDL 12, and BDL 13 were 8 mm, 5 mm, 3 mm, and 6 mm, respectively.

Genotypic identification LAB producing bacteriocin

The rapid identification of LAB has been determined by primers and probes targeting the 16S rDNA spacer regions (Balca´zar et al. 2007; Yu et al. 2015; Michel et al. 2016). This 16S rDNA gene of the selected strain was amplified by PCR, by which a 1500 bp gene fragment was sequenced and submitted to Genbank. The Blast search performed against GenBank revealed a large number of similar 16S rDNA gene sequences. The BLAST results of most promising bacterial isolates showed >99% similarities between available GenBank entries as displayed in Table 3, the strain BDL8 was clearly identified *Leuconostoc mesenteroides*, whereas others (BDL11, BDL 12 and BDL13 strains) were identified as *Lactobacillus plantarum*. In addition, a neighbor-joining phylogenetic tree based on 16S rDNA sequences of BDL8, BDL 11, BDL 12, and BDL 13 strains were constructed with other closely related species of *L. mesenteroides* and *L. plantarum* obtained from NCBI (Figure 1). This phylogenetic analysis revealed that the strains BDL11, BDL12, and BDL 13 were clustered together with *L. plantarum*, strain BDL8 with *L. mesenteroides* which are completely separated from *Pseudomonas husainii* as an outgroup by 1000 bootstrap values.

In comparison with previous studies carried out by researches, both of *L. plantarum* and *L. mesenteroides* have been isolated from fermented products, (Choi et al. 2015; Oguntoyinbo and Narbad 2015; Berbegal et al. 2016; Du et al. 2018; Jimenez et al. 2018; Lee and Kim 2019; Nurhikmayani et al. 2019). Moreover, *L. plantarum* has also reported from digestive tracts of shrimp (Kongnum et al. 2012), human breast milk (Jiang et al. 2016) and Italian rye-grass (Vijayakumar et al. 2015), whereas, genus of *Leuconostoc* mesenteroides mainly isolated from meat products (Kaihei et al. 2011) and *Oreochromis niloticus* (Paray et al. 2018).

Antimicrobial spectrum activity of crude bacteriocins

The antimicrobial spectrum measurements of crude bacteriocin produced by 4 strains were displayed in Table 4. The indicator microorganisms for its analysis include Gram-positive bacteria (*S. mutans*, *S aureus*, *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa*, *E. coli*, *S. typhi*). All crude bacteriocins showed bacteriocin activities against all indicator microorganisms tested, but the values of activity varied among the strains.

Table 2. Inhibition zone diameter of LAB isolates against *Listeria monocytogenes* using agar well diffusion assay

Strain	Inhibition zone diameter (mm)
BDL01	0
BDL05	0
BDL08	3 ± 0.01
BDL09	0
BDL11	8 ± 0.02
BDL12	5 ± 0.01
BDL13	6 ± 0.02
BDL15	0
BDL18	0
BDL20	0

Note: Mean values (n = 3) for each experiment.

Table 3. Genotypic identification LAB producing bacteriocin

OTU	Strain	Species of LAB homolog	Identity (%)	Accession number	Base pairs
1	BDL08	*Leuconostoc mesenteroides* strain ATCC 8293	99.50	NR_040814.1	1,448bp
		Leuconostoc pseudomesenteroides strain NRIC 1777	99.24	NR_074957.1	1549 bp
2	BDL11	*Lactobacillus plantarum* strain CIP 103151	99.59	NR_104573.1	1,527bp
		Lactobacillus plantarum strain NRRL B-14768	99.32	NR_042394.1	1474 bp
3	BDL12	*Lactobacillus plantarum strain* CIP 103151	99.52	NR_104573.1	1,527bp
		Lactobacillus plantarum strain JCM 1149	99.35	NR_117813.1	1466 bp
4	BDL13	*Lactobacillus plantarum strain* CIP 103151	99.48	NR_104573.1	1,527bp
		Lactobacillus plantarum strain JCM 1149	99.30	NR_117813.1	1466 bp

Table 4. The antimicrobial spectrum crude bacteriocin of isolates against 7 different microorganism indicators.

Indicator microorganisms	Bacteriocin activity (AU/mL)			
	L. plantarum BDL11	*L. plantarum* BDL12	*L. plantarum* BDL13	*L. mesenteroides* BDL008
Streptococcus mutans ATCC 25175	40	30	35	25
Staphylococcus aureus ATCC 29213	38	25	34	20
Bacillus cereus ATCC 11778	38	15	25	10
Pseudomonas aeruginosa ATCC 27853	25	10	15	7
Escherichia coli ATCC 25922	20	10	10	10
Salmonella typhi ATCC 6539	18	8	8	5

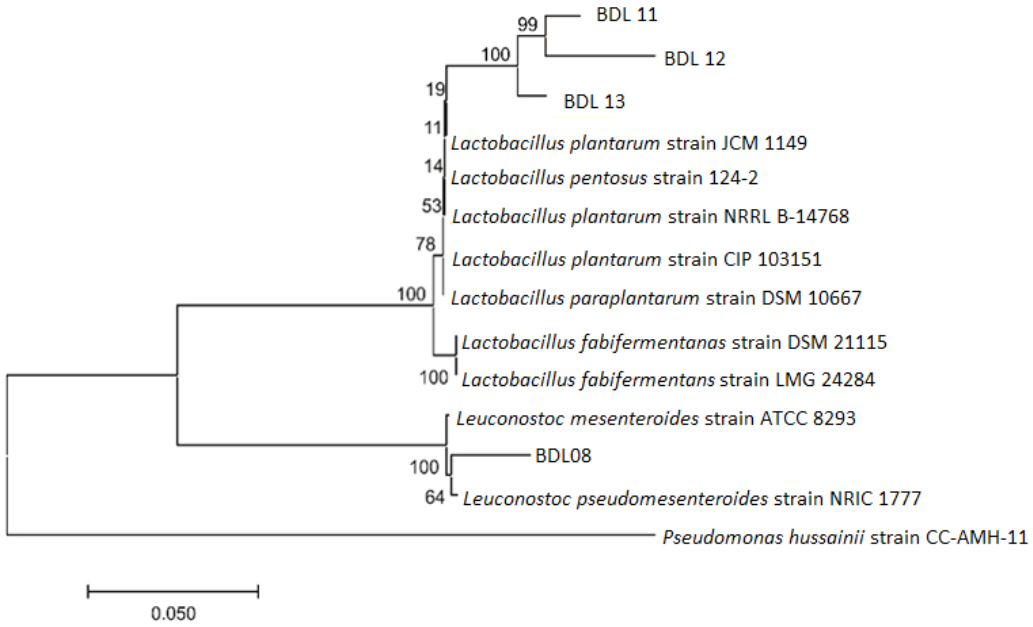

Figure 1. Phylogenetic tree developed using 16S rDNA gene sequences which are available in the GenBank database, using the method Neighbor-joining with bootstrap of 1000 replication

From the observations, it was found that crude bacteriocin activities were lower against Gram-negative bacteria compared with Gram-positive bacteria. This result was similar to previous research reported by Xi et al. (2018) who observed the *Enterococcus faecalis* TG2. The bacteriocins activity analyses were measured in the range of 25-40 AU/mL and 5-25 AU/mL against Gram-positive and Gram-negative bacteria, respectively. The pathogenic bacterium S. mutans was most sensitive to those crude bacteriocins LABs, whereas *S. typhi* was most resistant. The result of this sensitivity test was similar to previous studies (Kawada-Matsuo and Komatsuzawa 2017; Kim et al. 2019). The strongest crude bacteriocin activity to all indicators was observed in *L. plantarum* BDL11, whereas, the weakest it was observed in *L. mesenteroides* BDL008. Therefore, the crude bacteriocin characters of *L. plantarum* BDL11 were further analyzed their stabilities.

Generally, bacteriocins from lactic acid bacteria were regarded to have no antibacterial activity against Gram-negative bacteria (Kang and Lee 2005, Drider et al. 2006; Powell et al. 2007). This phenomenon was caused the lack of cell wall lipoteichoic acid on the Gram-negative bacterial surface (Ahn et al. 2017). However, these crude bacteriocins produced by 4 LAB isolates also had an

inhibitory effect on three Gram-negative bacteria, including *E. coli* ATCC 25922, *B. cereus* ATCC 11778, and *S. typhi* ATCC 6539. Similar reports have shown that some bacteriocins produced by LAB, could inhibit both Gram-positive and Gram-negative bacteria. The bacteriocins LXA produced by *Lactobacillus coryniformis* XN8 has been reported to have antibacterial activity against Gram-positive bacteria (*S. aureus*, *Enterococcus sakazakii*) and Gram-negative bacteria (*E. coli*, *Salmonella* sp) (Yi et al. 2016). Bacteriocin produced by *L. plantarum* SJ33 has also been reported to inhibit the growth of Gram-positive bacteria (*S. aureus*, *Aeromonas hydrophila*, *Clostridium perfringens*, *Clostridium sporogenes*, *B. subtilis*, *B. cereus*) and Gram-negative bacteria (*E. coli*, *Vibrio parahaemolyticus*, *Proteus vulgaris*, *Klebsiella pneumonia*, *P. aeruginosa*) (Mohapatra and Jeevaratnam 2019).

Characteristics of the crude bacteriocins of 4 isolates

The stability of the crude bacteriocin characters obtained from *L. plantarum* BDL11 against several treatments was summarised in Table 5. The activity of crude bacteriocin strain remained active in the range of pH values of 2-10. There was high bacteriocin activity at pH 2, and lower activity at pH 8 and 10. In addition, heat treatment up to 100ºC for 30 min did not affect the stability of crude bacteriocin. Similarly, treatment of NaCl concentrations did not affect the bacteriocin activities. These results were in accordance with previous studies on bacteriocins of *Lactobacillus sakei* GM3 (Avaiyarasi et al. 2016), *Lactobacillus fermentum* BZ532 (Rasheed et al. 2020) and *Lactobacillus plantarum* SLG10 (Pei et al. 2020). Those stability characters of the crude bacteriocin especially for pH, heat, and NaCl treatments were useful for being applied as biopreservative agents to control pathogens and spoiling bacteria in food products (Yang et al. 2014; Ahmad et al. 2017). In addition, its antibacterial activity was inactivated by proteolytic enzymes (trypsin, chymotrypsin, and proteinase K), indicating it is proteinaceous in nature (Mohapatra and Jeevaratnam 2019).

Table 5. The antibacterial activity of the crude bacteriocin obtained from *L. plantarum* BDL11 against several treatments

Treatments		Antibacterial activity (AU/mL)
Control	7	65
pH	2	120
	4	65
	6	32
	8	25
	10	25
Temperature	60˚C	65
	80˚C	33
	100˚C	33.5
NaCl	1.0%	65
	2.0%	57.8
	3.0%	57.5
	4.0%	57.5
	5.0%	57.3
Proteolytic enzymes	Trypsin	0
	Chymotrypsin	0
	Proteinase K	0

Application of biopreservatives to inhibit spoilage bacteria as well as pathogenic bacteria in food products have been previously reported by several researchers (da Silva Sabo et al. 2017; del Castillo-Santaella et al. 2018; Ho et al. 2018; Skariyachan and Govindarajan 2019). These biopreservatives can increase food shelf-life and avoid cases of foodborne diseases. Some of the bacteria that cause foodborne diseases were detected in food products including *S. mutans*, *S. aureus*, *P. aeruginosa*, *E. coli*, *B. cereus* and *S. typhi* (Gabriel et al. 2017; Bundidamornet et. al. 2018; Gnanasekaran et al. 2019; Incili et al. 2019). In comparison with synthetic preservatives such as benzoic acid, tertiary-butyl hydroquinone (TBHQ), formalin, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA), the biopreservatives were found safer for human use because they did not form mutagenic compounds that caused cancer (Li et al. 2017; Piper and Piper 2017; Dehghan et al. 2018; Mohamed et al. 2018; Mohammadzadeh-Aghdash et al. 2018; Abd-Elhakim et al. 2020). Whereas, the food products are generally preserved using a combination of biopreservation and other treatments, including pH, heat, and NaCl. From the investigations, the crude bacteriocin produced by the strain has several unique characteristics that were stable in those treatments.

Based on the investigation results, it can be concluded that the crude bacteriocin-producing BDL11 strain which isolated from traditional Indonesian fermented product (dadih soya) had highest bacteriocin activity. In addition, this strain was identified as *L. plantarum* on the basis of 16S rDNA gene sequence. Its crude bacteriocin had broad antibacterial activities against both Gram-positive and Gram-negative bacteria, stable in several treatments of temperature, pH, and NaCl. These findings indicate that crude bacteriocin *L. plantarum* BDL11 could be used as a potentially effective and natural food biopreservative. More research is needed to understand the metabolism and production of bacteriocin using low-cost medium for industrial food production.

ACKNOWLEDGEMENTS

This research was supported by RTA UGM 2020 (Contract No: 2488/UN1.P.III/DIT-LIT/PT/2020)

REFERENCES

Abbasiliasi S, Joo Shun T, Ibrahim TAT, Ramanan RN, Vakhshiteh F, Mustafa S, Ling TC, Rahim RA, Arif AB. 2012. Isolation of *Pediococcus acidilactici* Kp10 with ability to secrete bacteriocin-like inhibitory substance from milk products for applications in food industry. BMC Microbiol 12 (1): 260. DOI: 10.1186/1471-2180-12-260.

Abd-Elhakim YM, Hashem MMM, Abo-EL-Sooud K, Ali HA, Anwar A, El-Metwally AE, Mahmoud EA, Moustafa GG. 2020. Involvement of tumor necrosis factor-α, interferon-gamma-γ, and interleukins 1β, 6, and 10 in immunosuppression due to long-term exposure to five common food preservatives in rats. Gene 7425: 144590. DOI: 10.1016/j.gene.2020.144590.

Ahmad V, Khan MS, Jamal QMS, Alzohairy MA, Al Karaawi MA, Siddiqu MU. 2017. Antimicrobial potential of bacteriocins: In

therapy, agriculture and food preservation. Intl J Antimicrob Agents 49 (1): 1-11. DOI: 10.1016/j.ijantimicag.2016.08.016.

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.

An J, Zhu W, Liu Y, Zhang X, Sun L, Hong P, Wang Y, Xu C, Xu D, Liu H. 2015. Purification and characterization of a novel bacteriocin CAMT2 produced by *Bacillus amyloliquefaciens* isolated from marine fish *Epinephelus areolatus*. Food Control 51: 278-282. DOI: 10.1016/j.foodcont.2014.11.038.

Avaiyarasi ND, Ravindran AD, Venkatesh P, Arul V. 2016. In vitro selection, characterization and cytotoxic effect of bacteriocin of *Lactobacillus sakei* GM3 isolated from goat milk. Food Control 69: 124-133. DOI: 10.1016/j.foodcont.2016.04.036.

Balca´zar J, de Blas I, Ruiz-Zarzuela I, Vendrell D, Girone´s O, Muzquiz JL. 2007. Sequencing of variable regions of the 16S rRNA gene for identification of lactic acid bacteria isolated from the intestinal microbiota of healthy salmonids. Comp Immunol Microbiol Infect Dis 30 (2): 111-118. DOI: 10.1016/j.cimid.2006.12.001.

Barman S, Ghosh R, Mandal NC. 2018. Production optimization of broad-spectrum bacteriocin of three strains of *Lactococcus lactis* isolated from homemade buttermilk. Ann Agrar Sci 16 (3): 286-296. DOI: 10.1016/j.aasci.2018.05.004.

Berbegal C, Pena N, Russo P, Grieco F, Pardo I, Ferrer S, Spano G, Capozzi V. 2016. Technological properties of *Lactobacillus plantarum* strains isolated from grape must fermentation. Food Microbiol 57: 187-194. DOI: 10.1016/j.fm.2016.03.002

Bundidamorn D, Supawasit W, Trevanich S. 2018. A new single-tube platform of melting temperature curve analysis based on multiplex real-time PCR using EvaGreen for simultaneous screening detection of Shiga toxin-producing *Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes* in food. Food Control 94: 195-204. DOI: 10.1016/j.foodcont.2018.07.001.

Changcheng L, Huang L, Hwang C. 2017. Effect of temperature and salt on thermal inactivation of *Listeria monocytogenes* in salmon roe. Food Control 73 (B): 406-410.

Choi EA, Chang HC. 2015. Cholesterol-lowering effects of a putative probiotic strain *Lactobacillus plantarum* EM isolated from kimchi. LWT - Food Sci Technol 62 (1): 210-217. DOI: 10.1016/j.lwt.2015.01.019.

da Silva Sabo S, Pérez-Rodríguez N, Domínguez JM, de Souza Oliveira RP. 2017. Inhibitory substances production by *Lactobacillus plantarum* ST16Pa cultured in hydrolyzed cheese whey supplemented with soybean flour and their antimicrobial efficiency as biopreservatives on fresh chicken meat. Food Res Intl 99 (1): 762-769. DOI: 10.1016/j.foodres.2017.05.026.

de Cesare A, Vitali S, Tessema GT, Trevisani M, Fagereng TM, Beaufort A, Manfreda G, Skjerdal T. 2018. Modelling the growth kinetics of *Listeria monocytogenes* in pasta salads at different storage temperatures and packaging conditions. Food Microbiol 76: 154-163. DOI: 10.1016/j.fm.2018.04.013.

del Castillo-Santaella T, Cebrián R, Maqueda M, Gálvez-Ruiz MJ, Maldonado-Valderrama J. 2018. Assessing in vitro digestibility of food biopreservative AS-48. Food Chem 246: 249-257. DOI: 10.1016/j.foodchem.2017.10.149.

Dehghan P, Mohammadi A, Mohammadzadeh-Aghdash H, Dolatabadi JEN. 2018. Pharmacokinetic and toxicological aspects of potassium sorbate food additive and its constituents. Trends Food Sci Tech 80: 123-130. DOI: 10.1016/j.tifs.2018.07.012.

Drider D, Fimland G, Héchard Y, McMullen LM, Prévost H. 2006. The continuing story of class IIa bacteriocins. Microbiol Mol Biol Rev 70 (2): 564-582. DOI: 10.1128/MMBR.00016-05.

Du R, Zhao F, Pan L, Han Y, Xiao H, Zhou Z. 2018. Optimization and purification of glucan sucrase produced by *Leuconostoc mesenteroides* DRP2-19 isolated from Chinese Sauerkraut. Prep Biochem Biotech 48 (6): 465-473. DOI: 10.1080/10826068.2018.1466149.

Fei YS, Wei W, Li B, Jie HY, Ping DY, Jin X, Qin LF. 2016. Antimicrobial resistance, virulence profile, and molecular characterization of *Listeria monocytogenes* isolated from ready-to-eat food in China, 2013-2014. Biomed Environ Sci 29 (6): 448-452. DOI: 10.3967/bes2016.058.

Fernández-Cruz ML, Martín-Cabrejas I, Pérez-del Palacio J, Gaya P, Díaz-Navarro C, Navas JM, Arqués JL. 2016. In vitro toxicity of reuterin, a potential food biopreservative. Food Chem Toxicol 96: 155-159.

Field D, Ross RP, Hill C. 2018. Developing bacteriocins of lactic acid bacteria into next-generation biopreservatives. Curr Opin Food Sci 20: 1-6. DOI: 10.1016/j.cofs.2018.02.004.

Gabriel AA, Vera DD, Lazo Omy, Azarcon VB, de Ocampo CG, Marasigan JC, Sandel GT. 2017. Ultraviolet-C inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, and *Salmonella enteritica* in liquid egg white. Food Control 73 (B): 1303-1309. DOI: 10.1016/j.foodcont.2016.10.060.

Gänzle MG. 2015. Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr Opin Food Sci 2: 106-117. DOI: 10.1016/j.cofs.2015.03.001.

Gnanasekaran G, Lee JH, Kim H, Cho SH. 2019. The complete genome sequence and comparative genome analysis of the multi-drug resistant food-borne pathogen *Bacillus cereus*. Genomics S0888-7543 (19): 30079-5. DOI: 10.1016/j.ygeno.2019.05.003.

Hamidiyan N, Salehi-Abargouei A, Rezaei Z, Dehghani-Tafti R, Akrami-Mohajeri F. 2018. The prevalence of *Listeria* spp. food contamination in Iran: A systematic review and meta-analysis. Food Res Intl 107: 437-450. DOI: 10.1016/j.foodres.2018.02.038.

Haubert L, dos Santos Cruxen CE, Fiorentini AM, Padilha da Silva WP. 2018. Tetracycline resistance transfer from foodborne *Listeria monocytogenes* to *Enterococcus faecalis* in Minas Frescal cheese. Intl Dairy J 87: 11-15. DOI: 10.1016/j.idairyj.2018.07.014.

Hiko A, Bushura E, Belina D. 2019. Occurrence of Listeria in food chilling facilities of the different campuses in Haramaya University, Ethiopia. Sci Afr 3: e00074. DOI: 10.1016/j.sciaf.2019.e00074.

Ho VTT, Lo R, Bansal N, Turner MS. 2018. Characterisation of *Lactococcus lactis* isolates from herbs, fruits and vegetables for use as biopreservatives against *Listeria monocytogenes* in cheese. Food Control 85: 472-483. DOI: 10.1016/j.foodcont.2017.09.036.

Huang J, Luo Y, Zhou B, Zheng J, Nou X. 2019. Growth and survival of *Salmonella enterica* and *Listeria monocytogenes* on fresh-cut produce and their juice extracts: Impacts and interactions of food matrices and temperature abuse conditions. Food Control 100: 300-304. DOI: 10.1016/j.foodcont.2018.12.035.

Incili GK, Koluman A, Aktüre A, Ataşalan A. 2019. Validation and verification of LAMP, ISO, and VIDAS UP methods for detection of *Escherichia coli* O157:H7 in different food matrices. J Microbiol Methods 165: 105697. DOI: 10.1016/j.mimet.2019.105697.

Jiang M, Zhang F, Wan C, Xiong Y, Shah NP, Wei H, Tao, X. 2016. Evaluation of probiotic properties of *Lactobacillus plantarum* WLPL04 isolated from human breast milk. J Dairy Sci 99 (3): 1736-1746. DOI: 10.3168/jds.2015-10434.

Jimenez E, Alba Yepez A, Perez-Cataluna A, Vasquez, ER, Davila DZ, Vignolo G, Aznar R. 2018. Exploring diversity and biotechnological potential of lactic acid bacteria from tocosh-traditional Peruvian fermented potatoes - by high throughput sequencing (HTS) and culturing. LWT 87: 567-574. DOI: 10.1016/j.lwt.2017.09.033.

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.

Kaihei Oki K, Rai AK, Sato S, Watanabe K, Tamang JP. 2011. Lactic acid bacteria isolated from ethnic preserved meat products of the Western Himalayas. Food Microbiol 28 (7): 1308-1315. DOI: 10.1016/j.fm.2011.06.001.

Kang JH, Lee MS. 2005. Characterization of a bacteriocin produced by *Enterococcus faecium* GM-1 isolated from an infant. J Appl Microbiol 98 (5): 1169-1176. DOI: 10.1111/j.1365-2672.2005.02556.x.

Kawada-Matsuo M, Komatsuzawa H. 2017. Role of *Streptococcus mutans* two-component systems in antimicrobial peptide resistance in the oral cavity. Jpn Dent Sci Rev 53 (3): 86-94. DOI: 10.1016/j.jdsr.2016.12.002.

Kim N, Kim WJ, Kang S. 2019. Anti-biofilm effect of crude bacteriocin derived from *Lactobacillus brevis* DF01 on *Escherichia coli* and *Salmonella typhimurium*. Food Control 98: 274-280. DOI: 10.1016/j.foodcont.2018.11.004.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120. DOI: 10.1007/BF01731581.

Kongnum K, Hongpattarakere T. 2012. Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of white shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*.

Fish Shellfish Immun 32 (1): 170-177. DOI: 10.1016/j.fsi.2011.11.008.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33: 1870-1874. DOI: 10.1093/molbev/msw054.

Kumariya R, Garsa AK, Rajput YS, Sood SK, Akhtar N, Patel S. 2019. Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. Microb Pathog 128: 171-177.

Lee S, Kim M. 2019. *Leuconostoc mesenteroides* MKSR isolated from kimchi possesses α-glucosidase inhibitory activity, antioxidant activity, and cholesterol-lowering effects. LWT 116: 108570. DOI: 10.1016/j.lwt.2019.108570.

Li J, Bi Y, Sun S, Peng D. 2017. Simultaneous analysis of tert-butylhydroquinone, tert-butylquinone, butylated hydroxytoluene, 2-tert-butyl-4-hydroxyanisole, 3-tert-butyl-4-hydroxyanisole, α-tocopherol, γ-tocopherol, and δ-tocopherol in edible oils by normal-phase high-performance liquid chromatography. Food Chem 234: 205-211. DOI: 10.1016/j.foodchem.2017.04.176.

Mazzoli R, Bosco F, Mizrahi I, Bayer EA, Pessione E. 2014. Towards lactic acid bacteria-based biorefineries. Biotechnol Adv 32 (7): 1216-1236. DOI: 10.1016/j.biotechadv.2014.07.005.

Md Sidek NL, Tan JS, Abbasiliasi S, Wong FWF, Mustafa S, Ariff AB. 2016. Aqueous two-phase flotation for primary recovery of bacteriocin-like inhibitory substance (BLIS) from *Pediococcus acidilactici* Kp10. J Chromatogr B 1027: 81-87. DOI: 10.1016/j.jchromb.2016.05.024.

Michel E, Monfort C, Deffrasnes M, Guezenec S, Lhomme E, Barret M, Sicard D, Dousset X, Onno B. 2016. Characterization of relative abundance of lactic acid bacteria species in French organic sourdough by cultural, qPCR and MiSeq high-throughput sequencing methods. Intl J Food Microbiol 239: 35-43. DOI: 10.1016/j.ijfoodmicro.2016.07.034.

Mohamed TY, Nassar MY, Amin AS, Elnadi MM. 2018. Spectrophotometric determination of butylated hydroxyanisole in pure form and cream formulation via an oxidation-reduction reaction. Chemical Data Collections 15-16: 229-237. DOI: 10.1016/j.cdc.2018.05.002.

Mohammadzadeh-Aghdash H, Sohrabi Y, Mohammadi A, Shanehbandi D, Dehghan P, Dolatabadi JEN. 2018. Safety assessment of sodium acetate, sodium diacetate and potassium sorbate food additives. Food Chem 257: 211-215. DOI: 10.1016/j.foodchem.2018.03.020.

Mohapatra AR, Jeevaratnam K. 2019. Inhibiting bacterial colonization on catheters: Antibacterial and antibiofilm activities of bacteriocins from *Lactobacillus plantarum* SJ33. J Glob Antimicrob Resist 19: 85-92. DOI: 10.1016/j.jgar.2019.02.021.

Nguyen DTL, Hoorde, KV, Cnockaert M, De Brandt E, De Bruyne K, Thanh Le B, Vandamme P. 2013. A culture-dependent and -independent approach for the identification of lactic acid bacteria associated with the production of nem chua, a Vietnamese fermented meat product. Food Res Intl 50 (1): 232-240.

Nurhikmayani R, Daryono BS, Retnaningrum E. 2019. Isolation and molecular identification of antimicrobial-producing Lactic Acid Bacteria from chao, South Sulawesi (Indonesia) fermented fish product. Biodiversitas 20 (4): 1063-1068. DOI: 10.13057/biodiv/d200418

Oguntoyinbo FA, Narbad A. 2015. Multifunctional properties of *Lactobacillus plantarum* strains isolated from fermented cereal foods. J Funct Foods 17: 621-631. DOI: 10.1016/j.jff.2015.06.022.

Paray BA, Rather IA, Al-Sadoon MK, Hamad AF. 2018. Pharmaceutical significance of *Leuconostoc mesenteroides* KS-TN11 isolated from Nile Tilapia, Oreochromis niloticus. Saudi Pharm J 26 (4): 509-514. DOI: 10.1016/j.jsps.2018.02.006.

Pasteris SE, Pingitore VE, Ale CE, Nader-Maias ME, 2014. Characterization of a bacteriocin produced by *Lactococcus lactis* subsp. lactis CRL 1584 isolated from a *Lithobates catesbeianus* hatchery. World J Microbiol Biotechnol 30 (3): 1053-1062. DOI: 10.1007/s11274-013-1524-9.

Pei J, Jin W, Abd El-Aty AM, Baranenko DA, Gou X, Zhang H, Geng J, Jiang L, Chen D, Yue T. 2020. Isolation, purification, and structural identification of a new bacteriocin made by *Lactobacillus plantarum* found in conventional kombucha. Food Control 110: 106923. DOI: 10.1016/j.foodcont.2019.106923.

Piper JD, Piper PW. 2017. Benzoate and sorbate salts: a systematic review of the potential hazards of these invaluable preservatives and the expanding spectrum of clinical uses for sodium benzoate. Compr Re Food Sci F 16 (5): 868-880. DOI: 10.1111/1541-4337.12284.

Powell JE, Witthuhn RC, Todorov SD, Lmt D. 2007. Characterization of bacteriocin ST8KF produced by a kefir isolate *Lactobacillus plantarum* ST8KF. Intl Dairy J 17 (3): 190-198. DOI: 10.1016/j.idairyj.2006.02.012.

Rasheed HA, Tuoheti T, Zhang Y, Azi F, Tekliye M, Dong M. 2020. Purification and partial characterization of a novel bacteriocin produced by bacteriocinogenic *Lactobacillus fermentum* BZ532 isolated from Chinese fermented cereal beverage (Bozai). LWT 124: 109113. DOI: 10.1016/j.lwt.2020.109113.

Renschler MA, Wyatt A, Anene N, Robinson-Hill R, Pickerill ES, Fox NE, Griffith JA, McKillip JL. 2020. Using nitrous acid-modified de Man, Rogosa, and Sharpe medium to selectively isolate and culture lactic acid bacteria from dairy foods. J Dairy Sci 103 (2): 1215-1222. DOI: 10.3168/jds.2019-17041.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4 (4): 406-4025.

Sanger F, Nicklen S, Coulson AR. 1977. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 4 (12): 5463-5467.

Singh NP, Tiwari A, Bansal A, Thakur S, Sharma G, Gabrani R. 2015. Genome level analysis of bacteriocins of lactic acid bacteria. Comput Biol Chem 56: 1-6. DOI: 10.1016/j.compbiolchem.2015.02.013.

Skariyachan S, Govindarajan S. 2019. Biopreservation potential of antimicrobial protein-producing *Pediococcus* spp. towards selected food samples in comparison with chemical preservatives. Intl J Food Microbiol 29116: 189-196. DOI: 10.1016/j.ijfoodmicro.2018.12.002.

Tan JS, Abbasiliasi S, Ibrahim TAT, Kadkhodaei S, Suan Ng H, Vakhshiteh F, Ajdari Z, Mustafa S, Ling TC, Rahim RA, Ariff AB. 2014. Primary recovery of thermostable lipase 42 derived from recombinant *Escherichia coli* BL21 in aqueous two-phase flotation. Sep Pur Technol 133: 328-334. DOI: 10.1016/j.seppur.2014.06.048.

Vijayakumar M, Ilavenil S, Kim DH, Arasu MV, Priya K, Choi KC. 2015. In-vitro assessment of the probiotic potential of *Lactobacillus plantarum* KCC-24 isolated from Italian rye-grass (*Lolium multiflorum*) forage. Anaerobe 32: 90-97. DOI: 10.1016/j.anaerobe.2015.01.003.

Vitas AI, Díez-Leturia M, Tabar L, González D. 2014. Improving the methodology for *Listeria monocytogenes* detection in smoked salmon by using the wet pooling test. Intl J Food Microbiol 184: 109-112. DOI: 10.1016/j.ijfoodmicro.2013.11.036.

Wong FWF, Ariff AB, Abbasiliasi S, Stuckey DC. 2017. Recovery of a bacteriocin-like inhibitory substance from *Pediococcus acidilactici* Kp10 using surfactant precipitation. Food Chem 232: 245-252. DOI: 10.1016/j.foodchem.2017.03.102.

Xi Q W, Wang J, Du RP, Zhao FK, Han Y, Zhou ZJ. 2018. Purification and characterization of bacteriocin produced by a strain of *Enterococcus faecalis* TG2. Appl Biochem Biotech 184 (4): 1106-1119. DOI: 10.1007/s12010-017-2614-1.

Yang SC, Lin CH, Sung CT, Fang JY. 2014. Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. Front Microbiol 5 (241): 1-10. DOI: 10.3389/fmicb.2014.00241

Yépez A, Luz C, Meca G, Vignolo G, Mañes J, Aznar R. 2017. Biopreservation potential of lactic acid bacteria from Andean fermented food of vegetal origin. Food Control 78: 393-400. DOI: 10.1016/j.foodcont.2017.03.009.

Yi L, Dang J, Zhang L, Wu Y, Liu B, Lü X. 2016. Purification, characterization and bactericidal mechanism of a broad spectrum bacteriocin with antimicrobial activity against multi drug resistant strains produced by *Lactobacillus coryniformis* XN8. Food Control 67: 53-62. DOI: 10.1016/j.foodcont.2016.02.008.

Yu J, Wang HM, Zha MS, Qing YT, Bai N, Ren Y, Xi X, Liu WJ, Menghe BLG, Zhang HP. 2015. Molecular identification and quantification of lactic acid bacteria in traditional fermented dairy foods of Russia. J Dairy Sci 98 (8): 5143-5154. DOI: 10.3168/jds.2015-9460.

Zou J, Jiang H, Cheng H, Fang J, Huang G. 2018. Strategies for screening, purification and characterization of bacteriocins. Intl J Biol Macromol 117: 781-789. DOI: 10.1016/j.ijbiomac.2018.05.233.