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Detection of Plantaricin-Encoding Gene and Its Partial Purification in *Lactobacillus plantarum* BP102

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Abstract. *Lactobacillus plantarum BP102 isolated from garlic bulb tissue has probiotic properties, including producing bacteriocin called plantaricin. This study aimed to detect the gene encoding bacteriocin produced by Lactobacillus plantarum BP102, and to evaluate the bacteriocin activity at each stage of partial purification. After the end of the log phase of L. plantarum BP102 was determined, and the bacteriocin-encoding genes were checked by PCR technique. Partial purification of bacteriocin was elucidated including pH-neutralized cell-free-supernatant (CFS), precipitation using 80% of ammonium sulfate, and dialysis (cut-off 10 kDa), then the bacteriocin activity in every partial purification stage was evaluated. The molecular weight of plantaricin was estimated using SDS-PAGE analysis. Lactobacillus plantarum BP102 harbored the gene encoding plantaricin (pln) biosynthesis, namely plnEF and plnK genes. The activity of crude bacteriocin was inactivated by the presence of proteinase-K enzyme. The protein concentration was gradually decreased along with the purification process. The bacteriocin activity was demonstrated at each step of the purification process (CFS, precipitation, and dialysis) against Bacillus cereus by 9.23 ± 0.20 mm, 7.86 ± 0.15 mm, and 7.6 ± 0.10 mm, respectively; while, Escherichia coli by 10.3 ± 0.55 mm, 7.4 ± 0.1 mm, and 6.86 ± 0.45, respectively. The molecular weight of partially purified bacteriocin BP102 was found to be approximately 15.9 kDa. The overlaid part of the gel showed a slight inhibition against E. coli due to a low protein concentration. This bacteriocin purification process should be further optimized to improve the bacteriocin activity that could be useful for food preservation.*

Keywords: *Lactobacillus plantarum, partially purified, plantaricin, precipitation, probiotics*

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INTRODUCTION

Lactic acid bacteria (LAB) are typically acknowledged as having the potential to improve health and be used in food applications (Barcenilla et al., 2022; Jean et al., 2023). Lactic acid bacteria (LAB) are Gram-positive, acid-fast, non-spore-forming bacteria and produce lactic acid (acetic acid), ethanol, CO_2, formic acid, peroxides, and bacteriocins. LAB can be used as a functional culture because it contributes to organoleptic properties, nutrition, and health and can control pathogenic bacteria in food. LAB produced bacteriocins which are antimicrobial peptides synthesized via ribosomes (Setiarto et al., 2023).

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Bacteriocins kill target bacteria by binding to specific membrane proteins called bacteriocin receptors. Peptide and protein interactions cause membrane leakage and death (Lyu et al., 2023).

Based on their biochemical properties, bacteriocins are classified into four groups, namely class I (lantibiotics), class II (non-antibiotics) which are small and heat-stable peptides, class III (large bacteriocins) which are heat labile, and class IV (complex bacteriocins containing lipid or carbohydrate moiety) (Mekala et al., 2023). Bacteriocins of lactic acid bacteria have advantages such as being safe for human food additives and stable over a wide pH and temperature range (Even et al., 2002; Azhar et al., 2023). Therefore, bacteriocins can be used as starter cultures and food additives (Meral Aktaş et al., 2023; Usman & Ruqqayah, 2023). Other studies reported the use of bacteriocin from *Lactobacillus plantarum* as a starter culture to increase food security (Eric Donald et al., 2023; Xu et al., 2023).

Lactobacillus plantarum produces plantaricin-type bacteriocins such as plantaricin (pln) F (Ahaddin et al., 2021), pln Y (Chen et al., 2014), pln EF (Zhao et al., 2021), pln JK (Seddik et al., 2017), pln UG1 (Altalhi, 2008), pln W (Holo et al., 2001). Bacteriocin Lac-B23 produced by *L. plantarum* J23 has the character of pH stability, sensitivity to trypsin, proteinase-K, proteinase, and an estimated molecular weight of about 6.73 kDa (Zhang et al., 2018). *L. plantarum* SLG10 produces a new bacteriocin SLG10 that has antibacterial activity against Gram-positive and Gram-negative bacteria, with an estimated molecular weight of about 1422 Da (Pei et al., 2020). De Giani et al. (2019) found that plantaricin P1053 from *L. plantarum* has antibacterial activity against Staphylococcus aureus and *Escherichia coli*, the estimated molecular weight is 1053 Da. Although some side effects or safety concerns have been reported, the potential use of probiotics still attracts attention, therefore, it is necessary to assess the safety of new probiotic strains before using them (Deng et al., 2023). Other studies reported the use of bacteriocin from *L. plantarum* as a starter culture to increase food security (Eric Donald et al., 2023; Xu et al., 2023). *L. plantarum* BP102 was used in this study as a superior LAB isolate isolated from garlic bulbs (Wardhani, 2019). The aims of this study were to detect the bacteriocin-encoding gene and to evaluate the antimicrobial activity of the partiallypurified bacteriocin produced by *L. plantarum* BP102. The results of this study will contribute to microbial biotechnology so that it is expected to become a natural starter culture candidate in the future.

MATERIALS AND METHODS

Time and Location of Research

This research was conducted from December 2020 to June 2021 at the Microbology Laboratory and Molecular Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya.

Bacterial Culture Preparation

Lactobacillus plantarum BP102 is a collection of the Laboratory of Microbiology, Faculty of Mathematics and Science, Brawijaya University. *L. plantarum* BP102 is a plant endophytic bacterium from garlic (Wardhani, 2019). Both were cultured in de Man Rogosa Sharpe (MRS) broth and incubated as a pre-culture at 37 °C for 24 h. The pathogenic bacteria, Gram-negative bacteria *Escherichia coli* and Gram-positive bacteria *Bacillus cereus* is a collection of the Laboratory of Microbiology, Faculty of Mathematics and Science, Brawijaya University. All bacteria used in this study were cultured on nutrient agar (NA)

media (Merck, Darmstadt, Germany).

Detection of Bacteriocin-Encoding Genes

DNA extraction follows the heat treatment method (Héchard & Sahl, 2002). *L. plantarum* isolates were cultured on agar medium for 24 h, suspended in 200 µL ddH2O, and heated at 95 ºC for 20 minutes. The samples were centrifuged at 10.000 rpm for 5 minutes at 4 ºC. The supernatant was transferred to a new microtube. Bacteriocin-encoding genes were detected using the PCR technique with primer (Table 1). The amplification cycle consisted of pre-denaturing for 5 minutes at 95 °C, denaturing for 1 minute at 95 °C, annealing for 1 minute at 60 °C, extension for 1 minute at 72 °C, post extension for 5 minutes at 72 °C. The primers for the detection of bacteriocin-encoding genes are shown in Table 1. Electrophoresis of 1.5% (w/v) agarose gel stained with 1 µL of Gel red (Biotium, Australia) and visualized in Gel Documentation Imaging (E-Box, France).

Table 1. Primer sequences of plantaricin

Genes	Primer	Sequences (5'-3')	Amplicon Size (bp)	Source
plnA	PlanA-F	GTA CAG TAC TAA TGG GAG	450	Diep et al., 1995
	PlanA-R	CTT ACG CCA ATC TAT ACG		
plnEF	PlanEF-F	GGC ATA GTT AAA ATT CCC CCC	428	Diep et al., 1995
	PlanEF-R	CAG GTT GCC GCA AAA AAA G		
plnJ	PlanJ-F	TAA CGA CGG ATT GCT CTG	475	Diep et al., 1995
	PlanJ-R	AAT CAA GGA ATT ATC ACA TTA GTC		
plnK	PlanK-F	CTG TAA GCA TTG CTA ACC AAT C	246	Diep et al., 1995
	PlanK-R	ACT GCT GAC GCT GAA AAG		
plnO	PlanO-F	GTT CGT CCG GAA CGT TTT GT	300	Diep et al., 1995

Growth Curve Determination

The single colony of *L. plantarum* BP102 was grown on 10 mL MRS broth (Merck, Darmstadt, Germany) and incubated for 32 h. Sampling was carried out every 2 h in duplicates. The number of cells was counted with a hemocytometer.

Preparation of Cell-Free Supernatant (CFS)

50 mL preculture of *Lactobacillus planarum* BP102 was transferred to 450 mL MRS broth medium (Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h. The cultures were centrifuged at 10.000 rpm for 10 min at 4 °C to collect cell-free supernatant (CFS). The acidity level (pH) of CFS was adjusted to 6.5-7.0 by adding 1 N NaOH, then filter sterilized using a millipore membrane (Merck, Darmstadt, Germany) with a pore diameter of 0.22 µm (Mulyawati et al., 2019b).

Confirmation of Proteinaceous Character

The character of bacteriocin protein was confirmed by proteinase-K (1 mg/mL). The CFS bacteriocin 250 µL was mixed with 750 µL of proteinase-K (1 mg/mL) at pH 7 and incubated at 37 °C. The filtrate was sterilized into a sterile tube using a 0.22 µm diameter Millipore (Merck, Darmstadt, Germany). Antibacterial supernatant 20 µL was dropped

onto a sterile blank 6 mm diameter disc. The Blank disc was placed on NA medium inoculated with 100 µL of the indicator bacteria *E. coli* and *B. cereus* (10^6 cell/mL) and incubated at 37 °C for 24 h (Andarilla et al., 2011). Each sample was repeated three times and 1 control (compare sample).

Procedure to Measure The Bacterial Activities of Bacteriocin

The antibacterial activity of bacteriocins at each stage of purification (CFS, precipitate and dialysis) was carried out with three replications. A total of 60 µL (CFS, precipitate and dialysis) was dropped onto a sterile blank 6 mm diameter blank disk. The blank disc was placed on NA medium inoculated with 100 µL of the indicator bacteria *E. coli* and *B. cereus* (10^6 cell/mL) and incubated at 37 °C for 24 h. The diameter of the resulting inhibition zone around the blank disk was measured using a digital caliper (mm) as bacteriocin activity after being incubated for 24 h at 37 °C. The inhibitory activity assay was conducted in triplicates. The formula of the inhibition index is as follows.

$$\text{Inhibition index (mm)} = \frac{\text{clear zone diameter} - \text{blank disk diameter}}{\text{blank disk diameter}}$$

....(Putri et al., 2019)

Partial Purification of Bacteriocin

Partial purification was performed by precipitation of ammonium sulfate. The CFS was transferred to a 250 mL conical flask and gradually precipitated with 80% ammonium sulfate. For 24 hours, the mixture was incubated at 4 °C with a stirrer. The mixture was centrifuged for 10 minutes at 10.000 rpm and 4 °C. The precipitated bacteriocins on the tube wall were resuspended in 1 mL of 0.2 M buffered phosphate saline (PBS) pH 6.5. The suspensions were then transferred to a semi-permeable membrane with a lower molecular weight cut-off of 10 kDa, dialyzed against 0.05 M of PBS, and incubated at 4 ºC while stirring. At -20°C, the partially purified bacteriocins were kept.

Screening of Bacteriocin Activity in Each Purification Step Includes CFS, Precipitates, and Dialysis

The agar diffusion method is used to test the bacteriocin activity in each step of the purification process, including the screening of CFS, precipitates, and dialysis. The sample tested was CFS with a neutral pH of 60 µL, the sample was dropped onto a sterile blank disk with a diameter of 6 mm. The same treatment was for precipitation and dialysis. The blank discs were placed on NA media inoculated with 100 µL of the indicator bacteria *E. coli* and *B. cereus* (10^6 cell/mL) using a cotton swab and incubated at 37 °C for 24 h. The diameter of the zone of inhibition was measured as bacteriocin activity using a digital caliper. The inhibitory activity assay was performed three times (Putri et al., 2019).

Determination of Molecular Weight with SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide)

The protein molecular weight (CFS, precipitation, and dialysis) was determined by the sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis method. The protein samples were added with reduction buffer (1:1 v/v) (4.6% SDS, 10% mercaptoethanol, 20% glycerol, 1.5% Tris-HCl pH 6.8, 1% bromophenol blue). The sample was heated at 70 ºC for 5 minutes. The sample (20 µL) was placed in the SDS-PAGE. The stacking gel had an acrylamide concentration of 8%, while the separating gel had a concentration of 16.5%. A Mini-PROTEAN Tetra Cell (Bio-Rad Laboratories, Inc., Berkeley,

California) was used for electrophoresis for 2 hours at 110 V. Each sample was put into two separate wells. Two pieces of the gel were separated. The first part of the gel was stained with Coomassie Blue R250 for 15 minutes and then decomposed with a decolorizing solution (methanol, glacial acetic acid, dH2O) in a shaking incubator overnight at room temperature. The second part of the SDS-PAGE gel was used to test bacteriocin activity.

The 1% soft agar overlay method was used to confirm the antibacterial activity of the protein bands. The gel containing protein bands was cut and soaked in ddH2O for 1 hour, and the solution was replaced by 100 mL every 30 minutes. Then, 100 µL of *E. coli* (10^6 cell/mL) indicator bacteria were inoculated using a cotton swab. The clear zone formed around the band was observed. Protein concentration was measured using nanodroplets. The results of the dialysis protein were heated to 100 °C for 5 minutes and the antagonist assay was performed by inoculating *E. coli* and *B. cereus* (10^6 cell/mL) using a cotton swab on NA medium and then incubated at 37 °C for 24 h. This was evaluated to determine whether heat treatment during SDS-PAGE sample preparation could reduce or eliminate bacteriocin activity.

RESULTS AND DISCUSSION

Bacteriocin-Encoding Genes

The PCR analysis used five pairs of bacteriocin-specific primers *plnA, plnEF, plnJ, plnK,* and *plnO*. The results showed that the *Lactobacillus plantarum* had the bacteriocin gene plnEF with a size of ±428 bp and plnK with a size of ±246 bp. The bacteriocin-encoded gene detection found that bacteriocin type belonged to plantaricin (pln) which is a second type B bacteriocin with two peptides. Its activity is regarding the two distinct peptide actions (Barbosa et al., 2021; Kawahara et al., 2022). According to Zacharof & Lovitt (2012), *L. plantarum* generated at least six different bacteriocins.

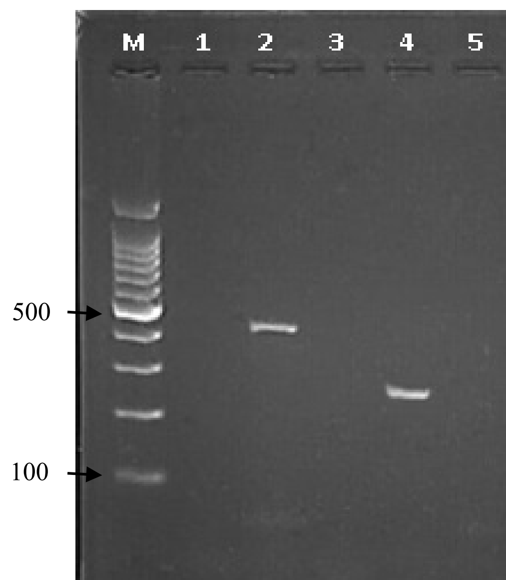

Figure 1. Results of PCR analysis of *L. plantarum* BP102. M: marker, 1: *plnA*, 2: *plnEF*, 3: *plnO*, 4: *plnK*, 5: *plnJ*

The Growth Curve of *Lactobacillus plantarum* BP102

Determination of the end of the exponential phase of *L. plantarum* BP102 was carried out as the basis for determining the incubation time to then take the crude bacteriocin extract in the form of CFS. The growth curve of *L. plantarum* BP102 revealed that the incubation time was 14 h. The number of cells growing on the media reached its peak at 14 h, in addition, the bacteria also were believed to produce high metabolite at the same time (Figure 2). The lag phase on this curve is not detected, it is suspected that this phase lasts a short time and is not observed during the hours of observation, in this phase the bacteria adapt to the environment. The lag phase can be affected by temperature, pH, and media conditions (Matejčeková et al., 2016; Averina et al., 2020). The stationary phase is 14-16 h

and the dead phase is 16-32. The log phase shows cells dividing at a constant rate and balanced growth. The stationary phase shows the accumulation of metabolites resulting from cell metabolic activity and reduced nutrient content. So that competition occurs and causes some cells to die and others to continue to grow in relatively constant numbers.

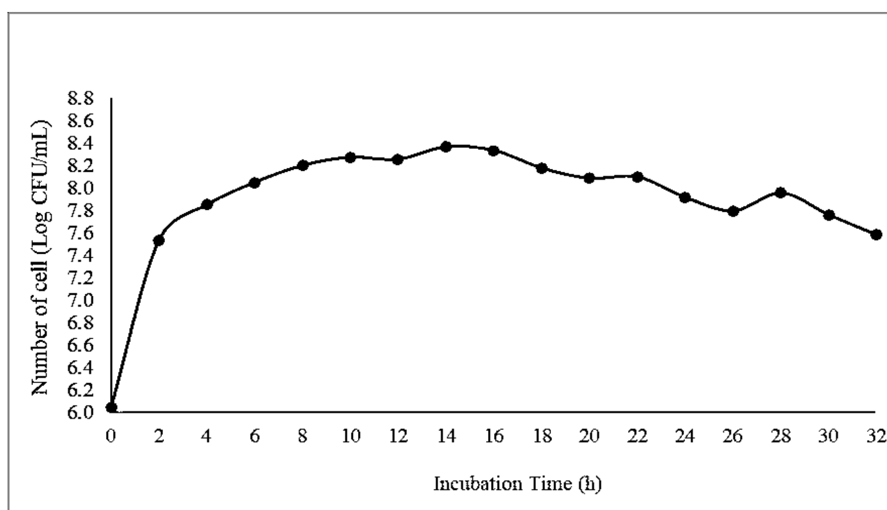

Figure 2. The Lactobacillus plantarum BP102 growth curve

Bacteriocin Protein Character

The initial characterization observed bacteriocin activity, namely CFS of *L Lactobacillus plantarum* BP102 which was added with proteinase-K. As a result, it did not produce an inhibition zone in both *Escherichia coli* and *Bacillus cereus* pathogenic bacteria. The CFS without proteinase-K as a control resulted in a clear zone. This indicated that the antibacterial activity caused by bacteriocin activity. Antibacterial activity was lost after the addition of proteolytic enzymes that can damage proteins (Bizani & Brandelli, 2002). The sensitivity of bacteriocin as a food biopreservative to different protease enzymes, including proteinase-K, trypsin, -amylase, and papainase, was examined. However, bacteriocins are generally more sensitive to proteinase-K (Todorov & Dicks, 2006). *L. plantarum* BP102 bacteriocin has a broad antibacterial spectrum and can inhibit both Gram-positive and Gram-negative pathogenic bacteria, namely *E.coli* and *B. cereus*. This is in line with the research by (Aruna et al., 2016) which produced a broad spectrum of bacteriocins against Gram-positive and Gram-negative bacteria.

Bacteriocin Activity Against Pathogenic Bacteria

E. coli growth was inhibited by bacteriocin activity (10.3 ± 0.55 mm; 7.4 ± 0.1 mm; 6.86 ± 0.45 mm; 6.3± 0.15, respectively) (Picture 3). Bacteriocin of *L. plantarum* B102 derived from CFS was significantly higher ($p<0.05$) compared to bacteriocin from the precipitation step. Yet, it showed no significant difference compared with dialysis results. The antibacterial activity of *L. plantarum* BP102 against *E.coli* and *B. cereus* from the highest to lowest were CFS, precipitation and dialysis (Figure 3). The inhibition index was in line with the results of protein concentration, namely CFS: 18.84 µg/mL, precipitation: 13.88 µg/mL, dialysis: 5.06 µg/mL.

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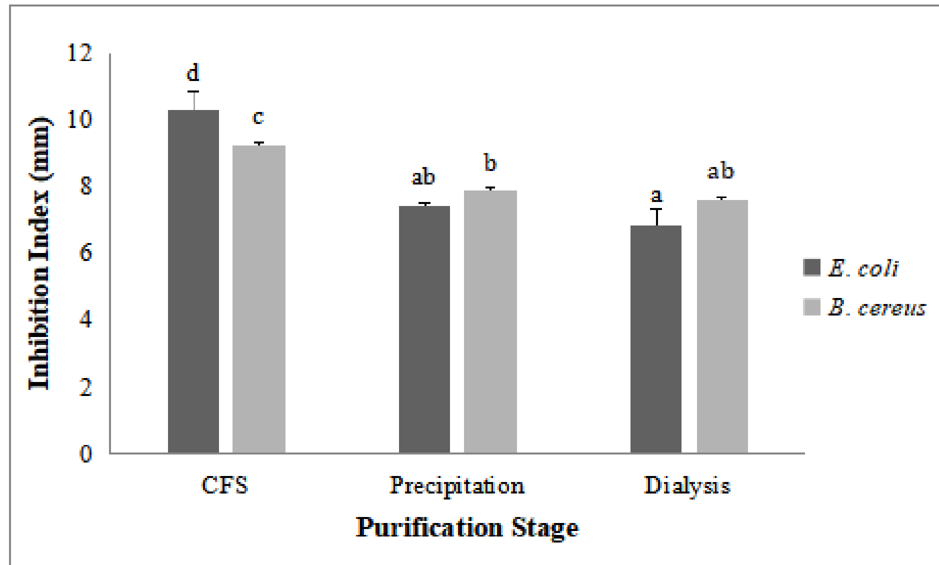

Figure 3. The antibacterial activity of CFS, precipitated bacteriocins, dialysis, *Lactobacillus plantarum* BP102

The plantaricin produced by L. plantarum BP102 has broad-spectrum antimicrobial ability against Gram-positive and Gram-negative bacteria and is effective against pathogenic bacteria that cause food spoilage and food poisoning. It also exhibits antimicrobial activity against *E. coli* and *B. cereus*. According to (Hechard & Sahl, 2002), bacteriocins kill bacteria by creating pores in the cell membrane, eventually leading to the cells leaking and dying. This is indicated by the formation of a clear zone around the blank disk. While the results of purification with heating treatment were tested on both pathogenic bacteria *E. coli* and *B. cereus* the results were not significantly different. It can be assumed that there is no effect of heating during sample preparation whose molecular weight will be searched using the SDS-PAGE method.

The use of the medium, specifically MRS, may have impacted on the high level of CFS activity that was generated. MRS serves as a source of nutrients that lactic acid bacteria need, including carbon, nitrogen, some amino acids, vitamins, growth factors, and minerals. Lactic acid production may be impacted by the media's composition when used for LAB cultivation. Yeast extract and peptones are the foundation of the De-Man Rogosa and Sharpe (MRS) medium, a sophisticated, nutrient-rich medium. Because it is simple to prepare and reasonably priced when compared to other synthetic chemical media, this growth medium is typically used for enrichment, cultivation, and isolation of LAB cultures, both as liquid media and agar media. MRS media is the standard medium used for culturing lactic acid bacteria (LAB), showing consistent growth for LAB growth (Parlindungan et al., 2021). The LAB species that can grow on MRS media include *Leuconostoc*, *Pediococcus*, *Lactobacillus*, *Enterococcus*, and *Weisella* (Wafula et al., 2023). According to the theory after partial purification with ammonium sulphate 80% the protein concentration may increase. The stage of protein precipitation is the concentration or binding of protein so it is expected that the protein concentration will be higher (Hanke et al., 2022). The addition of 80% ammonium sulphate with the help of stirring using a magnetic stirrer aims to increase the rate of dissolving ammonium

sulphate and homogenize the ammonium sulphate throughout every part. The stirring process produces foam in the solution. According to (Baez et al., 2011) the formation of foam will cause a change in the conformation of the protein molecule, the stirring process needs to be done slowly. Due to this process, protein concentration may be decreased.

The concentration of protein by adding salt to the protein solution was a common method. The salt that is often used for bacteriocin protein precipitation is ammonium sulfate. The advantages of using ammonium sulfate are that it has high solubility, does not affect enzyme activity, is affordable, and is better than other organic solvents (Kanwar et al., 2002). A high concentration of 80% salt will reduce protein solubility. It was because the electric charge around the protein increased that attracted water and protein molecules. Partial purification with 80% ammonium sulfate is widely used for bacteriocin precipitation (Cui et al., 2020; Lei et al., 2020; Heidari, 2021).

Bacteriocin production was influenced by several factors, such as carbon, nitrogen, phosphate, and fermentation conditions (pH, temperature, agitation), as well as optimization of bacteriocin production. The maximum incubation time for bacteriocin activity was 12 h and the maximum temperature was 30 °C. The optimal pH for bacteriocin production was influenced by the culture medium and the producing species (Jawan et al., 2020). Nisin Z produced by *Lactobacillus lactis* was optimal at pH 6 in xylose, but at pH 5.5 in glucose media (Matsusaki et al., 1996). Maximum bacteriocin production by *Lactobacillus acidophilus* was at pH 5, while *L. plantarum* was at pH 6 (Tahara et al., 1996).

The activity of bacteriocin decreased after the dialysis stage due to the release of protein so its activity was reduced. It was related to the molecular weight component in the dialysis stage with a cut-off membrane of 10 kDa. Proteins and salts of less than 10 kDa were separated in the dialysis stage by a 10 kDa cut-off membrane. Thus the bacteriocins produced are proteins, polypeptides, and peptides with molecular weights above 10 kDa which are estimated to contain plantaricin (pln) peptides. A decrease in protein concentration after the purification stage was also reported by Alang et al. (2020) for enterocin from *Enterococcus faecalis* K2B1 and Mulyawati et al. (2019b) for plantaricin from *L. plantarum* SB7.

Protein Molecular Weight

The results of SDS-PAGE analysis showed that the estimated molecular weight of the dialysis was 15.9 kDa (Figure 4). Bacteriocin CFS and the precipitation did not produce protein bands. Based on these results, *L. plantarum* BP102 isolate was suspected to produce class II bacteriocin. *L. plantarum* BP102 contains plantaricin genes, namely *plnEF* and *plnK*. The *plnEF* and plnK proteins were characterized by the estimated molecular weight of less than 10 kDa. While the estimated molecular weight of *L. plantarum* BP102 was more than 10 kDa, it was suspected that the protein from SDS-PAGE was a protein from another plantaricin gene. The molecular weight estimation of bacteriocins depended on the producing strain. Plantaricin from *L. plantarum* produced an estimated molecular weight of 2.2 kDa (Lin et al., 2021), 7.34 kDa (Ahaddin et al., 2021), 10 kDa (Siregar et al., 2021), 14.13 kDa (Fadel et al., 2015), sacacin from L. sake C2 estimated molecular weight of 5.5 kDa (Gao et al., 2010). Imai et al. reported that the protein-encoding *plnEF* with a molecular weight of 17 kDa. This indicated that the protein band profile detected in this study was thought to be derived from class II

bacteriocins. This was in accordance with the detection of bacteriocin coding genes, one of which is *plnEF* and *plnK*.

CFS is a cell-free supernatant, all proteins and non-proteins are still present (Tenea & Barrigas, 2018), so several protein bands from SDS-PAGE should have been detected. This also applied to precipitation, in this process all proteins were precipitated and it was suspected that there were many other proteins but in this process, there were no non-proteins (Khochamit et al., 2015). Therefore, it was suspected that the precipitation results should also have a protein band profile detected. CFS protein band profiles and undetected precipitation were also experienced by the study and the results of dialysis detected a protein band profile (Mulyawati et al., 2019b; Alang et al., 2020). Protein bands formed from the results of the dialysis step can be detected through SDS-PAGE analysis. This was because the resulting protein was pure and not mixed with salt or other compounds. The concentration of bacteriocin protein produced was thought to be sufficient to form protein bands using SDS-PAGE. Another study also detected protein bands from the results of the purification (dialysis) stage using SDS-PAGE (Ahaddin et al., 2021; Yadav & Tiwari, 2021).

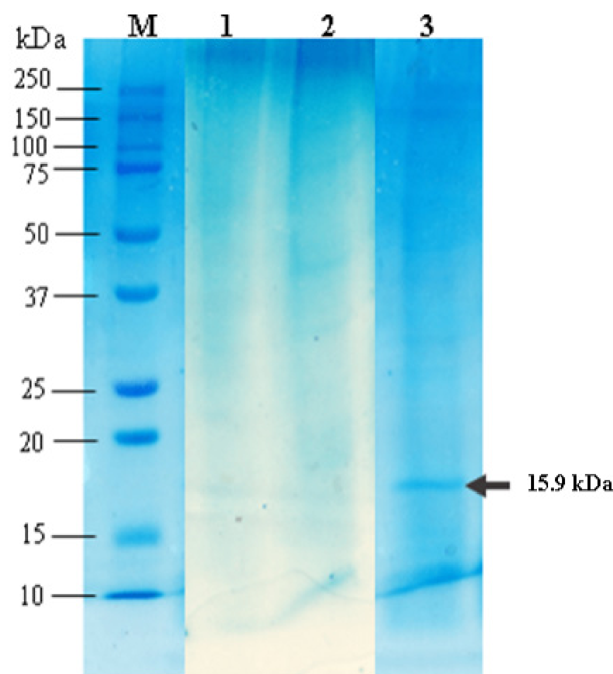

Figure 4. Visualization of the protein band profile from SDS-PAGE. The protein bands that appear are indicated by arrows. M= protein marker; 1= CFS; 2= Precipitation; 3= dialysis

The agar overlay method was used to confirm the bacteriocin activity of the SDS-PAGE gel (Figure 5). The gel containing *L. plantarum* BP102 dialysis bacteriocin revealed the zone of inhibition. The zone of inhibition was slightly on the gel described by the pathogenic bacteria *E. coli*. The inhibitory activity of bacteriocin *L. plantarum* BP102 was slightly due to the low partial purification protein concentration of 5.06 (µg/mL). Mulyawati et al. showed that *L. plantarum* SB7 produced class III bacteriocins with an estimated

molecular weight of around 48 kDa, a protein concentration of 12.56 µg/mL, and the results of the confirmatory test of bacteriocin using the overlay method could be seen clearly. The protein for CFS and precipitation was not detected, this is presumably because there were contaminants, other molecules, and salts, so the protein was not pure. While the results of partial purification detected the protein band profile, it was suspected that other molecules and salt had been lost (the protein was purified) after the dialysis process. The effect of heating the protein for an extended period of time, which results in protease deactivation, is one of many factors that affect the protein's SDS-PAGE band. However, some proteins can withstand 100 °C for a long time. Contaminants may occur in silver-colored gels and occasionally in Coomassie Blue-colored gels, other chemicals, such as certain cationic biocides (Mir et al., 2021).

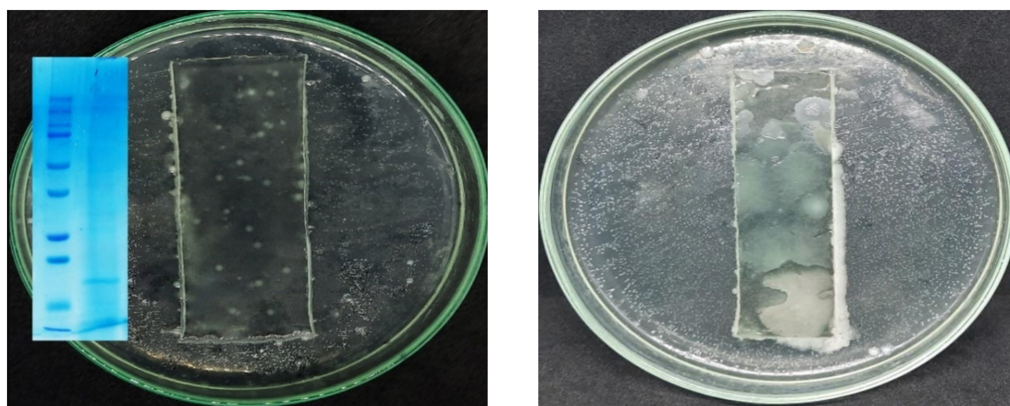

Figure 5. Confirmation test of protein band inhibition activity through agar overlay. (A) Slices of SDS-PAGE gel with dialyzed protein bands, (B) Slices of SDS-PAGE gels without protein bands

The protein concentration, 5.06 g/mL, was quite low. The Amicon Ultra-0.5 centrifugal filter devices for protein or DNA purification and concentration can be used to maximize protein concentration. The decrease in protein concentration was also caused by the weakness of the dialysis bag that comes out a lot. The antibacterial ability and high protein concentration of CFS showed potential as starter culture candidates and food preservatives. Therefore, bacteriocin from *L. plantarum* BP102 could be used as a promising biopreservative candidate in the food industry.

CONCLUSION

Lactobacillus plantarum BP102 as a starter culture candidate for fermentation products. This is because it is able to produce metabolite compounds, namely bacteriocins which accommodate the genes encoding plantaricin (pln) biosynthesis (namely the plnEF and plnK genes), and has bacteriocin activity which is able to inhibit the growth of pathogenic bacteria.

AUTHOR CONTRIBUTION

E.M.S. designing, collecting, and analyzing data and writing scripts. Y.D.J. and I.R. helps design and oversee all research processes.

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CONFLICT OF INTEREST

The published results of our research do not contain any conflicts of interest, either between researchers or with third parties.

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