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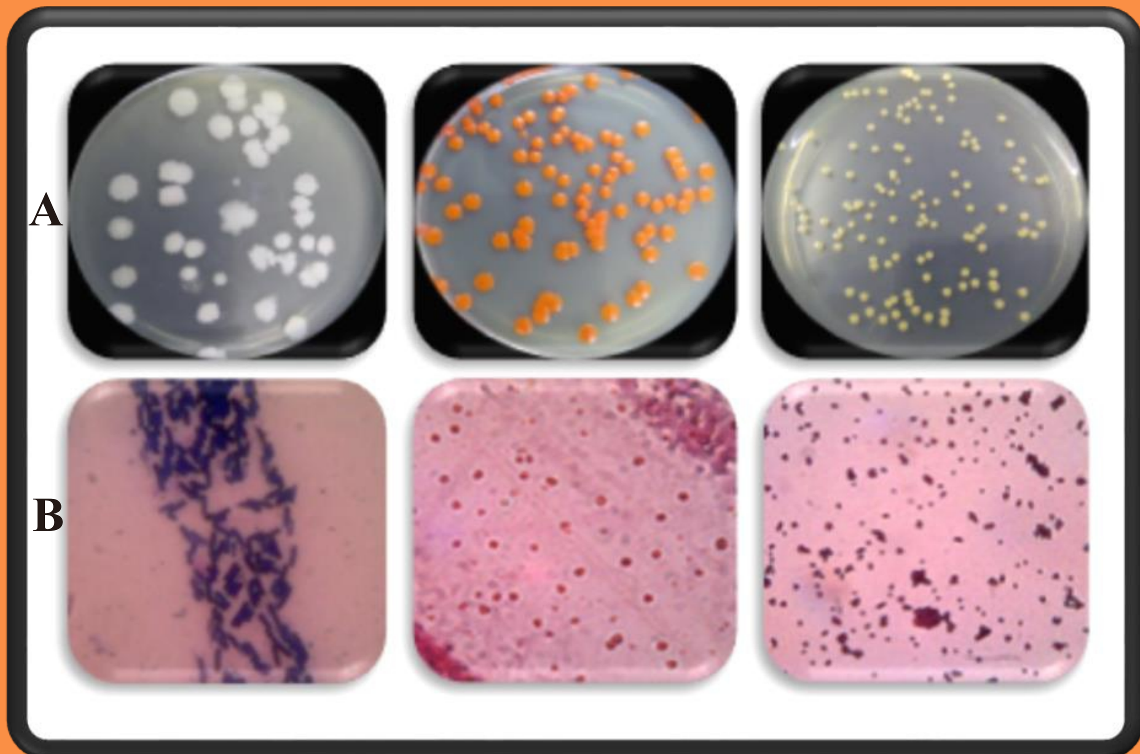

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Keterangan foto cover depan (*Notes of cover picture*): Bentuk koloni isolat bakteri Bt, BLSP-4, dan BLSP-3: (A) pada media pertumbuhan NA dan (B) pada pengamatan secara mikroskopis dengan perbesaran 100x (*Bacterial colony shapes of Bt, BLSP-4 and BLSP-3, respectively: (A) bacterial colony in growth medium NA (B) bacterial colony* on 100 x microscopic magnification), sesuai dengan halaman 15.

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CLONING, EXPRESSION, AND PARTIAL PURIFICATION OF PLANTARICIN W LOCUS PRODUCED BY *Lactobacillus plantarum* S34
[Kloning, Ekspresi, dan Purifikasi Parsial Lokus Plantarisin W Diproduksi oleh *Lactobacillus plantarum* S34]

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ABSTRAK
Lactobacillus plantarum S34 dilaporkan mempunyai aktivitas antibakteri yang terkait dengan produksi bakteriosin. Bagian dari gen yang menyandikan salah satu lokus bakteriosin yang diproduksi oleh *L. plantarum* S34, disebut dengan plantarisin W (*plnW*), diamplifikasi dari plasmid dan dikloning menggunakan sistem vektor pGEM®-T Easy ke dalam *Escherichia coli* DH5α. Sekuens nukleotida *plnW* (± 405 pb) diidentifikasi sebagai protein integral membran. Lebih lanjut, *plnW* diekspresikan secara heterologus sebagai fusi protein dengan His(6)-tag tioredoksin menggunakan vektor ekspresi pET-32a(+) ke dalam *E. coli* BL21 (DE3) pLysS. Protein fusi rekombinan *plnW* terdapat dalam sitoplasma sel, tetapi selain fraksi terlarut terdapat juga fraksi tidak terlarut berupa badan inklusi. Purifikasi parsial dilakukan menggunakan kromatografi afinitas ligan Co^{2+} untuk fraksi terlarut dan metode elektroelusi gel poliakrilamid untuk fraksi tidak terlarut. Massa molekul berukuran kurang lebih 33 kDa terdeteksi berdasarkan pemisahan SDS-PAGE dan dikonfirmasi dengan Western blot sebagai protein fusi rekombinan *plnW*. Protein yang sudah terpurifikasi bermanfaat untuk mengetahui kaitan antara struktur dan fungsi bakteriosin.

Kata kunci: bakteriosin, badan inklusi, *Lactobacillus plantarum* S34, plantarisin W.

ABSTRACT
Lactobacillus plantarum S34 was reported to have antibacterial activities which associated to bacteriocins production. Part of the gene encoding one of the bacteriocin loci that produced by *L. plantarum* S34, termed plantaricin W (*plnW*), was amplified from plasmid and cloned into *Escherichia coli* DH5α using pGEM®-T Easy vector system. *PlnW* nucleotide sequence (± 405 bp) was identified as a putative integral membrane protein. Moreover, *plnW* was heterologously expressed as a fusion protein with His(6)-tagged thioredoxin using pET-32a(+) expression vector into *E. coli* BL21 (DE3) pLysS. Recombinant *plnW* fusion protein was accumulated in the cell cytoplasm, however, along with soluble fractions, insoluble aggregates identified as inclusion bodies were also exhibited. Partial purifications were conducted for the soluble and insoluble fractions using ligand Co^{2+} affinity chromatography and polyacrylamide gel electroelution method, respectively. Molecular mass of approximately 33 kDa was detected based on SDS-PAGE separation and confirmed by Western blot as a recombinant *plnW* fusion protein. The purified protein will be useful for deciphering the bacteriocin structural and functional relationships.

Key words: bacteriocin, inclusion body, *Lactobacillus plantarum* S34, plantaricin W.

INTRODUCTION

Lactic acid bacteria (LAB), group of bacteria acquired generally recognized as safe (GRAS) status, have been known for their valuable role as culture starters and natural preservatives in fermented products since they can inhibit the growth of spoilage microorganisms. One of the antimicrobial agents produced by LAB is bacteriocin, the ribosomally synthesized and extracellularly released peptide or protein which has either narrow or broad spectrum antibacterial effects (Gálvez *et al.*, 2007; Karpiński and Szkaradkiewicz, 2013; Yang *et al.*, 2014). Studies on bacteriocins have been focused on identification, characterization of gene clusters, expression, production, mode of action, biosynthesis, bioactivities, and elucidation of their functional structures (Field *et al.*, 2007; Todorov, 2009).

Lactobacillus plantarum, an adaptable LAB species that can be found in different kinds of carbohydrate rich niches, is able to produce a number of bacteriocins that varies within strains. *L. plantarum* C11 has been reported as one of the earliest bacteriocin producer strains which already genetically characterized in terms of biosynthesis and gene regulator determinants (Diep *et al.*, 1996). In addition, other *L. plantarum* strains including, NC8 (Maldonado *et al.*, 2003), WCFS1 (Kleerebezem *et al.*, 2003), J51 (Navarro *et al.*, 2008), J23 (Rojo-Bezares *et al.*, 2008), V90 (Diep *et al.*, 2009), JDM1 (Zhang *et al.*, 2009), and ST-III (Wang *et al.*, 2011), have been found to retain similar bacteriocin loci in their genome. These loci, termed plantaricins (*pln*), can be either plasmid or chromosomally encoded. Recently, plantaricins have

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been received great attention due to their diversities and potential development of new antimicrobial additives (Diep *et al*., 2009; Sáenz *et al*., 2009).

There are about 22–25 genes within *pln* gene clusters of *L. plantarum* which being arranged into 5–6 operons under the regulation of multiple promoters and responsible for the biosynthesis of various bacteriocins. Two-peptide bacteriocins derived from plantaricin loci have been reported such as plantaricin A (encoded by two peptides *α* and *β*), plantaricin S (encoded by *plsA* and *plsB*), plantaricin W (encoded by *plwα* and *plwβ*), plantaricin NC8 (encoded by *plNC8α* and *plNC8β*), and plantaricin J51 (encoded by *orf3* and *orf4*) (Issen -Meyer *et al*., 1993; Stephens *et al*., 1998; Holo *et al*., 2001; Diep *et al*., 2009).

There are some important operons including *plnABCD*, *plnMNOP* and *plnGHSTUVWXY*. *PlnTUVWXY* codes putative integral membrane proteins acquiring protease CAAX motif which belong to Abi family and associated to bacteriocin self-immunity. These putative transmembrane segments are essential for the integration of immunity proteins into the membrane of the bacteriocin producer. Despite the extensive studies of the operon, the exact role of *plnTUVWXY* remains to be elucidated (Diep *et al*., 2009; Sáenz *et al*., 2009; Tai *et al*., 2015).

Lactobacillus plantarum S34 is one of LAB isolates originated from traditional fermented beef product named *bekasam*, commonly found at Way Kanan, Lampung, Indonesia. Recently, 16S rRNA sequencing analysis shows that *L. plantarum* S34 has 99% nucleotide homology with *L. plantarum* WCFS1 [GenBank Accession No. AL935263]. *L. plantarum* S34 is selected for further molecular genetic study since it shows potential antibacterial activities against representative pathogens such as *Escherichia coli*, *Salmonella typhi*, and *Bacillus subtilis*, which associated to bacteriocins production (Mustopa and Fatimah, 2014). This study aims to gain high yield protein from one of plantaricin loci of *L. plantarum* S34 designated plantaricin W (*plnW*), as a production model of bacteriocin integral membrane protein through heterologous expression in *Escherichia coli*.

MATERIALS AND METHODS

Plasmid DNA Isolation

Plasmid DNA of *L. plantarum* S34 were isolated by alkaline lysis method based on Sambrook *et al*., 1989; and O'Sullivan and Klaenhammer, 1993, with several modifications. Pellets were collected from *L. plantarum* S34 overnight cultures (MRS medium) and resuspended in alkaline lysis I solution (25 mM Tris-HCl pH 8.0, 50 mM glucose, and 10 mM EDTA) containing 10 mg mL^{-1} lysozyme and incubated at 37°C for 2 hours. Freshly prepared alkaline lysis II solution (0.2 N NaOH and 1% SDS) were mixed and incubated at RT for 5 minutes. Next, ice-cold alkaline lysis III solution (5 M potassium acetate, glacial acetic acid, and H_2O) were mixed and incubated on ice for 10 minutes followed by centrifugation at 13,000 *rpm* at 4°C for 10 minutes. The upper phase were mixed (1:1) with PCI (phenol:chloroform:isoamyl alcohol = 25:24:1) followed by centrifugation at 13,000 *rpm* at 4°C for 10 minutes. The upper phase were precipitated with isopropanol (1:1) by incubation at -20°C for 2 hours and followed by centrifugation at 13,000 *rpm* at 4°C for 10 minutes. The pellets were rinsed with 70% ethanol, air-dried and resuspended in H_2O containing 0.1 mg mL^{-1} RNase. Plasmid DNA isolation from *E. coli* DH5α, *E. coli* TOP10 and *E. coli* BL21 (DE3) plysS were performed using QIAGEN plasmid MINI and MIDI kits (Qiagen, Germany), according to manufacture's instructions.

Cloning

PlnW gene was amplified from native plasmid of *L. plantarum* S34 by PCR using Platinum *taq* DNA polymerase (Invitrogen, USA) and primers previously described by Sáenz *et al*., 2009 (forward primer plnWMY42-F: 5'GATCAGCCACGATAC-CAAC-3'; reverse primer plnWMY42-R: 5'-CTAAAGAAAAAGCCCCTGAAAC-3'). The PCR cycles were performed as follow: initial denaturation at 94°C for 3 minutes, 35 cycles of amplification consisting: denaturation at 94°C for 1 minute, annealing at 58.5°C for 1 minute, and extension at 72°C for 90 seconds. The final extension was performed at 72°C for 6 minutes. PCR product (± 750 bp) was confirmed by 1.0% agarose gel electrophoresis. PCR purifications were done using

Qiaquick Gel Extraction kit (Qiagen, Germany) according to manufacture's instructions. Cloning of *plnW* gene into *E. coli* DH5α (Novagen, USA) was performed using pGEM®-T Easy vector system (Promega, USA) according to manufacture's instructions. The molecular size of DNA target (± 750 bp) was confirmed by restriction enzymes (*Nco* I and *Sal* I). Sequence confirmation was performed using T7 promoter and SP6 terminator primers and analyzed using BioEdit software.

Based on the obtained sequence, new primer pairs were designed containing *Bam*H I and *Hind* III restriction sites (forward primer plnWS34BHinF: 5'-GACTGGATCCATGTTACAGAAGAATTTACG-3'; reverse primer plnWS34BHinR: 5'-GATCAAGCTTTCAAATGACGGCATCGAGTG-3'). Plasmid derived from pGEM-plnW were used as template for the PCR cycles as mentioned above with annealing temperature at 55°C for 1 minute. PCR product (± 405 bp) was confirmed and purified.

The target gene was ligated into pET32a (+) (Novagen, USA) expression vector, in-frame with His(6)-tagged thioredoxin using restriction enzymes and T4 ligase (Takara Bio Inc, Japan). The assembled vector was firstly sub-cloned into *E. coli* T0P 10 (Novagen, USA) and then transformed into *E. coli* BL21 (DE3) pLysS (Novagen, USA) as expression host using standard method described by Sambrook *et al.*, 1989. LB agar medium (1.5% w/v) containing appropriate antibiotics (100 µg mL^{-1} ampicillin for *E. coli* T0P 10; 100 µg mL^{-1} ampicillin and 25 µg mL^{-1} chloramphenicol for *E. coli* BL21 (DE3) pLysS) were used as selection markers. The bacterial transformants were selected and confirmed by PCR and nucleotide sequencing. Furthermore, amino acid alignments were compared with the reference strain sequences (AL935263.2, DQ323671.2, CP002222.1, FJ809773.1, CP001617.1, X94434.2, and DQ340868.2) using Basic Local Alignment Search Tool (BLAST) program (*http://www.ncbi.nlm.nih.gov/BLAST*).

Expression

The expression of *plnW* protein recombinant was performed according to Putri *et al.*, 2014, by inoculating 10% (v/v) of an overnight culture of *E. coli* BL21(DE3) pLysS harboring assembled plasmid into 200 mL of LB broth containing 100 µg mL^{-1} ampicillin at 37°C with agitation at 150 *rpm*. As inducer, 0.5 mM of isopropyl-β-D-thiogalactopyranoside (IPTG - Thermo Scientific, USA) were added when $OD_{600} \approx 0.6$, followed by further incubation at 22°C with agitation at 150 *rpm* for 5 hours. The cells were harvested and after three times of freezing and thawing, pellets were resuspended in lysis buffer (10 mM Tris-HCl pH 8.5, 100 mM NaCl, and 0.25% (v/v) Tween-20) and subjected into on-ice sonication (5 cycles of 15 seconds pulse on and 60 seconds pulse off) for cell disruption. The cell lysate were centrifuged at 13,000 *rpm* at 4°C for 30 minutes. Both of the obtained supernatants (soluble fractions) and pellets (insoluble fractions) were subjected for further purification methods.

Partial Purification of Soluble Fraction

The soluble fractions were partially purified using immobilized metal affinity chromatography (IMAC) resin charged with cobalt (Co^{2+}) (TALON, Clontech, Takara Bio) as previously described by Utama *et al.*, 2000. Briefly, soluble fractions were applied on lysis buffer-calibrated resin by gently binding it for 3 hours at 4°C. The resin-bound protein was collected by brief centrifugation followed by two times washing with lysis buffer containing 10 mM imidazole. The recombinant protein was purified and eluted with lysis buffer containing 400 mM imidazole. The purified protein was denaturated and analyzed using 12% SDS PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) as described by Laemmli, 1970, stained with staining solution (0.5% (w/v) Coomassie Brilliant Blue G (CBB-G, Sigma, USA), 50% (v/v) methanol, and 10% acetic acid), and destained with destaining solution (50% (v/v) methanol and 10% acetic acid). Precision Plus Protein Standards (Bio Rad, USA) was used as molecular weight marker.

Partial Purification of Insoluble Fraction

The remaining insoluble fractions were washed three times with washing buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 2 M Urea, and 1% Triton X-100) according to Liu *et al.*, 2011, before solubilized

with solubilizing buffer (100 mM Tris-HCl pH 12.5 and 2 M Urea) by an overnight constant mixing at 4°C, according to Patra *et al.*, 2000. The denaturing agents were removed from solubilized protein by step-wise dialysis system, in which Urea concentration was gradually reduced, according to Tsumoto *et al.*, 1998, with several modifications. Firstly, the solubilized protein was dialyzed against denaturing removal buffer 1a (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, and 1 M Urea) followed by denaturing removal buffer 1b (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, and 0.5 M Urea). Secondly, protein refolding was performed by dialysis against refolding buffer 2a (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 0.4 M Urea, and 0.25 mM 2-mercaptoethanol) followed by stabilizer buffer 2b (50 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 0.4 M Urea, 0.25 mM 2-mercaptoethanol, and 1% (v/v) glycerol). Each dialysis was performed by gentle agitation for 24 hours at 4°C using cellulose membrane dialysis tubing (12,400 MWCO, SIGMA, Germany) pre-heated with 1 mM EDTA and H_2O for 10 minutes, subsequently.

The refolded protein was pooled and directly subjected into preparative SDS-PAGE for further partial purification using electroelution method according to Mohammadian *et al.*, 2010 with some modifications. Briefly, after being stained and distained with the same method above, the target band was precisely excised and sliced into small pieces using clean scalpel. Gel fragments were carefully put into membrane dialysis tubing containing electroelution buffer (25 mM Tris pH 8.3, 0.192 M glycine, and 0.1% SDS). The membrane tube was then placed into electrophoresis apparatus filled with electroelution buffer and the electroelution was performed at 60 V at 4°C until CBB-G was being removed and the gel become colorless.

Protein precipitation was done by adding four times volume of cold acetone to the electroeluted protein and incubated on ice for 30 minutes. The precipitated protein was separated by centrifugation at 10,000 *rpm* at 4°C for 10 minutes, allowed to dry and dissolved in 20 µL of 8 M Urea in dilution buffer (50 mM Tris pH 7.9, 20% glycerol, 0.1 mM EDTA, 1 mM DTT, 0.15 M NaCl, and 0.1% SDS), followed by incubation at room temperature for 20 minutes. The solution was diluted 10 times using dilution buffer and permitted to renaturation for 1 hour at room temperature. The purified protein was then analyzed by 12% SDS-PAGE for molecular weight confirmation.

Western Blot Analysis

Western blot analysis was performed using Anti-His(C-term)-HRP Antibody (Invitrogen, USA), according to the manufacture's instructions. Briefly, the obtained solubilized lysate were separated using 12% SDS-PAGE without staining and then electrophoretically transferred (30 V, 40 mA; overnight incubation at 4°C) to nitrocellulose membrane (blotting) in transfer buffer (25 mM Tris pH 8.3, 192 mM glycine, and 20% (v/v) methanol). The membrane was blocked by gentle agitation in 10 mL blocking buffer (PBS/T: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, and 0.05% (v/v) Tween-20; and 5% (w/v) nonfat, dry milk) for 1 hour at room temperature. The membrane was then washed two times with PBS/T for 5 minutes each, and transferred to 10 mL blocking buffer containing 3 µL Anti-His(C-term)-HRP Antibody followed by overnight incubation with gentle agitation at 4°C. After the membrane was washed two times with PBS/T for 5 minutes each, the target band was detected by Novex HRP Chromogenic Substrate (Invitrogen, USA) consist of TMB (3,3',5,5'-tetramethylbenzidine) which forms a blue precipitate upon reaction with HRP (horseradish peroxidase).

RESULT

Cloning and Expression

The PCR target product (± 750 bp) was successfully cloned into *E. coli* DH5α using pGEM®-T Easy vector system and confirmed by restriction enzymes (*Nco* I and *Sal* I) (Figure 1). Sequence analysis showed that there was additional region (partial *plnV* locus) within the amplified product (data not shown) possibly due to nonspecific amplification in PCR. New primer pairs with additional restriction sites (*Bam*H I and *Hind* III) were designed in order to amplify specific region of *plnW* locus. The desired PCR product (± 405 bp) harboring *plnW* region was successfully amplified

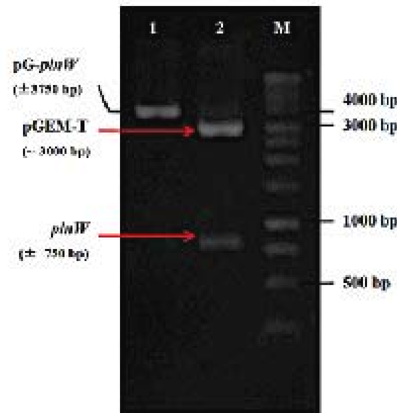

Figure 1. DNA target (± 750 bp) was confirmed using restriction enzymes (*Nco* I and *Sal* I) and visualized in 1.0% gel electrophoresis. (*DNA target (± 750 bp) dikonfirmasi menggunakan enzim restriksi (Nco I and Sal I) dan divisualisasi menggunakan gel elektroforesis 1%*).

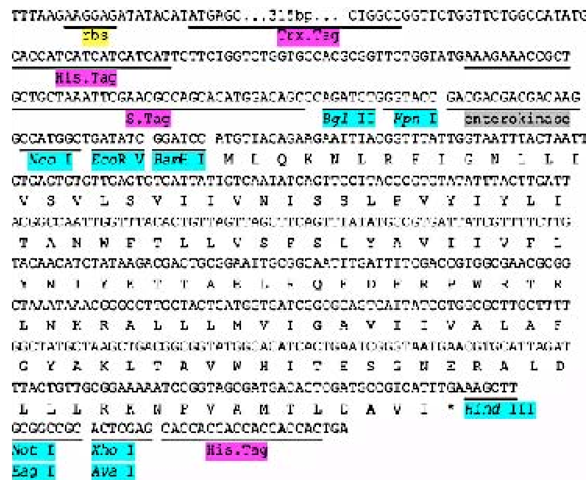

Figure 2. Sequence analysis of recombinant *plnW* gene in fusion with His(6)-tagged thioredoxin. (*Analisis sekuens fusi rekombinan gen plnW dengan His(6)-tagged thioredoxin*)

and transformed into *E. coli* TOP 10 and *E. coli* BL21 (DE3) pLysS using pET32a(+) as expression vector and confirmed by nucleotide sequencing (Figure 2).

The amino acid multiple alignments showed that the obtained *plnW* amino acid sequence had similar identity compared to the previously published *plnW* amino acid sequences (strains WCFS1, J23, STIII, V90, JDM1, C11, and J51; *http://www.ncbi.nlm.nih.gov*) (Figure 3). However, only 135 out of 228 amino acid from the whole part of *plnW* locus could be confirmed in this work. The PCR amplification using primer pairs targeted the whole part of *plnW* was unsuccessful (data not shown). Nonetheless, partial *plnW* locus could also be seen on the other *L. plantarum* strains such as C11 (219 amino acid) and J51 (150 amino acid).

Partial Purification of Soluble and Insoluble Fractions

The recombinant fusion protein derived from soluble fractions which partially purified using IMAC resin was visualized by 12% SDS-PAGE stained by CBB-G (Figure 4A). The fusion protein derived from insoluble fractions was partially purified and confirmed by CBB-G (Figure 4B). The

partially purified fusion protein derived from insoluble fractions exhibited higher purity and concentration compared to the one derived from soluble fractions.

Figure 3. Multiple alignments of *plnW* amino acid isolated in this study compared to the previously published *plnW* locus derived from several reference strains of *L. plantarum*. (*Multiple Alignment asam amino plnW yang diisolasi pada penelitian ini dengan locus plnW dari beberapa strain L. plantarum yang sudah dipublikasi sebelumnya*).

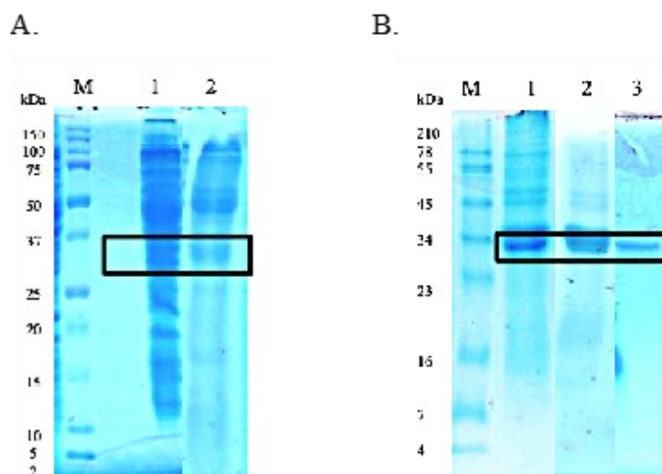

Figure 4. SDS-PAGE analysis of recombinant *plnW* fusion protein; (A). Partial purification of soluble fractions using IMAC: M. Marker; 1. Cell lysate; 2. Protein target (± 33 kDa) eluted with 400 mM imidazole; (B). Partial purification of insoluble fractions using electroelution: M. Marker; 1. Solubilized protein; 2. Dialyzed protein; 3. Protein target (± 33 kDa) partially purified with electroelution, precipitated, and diluted with dilution buffer. (*Analisis SDS-PAGE fusi rekombinan protein plnW: (A). Purifikasi parsial fraksi terlarut menggunakan IMAC: M. Marker; 1. Cell lysate; 2. Protein target (± 33 kDa) dielusi dengan 400 mM imidazole; (B). Purifikasi parsial fraksi tidak terlarut menggunakan elektroelusi: M. Marker; 1. Protein tersolubilisasi; 2. Protein terdialisis; 3. Protein target (± 33 kDa) yang dipurifikasi parsial dengan elektroelusi, dipresipitasi dan didilusi dengan bufer dilusi)*.

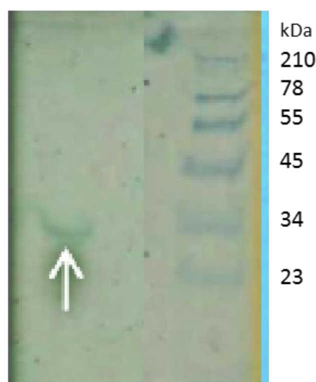

Figure 5. The target protein (± 33 kDa), which indicated by white arrow, was successfully visualized by Novex HRP Chromogenic Substrate (Invitrogen, USA). [*Protein target (± 33 kDa), yang ditunjukkan dengan tanda panah putih, berhasil divisualisasi dengan Novex HRP Chromogenic Substrate (Invitrogen, USA)*]

Western Blot Analysis

Anti-His(C-term)-HRP Antibody allowed the detection of the recombinant fusion protein (His (6) tag-*plnW*) containing a C-terminal polyhistidine tag with a free carboxyl group within the solubilized protein. The result showed that the desired fusion protein (± 33 kDa) was successfully detected (Figure 5).

DISCUSSION

Bacteriocins production is one of the alternative solutions toward the increasing number of antibiotic-resistant pathogens and the side effect of chemical preservatives. Nevertheless, bacteriocins quorum sensing-dependent production in wild environments during late lag growth phase is often result in low concentration (Diep *et al*., 2009). One approach that can be done to address this issue is using recombinant technology to produce the target bacteriocin abundantly and more stable. The excessive production of recombinant bacteriocin also beneficial for further elucidation of genetic structural and functional relationships, thus, will lead to better understanding of their mechanism of action at molecular level (Meyer *et al*., 2010).

However, overproduction of bacteriocins, including plantaricins, may also cause lethal effect to the host cell due to their toxicity. Thus, currently there is no ideal system for the expression of native plantaricin. It is note worthy that the expression host plays important role on the quality and quantity of the produced recombinant plantaricin. *E. coli* is commonly used as expression host since it is easy to handle. Mustopa *et al.,* 2016, reported that plantaricin E and F derived from *L. plantarum* S34 have been successfully cloned and expressed into *E. coli*.

Therefore, this study was heterologous production system of recombinant plantaricin W, one of the bacteriocin loci encoded by plasmid-borne genes derived from *L. plantarum* S34, in *E. coli.* Plantaricin W (*plnW*) has been identified as a putative integral membrane protein located downstream of the structural genes. This locus generally involved in a wide variety of cellular functions, one of them is self-immunity system, which protect the producer bacteria from being killed by its own bacteriocin.

This study suggests a serial method of isolation, cloning, expression, and partial purification of recombinant *plnW*. Although a truncated region has been identified, the sequence analysis shows a high identity compare to the reference strains aligned (Figure 3). These genomic discrepancies may be due to the alteration of gene expression within *L. plantarum* strains, which derived from distinctive niches, as the result of environmental adaptation.

It is important to consider some drawbacks such as potential toxicity of *plnW* to the host, lack of secretion system for efficient release of *plnW*, and limited ability to facilitate extensive disulfide bond formation due to overproducing of *plnW* during

heterologous expression in *E. coli* BL21 (DE3) pLysS. Therefore, the fusion system (His(6)-tagged thioredoxin within pET-32a(+) expression vector) is used to prevent inclusion body formation and to generate standard purification method. His-tags enable purification method based on metal ions affinity which allows single step purification from crude extract cells, while thioredoxin is a hydrophilic tag which can increase the protein solubility.

The heterologous expression method of *plnW* in *E. coli* was optimized as previously reported by Putri *et al.*, 2014. In order to increase the solubility, *plnW* was expressed at lower temperature and induced by low concentration of IPTG. Our result showed that the purification using affinity chromatography was insufficient to obtain high yield of recombinant *plnW*. The fusion protein could not effectively bound to the resin, and most of it was remained in cell lysate, thus resulted in low purity.

Unfortunately, the formation of inclusion body still become bottleneck during the expression of recombinant *plnW*. We used several strategies to recover the active *plnW*, including solubilization of inclusion body, refolding of solubilized protein and partial purification of refolded protein. Washing step using low concentration of Triton X-100 helped in removing several impurities. Mild solubilization using low concentration of Urea at alkaline condition could solubilize the inclusion body aggregates while preserving the native-like protein structure. We also described step-wise dialysis refolding method, which controls the folding pathway by gradually adjusting the concentration of the denaturant to induce sequential folding.

Partial separation of denatured protein was best achieved by preparative SDS-PAGE followed by electroelution of the protein which results in high-throughput recovery of the proteins from inclusion body. However, additional purification step such as thioredoxin removal using enterokinase enzyme is still needed.

CONCLUSION

This study suggests heterologous expression and partial purification system of plantaricin W derived from *L. plantarum* S34 using *E. coli* as expression host, which allows convenient and efficient way of recovering properly folded recombinant protein from inclusion body aggregates. The characterization of this locus will be useful for the exploration of *L. plantarum* S34 antibacterial properties. More information on the genetic structures of plantaricin loci of indigenous lactic acid bacteria from Indonesia is needed. Further research on some important gene including for the proposing of new species or genus is mandatory.

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b. Buku
   Kramer PJ. 1983. *Plant Water Relationship*, 76. Edisi ke-(bila ada). Academic, New York.
c. Prosiding atau hasil Simposium/Seminar/Lokakarya.
   Hamzah MS dan SA Yusuf. 1995. Pengamatan Beberapa Aspek Biologi Sotong Buluh *(Sepioteuthis lessoniana)* di Sekitar Perairan Pantai Wokam Bagian Barat, Kepulauan Aru, Maluku Tenggara. *Prosiding Seminar Nasional Biologi XI,* Ujung Pandang 20-21 Juli 1993. M Hasan, A Mattimu, JG Nelwan dan M Litaay (Penyunting), 769-777. Perhimpunan Biologi Indonesia.
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