# CONSTRUCTION, EXPRESSION AND PURIFICATION OF RECOMBINANT PRE-MATURE PEPTIDE OF PLANTARICIN F FROM *Lactobacillus plantarum* S34 IN *Escherichia coli*

## *Konstruksi, Ekspresi, dan Purifikasi Peptida Prematur Plantarisin F dari* Lactobacillus plantarum *S34 pada* Escherichia coli

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## ABSTRACT

Plantaricin is one of bacteriocins that have the potential to be used as food preservative. Plantaricin is safe for human consumption because it can be easily degraded by proteolytic enzymes. The objective of this study was to express and purify recombinant pre-mature peptide of plantaricin F from *Lactobacillus plantarum* S34 in *Escherichia coli*. Plantaricin gene-specific primer was used to obtain pln F structural gene amplicon from *L. plantarum* S34. This amplicon was cloned in pET32a vector and expressed in *E. coli* BL21 (DE3) pLysS. Pre-mature plantaricin F peptide was expressed as Histagged-fusion protein and separated by $Co^{2+}$-chelating affinity chromatography. *L. plantarum* S34-derived pre-mature plantaricin F peptide fused with thioredoxin-$(His)_{6tag}$ had successfully been expressed in *E. coli* BL21 (DE3) pLysS using pET32a as an expression vector. The fused recombinant pln F as pre-mature state expressed had a molecular mass of $\pm$24 kDa, meanwhile the fused recombinant that contained only the leader peptide of pln F appeared as $\pm$20 kDa based on SDS-PAGE separations. The optimal production of fused recombinant pln F as soluble fraction was obtained when culture condition was added with 0.5 mM of IPTG and incubated at 22°C for 5 hours (OD~1). Furthermore, the expression of fused recombinant pln F as its pre-mature peptide pointed out that the pln F's leader peptide could be proteolytically cleaved by a system in heterologous cells. Overall, heterologous pln F production as pre-mature peptide fused with thioredoxin-$(His)_{6tag}$ had been well established. From this research, we expect plantaricin F can be expressed and purified in *E. coli*.

[***Keywords***: Plantaricine, recombinant pln F, leader peptide, mature peptide, pET32a vector]

## ABSTRAK

*Plantarisin merupakan salah satu bakteriosin yang berpotensi sebagai bahan pengawet pangan. Plantarisin aman untuk dikonsumsi manusia karena dapat dengan mudah terdegradasi oleh enzim proteolitik. Tujuan penelitian ini adalah mengekspresikan dan memurnikan protein plantarisin F prematur rekombinan dari* Lactobacillus plantarum *S34 pada* Escherichia coli. *Primer spesifik untuk mengamplifikasi plantarisin F digunakan untuk memperoleh gen pln F dari* L. plantarum *S34. Gen pln F yang didapat kemudian diklon pada vektor pET32a dan diekspresikan pada* E. coli *BL21 (DE3) pLysS. Peptida plantarisin F prematur dinyatakan sebagai protein* Histagged-fusion *dan dipisahkan oleh* $Co^{2+}$-chelating *kromatografi afinitas. Plantarisin F prematur yang difusikan dengan teoredoksin-$(His)_{6tag}$ telah berhasil diekspresikan pada* E. coli *BL21 (DE3) pLysS menggunakan pET32a sebagai plasmid ekspresi. Plantarisin F prematur rekombinan yang difusikan dengan teoredoksin-$(His)_{6tag}$ memiliki berat molekul $\pm$24 kDa, sementara itu plantarisin F rekombinan yang hanya mengandung* leader peptide *berbobot $\pm$20 kDa berdasarkan hasil pemisahan pada SDS-PAGE. Produksi optimal plantarisin F rekombinan sebagai fraksi terlarut (*soluble peptide*) didapat pada kondisi kultivasi sel inang* E. coli *rekombinan yang ditambahkan IPTG 0,5 mM pada suhu 22°C dengan periode kultivasi 5 jam (OD ~ 1). Ekspresi plantarisin F rekombinan sebagai peptida prematur mengindikasikan bahwa* leader peptide *dapat dipotong secara proteolitik oleh suatu sistem di dalam sel inang yang heterolog. Dapat disimpulkan bahwa plantarisin F rekombinan dalam bentuk peptida prematur yang terfusikan telah berhasil diproduksi. Implikasi penelitian ini adalah plantarisin F dapat diekspresikan dan dimurnikan pada* E.coli.

[*Kata kunci*: Plantarisin, pln F rekombinan, *Leader peptide*, mature peptide, vektor pET32a]

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## INTRODUCTION

Lactic acid bacteria (LAB), a group of gram-positive bacteria, have received special attention to date because of their ability to produce bacteriocins that can be applied as a natural preservative agent (da-Silva *et al*. 2014). The usages of LAB and their metabolic products as anti-food contaminations were due to the GRAS (Generally Regarded as Safe) state received (Zacharof and Lovitt 2012). Furthermore, LAB have been isolated from various kinds of traditional Indonesian fermented foods such as shrimp paste ‑terasi·, fermented fish and meat ‑bekasam·, and soy mash ‑tempe· habitated with *Tetragenococcus halophilus*, *Tetragenococcus muricatus*, and *Lactobacillus plantarum* (Roling *et al*. 1996; Kobayashi *et al*. 2003; Candra *et al*. 2007; Mustopa *et al*. 2010).

Class IIb bacteriocins produced by LAB are mostly applied in food to prevent microbial contamination due to their strong antibacterial activity (Abee *et al*. 1995; Cleveland *et al*. 2000). These unique peptides have a conserved N-terminal, named GxxxG motifs in its mature peptide form (Fimland *et al*. 2008). These are such as plantaricins EF (PlnEF) and plantaricins JK (PlnJK) that belong to a large group of small, heat-stable, cationic, non-lantibiotic peptides (Pal and Sheela 2014) and produced by different strains of *L. plantarum* (Anderssen *et al*. 1998).

The conventional procedures to collect native plantaricin peptides directly from *L. plantarum* using ammonium sulphate precipitation followed by chromatography-based purifications have recently been established with excellent biological activity and high in protein purity obtained (Atrih *et al*. 2001; Gong *et al*. 2010; Hata *et al*. 2010; Zhu *et al*. 2014). However, the procedures are hard to be applied to produce native plantaricins directly isolated from natural producers with high yield (Tiwari and Srivastava 2008).

These conventional methods if applied in industrial process for large scale production would be contra-productive because of time consuming, laborious process, high cost and difficulty in the purification steps (Li and O′Sullivan 2002). To overcome the constraints, efforts had been done for example to heterologously produce plantaricins in quite different cells, such as *Escherichia coli* (Fang *et al*. 2010) that offer more rapid, high yield and economical production of recombinant proteins (Sivashanmugam *et al*. 2009).

Contrary with another type of class II bacteriocins such as lactation, enterocin, mesentericin, nisin, divercin and sakacin which had been well established expressed heterologously in any type of cells (Richard *et al*. 2004; Fimland *et al*. 2008; Rogne *et al*. 2009; Basanta *et al*. 2010; Fang *et al*. 2010; Borrero *et al*. 2012). In attempt to increase the yield of bacteriocin peptides, their heterologous expression in procaryotic species have been widely studied (Richard *et al*. 2004; Kloke *et al*. 2005). But until now, little study is known to report heterologous expression of plantaricin especially in the form of their pre-mature peptide state (Fimland *et al*. 2008; Fang *et al*. 2010). The objective of this study was to express and purify recombinant pre-mature peptide of plantaricin F from *L. plantarum* S34 in *E. coli*.

## MATERIALS AND METHODS

### Construction of Recombinant Vector

A plantaricin F gene isolated from bekasam-derived *L. plantarum* S34 was subcloned into pGEM-T (pGEM-T-pln EF) then the pln F gene was amplified from pGEM-T-pln F by PCR using a primer pair 5'-GACT<u>GGATC</u> CATGAAAAAATTCTA GTTTT-3' as sense primer and 5'-GATC<u>AAGCTT</u>CTATCCGTGGAT GAATCC TC-3' as anti-sense primer. The primers contained upstream *Bam*HI and downstream *Hind*III restriction site (underlined nucleotides). To prevent protein fusion between the recombinant pln F with C-terminal His tag protein, strong stop codons TGA were incorporated at 3-end of the anti-sense primer. All DNA manipulations were carried out according to the procedures described by Sambrook *et al*. (2001). PCR profile was 94°C, 1 minute of denaturation; 63°C, 1 minute of annealing; extension 72°C for 30 seconds and repeated for 35 cycles. The PCR product and vector pET32 a (Novagen) were cut with *Bam*HI and *Hind*III enzymes. After purification with a Qiaquick® PCR purification kit (Qiagen, ICI Americas Inc), the digested pln F gene and vector were ligated using T4 DNA ligase (Takara) and the ligation mixtures were transformed into competent *E. coli* BL21 (DE3) pLysS (Novagen). Recombinant plasmid-containing bacteria were initially selected on LB agar with ampicillin (100 µg mL$^{-1}$) then the recombinant bacteria growth on the selection medium was subjected to PCR colony using the same primer as described above. The recombinant pET32a-pln F from PCR colony test was sequenced to confirm the inserted pln F gene nucleotide sequence.

## Expression of Recombinant pln F in *E. coli*

The expression method previous study (Li *et al.* 2010). Recombinant plasmid that had been constructed was sub-cloned into competent *E. coli* BL21 (DE3) pLysS. The recombinant bacteria were cultured in 200 ml LB broth containing ampicillin (100 μg mL$^{-1}$) with shaked at 150 rpm at 37°C. When $OD_{600}$ reached 0.4, cultures were induced by IPTG (Isopropylβ-D-1-thiogalactopyranoside). To optimize recombinant expression, three conditions were applied, i.e. (1) IPTG inducer concentrations (0.1, 0.5, and 1 mm); (2) induction times (1, 2, 3, 4, and 5 hours); and (3) induction temperatures (22°C, 25°C, and 37°C). Furthermore, the optimized condition was used to produce and purify recombinant pln F from *E.coli* BL21 (DE3) pLysS.

Five hours after IPTG induction, recombinant bacteria were harvested by centrifugation at 8000 g, 4°C for 10 minutes. The pellets from recombinant bacteria were resuspended with lysis buffer pH 8 containing 20 mM Tris-HCl, 500 mM NaCl and 20 mM imidazole directed to sonication (0.5 cycles of 30 outputs, 15 s pulse on and 60 s pulse off). The lysate was then centrifuged at 18,000 g, 4°C for 30 minutes. After that, the supernatant was collected and the pellet was resuspended in the same lysis buffer. Pellet and supernatan fractions were separated using 12% SDS polyacrylamide gel electrophoresis (SDS PAGE).

## Purification of Recombinant pln F

The purification method was done according to a previous study by Utama *et al.* (2000) and Li *et al.* (2010) with modification. Supernatant fraction was resuspended with resin Co$^{2+}$ (TALON) and bound on a rotator for 3 hours at 4°C. After that, the sample was centrifuged at 7000 g, 4°C for 7 minutes. The resulting supernatant was discarded, and the resin was resuspended in wash buffer (Tris HCl 20 mM pH 8, 500 mM NaCl, 60 mM imidazole). Subsequently, the supernatant was centrifuged at 7000 g, 4°C for 5 minutes. The washing process was repeated two times. The resin was then resuspended with elution buffer (Tris HCl 20 mM pH 8, 500 mM NaCl, 250 mM imidazole) and bound on a rotator at 4°C for 12 hours. Subsequently, the fraction was centrifuged at 7000 g, 4°C for 5 minutes. The supernatant was taken and then dialyzed using dialyzed buffer (500 mM Tris HCl, pH 8,10 mM CaCl$_2$, 1% Tween-20 (v/v)).

## SDS-PAGE

This method was used to determine whether recombinant pln F was expressed or not. The fractions from expression and purification of recombinant pln F were collected, then the mixture was dissolved with loading buffer (50 mm Tris HCl, pH 6.8, 2% SDS, 10% glycerol, 1% mercapto ethanol, 12.5 M EDTA, 0.02% Bromophenol blue, H$_2$O). The fraction was boiled for 15 minutes, then separated by electrophoresis using 12% SDS-PAGE of a vertical electrophoresis (Mupid-eXu, Advance Japan). The gel was then stained with coomassie blue staining solution overnight distained with staining buffer for 30 minutes, three times. The gel picture was then taken using a digital scanner (Canon MP258, Canon).

## RESULTS AND DISCUSSION

### Expression of Recombinant pln F in *E. coli*

pET32a containing Trx-plnF fusion protein for the expression was shown in Figure 1. A pre-mature pln F fragment containing *Bam*HI and *Hind*III restriction sites with 159 bp in size was obtained by PCR amplification (Fig. 1a). Then the PCR product was sub-cloned into the pET32a expression vector, and the insertion was confirmed by double digestion using *Bam*HI and *Hind*III restriction enzymes. Recombinant DNA sequence inserted was verified by DNA sequencing.

We had constructed and expressed pln F which consisted of leader and mature peptide (Fig. 1b) as protein fusion with thioredoxin-(His)$_{6tag}$ at N-terminus in *E.coli* with a high degree of solubility as seen in the soluble fraction of supernatant obtained from lysed-recombinant *E.coli*. Fimland *et al.* (2008) had produced recombinant mature pln F fused to a fusion partner protein called GB1 using pGEV2 with 1 mM IPTG for induction, but the recombinant protein obtained was forming inclusion body. This contrary result with our study indicated that the difference in fusion partner protein, vector expression and IPTG used would impact on the degree of solubility of recombinant pln F produced. Interestingly, because of recombinant pln F in our study expressed as pre-mature peptide, we predicted that the leader peptide itself may also have function as a guide to chaperon like-protein on translocation machinery thus helping proper folding of pln F (Mathiesen *et al.* 2008).
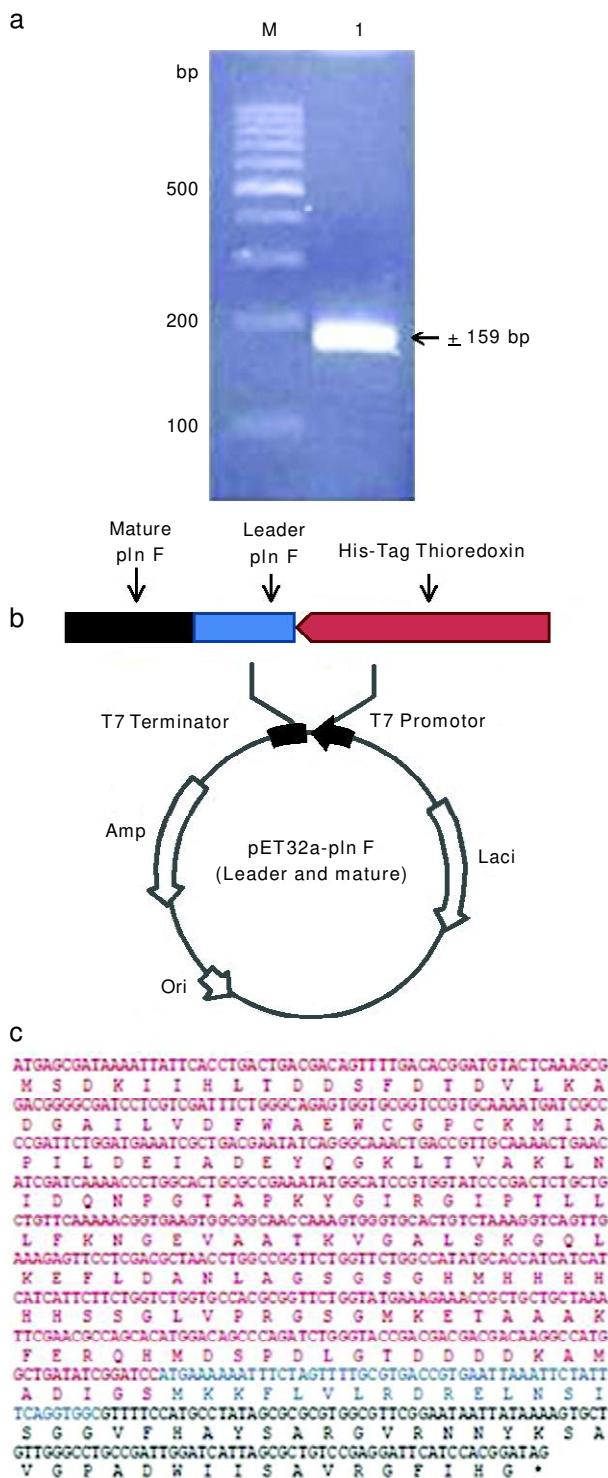
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a



b

c

**Fig. 1.** (a) Results of PCR amplification of pln F gene. M is 100 bp DNA ladder marker (bp), lane 1 is pre-mature pln F gene construction of pET32a-pln F; (b) fragment with size of about 159 bp inserted into *Bam*HI and *Hind*III sites of pET32a vector; (c) nucleotide and amino acid sequence of fused recombinant pln F, the blue colour is thioredoxin-(His)$_{6tag}$ fusion partner protein followed with S-Tag and enterokinase cleavage site (1-165 aa), the red colour is leader peptide of pln F (166183 aa), the green color is mature peptide of pln F (184-217 aa), the asterix is stop codon.

After heat shock treatment to deliver pET32a-pln F into the *E. coli* BL21 (DE3) pLysS, the targeted recombinant protein of pln F appeared as two dominant bands. The first band was ±24 kDa which composed of recombinant pre-mature peptide of pln F and thioredoxin-(His)$_{6tag}$ fusion protein and the second band was ±20 kDa contained only leader peptide. The fusion protein indicated that some of mature peptides of pln F have been proteolytically cleaved from its pre-mature state by a system in *E.coli* (Fig. 2a) and those two protein bands were not appeared in recombinant *E.coli* which was only transformed by pET32a(+) with no insert of pre-mature pln F gene. Expression of recombinant protein fused with partner protein such thioredoxin-(His)$_{6tag}$ would give several advantages compared to the unfused recombinant protein. As described by Cao *et al*. (2005), Khow and Suntrarachun (2012), and Tao *et al*. (2014), those benefits included (1) helping to increase solubility and proper folding of recombinant protein expressed, (2) protecting them from being degraded by intracellular protease and (3) simplifying the purification of recombinant protein.
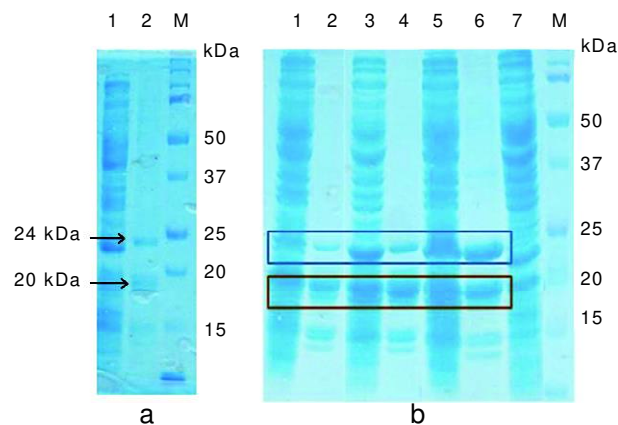


**Fig. 2.** Result of SDS-PAGE separations of the recombinant pln F; (a) expression of recombinant pln F at 37°C with 0.5 mM IPTG, lane 1 was supernatant fraction from lysed-recombinant *E. coli*; line 2 was an elution fraction from TALON-treated supernatant fraction, (b) the effect of different temperatures on recombinant pln F expression; lane 1, 2 and 3 were supernatant of lysed-recombinant *E. coli* incubated at 37°C, 25°C and 22°C, respectively; lanes 2, 4 and 6 were elution fraction of TALON-treated supernatant of lysed-recombinant *E. coli* incubated at 37°C, 25°C and 22°C, respectively; lane 7 was supernatant of *E. coli* contained pET32 vector with no insert pln F gene. Blue and red squares were two dominant bands represented the fused recombinant pln F with or without its mature peptide. M was peptide molecular weight marker.
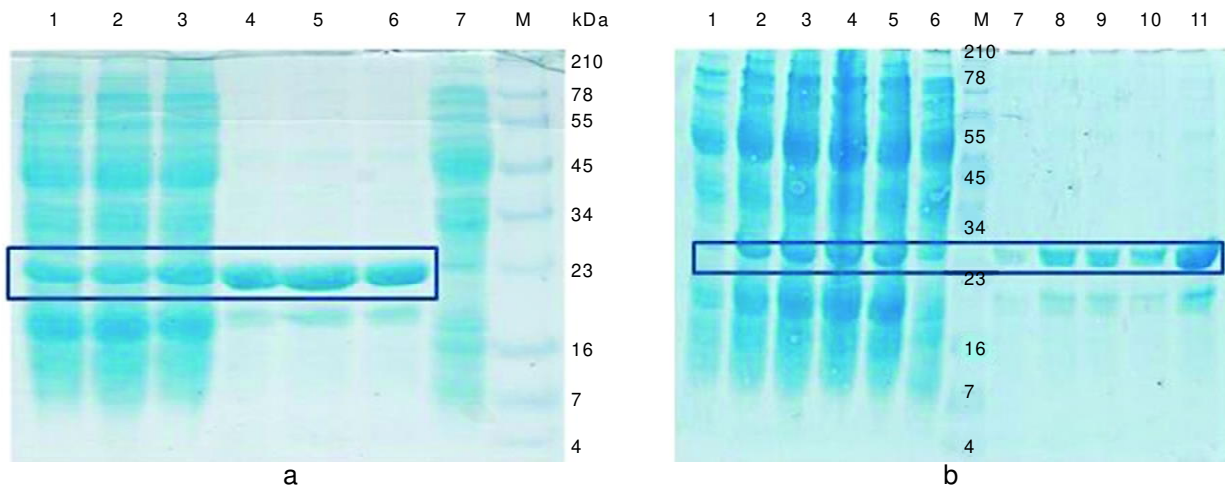
**Fig. 3.** The effects of IPTG and incubation time on recombinant pln F expression at induction temperature of 22°C, (a) lanes 1 to 3 were supernatants from the lysed-recombinant *E. coli*; lanes 4 to 5 were elution fractions of TALON-treated supernatant fraction after *E. coli* induction with IPTG at 0.1, 0.5 and 1 mM, (b) the effect of different incubation times. Lanes 1 to 5 were the supernatants of lysed-recombinant *E.coli* expressed for 1, 2, 3, 4, and 5 hours after addition of 0.5 mM IPTG; Lane 6 was pET32a with not insert pln F gene; lanes 7 to 11 were elution fractions of TALON-treated supernatant fraction expressed for 1, 2, 3, 4, and 5 hours after addition of 0.5 mM IPTG. M was molecular marker mass peptide (BIO-RAD).

To obtain maximum production of recombinant pln F we tested different values of temperature incubation, IPTG concentration and incubation time. As shown in Figure 2 the recombinant pln F expression was temperature dependent with a protein level reached maximum value at 22°C (Fig. 2b, lane 6). For IPTG optimization, the yield of recombinant pln F protein was highest when 0.5 mM IPTG was added into the medium (Fig. 3a, lane 5). Meanwhile, 5 hours culture of recombinant *E.coli* containing pET32a-pln F at 22°C after 0.5 mM IPTG addition gave the best protein recombinant expression as pointed out in Figure 3b. The optimum condition obtained (induction temperature was 22°C, induction time was 5 hours, and IPTG concentration was 0.5 mM) then applied for production and purification of fused recombinant pln F.

In attempt to obtain the optimum production of recombinant pre-mature pln F, here we also had optimized critical parameters that contribute directly to the successfulness of protein expression in *E.coli* such as induction temperature, induction time and IPTG concentration addition (Sambrook and Russell 2001; Sivashanmugam *et al*. 2009). As we expected, the induction temperature where recombinant pln F was produced by recombinant *E. coli* had a prominent effect on protein expression yield and solubility. Induction in 37°C resulted in very thin recombinant protein band on SDS-PAGE result,

meanwhile lowering the induction temperature to 22°C (IPTG was 0.5 mM and induction time was 5 hours) resulted in a thicker recombinant protein band. Recombinant mature pln F obtained from Fimland *et al*. (2008) was produced as inclusion body due to inappropiate induction temperature applied (37°C). Another study conducted by Rogne *et al*. (2009) also obtained similar result for recombinant pln J and K collected from 37°C incubated-recombinant *E.coli*. Tao *et al*. (2014) stated that lowering the induction temperature during IPTG added-medium culturing would help to increase solubility of recombinant protein expressed as much as $\pm 80\%$, although it would also impacte directly on the capability of recombinant cells to proliferate of being slow down compared to high temperature (Chen *et al*. 2012).

Construction and heterologous expression of mature pln F had well been established by other authors with excellent results (Fimland *et al*. 2008; Fang *et al*. 2010), yet no information was provided related to its heterologous expression as pre-mature peptide of pln F until today. We assumed that the expression of pln F as its pre-mature state, if any, would give essential information regarding biological function of homologous leader peptide in heterologous cells. For examples some leader peptides had been proven being acting as a molecular guide in properly directing of pre-mature peptides to the secretory machinery system, the process is called

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protein maturation and secretion (Belkum *et al*. 1997; Mathiesen *et al*. 2008).

As explained above, pre-mature peptide of pln F had been successfully constructed in pET32a vector and expressed in *E.coli* BL21 (DE3) pLysS. The functionality of leader sequence within premature recombinant pln F had a size $\pm$ 20 kDa bellowed from fused pre-mature of recombinant pln F band (Fig. 3a). We predicted this additional protein band was a union of thioredoxin-$(His)_{6tag}$ fusion partner protein with S-tag and enterokinase cleavage site (total size was 18.15 kDa) followed by leader peptide sequence (size was 1.98 kDa) without recombinant mature of pln F. We predict that recombinant mature pln F may be secreted from intracellular part of the *E.coli* into the culture medium. According to Ennahar *et al*. (1996) and Kelly *et al*. (1996), the function of leader sequences of bacteriocins was not only as an inhibitor of antibacterial activity of the bacteriocins against their producer host cells, but also provided a recognition signal to the ABC transporter in maturation and secretion processes. The incomplete proteolytic digestion of a fused recombinant pre-mature peptide of pln F in our study indicated the lack of recognition of pre-peptide of bacteriocin by

domain of ABC transporter in heterologous cells (Belkum *et al*.1997).

## Purified Recombinant pln F

To purify the recombinant pln F, the soluble fraction in each case was subjected to $Co^{2+}$ affinity coloum purification. The bound proteins were eluted in 200 mM of imidazole. At this optimum concentration of imidazole, the purity of target protein could be recovered significantly. As shown in Fig. 4 the fused recombinant pln F had been purified, in two forms of dominant protein bands, from the supernatant of intracellular part of lysed cells although some of unspecific bands were also co-purified in our procedure. This result highlighted that both forms of fused recombinant pln F had succeeded to be purified using this method.

Pre-mature plantaricin F peptide fused with 6× histidine theriodoxin has been successfully expressed and purified in *E. coli* BL21 (DE3) pLysS by using pET32a as an expression vector. In this study, the expression and purification of recombinant pln F in *E. coli* BL21 (DE3) pLysS was investigated. The expressed recombinant pln F has a molecular mass of 24 kDa when separated on SDS-PAGE. The recombinant pln F produced was temperature dependent and the level of recombinant plnF production could be enhanced when the host was induced by 0.5 mM IPTG and 25°C incubation and reached optimal at 22°C incubation.

## CONCLUSION

Recombinant pre-mature pln F peptide was successfully expressed in *Escherichia coli* BL21 (DE3) pLysS by using pET32a as an expression vector. The optimum condition of expression was 0.5 mM of IPTG for 5 hours at 22°C. This recombinant peptide was successfully purified from *E. coli* cell.
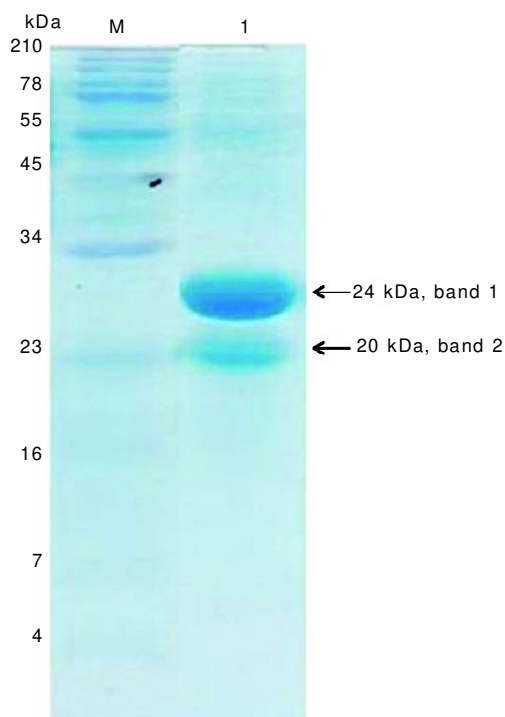
## ACKNOWLEDGEMENTS

**Fig. 4.** Result of recombinant pln F purification using TALON binding method. Lane 1 was two dominant peptides of recombinant pln F. M was peptide molecular weight marker.

# REFERENCES

Abee, T., E.L. Kro and C. Hill. 1995. Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. Int. J. Food. Microbiol. 28: 169-185.

Anderssen, E.L., D.B. Diep, I.F. Nes, V.G.H. Eijsink and J.N. Meyer. 1998. Antagonistic activity of *Latobacillus plantarum* C11: two new two-peptide bacteriocins, plantaricin EF and JK, and the induction factor plantaricin A. Appl. Environ. Microbiol. 64: 2269-2272.

Atrih, A., N. Rekhif, A.J.G. Moir, A. Lebrihi and G. Lefebvre. 2001. Mode of action, purification and amino acid sequence of plantaricin C19, an anti-*Listeria* bacteriocin produced by *Lactobacillus plantarum* C19. Int. J. Food. Microbiol. 68: 93-104.

Basanta, A., C. Herranz, J. Gutierrez, R. Criado, P.E. Hernandez and L.M. Cintas. 2010. Use of the yeast *Pichia pastoris* as an expression for host secretion of enterocin L50, a leaderless two-peptide (L50A and L50B) bacteriocin from *Enterococcus faecium* L50. Appl. Environ. Microbiol. 76: 3314-3324.

Belkum, M.J.V., W.W. Randy and E.S. Michael. 1997. Double-glycine-type leader peptides direct secretion of bacteriocins by ABC transporters: colicin V secretion in *Lactococcus lactis*. Mol. Microbiol. 23: 1293-1301.

Borrero, J., G. Kunze, J.J. Jimenez, E. Boer, L. Gutiez, C. Herranz, L.M. Cintas and E.P. Hernandez. 2012. Clonig production and functional expression of the bacteriocin enterocin A, produced by *Enterococcus faecium* T136, by the yeast, *Pichia pastoris*, *Kluyveromyes lactis*, *Hansenula polymorpha* and *Arxula adeninivorans*. Appl. Environ. Microbiol. 78: 5956-5961.

Candra, J.I., W. Zahiruddin and Desniar. 2007. Isolation and characterization of lactic acid bacteria from bandeng (*Chanos chanos*) bekasam. Aquatic Prod. Technol. Bull. 10: 1424.

Cao, W., Y. Zhou, Y. Ma, Q. Luo and D. Wei. 2005. Expression and purification of antimicrobial peptide adenoregulin with C-amidated terminus in *Escherichia coli*. Protein Expr. Purif. 40: 404-410.

Chen, H., F. Tian, S. Li, Y. Xie, H. Zhang and W. Chen. 2012. Cloning and heterologous expression of a bacteriocin sakacin P from *Lactobacillus sakei* in *Escherichia coli*. Appl. Microbiol. Biotechnol. 94: 1061-1068.

Cleveland, J., T.J. Montville, I.F. Nes and M.L. Chikindas. 2000. Bacteriocins: safe, natural antimicrobials for food preservation. Int. J. Food. Microbiol. 71: 1-20.

da-Silva, S.S., V. Michele, M.D.G. Jose and P.D.S.O. Ricardo. 2014. Overview of *Lactobacillus plantarum* as a promising bacteriocin producer among lactic acid bacteria. Food. Res. Int. 64: 527-536.

Ennahar, G., A.A. El-Essawy, M. Uyttendaele and J. Debevere. 1996. Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: characterization, production and bactericidal action of plantaricin UG1. Int. J. Food. Microbiol. 30: 189-215.

Fang, Y., Y. Zhan-qiao and Z. Ri-jun. 2010. Heterologous expression of plantaricin genes pln E and pln F in *E. coli*. J. China Anim. Husb. Vet. Med. 37: 55-59.

Fimland, P.R., F. Gunnar, N. Jon and E. Per. 2008. Three-dimensional structure of the two peptides that constitute the two-peptide bacteriocin plantaricin EF. J. Biochem. Biophys. Acta. 1784: 1711-1719.

Gong, H.S., X.C. Meng and H. Wang. 2010. Plantaricin MG active against Gram-negative bacteria produced by *Lactobacillus plantarum* KLDS1.0391 isolated from õjiaokeö, a traditional fermented cream from China. Food Control. 21: 89-96.

Hata, T., T. Rie. and O. Sadahiro. 2010. Isolation and characterization of plantaricin ASM1: A new bacteriocin produced by *Lactobacillus plantarum* A-1. Int'l. J. Food. Microbiol. 137: 94-99.

Kelly, W.J., R.V. Asmundson and C.M. Huang. 1996. Isolation and characterization of bacteriocin-producing lactic acid bacteria from ready-to-eat food products. Int. J. Food. Microbiol. 33: 209-218.

Khow, O. and S. Suntrarachun. 2012. Strategies for production of active eukaryotic proteins in bacterial expression system. Asian J. Pac. Trop. Biomed. 2: 159-162.

Klocke, M., K. Mundt, F. Idler, S. Jung and J.E. Bachausen. 2005. Heterologous expression of enterocin A, a bacteriocin from *Enterococcus faecium*, fused to a cellulose-binding domain in *Escherichia coli* results in a functional protein with inhibitory activity against *Listeria*. Appl. Microbiol. Biotechnol. 67: 532-538.

Kobayashi, T., M. Kajiwara, M. Wahyuni, T. Kitakado, N.H. Sato, C. Imada and E. Watanabe. 2003. Isolation and characterization of halophilic lactic acid bacteria isolated from õterasiö shrimp paste: A traditional fermented seafood product in Indonesia. J. Gen. Appl. Microbiol. 49: 279-286.

Li, H. and D.J. OøSullivan. 2002. Heterologous expression of the *Lactococcus lactis* bacteriocin, nisin, in a dairy *Enterococcus* strain. Appl. Environ. Microbiol. 68: 3392-3400.

Li, C., H.M. Blencke, V. Paulsen, T. Haug and K. Stensvag. 2010. Powerful work horses for antimicrobial peptide expression and characterization. Bioeng. Bugs. 1: 217-220.

Mathiesen, G., A. Sveen, J.C. Piard, L. Axelsson and V.G.H. Eijsink. 2008. Heterologous protein secretion by *Lactobacillus plantarum* using homologous signal peptides. J. Appl. Microbiol. 105: 215-226.

Mustopa, A.Z., R. Balia, W.S. Putranto, M. Ridwan and M. Solehudin. 2010. Screening of lactic acid bacteria isolated from beef bekasam to produce bacteriocins to inhibit pathogenic bacteria. Proceedings of the National Seminar, Faculty of Animal Husbandry, Padjadjaran University. 2: 679-685.

Pal, G and S. Sheela. 2014. Cloning and heterologous expression of plnE, -F, -J and -K genes derived from soil metagenome and purification of active plantaricin peptides. Appl. Microbiol. Biotechnol. 98: 1441-1447.

Richard, C., D. Drider, K. Elmorjani, D. Marion and H. Prevost. 2004. Heterologous expression and purification of active divercin V41, a class IIa bacteriocin encoded by a synthetic gene in *Escherichia coli*. J Bacteriol. 186: 4276-4284.

Rogne, P., C. Haugen, G. Fimland, J. Nissen-Meyer and P.E. Kristiansen. 2009. Three-dimensional structure of the two-peptide bacteriocin plantaricin JK. Peptides 30: 1613-1621.

Roling, W.F.M. and H.W. Verseveld. 1996. Characterization of *Tetragenococcus halophilus* populations in Indonesian soy mash (kecap) fermentation. Appl. Environ. Microbiol. 62: 1203-1207.

Sambrook, J., E.F. Fritsch and T. Maniatis. 2001. Molecular Cloning: A laboratory manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.

Sambrook, J. and D. Russell. 2001. Molecular cloning: A laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, New York.

Sivashanmugam, A., M. Victoria, C. Chunxian, Z. Yonghong, W. Jianjun and L. Qianqian. 2009. Practical protocols for production of very high yields of recombinant proteins using *Escherichia coli*. Protein Sci. 18: 936-948.

Tao, Y., M.Z. Dong and W. Ya. 2014. Expression, purification and antibacterial activity of the channel catfish hepcidin mature peptide. Protein Expr. Purif. 94: 73-78.

Tiwari, S.K. and S. Srivastava. 2008. Purification and characterization of plantaricin LR14: A novel bacteriocin produced by *Lactobacillus plantarum* LR/14. Appl. Microbiol. Biotechnol. 79: 759-767.

Utama, A., S. Hiroyuki, M. Shigeru, H. Futoshi, M. Kouichi, I. Akira, H. Masahiro, T. Kazuhiro and M. Tatsuo. 2000. Identification and characterization of the RNA helicase activity of Japanese encephalitis virus NS3 protein. FEBS Lett. 465: 74-78.

Zacharof, M.P. and R,W. Lovitt. 2012. Bacteriocins produced by lactic acid bacteria. APCBEE Procedia. 2: 50-56.

Zhu, X., Z. Yizhen, S. Yalian and G. Qing. 2014. Purification and characterisation of plantaricin ZJ008, a novel bacteriocin against *Staphylococcus* spp. from *Lactobacillus plantarum* ZJ008. J. Food. Chem. 165: 216-223.