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Expression and Characterization of Recombinant Protein of J-SU pGEX either by Single or Double Cell Lysis

Ekspresi dan Karakterisasi Protein Rekombinan J-SU pGEX dengan Pemecahan Sel Tunggal dan Ganda

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Abstrak

Penelitian ini dimaksudkan untuk optimasi produk protein rekombinan Superficial Unit dari virus Jembrana (JSU) yang dieksperikan melalui pemecahan sel secara tunggal dan ganda dengan sistem pGEX dalam skala flask 100ml media kultur. Dua metode pemecahan sel yang digunakan yaitu *Freeze and Thaw* (FT) sebagai pemecahan tunggal dan gabungan FT dan Sonikasi sebagai pemecahan ganda. Sel inang (*E. Coli* pembawa konstruk JSU pGEX) ditumbuhkan dengan induksi IPTG pada 37ºC dengan pengocok berkecepatan 200rpm sampai mencapai kepadatan sel 0,8. Sel atau pelet dikoleksi dengan sentrifugasi, pelet dipecah dengan 2 perlakuan pemecahan sel tunggal dan ganda. Hasil pemecahan sel disentrifugasi untuk dikoleksi peletnya sebagai *inclusion body*. Solubilisasi dilakukan terhadap *inclusion body* dengan solubilisasi buffer dan diperoleh substrat protein JSU kemudian dimurnikan melalui Gluthation sepharose 4B (500µl resin) dengan metode *batch capture*. Hasil karakterisasi dengan SDS PAGE dan *Western Blotting* menunjukkan ukuran protein JSU pGEX yang tepat yaitu 60kDa pada kedua sistem pemecahan sel. Namun demikian, pemecahan sel secara tunggal menghasilkan protein murni JSU pGEX lebih besar (0.812ng/ul) dibanding pemecahan sel secara ganda (0.486ng/ul). Dari penelitian ini dapat disimpulkan bahwa protein rekombinan JSU pGEX terekspresi lebih baik dengan metode pemecahan sel *Freeze and Thaw*.

Kata kunci: Protein rekombinan, JSU pGEX, ekspresi, pemecahan sel

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Introduction

Jembrana disease is an acute and severe disease, endemic in Bali cattle (*Bos javanicus*). The disease caused a 20% case fatality rate after a short incubation period in Bali cattle (Moll, 1998). However, in the later publication the Jembrana Disease Virus (JDV) is also infecting to other cattle types and buffalo under experiment and they become infected under field condition (Soeharsono *et al*., 1995). Up to present, vaccination by using crude vaccine has been applied to prevent the Jembrana disease in Bali cattle (Hartaningsih and Wilcox, 1996; Hartaningsih *et al*., 2001). The crude vaccine is provided from infected tissue of Bali cattle by Jembrana viruses. Availability of this crude vaccine is limited compared to recent population of Bali cattle in the field. Despite limited in production, the crude vaccine is also costly, less effective and demanding in vaccine maintenance.

Entering biotechnology era, it is possible to develop a conventional vaccine such as Jembrana vaccine by recombinant DNA technology to produce recombinant viral protein as Jembrana vaccine. Development of recombinant protein for potential vaccine was

initiated by colleagues at Murdoch University for JDV. Initiation of producing a recombinant Jembrana vaccine is therefore expected to solve some of the problems associated with disadvantages of the current crude vaccine. This recombinant Jembrana vaccine is a safer, effective and lower production cost. JDV is identified as retrovirus disease belongs to lentivirus group of Retroviridae family (Chadwick *et al*., 1995; Wilcox *et al*., 1995). The genome of JDV is 7732bp in length (Chadwick *et al*., 1995). The JDV consists of at least three major genes (*gag*, *pol* and *env*) encoding proteins that needed for virus replications (Chadwick *et al*., 1995). The *env* gene encodes TM and SU proteins on the trans-membrane and surface of the virus, respectively and most attempts to induce a protective immunity against lentivirus infection.

The Jembrana Superficial Unit (JSU) is one of potential candidate recombinant Jembrana vaccines that constructed in pGEX system. This JSU pGEX implemented GST tag as protein fusion. In its expression, the JSU therefore needs anti GST to recognize the JSU recombinant protein. This JSU was expressed through a host cell of *Escherichia coli* (*E. coli*) for producing recombinant protein.

A study on laboratory expression and purification of JSU pGEX performed a complicated procedure following the GST (glutathione-s-transferase) system (Amersham-pharmacia biotech, 1997). It is known that most of recombinant protein is always purified from *E. coli* after cloning of the gene into expression vectors (Studier and Moffatt, 1986; Studier *et al*., 1990). In prior to purification, there is a process of disrupting the cell membrane or termed as cell lysis by adding lysozyme to break the cell wall of *E. coli*. Cell lysis is the first step in cell fractionation and protein purification (Anonymous, 2008a). Many techniques are available for disruption of cells, including physical and detergent-based methods. The physical disruption of cells is such as Freeze and Thaw, and Sonication methods. The freeze and thaw methods involve exposure into liquid nitrogen and melt the protein substrate into warm water. Sonication lysis is breaking open cells using high frequency sound wave, it causes disruption of the bacterial cell wall (Anonymous, 2008b).

This present study was designed on implementation of two physical methods of cell lysis in the expression of JSU pGEX to optimize the protein yields. The effect of cell lysis methods was associated with the concentration of recombinant protein yields.

Materials and Methods

Cell Growth and IPTG Induction

Construct of J-SU pGEX in glycerol was taken out (10ul) and cultured into 5ml of Luria Broth (LB) medium (10.0 g l^{-1} tryptone, 5 g l^{-1} NaCl, 5.0 g l^{-1}yeast extract) containing 100µg/ml amphicillin and incubated at 37ºC by shaker at 200rpm overnight. The overnight culture was inoculated into 100ml LB medium (in ratio of 1:20) and cultured at 37ºC with shaker at 200rpm to achieve an optical density (OD_{600}) of 0.8 then induced with 0.1mM IPTG (Isopropyl-f1-D-thiogalactopyranoside) for another an hour. Induction of IPTG was aimed to stimulate the protein expression (Mills, 2001). The inoculated culture was centrifuged at 5000 rpm at 4ºC for 10 minutes to collect pellets and then kept at -70ºC.

Cell Lysis

Two cell lyses performed in the treatments were:

Freeze and Thaw (Single Lysis)

The pellets taken out from a freezer (-70ºC) was exposure of liquid Nitrogen (liquid N2) for 30 seconds then thawed into a water batch at 42ºC for 1 minute. This lysis of freeze and thaw (FT) was repeated for 5 times. Volume of 10 ml lysis buffer (100mM tris HCL pH 8; 5mM EDTA; 10% triton X 100) was added into the digested cells.

Freeze-Thaw (FT) and Sonication (Double Lysis)

This treatment was undertaken in combination of FT and Sonication treatments. The pellets was first digested by FT method as above (no 1) then followed by sonication for 3

cycles (15 seconds digesting by ultra sonic with 1 minute interval). Sonication was conducted under condition of a lower temperature at -4ºC or sample was held on ice.

The collected substrate from both treatments (single and double lyses) was then centrifuged using 8000rpm at 4ºC for 20 minutes to collect pellets. The pellets were inclusion body while the supernatant was termed as cell lysate (CL).

Solubilization of Inclusion Body

In prior to solubilization, the pellet (inclusion body) was washed with washing buffer (50mM tris HCL pH 7.5; 50mM NaCl; 5% glycerol) three times, each washing was conducted by centrifugation of 8000rpm at 4ºC for 15 minutes. After 3 times washing, the collected pellets (inclusion body) were solublelized with solubilization buffer (2M tris pH 12; 2M Urea; 20mM DTT; 1mM PMSF) on a rocker overnight to homogenize the substrate.

Purification of Protein

The collected substrate from sulubilization was then purified using Gluthation sepharose 4B (in 500µl resin) by a batch capture method on a rotator and then spun down to collect the substrate. First substrate collected from this purification was termed as inner volume (IV). The left resin was washed using washing buffer solution for twice and termed as Washing 1 and 2 (W1 and W2). The washed resin was eluted by elution buffer three times then termed as Elusion 1, 2 and 3 (E 1, E2 and E3). The collected elution was as final purified protein.

Characterization of Protein

The purified protein was identified on SDS PAGE (2 gels), one gel was for SDS protein identification and another gel was directly transferred on a Nitrocellulose membrane for Western Blot (WB) using primary goat anti-GST antibody and secondary anti body of anti-goat IgG (Rabbit) HRP conjugated (Amersham Biosciences). Characterization of purified protein was identified with a precision Plus Protein™ dual color standards (BIORAD) to check the molecular weight of JSU pGEX.

Quantification of Protein

Purified protein was quantified by a GeneQuant machine at a wave length of λ 280 nm for protein concentration.

Results and Discussion

Expression and Characterization of JSU

In this study, expression of JSU pGEX was performed through *Escherichia coli* of BL21 strain. Characterization of JSU pGEX was conducted by using SDS-PAGE and WB on both cell lysis treatments where each lysis was repeated 3 times. The results showed that the size of JSU pGEX was performed at the right molecular weight (± 60 kDa) at both treatments. Visualization of both SDS PAGE and WB derived from both cell lyses were presented (as a representative of 3 repetitions) on Figure 1 and Figure 2 of single and double treatments, respectively.

As showed on Figure 1 and 2, that JSU pGEX was well expressed through *E. coli* of BL21 strain at a right molecular weight of ±60 kDa. As mentioned by Ramos *et al*., (2004) that BL21 strain of *E. coli* is suitable for expression of recombinant protein. In pGEX system, GST tag was used as protein fusion with molecular size of 26kDa (Amersham-pharmacia biotech, 1997) while the JSU protein has molecular weight of ± 34kDa (Chadwick *et al*., 1995). Therefore, the JSU pGEX has size of ±60kDa as exactly the same size as found in this study.

It seems that cell digestion by FT method as a single treatment of cell lysis in this study was quite suitable for this JSU pGEX expression. This finding was proven by more thick and sharp bands on WB after SDS PAGE on Figure 1b (see at E1 and E2). Figure 1b, bands were showed thicker on CL and W1, 2 compared to that on all elution (E1, 2 and 3). It could be understood that at first expression was showed as the thickest band on CL since the protein was just expressed in supernatant as crude condition, and slightly degraded in thickness as at W1 and 2. The purified protein was the left protein bound on resin and after elution then the protein released as E1, 2 and 3

and again the bands showed slowly degradation in band thickness.

From the expression of JSU pGEX by double cell lysis, it seems that the protein in WB was depleted (Figure 2a. and 2b.). The Physical digestion by double cell lysis illustrated that many proteins were lysed as shown on SDS PAGE (Figure 2a), even though there was a specific protein of JSU pGEX (±60kDa) and exposed less thick (Figure 2b) compared to the single cell lysis (Figure 1b).

Specific expression of the size of JSU pGEX (±60kDa) at WB was possibly due to the role of primary and secondary antibodies used in this study.

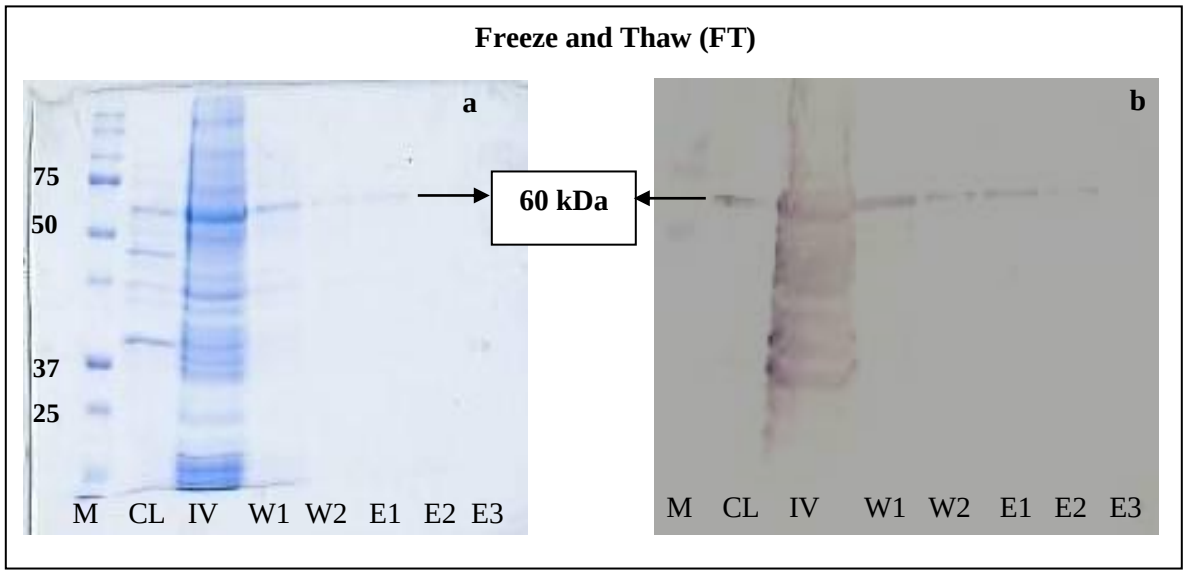

Figure 1. Characterization of JSU-pGEX derived from single cell lysis (Freeze-Thaw): a. SDS-PAGE; b. Western Blotting.
(M= Marker; CL= Cell lysate; IV= Inner volume; W1-W2=Washing1-2; E1-3= Elusion 1-3).

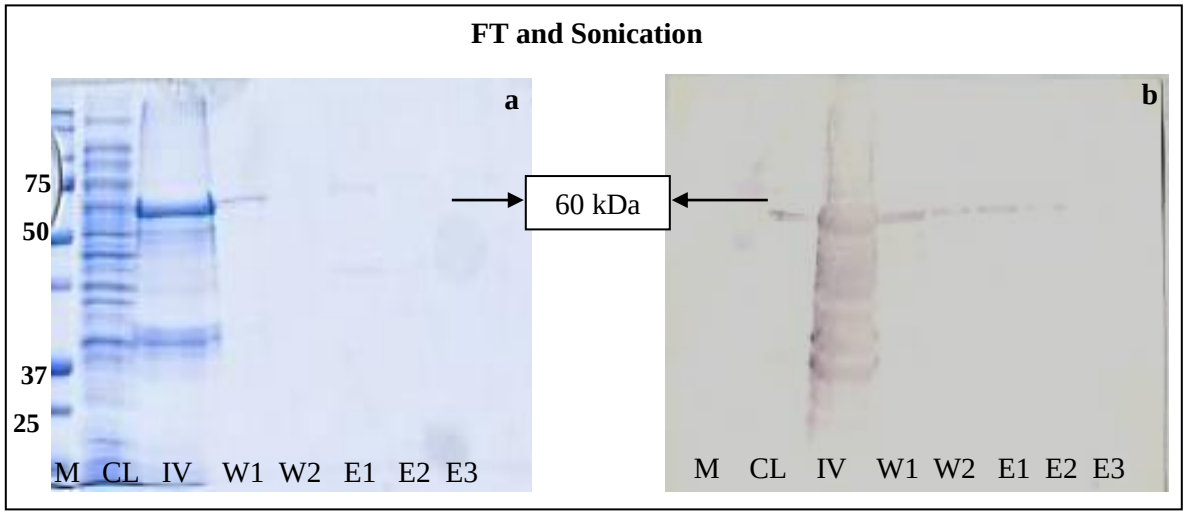

Figure 2. Characterization of JSU-pGEX derive from double cell lysis (Sonication and FT): a. SDS-PAGE; b. Western Blotting.
(M= Marker; CL= Cell lysate; IV= Inner volume; W1-W2=Washing1-2; E1-3= Elusion 1-3)

Quantification of JSU

Expression of some recombinant viral proteins often produces in the form of inclusion body where it needs to be solublelized to get soluble protein or active protein. It is also occurring on JSU recombinant protein. As recommended by Singh and Panda (2005) that adding solubilization buffer into inclusion body is a method of protein solubilization to obtain soluble protein. This study was also using the solubilization buffer to solublelize the inclusion body of JSU pGEX recombinant protein.

In this study, the purified soluble protein of JSU pGEX resulting from protein purification was quantified by using a GeneQuant machine for protein calculation. Purified protein was obtained from three repetitions where each repetition was eluted three times, namely E1, E2 and E3. A total purified protein was averaged from those total three repetitions. Each elution (E) of those three repetitions from both derived cell lyses was presented on Table 1.

Table 1 showed that averagely, purified recombinant protein JSU pGEX derived from single cell lysis resulted a higher concentration (0.790 ng/µl) compared to that double cell lysis (0.447 ng/µl). Double cell lysis was a combination of FT method and sonication (physical method). It seems that the effect of double cell lysis might heat and trigger to protein degradation, it is due to the effect of high temperature resulted from three cycles of sonication. Most of protein is composed by molecules that easily damage when it is not in the right physiology condition. Fractionation of cells is therefore should be performed at the lower temperature (0-4^{o}C) or on ice (Anonymous, 2008a) and at the certain condition of buffer and pH (Anonymous, 2009a). It seems that a higher physical cell lysis would more deplete the protein when it is not handled in the right way. As known, there are two types of protein degradation: by lysosomal and cytosolic, however the protein degradation is energy consuming process (Anonymous, 2009b). In this study, the effect of a physical cell lysis (sonication) might change the higher temperature into energy.

However based on the result of this study, it can be suggested that exposure of three cycles using ultra sound of sonication should be performed extra carefully and gently where protein should be managed on a lower temperature to avoid protein degradation. Overall, the protein was expressed in higher yield by a single cell lysis compared to that by double cell lysis.

Table 1. Concentration of JSU pGEX derived either by single or double cell lysis from 100ml culture.

Repetition	Elusion (E)	Concentration ng/µl	
		Freeze & Thaw (FT)	FT and Sonication
1	E1	0.952	0.330
	E2	0.932	0.630
	E3	0.752	0.303
	Average	**0.879**	**0.421**
2	E1	1.006	0.540
	E2	0.429	0.410
	E3	0.301	0.335
	Average	**0.579**	**0.428**
3	E1	1.158	0.268
	E2	0.859	0.680
	E3	0.716	0.532
	Average	**0.911**	**0.493**
Total average		**0.790**	**0.447**

Conclusion

This research concluded that both cell lysis systems (single and double) showed at the right size of 60kDa JSU pGEX on SDS PAGE and Western Blotting. However, a single cell lysis system yielded a higher pure recombinant protein of JSU pGEX (0.812ng/ul) compared to that a double cell lysis system (0.486ng/ul).

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