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Pengaruh Suhu dan Komposisi Media Kultur Terhadap Ekspresi Protein Rekombinan JSU-pETsebagai Bahan Kandidat Vaksin Penyakit Jembrana pada sapi Bali

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ABSTRAK

Penyakit Jembrana hanya menyerang pada sapi Bali, hingga saat ini preventif dilakukan dengan menggunakan vaksin konvensional dari virus Jembrana yang dilemahkan. Kelemahan vaksin tersebut adalah menimbulkan efek samping, mahal dan tidak kontinu diproduksi. Vaksin rekombinan telah dibuat menggunakan gen *env-su* pengkode protein *Surface Unit* (SU) dari virus Jembrana kedalam plasmid pET, dan ditranformasikan kedalam sel inang *E. coli* BL21. Penelitian ini dimaksudkan untuk mengoptimalkan ekspresi protein Jembrana JSU-pET dengan menerapkan kombinasi suhu setelah induksi (37^{o}C dan 25^{o}C) dan komposisi media kultur (LB dan mLB/dimodifikasi). *Escherichia coli* BL21 pembawa plasmid JSU-pET dikultivasi pada kedua media kultur dan diinkubasi dalam kedua suhu diatas. Sel *E. coli* BL21 dipanen dengan sentrifugasi, pelet dilisiskan dengan sonikasi. Supernatan hasil sonikasi dimurnikan dengan Ni-NTA Resin Agarose. Rekombinan protein JSU yang dimurnikan, dikarakterisasi dan dikuantifikasi dengan spektrofotometer. Hasil penelitian menunjukkan bahwa protein JSU rekombinan terekspresi pada ukuran 37kDa pada semua perlakuan, Hasil protein JSU pada suhu kultur 25^{o}C menunjukkan *yield* lebih tinggi baik pada LB maupun mLB (0,618 ± 0,095 vs 0,704 ± 0,094) daripada dikultur pada 37^{o}C (0,598 ± 0,137 vs 0,553 ± 0,041) pada kedua media kultur yang sama. Temuan ini menunjukkan bahwa suhu kultur yang lebih rendah dapat meningkatkan ekspresi (*yield)* protein JSU baik pada LB atau mLB.

Kata kunci: Suhu, Media, *E. coli*, JSU-pET, Ekspresi

The Effects of Temperature and Culture Medium Composition on Protein Recombinant JSU-pET Expressionas a vaccine candidate for Jembrana Disease in Bali Cattle

ABSTRACT

Jembrana disease only attacks in Bali cattle, up topresentpreventive is conducted by using conventional vaccines of the attenuated Jembrana virus. Disadvantages of the conventional vaccine are expensive and uncontinuous in production also has side effect. Recombinant vaccine was created by using Surface Unit (SU) env-su gene of Jembrana virus into plasmid pET, and transformed into the E. coli BL21 host cell for protein expression of Jembrana SU (JSU). This research was aimed to optimize the expression of the JSUprotein recombinant by applying a combination treatment of temperature culture after induction and culture media composition of LB and mLB (modified). Escherichia coli BL21 containing plasmid JSU-pET was cultivated in those two culture media and incubated at those two applied temperatures. JSU pellets were lysed and supernatant was purified with Ni-NTA resin. The purified JSU protein was characterized and qualified by a spectrophotometer. The results showed that JSU protein was successfully expressed at the right size of 37kDa in all treatments. JSU expression showed that protein yield was higher at culture of (0.618 ± 0.095 vs 0.704 ± 0.094) than cultured at 37^{o}C (0.598 ± 0.137 vs. 0.553 ± 0.041) in both media of LB and mLB, respectively. These findings indicate that a lower culture temperature could increase protein yield both in LB and modified LB media.

Keywords: *Temperatures, medium, E. coli, JSU, expression*

INTRODUCTION

Jembrana disease (JD) areinfectious only in pure Bali cattle. Currently, the prevention for JD by using crude vaccine, made form attenuated live virus of JD was collected from infected Bali cattle lymph (Hartaningsih *et al*., 2001). The disadvantages of crude vaccine are not specific, had fever as a side effect and high cost in production (Hartaningsih *et al.,* 2001). Up to present, the source of licensed vaccine for veterinary medicine is often in the form of killed or inactivated microorganism, live attenuated vaccine (McVey, 2010; Unikrishnan*et al*.,2012).

The Jembrana virus was obtained from infected lymph of acute Bali cattle by Jembrana virus and attenuated chemically by using Triton-X. The crude vaccine could only protect Bali cattle from Jembrana disease for three months and need a high cost to produce it (Hartaningsih *et al*., 2001). At present, our laboratory of animal molecular genetics at LIPI has developed the candidate Jembrana vaccine based on protein recombinant technology.

The Candidate recombinant vaccine for Jembrana Disease was constructed from Jembrana virus genome (*env-su* and *tat* gene) at LIPI, *i.e.* JTAT-His and JSU-His. All of those vaccine were produced in *E. coli*. Recently, Jembrana recombinant vaccine of JTAT was field trialed in Bali cattle and did not cause fever as a side effect of vaccination (Margawati, 2017, project report). Another construct is Jembrana*env-su* gene encoding ofSuperficial Unit(JSU) proteinwhich was constructed and expressed in pET (JSU-pET) with fusion his-tag system and transformed into host *E. coli* BL21.

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Escherichia coli have been the most widely used host to produce large amounts of heterologous proteins within a short time at a low cost (Chae *et al*., 2017; Gopal, 2013). Genetically it has been well known, and various strains have been developed for specific purposes to produce veterinary vaccine for medicine (Rosano, 2014; Jorge, 2017).

However, *E. coli* may produce inclusion body with low yield of protein recombinant. Generally, the goal of proteinrecombinant production is not only to maximize the amount of protein recombinant but also to achieve a protein with high quality, i.e. a protein in an active, soluble and pure form. Many solutions have been studied to increase the yield and to get the soluble protein.

The expression of JSU-pET was not optimal yet, hence, it needs to develop the method to increase the expression of JSU-pET. The expression of recombinant protein was influenced by severalfactors, IPTG concentration, Cell Optical Density, culture media composition (Studier 2005; Uhoraningonga et al., 2018), and temperature (Song *et al*., 2012).The low temperature cultivation (<28ºC) after induction with IPTG will formed protein in a soluble form and increase the yield of protein (Singh *et al.,* 2015; Larentis *et al*., 2014; Carrio *et al.,* 2005) While media composition will give high density cell culture or the induce the death of *E. coli* cells (Farrel and Finkel 2003). According to Kram and Finkel (2015), The effect of media compositionswill lead the oxidative stress, increasing of pH, the death of cells during log phase and changes in long-term survival patterns

In this study, to achieve the high expression and yield of JSU protein recombinant, we only focused on the temperature and modified culture medium, were two factors concerned to increase the expression of JSU-pET recombinant protein.

MATERI DAN METODE

JSU construct and transformation

Jembrana Surface Unit (JSU) plasmid was transformed into*E. coli* BL21 DE3 PLys Host cells(*E. coli* BL21 DE3 PLys-JSU-pET) by a heat shock method (Froger and Hall, 2007).The *E. coli* host cell was competent by TSS method according to procedure of Chung *et al*. (1989).The transformed *E. coli* were plated onto LBplate containing Amphicillin (100µg/ml) then incubated overnight at 37ºC.

Cultivation

Cultures media of *E. coli* bearing JSU construct were prepared for each treatment. The *E. coli*cells from stock cultures (20% glycerol) were grownin 5 ml LB medium containing 100µg/ml ampicillin, in a rotary shaker (150rpm) for overnight at 37ºC.

Media compositions and temperatures treatment

Two medium compositions (LB and mLB/modified) and two temperatures of 25ºC and 37ºC were appliedafter IPTG induction.The composition of LB medium (50ml) was Bactotryptone, yeast extract and NaCl while mLB was supplemented by 5% Glucose and 1% MgCl2. Those LB and mLB were containing Amphicillin (100µg/ml) and prepared in 250ml flaskfor each treatment. Overnight culture (2ml) was inoculated into flask containing media (50ml), prepared for 4 flaks and shake on the rotary shaker (150rpm; 1 hour; 37ºC). When the optical density (OD) was measured until OD600=0.6, 0.6µmM IPTG was added to the flasks for induction. After induction, the culturewere incubated at 25ºC and 37ºC with shaking 150rpm for three hours.

Purification and characterization

The *E. coli* cells were collected by centrifugation (4.000rpm; 10ºC; 15 min). The *E. coli* pellets were suspended with 10mM immidazole then lyses by two methods i.eof freeze thaw and sonication (Margawati*et al*.,2009) followed by centrifugation (9000 rpm; 4ºC; 25 min). The supernatant bearing JSU protein recombinant was purified by binding into the Ni-NTA resin. The purified JSU protein recombinant was characterized by 12% SDS-PAGE. All the purified JSU protein recombinant was quantified by using a Genequant (Amersham, USA).

Analysis

The research was composed by combination treatments of temperature culture after induction (25ºC and 37ºC) and culture media composition of LB and mLB (modified). Data of purified JSU protein yield was analyzed descriptively.

HASIL DAN PEMBAHASAN

Transformation

The JSU protein recombinant was successfully transformed into *E. coli* BL21 host cells. The *E. coli* colonies grew in white color of the LB plate (100µg/ml Amphicillin), see Figure 1.

Transformation result of this study (Figure 1) showed that *E. coli* colonies bearing JSU construct showed in white colonies. It proved that the JSU constructs were successfully transformed into *E. coli* BL21. Phadmanabhan *et al*. (2011), the white colonies indicated an insertion of foreign DNA and loss of the

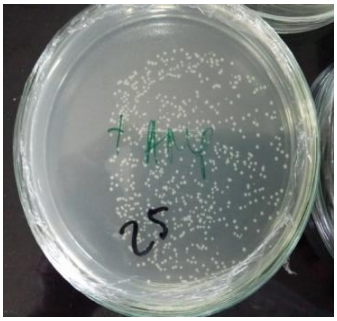

Figure 1. Performance of white colonies of *E. coli* BL21 on LB media plate

E. coli cells ability to hydrolyze the marker (β-Galactosidase).

Transformation medium (TSS) was used in this study containing 5% DMSO and PEG 6000. These reagents are highly increasing the efficiency of transformation process. Using the TSS method eliminates centrifugation and reduces incubation time compared to other methods (Chung *et al.*, 1989). Transformation efficiency can be optimized by supplementation CaCl2 and glycerol into pellets for increasing competent cells (Margawati *et al.*, 2017).

Optical Density

The growth of *E. coli* as a host cells was monitored by measuring of their optical density (OD) (Figure 2). The E. coli grew faster after 2-hour observation in mLB both at 25°C (purple block; OD=0.623) and 37ºC (green block; 0.626) compared to other treatments.

The optical density (OD) showed that *E. coli* grew faster in mLB37 and mLB25 rather than LB25 and LB37 (Figure 2). The modification in a culture medium (LB supplemented with glucose and Mg^{2+})will increased the OD both at 25ºC and 37ºC. Glucose is the best sugar source for *E. coli* growth therefore when the glucose runs out the growth decreases. As stated by Bren *et al*. (2016) and Caglar*et al*. (2017) in general, glucose is the best carbon source for *E. coli* growth. The glucose triggers faster growth than other sugar sources, and it is consumed firstly than other sugar mixtures in the medium.

The addition of Mg^{2+} is actually not influencing directly to the protein production (Caglar*et al.*2017). The addition of Glucose and Mg^{2+} will give a longer phase of E. coli growth (Nikaido, 2009). Culture temperature of 37ºC gives more quick growth of E. coli subsequently it could down growth very fast (Samelis & Sofos, 2002). It might be correlated with the availability of energy source from glucose. Meanwhile culture temperature of the 25°C could hold longer the energy source from glucose in *E. coli* growth.

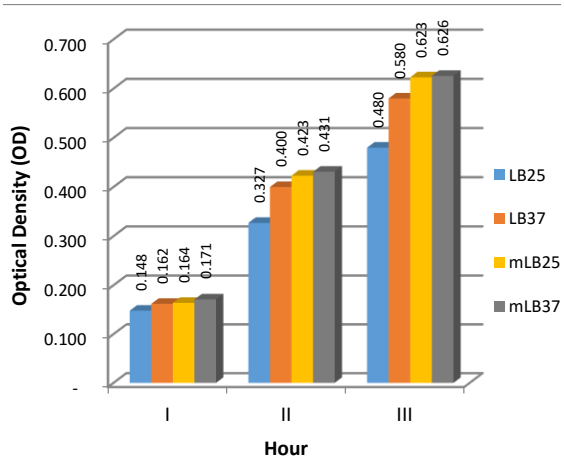

Figure 2. Graphof Optical Density (OD_{600}) *E. coli* at different culture temperatures and media

There were different colors of collected pellets after harvesting *E. coli* (Figure not shown). Those were yellowish pellets collected from LB medium of both cultured at 25ºC and 37ºC while white pellets were obtained from mLB medium at both culture temperatures (25ºC and 37ºC).

The white color of *E. coli* pellets was emerged in mLB at both culture temperatures (25ºC and 37ºC). Some researcher found that if the pellet in white color it indicates the protein recombinant is the form of inclusion body (Nelson *et al*., 2014). The yellowish color of *E. coli* pellets from LB medium at both 25ºC and 37ºC might be caused by compound of culture medium containing yeast extract. In this study the mLB medium was added Mg^{2+}. The content of culture medium needs more thorough study in expression and form of protein recombinant.

Protein Expression

The expression of purified JSU showed on the right size of 37kDa (Figure 3). The purified JSU protein recombinant was quantified then analyzed discriptively (Table 1). The average of JSU protein yield was higher in a temperature cultured at 25°C either in LB or in mLB (0.618±0.095 or 0.704±0.094) compared to that cultured at 37ºC either in LB or in mLB (0.598±0.137 or 0.553±0.041).

Characterization of JSU protein recombinant was successfully found on the right size of 37ºC in all treatments where the JSU protein was using 6x His-tag fusion (Figure 3). The size band of JSU emerged more clear in mLB25 and mLB37 than LB25 and LB37. It was suggested by Backlund *et al*. (2011) that there is a correlation of feeding glucose in protein recombinant production by fed-batch method.

Tabel 1. Average of quantified JSU protein recombinant in treatments

Medium	Temperatures	
	25ºC	37ºC
LB	0.618±0.095	0.598±0.137
mLB	0.704±0.094	0.553±0.041

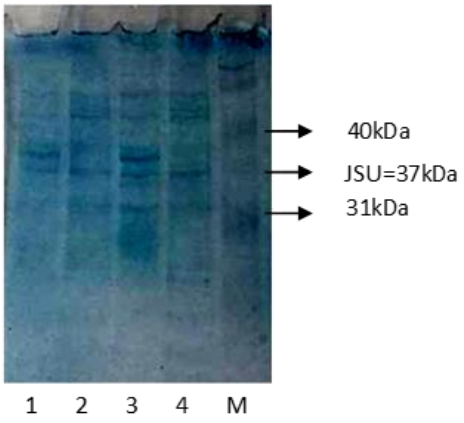

Figure 3. JSU protein recombinant expression on 12% SDS PAGE.1=LB25; 2=mLB25; 3=LB37; 4=mLB37; M=Protein LadderPrestained, BIORAD

In Table 1, it was shown thatthe average of JSU protein yield was higher at 25ºC either in LB or in mLB compared to that cultured at 37ºC either in LB or in mLB (Table 1). This finding showed that the best yield was achieved in the mLB medium at the culture temperature of 25ºC. It might be the Mg^{2+} supplementation increasing the expression the protein (Nikaido, 2009). In addition, it was stated that culturing at 25ºC could hold a longer growth of *E. coli*. According to Novy and Morris (2001), Glucose contained in the culture medium will increase the protein expression in pET30 system. This finding also similar with Wang *et al.* (2014), that mutant TC110 will grew quickly and produced much less acetate in 2×LB broth medium with 2% glucose.

CONCLUSION

JSU protein was succesfully expressed with the size of 37kDa in all of treatments. The yield from culture with temperature 25ºC showed higher yield compare with the yield from 370C culture, either cultured in LB or mLB, respectively. It was suggested that lower temperature could be applied to get a better yield of JSU recombinant protein rather than cultured in a high temperature both in LB and mLB media.

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