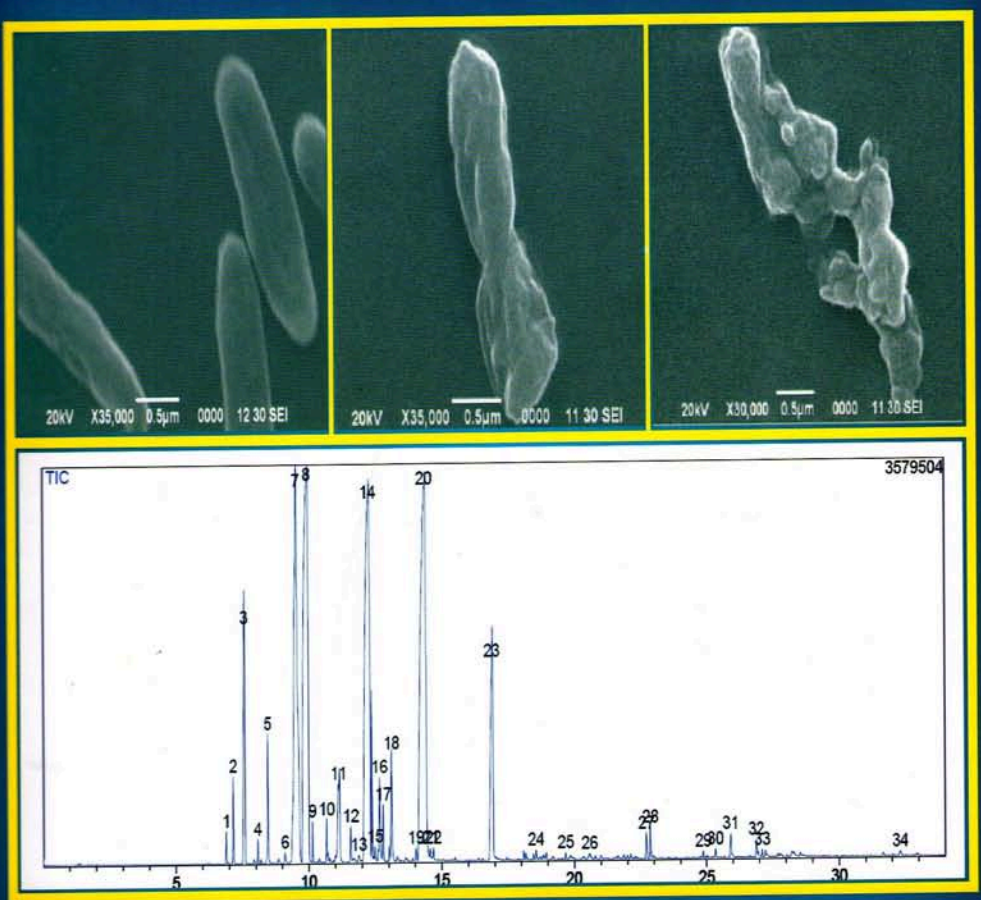
ISSN 0126-1754
Volume 9, Nomor 1, April 2008
Terakreditasi Peringkat A
SK Kepala LIPI
Nomor 14/Akred-LIPI/P2MBI/9/2006

Berita Biologi

Jurnal Ilmiah Nasional



Diterbitkan Oleh
Pusat Penelitian Biologi - LIPI

Berita Biologi merupakan Jurnal Ilmiah ilmu-ilmu hayati yang dikelola oleh Pusat Penelitian Biologi - Lembaga Ilmu Pengetahuan Indonesia (LIPI), untuk menerbitkan hasil karya-penelitian (original research) dan karya-pengembangan, tinjauan kembali (review) dan ulasan topik khusus dalam bidang biologi. Disediakan pula ruang untuk menguraikan seluk-beluk peralatan laboratorium yang spesifik dan dipakai secara umum, standard dan secara intemasional. Juga uraian tentang metode-metode berstandar baku dalam bidang biologi, baik laboratorium, lapangan maupun pengolahan koleksi biodiversitas. Kesempatan menulis terbuka untuk umum meliputi para peneliti lembaga riset, pengajar perguruan tinggi maupun pekarya-tesis sarjana semua strata. Makalah harus dipersiapkan dengan berpedoman pada ketentuan-ketentuan penulisan yang tercantum dalam setiap nomor.

Diterbitkan 3 kali dalam setahun yakni bulan April, Agusrus dan Desember. Setiap volume terdiri dari 6 nomor.

Surat Keputusan Ketua LIPI

Nomor: 1326/E/2000, Tanggal 9 Juni 2000

Dewan Pengurus

Pusat Penelitian Biologi - LIPI
Kompleks Cibinong Scienqe Centre (CSC-LIPI)
Jin Raya Jakarta-Bogor Km 46,
Cibinong 16911, Bogor - Indonesia
Telepon (021) 8765066 - 8765067
Faksimili (0251) 8765063
Email: herbogor@,indo.net.id
ksama_p2biologi(@,vahoo.com

Keterangan foto/ gambar cover depan: *Perbandingan tingkat kerusakan dinding sel Escherichia coli yang diperlakukan dengan minyak atsiri temu kunci* (Kaempferia pandurata), *dan kromatogramnya yang dihasilkan dengan GC-MS sesuai makalah di halaman 1* (Foto: koleksi Universitas Sriwijaya/ Institut Pertanian Bogor - Miksusanti).

LIPI

Berita Biologi

Jurnal Ilmiah Nasional

ISSN 0126-1754

Volume 9, Nomor 1, April 2008

Terakreditasi

SK Kepala LIPI

Nomor 14/Akred-LIPI/P2MBI/9/2006

Diterbitkan oleh

Pusat Penelitian Biologi - LIPI

Ketentuan-ketentuan untuk Penulisan dalam Jurnal Berita Biologi

1. Karangan ilmiah asli, *hasil penelitian* dan belum pernah diterbitkan atau tidak sedang dikirim ke media lain.
2. Bahasa Indonesia. Bahasa Inggris dan asing lainnya, dipertimbangkan.
3. Masalah yang diliput, diharapkan aspek "baru" dalam bidang-bidang
   - Biologi dasar *(pure biology),* meliputi turunan-turunannya (mikrobiolgi, fisiologi, ekologi, genetika, morfologi, sistematik dan sebagainya).
   - Ilmu serumpun dengan biologi: pertanian, kehutanan, peternakan, perikanan ait tawar dan biologi kelautan, agrobiologi, limnologi, agro bioklimatologi, kesehatan, kimia, lingkungan, agroforestri. *Aspek/pendekatan biologi* harus tampak jelas.
4. Deskripsi masalah: harus jelas adanya tantangan ilmiah *(scientific challenge).*
5. Metode pendekatan masalah: standar, sesuai bidang masing-masing.
6. Hasil: hasil temuan harus jelas dan terarah.
7. Kerangka karangan: standar.
   Abstrak dalam bahasa Inggris, maksimum 200 kata, spasi tunggal, ditulis miring, isi singkat, padat yang pada dasarnya menjelaskan masalah dan hasil temuan. *Hasil* dipisahkan dari *Pembahasan.*
8. Pola penyiapan makalah: spasi ganda (kecuali abstrak), pada kertas berukuran A4 (70 gram), maksimum 15 halaman termasuk gambar/foto; pencantuman Lampiran seperlunya.
   Gambar dan foto: harus bermutu tinggi, gambar pada kertas kalkir (bila manual) dengan tinta cina, berukuran kartu pos; foto berwarna, sebutkan programnya bila dibuat dengan komputer.
9. Kirimkan 2 (dua) eksemplar makalah ke Redaksi (alamat pada cover depan-dalam) yang ditulis dengan program Microsoft Word 2000 ke atas. Satu eksemplar tanpa nama dan alamat penulis (-penulis)nya. Sertakan juga copy file dalam CD (bukan disket), untuk kebutuhan Referee secara elektronik. Jika memungkinkan, kirim juga filenya melalui alamat elektronik (E-mail) Berita Biologi: herbogor@,indo.net.id dan ksama_p2biologi(3),vahoo.com
10. Cara penulisan sumber pustaka: tuliskan nama jurnal, buku, presiding atau sumber lainnya secara lengkap, jangan disingkat. Nama inisial pengarang tidak perlu diberi tanda titik pemisah.
    a. Jurnal
       Premachandra GS, H Saneko, K Fujita and S Ogata. 1992. Leaf Water Relations, Osmotic Adjustment, Cell Membrane Stability, Epicutilar Wax Load and Growth as Affected by Increasing Water Deficits in Sorghum. *Journal of Experimental Botany* 43, 1559-1576.
    b. Buku
       Kramer PJ. 1983. *Plant Water Relationship,* 76. Academic, New York.
    c. Presiding atau hasil Simposium/Seminar/Lokakarya dan sebagainya
       Hamzah MS dan SA Yusuf. 1995. Pengamatan Beberapa Aspek Biologi Sotong Buluh *(Sepioteuthis lessoniana)* di Sekitar Perairan Pantai Wokam Bagian Barat, Kepulauan Am, Maluku Tenggara. *Prosiding Seminar Nasional Biologi XI,* Ujung Pandang 20-21 Juli 1993. M Hasan, A Mattimu, JG Nelwan dan M Littay (Penyunting), 769-777. Perhimpunan Biologi Indonesia.
    d. Makalah sebagai bagian dari buku
       Leegood RC and DA Walker. 1993. Chloroplast and Protoplast. Dalam: *Photosynthesis and Production in a Changing Environment.* DO Hall, JMO Scurlock, HR Bohlar Nordenkampf, RC Leegood and SP Long (Eds), 268-282. Champman and Hall. London.
11. Kirimkan makalah serta copy file dalam CD (lihat butir 9) ke Redaksi. Sertakan alamat Penulis yang jelas, juga meliputi nomor telepon (termasuk HP) yang mudah dan cepat dihubungi dan alamat elektroniknya.

Berita Biologi menyampaikan terima kasih
kepada para Mitra Bestari/Penilai (Referee) nomor ini
9(1)-April 2008

Prof. Dr. Adek Zamrud Adnan (Farmasi, FMIPA-Universitas Andalas)
Dr. Andria Agusta (Pusat Penelitian Biologi-LIPI)
Dr. B Paul Naiola (Pusat Penelitian Biologi-LIPI)
Drs. Edy Mirmanto, MSc (Pusat Penelitian Biologi-LIPI)
Dr. Erdy Santoso (Puslitbang Hutan dan Konservasi Alam Departemen Kehutanan)
Dr. Hah Sutrisno (Pusat Penelitian Biologi-LIPI)
Dr. Herman Daryono (Puslitbang Hutan dan Konservasi Alam Departemen Kehutanan)
Dr. Iwan Saskiawan (Pusat Penelitian Biologi-LIPI)
Ir. Maria Imelda, MSc (Pusat Penelitian Bioteknologi-LIPI)
Dra. Nunuk Widhyastuti, MSi (Pusat Penelitian Biologi-LIPI)
Dr. Nuril Hidayati (Pusat Penelitian Biologi-LIPI)
Dr. Nyoman Mantik Astawa (Departemen Virologi FKH -Universitas Udayana)

DAFTAR ISI

MAKALAH HASIL RISET (ORIGINAL PAPERS)

RETRANSFORMATION AND EXPRESSION OF RECOMBINANT VIRAL PROTEIN OF JEMBRANA SU AND Tat (JSU AND JTat) IN pGEX SYSTEM
[Retransformasi dan Ekspresi Protein Virus Rekombinan JSU dan JTat Penyakit Jembrana dalam Sistem pGex]

Endang T Margawati*, Andi Utama and Indriawati
Research Centre for Biotechnology, The Indonesian Institute of Sciences (LIPI)
Jin Raya Bogor KM 46, Cibinong 16911
*E-mail: etmargawati@,cbn.net.id

ABSTRAK

Genom virus penyakit Jembrana setidaknya memiliki 3 gen besar yang menyandi protein dan beberapa di antaranya diperlukan untuk replikasi virus. Protein JSU dan JTat diduga dapat menginduksi kekebalan yang protektif pada sapi Bali terhadap penyakit Jembrana sehingga keduanya sangat berpotensi untuk dipakai sebagai vaksin rekombinan. Penelitian ini dirancang untuk meretransformasi protein rekombinan JSU dan JTat ke dalam *Escherichia coli* menggunakan sistem pGEX. Konstruk JSU dan JTat dalam pGEX dikoleksi plasmidnya dengan metode miniprep dan kemudian diretranformasikan ke dalam *E. coli* strain BL21 dan DH5a. JSU dan JTat hasil retransformasi diekspresikan pada medium LB untuk skala produksi kecil dengan sistem pGEX. Hasil penelitian ini meminjukkan bahwa kedua JSU dan JTat hasil retransformasi ke dalam *E. coli* strain BL21 terlihat tumbuh lebih baik pada medium LB jika dibandingkan retransformasi ke dalam *E. coli* strain DH5a. Hasil retransformasi JSU dan JTat dikarakterisasi dan diidentifikasi dengan *Western blotting* dan tampak menunjukkan ukuran protein yang benar, yaitu protein rekombinan JSU berukuran 60kDa dan JTat berukuran 36,7kDa. Protein rekombinan JSU muncul dengan pita tunggal dan lebih jelas jika dibandingkan dengan protein JTat. Konsentrasi protein JSU sedikit lebih rendah (1,883 mg ml^{1}) jika dibandingkan dengan JTat (l,981mg ml^{1}). Penelitian ini menunjukkan bahwa JSU pGEX masih tersimpan dan diekspresikan dengan baik, sementara JTat mungkin perlu dilakukan perakitan ulang untuk memantapkan ekspresinya.

Kata kunci: Jembrana, sapi Bali, protein rekombinant, JSU, JTat.

ABSTRACT

The genome of Jembrana disease virus (JDV) has at least three major genes encoding for the viral proteins and some of them use it for its replication. Recombinant proteins of JSU and JTat are expected to induce a protective immune response against the virus and are, therefore potential for the development of a protective recombinant vaccine for Jembrana disease. This research was designed to retransform both JSU and JTat into *Escherichia coli* and expressing the proteins using pGEX system. Constructs of pGEX bearing JSU and JTat genes were collected by a miniprep method and then retransformed either into *E. coli* of BL21 or DH5o strains. The retransformed JSU and JTat were expressed in a small scale production of LB medium using pGEX system. The results showed that both retransformed JSU and JTat into *E. coli* strain BL21 appeared to be better growing on LB medium as compared to that of transformed constructs into strain DH5a. In western blotting assay, both retransformed JSU and JTat produced the right size of 60kDa for JSU recombinant protein and 36.7kDa for JTat recombinant protein. Recombinant protein JSU showed a sharper and a single band as compared to that J-Tat protein. Protein concentration of JSU was slightly lower (1.883mg ml^{1}) as compared to that JTat (1.981mg ml^{1}). This result suggests that JSU construct in pGEX is well-expressed in *E. coli* whereas JTat might need to be reconstructed to enhance its expression.

Keywords: Jembrana disease, Bali cattle, recombinant proteins, JSU, JTat.

INTRODUCTION

Jembrana disease is an infectious, severe and acute viral disease in Bali cattle (Kertayadnya *et al.*, 1993). It was recognized initially in Jembrana districts in Bali, and was therefore termed as Jembrana disease virus (JDV). In Bali cattle, the JDV caused about 20% case fatality rate. The JDV was then identified as lentivirus of Retroviridae family (Chadwick *etal*, 1995; Wilcox *et al.*, 1995). The prevention of Jembrana disease (JD) in Bali cattle is currently conducted by vaccination. Until recently, a crude vaccine is prepared from infected organ of Bali cattle acutely affected by Jembrana disease. The crude vaccine is available for use to prevent the spread of the disease in Bali cattle and has been widely used in many parts of Indonesia. Some disadvantages of the crude vaccine are as follows. It still requires to sacrifice JDV-infected donor cattle in order to prepare the vaccine. As the vaccine is prepared from an infected organ, it is also difficult to instigate a proper quality control for the vaccine. In addition, the vaccine is still expensive to produce. The development

of a recombinant Jembrana vaccine is therefore expected to solve some of the problems associated with disadvantages of the current vaccine.

Jembrana disease viral genome consists of at least three major genes: *pol, gag* and *env.* Jembrana Surface Unit (JSU) protein is encoded by *env* gene, while *tat* gene that encodes for JTat protein, is a small accessory gene lies between *pol* and *env* genes. At least two proteins, JSU and JTat, are potential candidates for the development of a protective vaccine against Jembrana disease in Bali cattle (ACIAR, 2004). The JSU is the virus attachment protein which initiates the first interaction between the virus and the infected cells. Antibody capable of binding with the protein will be likely preventing the viral infection (Barnett *et al.,* 2001). Tat protein is a minor protein required by the virus to activate the transcription of the viral genome into mRNA which is necessary for the efficient replication of the virus in the infected host (Cota-Gomez *et al.,* 2002; Wu, 2004). Immune response against the protein is expected to be able to slow down or completely stop the viral replication.

The production of the recombinant proteins for JDV is possible as the genes encoding for the proteins have been well-characterized (Chadwick *et al.,* 1995) which in turn will enable the cloning of the genes in an appropriate expression systems such as pGEX. Cloning of JSU and JTat is normally performed using pGEX system in which the genes were fused with GST tag. Recombinant JSU protein has the molecular weight of ±34kDa and JTat has the molecular weight of±10.7kDa, whereas the GST tag *per se* has the molecular weight of ± 26kDa (Amershampharmacia biotech, 1997). Cloning of both JSU and JTat genes in pGEX system produces fused protein with a GST tag. The fused recombinant proteins, GST-JSU and GST-JTat are expected to have the molecular weights of ± 60kDa and ±36.7kDa respectively. Both JSU and JTat recombinant proteins are normally expressed in the form of inclusion body which needs to be solubilized to obtain native proteins. Previous studies have shown that JSU and JTat expression did not result in the right size of molecular weight after characterization by SDS-PAGE and Western blotting (Margawati *et al.,* 2004). It appears that the constructs were not stable or not well-maintained in glycerol. This study was therefore designed to re-transform both constructs of JSU and JTat into *Echerichia coli* and to express them in pGEX system.

MATEWALAND METHODS

Retransformation of Construct

Plasmid Isolation of JSU dan JTat

The origin of JSU and JTat pGEX constructs was provided by the virology laboratory of Murdoch University. Plasmid bearing *su* and *tat* genes were collected from JSU and JTat pGEX constructs by miniprep method. Colonies of the JSU and JTat constructs were cultured overnight in Luria Bertani (LB) medium (consists of bacto trypton, yeast extract; NaCl) supplemented with lOOug/ml amphicillin at shaking conditions of 200 rpm at 37°C. Bacterial *E. coli* pellets were collected by centrifugation at 4000 rpm in 10°C environment for 15 min. The collected pellets were lysed with three types buffer solutions, cell lysis buffer, neutralization buffer and centrifuged at 4000 rpm in 10°C environment for 15 min, and the the supernatant was collected. Into supernatant was added isopropanol and incubated at -20°C for 2 hours, and was then centrifuged at 4000 rpm at 10°C for 15 minutes to collect pellets. The pellets were washed with 80% ethanol and again centrifuged with 4000 rpm at 10°C for 15 min to collect the pellets (bearing the plasmid). The pellets on the bottom of a micro tube were air dried, and were then diluted in TE buffer.

Competency of Escherichia coli strain BL21 andDHSa

Competent host cells of *E. coli* (BL21 and dH5oc) are needed for retransformation. A method of TSS (Transformation Storage Solution, consists of LB Medium, PEG6000, DMSO and 1M MgC12) was used to prepare competent *E. coli* (Chung *et al.,* 1989). In prior to competing cells, *E. coli* colony was overnight cultured with shaking of 200 rpm at 37°C and the pellet was then collected by centrifugation of 4000 rpm 4°C for 5 minutes . The collected pellets (£. *coli)* were re suspended by adding a cold TSS solution. This TS^ contains *E. coli* competent and ready to be used.

Retransformation of JSU and JTat

An amount of 2ul TE buffer (containin

plasmid) was introduced into lOO,ul of competent cells in TSS by heat shock method for re-transformation. Into the retransformed *E. coli* was added **400(J,l** LB medium without amphicillin and incubated at 37°C with shaking at 200 rpm for 1 hour. The substrate medium was centrifuged with 4000 rpm at 4°C for 1 minute to collect supernatant (SN). The SN was spread out and grown on LB agar plate containing amphicillin.

Expression of Protein *Overnight Culture*

Colonies (containing retransformed JSU and JTat) were overnight cultured into 2-5 ml LB medium containing 100 |xg/ml amphicillin and incubated at 37°C with shaking of 200 rpm.

Inoculation and IPTG Induction

Inoculation was performed with a ratio of 1:20 (overnight culture: LB medium) in 100ml LB medium and incubated at 37°C with shaking of 200 rpm for 3 hours, or up to achieve 0.8 OD (optical density). Induction of 0.1 mM IPTG was implemented after the culture OD of 0.8 was achieved and the culture was kept for another 1 hour. Induction of IPTG was intended to stimulate protein expression (Mills, 2001).

*Harvesting **and** Lysis*

E. coli was harvested after 4 hours in a total culture, including the IPTG induction, by centrifugation of 5000 rpm at 4°C for 15 minutes. Pellet was digested by freezing and thawing method without addition of cell digester *(e.g* lysozyme) except supplementation of lysis buffer. The freezing and thawing were conducted for 5 cycles each of which consisted of sample exposure (containing pellet and lysis buffer) into liquid nitrogen (N_2) for 30 seconds and then thawing into water batch at 42°C for 60 seconds. This substrate was then centrifuged with 5000 rpm at 4°C for 15 minutes to collect the pellet (inclusion body).

Solubilization of Inclusion Body

Prior to solubilization, the pellet was washed three times by adding washing buffer and centrifuged with 12.000 rpm at 4°C for 15 minutes to collect the pellet. The pellet was overnight solubilized by adding buffer and kept at 4°C (Singh and Panda, 2005).

*Expression **and Purification***

The solublelized substrate (semi-purified protein) was purified by using gluthatione sepharose 4B with a batch capture method. The purified protein was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and directly transferred onto nitrocellulose membrane for western blotting (WB) assay. The JSU and JTat proteins were identified by monoclonal anti-GST antibody. The purified protein can be identified by checking their sizes, and using pGEX system JSU has ± 60kDa while JTat has ± 36.7kDa.

Quantification of Protein

The purified recombinant protein was quantified using a BCA™ Protein Assay Kit with BSA standard (Pierce).

RESULTS

Retransformation JSU and JTat

Plasmid containing both Jembrana *SU* and *tat* genes were transformed into *E. coli* strain BL and dH5a. The transformed *E. coli* cells were grown on LB agar plate containing amphicillin. Both transformed E.coli (strain BL and dH5oc) containing both JSU and JTat grew and formed colonies. However, the colonies derived from *E. coli* strain BL formed bigger colonies as compared to that colonies derived from dH5a on both JSU and JTat. Therefore, retransformed JSU and JTat derived from BL strain was used for protein expression.

Expression of JSU and JTat pGEX

The expression of retransformed JSU and JTat into *E. coli* strain BL was performed three times each of which was in a volume of 100 ml LB culture medium. The solublelized substrate (semi-purified protein) of both JSU and JTat was purified with gluthatione sepharose 4B by a batch capture method. JSU and JTat proteins were analysed by SDS-PAGE and then identified by WB using monoclonal anti-GST antibodies. Result of the characterization of both JSU and JTat on WB showed on the right sizes on both retransformed constructs: JSU=±60kDa (Figure 1) and JTat= ±36.7kDa (Figure 2). However, the band derived from the retransformed JSU resulted in a sharper and thicker band (Figure 1) as compared to that of JTat (Figure 2) on elusion 1,2,3,4 (El, E2, E3 and E4) of each construct. Moreover, WB of JTat showed very fine bands on E3 and E4.

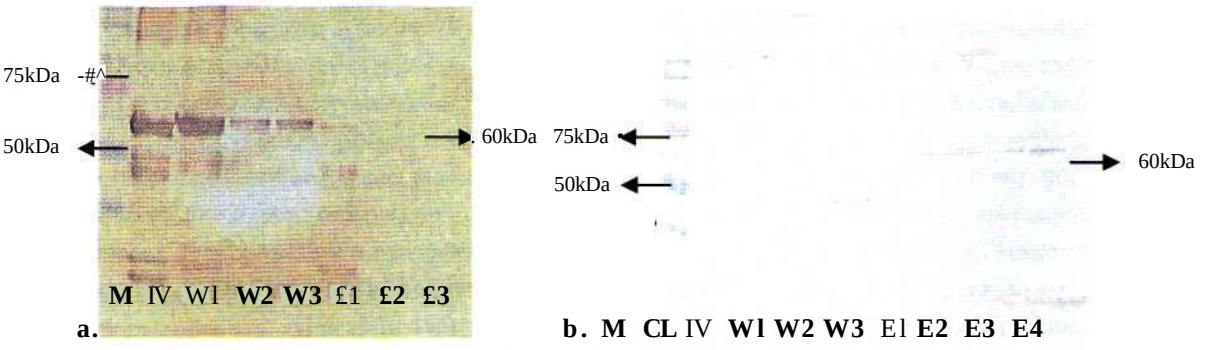


Figure 1. Characterization and identification of retransformed JSU pGEX by western blotting: a. Trial 1 and b. Trial 2. (M= Marker; CL= Cell Lysate; IV= Inner Volume; W1, W2, W3= Washingl, 2,3; E1, E2,W3, W4 = Elusion 1,2, 3, 4)

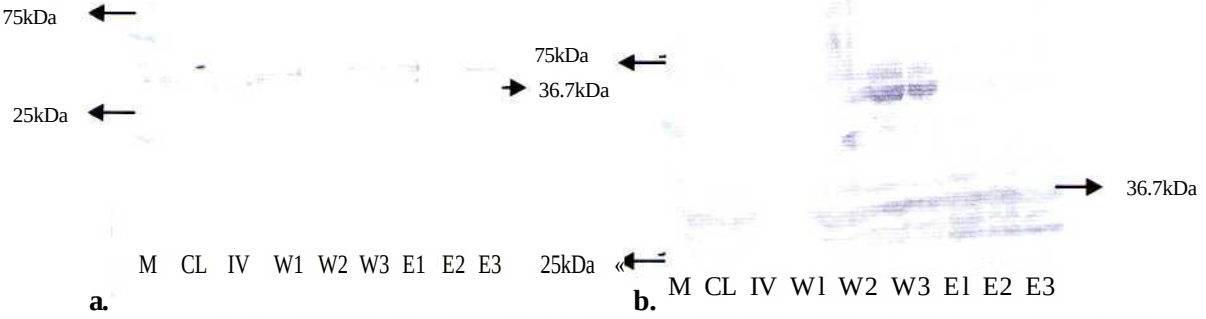


Figure 2. Characterization and identification of retransformed JTat pGEX by western blotting: a. Trial 1 and b.Trial 2. (M= Marker; CL= Cell Lysate; IV= Inner Volume; W1, W2, W3= Washingl, 2,3; E1, E2, W3, W4 = Elusion 1,2,3,4)

Protein Concentration

In this study, purified protein was still bound to the gluthation sepharose 4B matrix up to elusion 4 for JSU trial 1 (Figure lb), whereas the others were eluted up to elusion 3. Therefore, we considered to calculate the protein concentration until elusion 3 (E1, E2 and E3) of those retransformed JSU and JTat pGEX. The protein quantification was repeated twice for each retransformed construct. The average concentration of re-transformed JSU protein was a slightly lower (1.883mg ml^{-1}) as compared to that of JTat (1.98 lmg ml" '), (Table 1). However, JSU recombinant protein appeared to be better-purified as only a single and sharp band was detected (Figure 1) as compared to JTat (Figure 2). The data on the protein concentration for both recombinant proteins in each elution (E) and each average concentration from two trials were shown at Table **1**.

Table 1. Concentration of purified JSU and JTat proteins derived from retransformation into *E. coli* strain BL21

Trial	Elution (E)	Cone, (mg ml^{-1})	
		JSU	JTat
Trial 1 (Tl)	El E2 E3	2,181 1,905 1,317	2,769 1,5142,835
Average		**1,801**	**2,373**
Trial 2 (T2)	El E2 E3	1,952 2,032 1,908	1,967 1,507 1,289
Average		**1,964**	**1,588**
Avearage (T1+T2)		**1,883**	**1,981**

DISSCUSION

Retransformation and Expression of JSU dan JTat pGEX

Plasmid JSU and JTat pGEX were transformed into host cells, *E. coli* strain BL21 resulting in bigger

colonies on LB medium agar plate as compared to that of transformed into DH5a. This result showed that *E. coli* strain BL21 is more suitable for use in the protein expression of both JSU and JTat in pGEX system. As reported by Inoue *et al.* (1990), *E. coli* strain DH5a is usually used for plasmid transformation whereas *E. coli* strain BL21 is normally used for protein expression. Similar finding was also reported by Ramos *et al.* (2004) that the *E. coli* strain DH5a was used for all routine cloning experiments while the *E. coli* strain BL21 (DE3) was used for recombinant protein expression. In our previous study, *E. coli* strain JM109 was used for cloning experiment of recombinant protein JTat. It showed a similar result to strain DH5a and grew small colonies in LB agar plate medium. Therefore, both strains DH5a and JM109 are suitable for cloning experiment.

Transformation of plasmid into DH5a or JM109 is mainly intended for cloning purposes that is to check whether or not the plasmid bears the desired gene insert upon screening. Once the cloned plasmid is detected to bear the desired gene insert, it is then retransformed into *E. coli* strain BL.

In this study, the retransformation was performed by using the supernatant (SN) derived from centrifugation after heat shocking and then the SN was grown on LB agar plate medium. As reported in our previous study (Margawati *et al.*, 2006), the supernatant collected after heat shocking of plasmid J-Tat pGEX resulted in a better growing of the construct on LB agar plate medium and even yielded a higher concentration of purified recombinant protein. Another simple protocol was reported by Tu *et al.* (2005) that plasmid transformation or mixture of plasmid and competent cell can be directly spread out and grown on the agar plate medium without centrifugation.

Expression and Concentration of retransformed JSU and JTat

In this study, constructs of JSU and JTat were re-transformed into *E. coli* BL21 for protein expression. As stated by Inoue *et al.* (1990) and Ramos *et al.* (2004), recombinant protein can be expressed in *E. coli* strain BL21, while *E. coli* strain DH5a is suitable for routinely cloning experiment. Cells were lysed by adding lysis buffer followed by by freezing and thawing method. Basically, the lysis of cells is performed by employing non-ionic detergents to disrupt the cellular membrane after the wall of cells was broken by lysozyme treatment. As reported in the recent finding, cell lysis can be conducted in a simpler method by insertion of a bacteriophage lamda lysis gene cluster into pET22b expression (Jang *et al.*, 2007) to make a simpler and a more efficient process of protein purification.

Expression of recombinant JSU and JTat proteins often resulted in the form of inclusion body which needs further solubilization. Such solubilization can be achieved by adding solubilization buffer to obtain soluble protein (Singh and Panda, 2005) or semi-purified protein. The semi-purified protein was then further purified by gluthatione sepharose 4B with a batch capture method. The purity of the proteins was, then checked by SDS-PAGE and WB. Both JSU and JTat recombinant proteins appeared to be expressed properly using pGEX system which resulted in ± 60kDa protein for JSU (Figure 1) and ± 36.7kDa protein for JTat (Figure 2). The JSU appeared to be expressed better as it produced a sharper and a single band in SDS-PAGE as compared to that JTat. This sharp band of purified JSU was obtained from pooled elusion 1,2, 3 and even up obtained from elusion 4 (Figure lb). In contrast, JTat showed inconsistent band quality (sharp and thick) in every elution following analysis by SDS-PAGE and WB. Expression of JTat recombinant protein was not as clear as the JSU, and it appeared to be due to the quality of the construct. A good and stable JSU construct seems to be established using pGEX system before re-transformation. The JTat, however, appeared to unstable construct. As seen on the visualization of purified protein by western blotting, JSU produced a single band of ±60kDa (Figure 1) while JTat, produced multiple protein bands beside the JTat protein (36.7kDa) *per se* (Figure 2). Consequently, this expression consistently affected the concentration of the purified recombinant protein (retransformed)of JTat in pGEX which showed a slightly higher protein concentration (1.981mg ml^{-1}) as compared to that retransformed JSU (1.981mg ml^{-1}) (Table 1). The maintenance of constructs, therefore, needs to be taken into account by keeping the constructs in the right lower temperature of-70 up to -80°C and retransformed

periodically (monthly or in every a 3-month) for refreshing their micro-environment and keeping their survival.

SUMMARY

Retransformation of JSU and JTat was successfully conducted into to *E. coli* strain BL21 while transformation into *E. coli* strain DH5a appeared to be better for cloning purposes. Expression of JSU and JTat recombinant proteins in pGEX system resulted in the formation of inclusion body which needs for further solubilization. Both JSU and JTat were on the right sizes when detected by western blot assay which clearly showed 60kDa and 36.7kDa proteins, respectively for JSU and JTat. However, JSU seems to better expressed that Jtat as only a single band but a slightly lower concentration of purified protein as compared to that of JTat.

ACKNOWLEDGEMENT

This research was possible and supported by DIPA of Research Centre for Biotechnology-LIPI fund. We thank to colleagues of the Virology Laboratory at Murdoch University, Perth, Australia for providing the constructs. Appreciation is extended to Neneng Hasanah and Muhamad Ridwan, SSi for their excellent assistance.

REFERENCES

Amershampharmacia biotech. 1997. *GST Gene Fusion System.The3rd* edition, revision 2. Amershampharmacia biotech.

ACIAR. 2004. Production of a vaccine for the control of Jembrana disease in Indonesia. *Review Report.* ACIAR Project No. AS 1/2000/029.

Barnett AL, RA Davey and JM Canningham. 2002. Modular organization of the friend murine leukaemia virus envelope protein underlies the mechanism of infections. *Proceedings of the National Academic of Science, USA* **98 (7), 4113-4118.**

Chadwick BJ, RJ Coelen, GE Wilcox, LM Sammels and G Kertayadnya. 1995. Nucleotida sequence analysis of Jembrana disease virus: a new bovine lentivirus associated with an acute disease syndrome. *Journal of General Virology.* 76,1637-1650.

Chung CT, SL Niemela and RH Miller. 1989. One-step preparation of competent *Escherichia coli:* transformation and storage of bacterial cells in the same solution. *Proceedings of National Academic Science USA* **86,**2172-2175.

Cota-Gomez A, NC Flores, C Cruz, A Casullo, TY Aw, H Ichikawa, J Schaak, R Scheinmen and SC Flores. 2002. The human immunodeficiency virus-1 Tat protein activates human umbilical vein endothelial cell E-selectin expression via an NF-B-dependent mechanism. *Journal of Biology cal Chemistry 211,*14390-14399.

Inoue H, H Nojima and H Okayama. 1990. High efficiency transformation of *Escherichia coli* with plasmids. *Gene* **96 Issue I,** 23-28.

Jang B, Y Jung and D Lim. 2007. Development of simple cell lysis method for recombinant DNA using bacteriophage lamda lysis genes. *The Journal of Microbiology* **45(6),** 593-596.

Kertayadnya G, GE Wilcox, S Soeharsono, N Hartaningsih, RJ Coelen, RD Cook, ME Collin and J Brownlie. 1993. Characteristics of a retrovirus associated with Jembrana disease in Bali cattle. *Journal of General Virology 1 A,* 1765-1774.

Margawati ET, E Yetti, Indiawati, A Margiawan and A Istiarto. 2004. Produksi protein virus rekombinan untuk produksi vaksin Jembrana. *Laporan Teknik.* Proyek Penelitian Bioteknologi. T.A. 2004, 70-76.

Margawati ET, Indriawati and M Ridwan. 2006. Production of recombinant protein J-Tat p-GEX derived from different retransformed construct origins. *Jurnal Mikrobiologi Indonesia* 11(2), 91-94.

Mills AA. 2001. Changing colors in mice: an inducible system that delivers. *Genes and Development.* **15, 1461-1467.**

Ramos CRR, PAE Abreau, ALTO Nascimento and PL Ho. 2004. A high-copy T7 *Escherichia coli* expression vector for the production of recombinant proteins with a minimal N-terminal His-tagged fusion peptide. *Brazil Journal of Medical and Biological Research* 37(8), 1103-1109 ISSN 0100-879X.

Singh SM and AK Panda. 2005. Solubilization and refolding of bacterial inclusion body proteins. *Journal ofBioscience and Bioengineering* 99 (4), 303-310.

Tu Z, G He, KX Li, MJ Chen, J Chang, L Chen, Q Yao, DP Liu, H Ye, J Shi and X Wu. 2005. An improved system for competent cell preparation and high efficiency plasmid transformation using different *Escherichia coli* strains. *Microbial Biotechnology - Electronic Journal of Biotechnology (EJB)* 8(1).

Wilcox GE, BJ Chadwick and G Kertayadnya. 1995. Jembrana disease virus: a new bovine lentivirus producing an acute severe clinical disease in *Bos Javanicus* cattle. *Abstract. The 3rd International Congress on Veterinary Virology,* Interlaken, Switzerland 4-7 September 1994.

Wu T. 2004. HIV-1 gene expression: lessons from pro virus and non-integrated DNA. *Review,* http:// www, retro viro lo gy.com/containt/1/1/13.