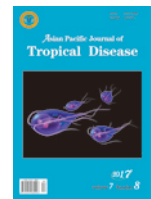


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Soluble expression and purification of hepatitis B core antigen (HBcAg) subgenotype B3 in *Escherichia coli* using thioredoxin fusion tagRahmah Waty^{1,2}, Apon Zaenal Mustopa^{2*}, Suharsono^{1,3}, Ratih Asmana Ningrum², Hidayah Murtiyaningsih²¹School of Biotechnology, Bogor Agricultural University, Bogor, Indonesia²Research Centre for Biotechnology, Indonesian Institute of Science (LIPI), Bogor, Indonesia³Research Center for Bioresources and Biotechnology, Bogor Agricultural University, Bogor

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ABSTRACT

Objective: To express HBcAg protein (hepatitis B virus subgenotype B3) in *Escherichia coli* in soluble form.**Methods:** HBcAg sequence of hepatitis B virus subgenotype B3 was cloned into plasmid pET32a and introduced to *E. coli* BL21 (DE3). The *E. coli* was grown in Luria-Bertani (LB) medium supplemented with ampicillin with agitation. Protein expression was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) at concentrations of 0.1 mmol/L, 0.3 mmol/L, and 0.5 mmol/L at room temperature (28 °C). The bacteria were dissolved in lysis buffer and lysed by freeze-thawing method then sonication. The fusion protein [thioredoxin A-(His)₆tag-HBcAg] was purified using immobilized metal affinity chromatography. The protein expression was analyzed by SDS-PAGE, dot blot, and western blot.**Results:** This research showed that DNA sequence of HBcAg could be propagated in pET32a and soluble protein was successfully expressed in *E. coli*. Induction with 0.3 mmol/L IPTG and 4-hour incubation was the best condition to express the HBcAg protein. SDS-PAGE and dot blot analysis showed that HBcAg protein could be expressed in *E. coli*. Western blot analysis showed that molecular weight of HBcAg fusion protein was about 38.5 kDa.**Conclusions:** This study confirmed that HBcAg protein could be expressed in soluble form in *E. coli*.

1. Introduction

Hepatitis B virus (HBV) infection is a serious health problem in the world. HBV is one of the major causes of liver diseases because of its long term effects. The HBV infection in liver can cause acute or chronic disease. It can be transmitted through contact with blood or other body fluids of hepatitis patient. It was estimated that 257 million people worldwide have been infected with HBV. In 2015, 887 000 deaths were caused by hepatitis and its complications such as cirrhosis and hepatocellular carcinoma[1]. Besides, the prevalence of HBV infections in Southeast Asia including Indonesia is high[2].

Genetic variability of HBV plays an important role in the development of HBV infection treatment. Based on nucleotide array, HBV is classified into eight genotypes (A–H) and each genotype is classified into some subgenotypes. Each genotype or subgenotype has a different geographic distribution. For example, some HBV/B subgenotypes from Southeast Asia (B3, B4, B5, B7, B8, and B9) are clearly separated with HBV/B1 and B2 subgenotypes which are found in Japan and China[3].

Indonesia is an archipelago that consists of thousands of islands and is a home to hundreds of ethnic populations. The distribution of HBV genotypes/subgenotypes in the Indonesian archipelago is related to the ethnic pattern of its population. There are three main genotypes of HBV in Indonesia, namely, HBV/B (70.9%), HBV/C (27.5%), and HBV/D (1.6%). HBV/B is the dominant genotype in Western Indonesia, especially HBV/B3 which is the predominant subgenotype in Java region. In East Indonesia, HBV/C is the predominant genotype followed by HBV/D[3,4].

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The therapy for chronic hepatitis B is aimed to decrease the viral replication and prevent the occurrence of more severe liver disease, such as cirrhosis or hepatocellular carcinoma[5]. There are many types of therapeutic vaccines for HBV infection. They are recombinant peptide-based vaccines, DNA-based vaccines, viral vector-based vaccines, and cell-based vaccines[6]. Nucleoside analogs drugs are able to delay the occurrence of complications of HBV infection. Tenofovir and entecavir are common antiviral drugs for chronic hepatitis B treatment. However, these drugs are not able to degrade all forms of DNA virus, such as cccDNA, so replication of HBV and liver damage still occur in the off-treatment period. In addition, treatment of HBV infection with antiviral drugs can lead to toxicity and resistance of the virus in long-term treatment[7]. Therefore, more effective and safe treatment alternatives are needed and antigen-based therapy is potent for chronic hepatitis B treatment[8].

HBV has a spherical structure that consist of two structural protein, hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg). The presence of HBsAg indicates the HBV infection. HBsAg-based vaccine can be used as prevention vaccine and therapeutic vaccine. However, monotherapy with HBsAg is unable to control replication of HBV and liver damage, so HBsAg-based vaccine was usually combined with other drugs for the treatment. Furthermore, HBsAg-based vaccine induces various HBsAg specific immunity, but it has no effect on induction of HBcAg-specific cytotoxic T lymphocyte (CTL). Whereas, immunogenicity of HBcAg is higher than that of HBsAg. HBcAg-based vaccine can induce both HBsAg-CTL and HBcAg-CTL in the liver[7].

Previous studies had successfully propagated and expressed HBcAg in prokaryote and eukaryote systems such as *E. coli* and *Pichia pastoris*, respectively[9]. The expression of HBcAg protein in *E. coli* has been done before; furthermore, this protein can be expressed in fusion form with other proteins such as the EDIII-2 protein, but it is still expressed in insoluble form. This makes the process of protein purification complicated because the protein purification method must be followed by refolding process[10]. Meanwhile, HBcAg expression in *P. pastoris* needs longer time with more complex cell lysis procedures[9]. Thus, the objective of this study was to construct HBcAg encoding gene HBV/B subgenotype B3 and to express the recombinant protein in *E. coli* system in soluble form.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterial strains and vector used in this study are listed in Table 1. *E. coli* was grown in Luria-Bertani (LB) medium [10 g/L tryptone (Oxoid, England), 10 g/L sodium chloride (Merck, Denmark), 5 g/L yeast extract (Oxoid, England)] supplemented with ampicillin (100 µg/mL) (Bio Basic, Canada) at 37 °C with agitation.

Table 1

Bacterial strains and vector used in this study

Strains and vector	Relevant characteristics	Source of reference
Bacterial strains		
<i>E. coli</i> TOP10	Cloning host	Novagen
<i>E. coli</i> BL21 (DE3)	Expression host	Novagen
Vector		
pET32a	Ampicillin resistance, cloning vector, expression vector	Novagen

2.2. Construction of expression vector for HBcAg

The synthetic HBcAg gene (GeneArtThermoFisher Scientific, Germany) was designed based on GenBank accession number EF47392.1. The pMAT plasmid vector containing HBcAg gene (HBV/B subgenotype B3, 1839 Java) was used as the template for PCR reaction. The primers used in this study are listed in Table 2. The HBV core gene was amplified using primers HBcAg-BamHI-HindIII_F and HBcAg-BamHI-HindIII_R that contained *Bam*HI and *Hind*III restriction sites. The PCR was performed under following conditions: pre-denaturation at 94 °C for 3 min, then 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 30 s, and a final extension at 72 °C for 6 min.

Table 2

Oligonucleotide primers used for PCR amplification in this study

Primers	Sequence (5'→3')	Restriction site
HBcAg-BamHI-HindIII_F	ATTGGATCCATGGACATTGACCCGTAT	<i>Bam</i> HI
HBcAg-BamHI-HindIII_R	ACGCAAGCTTCTAACATTGAGATTCCCG	<i>Hind</i> III
T7 promoter_F	TAATACGACTCACTATAG	
T7 terminator_R	CAAAAAACCCCTCAAGAC	

The PCR product and pET32a were cut with restriction enzymes *Bam*HI and *Hind*III. The restriction products were run on 1% agarose gel and the desired band was purified with QIAquickGel Extraction Kit (Qiagen, Germany). The purified gene was ligated (using T4 DNA ligase) into pET32a which was cut by the same restriction enzymes (*Bam*HI and *Hind*III). The recombinant plasmid (pET32a-HBcAg) was transformed into *E. coli* TOP10 using heat-shock method[11]. The recombinant plasmid (pET32a-HBcAg) was isolated from *E. coli* TOP10 with Presto™ Mini Plasmid Kit (Geneaid) and transformed into *E. coli* BL21 (DE3) to express the recombinant protein. The recombinant plasmid from *E. coli* BL21 (DE3) was isolated with Presto™ Mini Plasmid Kit (Geneaid) and analyzed by conducting DNA sequencing.

2.3. Expression of HBcAg protein

The recombinant bacteria were grown in shaker flask overnight at 37 °C in 10 mL LB medium that contained ampicillin (100 µg/mL). The whole culture was inoculated into 200 mL fresh LB medium and grown at 37 °C until the optical density (OD₆₀₀) of the culture reached 0.3. Protein expression was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) (Thermo Scientific, Italy) to the

final concentrations of 0.1 mmol/L, 0.3 mmol/L, and 0.5 mmol/L and incubated at room temperature (28 °C). During incubation period (1–5 h), the cells were harvested by centrifugation at 5000 r/min for 10 min at 4 °C every an hour. The pellet was stored at –20 °C until used.

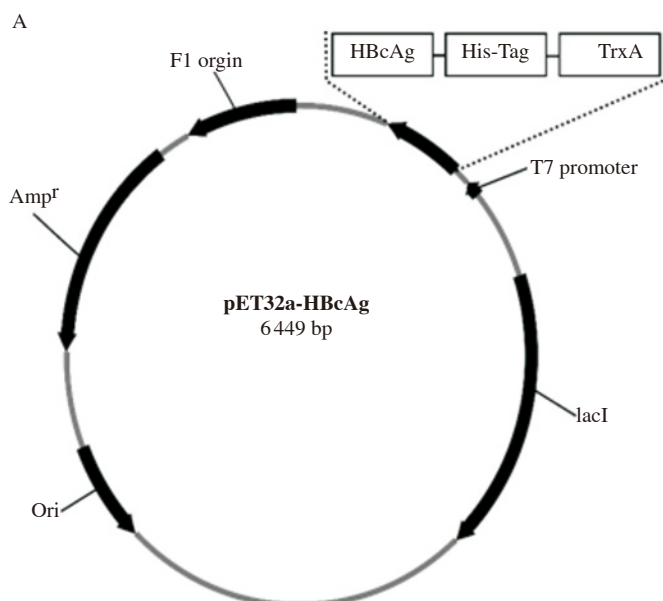
Cells were lysed using freeze-thawing method followed by sonication. The pellet was frozen and thawed three times for 30 min each process and was resuspended in 15 mL lysis buffer [10 mmol/L Tris-HCl (Bio Basic, Canada), pH 8.5, 100 mmol/L NaCl (Merck, Denmark), 0.25% Tween 20 (MP Biomedicals, France)]. The suspension was sonicated in ice. Supernatant was collected by centrifugation at 10000 r/min for 30 min at 4 °C then was applied to TALON® metal affinity resin (Clontech, Japan) under rotating agitation for 3 h at 4 °C. After extensive washing with the same buffer, the fusion protein was eluted with elution buffer [10 mmol/L Tris-HCl, pH 8.5, 100 mmol/L NaCl, 0.25% Tween 20, 400 mmol/L imidazole (Bio Basic, Canada)] overnight under rotating agitation at 4 °C. The eluent was dialyzed overnight in dialysis buffer (10 mmol/L Tris-HCl, pH 8.5, 100 mmol/L NaCl, 10% glycerol) at 4 °C.

2.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE analysis was performed by SDS 10% (v/v) polyacrylamide gel. The fusion proteins were suspended in SDS loading buffer and heated at 100 °C for 10 min. Then 10 µL of SDS loading buffer was loaded per 10 µL sample. After vertical electrophoresis, the gel was stained with Coomassie Brilliant Blue G (Sigma-Aldrich, China) overnight, and destained three times (30 min for each process). The molecular weight of fusion protein was predicted using DNAMAN version 9 (Lynnon Corp., Canada).

2.5. Dot blot analysis

A total of 10 µL of protein sample was spotted onto nitrocellulose



membrane. After the membrane dried, the membrane was incubated with blocking reagent (10% skim milk in TBS 1×) at room temperature for 1 h. The membrane was washed with TBS-T 0.1% Tween three times for 5 min each process and incubated with primary antibody (monoclonal HBc antibody) for 1 h. After washing out the primary antibody, the membrane was incubated with secondary antibody (anti-mouse) at room temperature for 1 h. The membrane was washed again with TBS-T 0.1% Tween three times for 5 min each process. Staining was done with NBT-BCIP solution for 1–3 min. After the result appeared, the membrane was immersed in distilled water.

2.6. Western blot analysis

The protein from SDS-PAGE was transferred to nitrocellulose membrane. The membrane was incubated with primary antibody (monoclonal HBc antibody), followed by secondary antibody (anti-mouse) that conjugated with substrate NBT-BCIP solution.

3. Results

The gene of HBcAg (HBV/B subgenotype B3) was inserted into expression vector pET32a to construct recombinant vector pET32a-HBcAg. The recombinant vector was transformed into *E. coli* TOP10 as cloning host and successfully introduced into *E. coli* BL 21 (DE3) as expression host. The expression vector was constructed to form a fusion protein containing Trx A (thioredoxin A) and (His)_{6tag} under control of T7 promoter (Figure 1A). The PCR product of pMAT-HBcAg showed that HBcAg sequence was 549 bp in size (Figure 1B). Recombinant plasmid pET32a-HBcAg sequence was verified by DNA sequencing. The result of DNA sequencing is showed in Figure 2.

The recombinant protein was successfully expressed using the prokaryotic expression system in soluble form. After transformation into *E. coli* BL21 (DE3), the fusion protein was overexpressed by

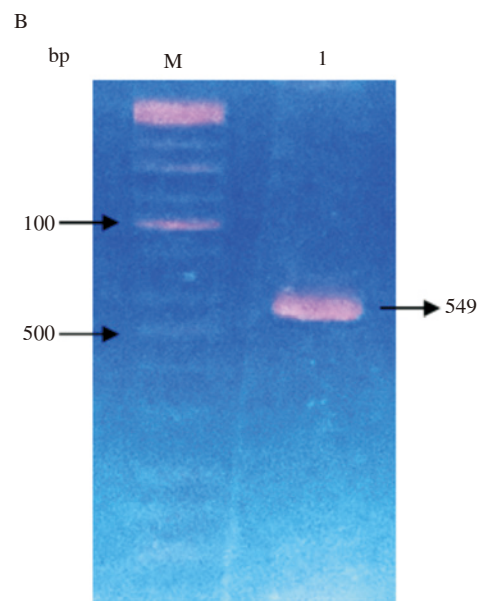


Figure 1. Construction of expression vector.

A: Schematic representation of the expression vector pET32a-HBcAg. HBcAg was expressed as a fusion protein with His-tag and thioredoxin A (TrxA); B: PCR product of pMAT-HBcAg with specific primers. M: 1 kb DNA ladder marker; Lane 1: HBcAg sequence was about 549 bp.

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1 ..... ATGAGCGATAAAATTATTCACCTGACTGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTC
1 ..... M·S·D·K·I·I·H·L·T·D·D·S·F·D·T·D·V·L·K·A·D·G·A·I·L·
76 ..... GTCGATTTCTGGGCAGAGTGGTGC GGTCGGTCCGTGCAAAATGATCGCCCCGATTCTGGATGAAATCGTGACGAATAT
26 ..... V·D·F·W·A·E·W·C·G·P·C·K·M·I·A·P·I·L·D·E·I·A·D·E·Y·
151 ..... CAGGGCAAACCTGACCGTTGCAAACTGAACATCGATCAAACCCTGGCACTGCGCCGAAATATGGCATCCGTGGT
51 ..... Q·G·K·L·T·V·A·K·L·N·I·D·Q·N·P·G·T·A·P·K·Y·G·I·R·G·
226 ..... ATCCCGACTCTGCTGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTGTCTAAAGGTCAGTTG
76 ..... I·P·T·L·L·L·F·K·N·G·E·V·A·A·T·K·V·G·A·L·S·K·G·Q·L·
301 ..... AAAGAGTTCCTCGACGCTAACCTGGCCGGTTCGGTTCTGGCCATATGCACCATCATCATCATTCTTCTGGT
101 ..... K·E·F·L·D·A·N·L·A·G·S·G·S·G·H·M·H·H·H·H·H·S·S·G·
376 ..... CTGGTGCCACGCGGTTCTGGTATGAAAGAAACCGCTGCTGCTAAATTCGAACGCCAGCACATGGACAGCCCAGAT
126 ..... L·V·P·R·G·S·G·M·K·E·T·A·A·A·K·F·E·R·Q·H·M·D·S·P·D·
451 ..... CTGGGTACCGACGACGACGACAAGGCCATGGCTGATATCGGATCCATGGACATTGACCCGTATAAAGAATTTGGA
151 ..... L·G·T·D·D·D·D·K·A·M·A·D·I·G·S·M·D·I·D·P·Y·K·E·F·G·
526 ..... GCTTCTGIGGAGTTACTCTTTTTTGCCTTCTGACTTCTTTCTTCTATTTCGAGATCTTCTCGACACCGCCTCT
176 ..... A·S·V·E·L·L·S·F·L·P·S·D·F·F·P·S·I·R·D·L·L·D·T·A·S·
601 ..... GCTCTGTATCGGGAGGCCTTAGAGTCTCCGGAACATTGTTACCTCACCATACGGCACTCAGGCAAGCTATTTG
201 ..... A·L·Y·R·E·A·L·E·S·P·E·H·C·S·P·H·H·T·A·L·R·Q·A·I·L·
676 ..... TGTGGGGTGAGTTGATGAATCTAGCCACCTGGGTGGGAAGTAATTTGGAAGACCCTGCATCCCGGGAATTAGTA
226 ..... C·W·G·E·L·M·N·L·A·T·W·V·G·S·N·L·E·D·P·A·S·R·E·L·V·
751 ..... GTCAGTTATGTCAATGTTAATATGGGCCTAAAAATCAGACAACCTATTGTGGTTTCACATTTCTGTCTTACTTTT
251 ..... V·S·Y·V·N·V·N·M·G·L·K·I·R·Q·L·L·W·F·H·I·S·C·L·T·F·
826 ..... GGAAGAGAACTGTTCTTGAATATTTGGTGTCTTTTGGAGTGTGGATTGCGCACACCTCTGCATATAGACCACCA
276 ..... G·R·E·T·V·L·E·Y·L·V·S·F·G·V·W·I·R·T·P·P·A·Y·R·P·P·
901 ..... AATGCCCTATCTTATCAACACTTCCGGAACCTACTGTTGTTAGACGACGAGGCAGGTCCCCTAGAAGAAGAACT
301 ..... N·A·P·I·L·S·T·L·P·E·T·T·V·V·R·R·R·G·R·S·P·R·R·R·T·
976 ..... CCCTCACCTCGCAGACGAAGGTCTCAATCGCCGGTTCGAGAAGATCTCAATCTCGGGAATCTCAATGTTAG
326 ..... P·S·P·R·R·R·R·S·Q·S·P·R·R·R·R·S·Q·S·R·E·S·Q·C·*·

```

Figure 2. Nucleotide and amino acid sequence of fused recombinant HBcAg. The blue color is thioredoxin-(His)_{6tag} fusion partner protein followed with S-tag and enterokinase cleavage site (1-165 aa), the red color is peptide of HBcAg (166-348 aa), the asterisk is the stop codon.

IPTG induction (final concentrations 0.1 mmol/L, 0.3 mmol/L, and 0.5 mmol/L) and different incubation times (1 h, 2 h, 3 h, 4 h, and 5 h). The optimum condition to express the HBcAg fused protein was 4 h induction with 0.3 mmol/L IPTG (Figure 3A and 3B).

Purification of HBcAg fused protein was done with TALON® metal affinity resin by using fused (His)_{6tag}. TALON® metal affinity resin would bind the protein in (His)_{6tag} region, thus the targeted protein could be purified. The fusion protein was about 38.5 kDa, consisting of 21 kDa HBcAg protein and 17.5 kDa fusion partner protein (Trx A-(His)_{6tag}) (Figure 3C).

Expression of HBcAg recombinant protein was verified by dot blot and western blot analyses. Dot blot analysis of the expression product and monoclonal antibody HBc showed positive result marked by purple spot in the membrane, and western blot analysis showed that molecular weight of HBcAg fusion protein was about 38.5 kDa (Figure 3D and 3E).

4. Discussion

The level of foreign gene expression depends on the identity of the host organism. Bacteria are organisms that usually used to produce many recombinant proteins. However, poor growth rate, protein inactivity, inclusion body formation, and even unexpressed recombinant protein are some problems that are often found when recombinant protein was expressed in prokaryotic system. *E. coli* is the commonly used prokaryote to produce recombinant protein. The advantages of using *E. coli* as host expression system are (1) culture may reach stationary phase in a few hours, (2) high density of cell cultures is easily achieved, (3) growth media can be made from inexpensive components, (4) transformation with exogenous gene is relatively easy and fast[12]. In this research, *E. coli* reached stationary phase in 5 h after induction indicated by OD₆₀₀ ≈ 1, so that the production of recombinant protein was relatively faster than that

in previous research in *P. pastoris* that required 4 days[9].

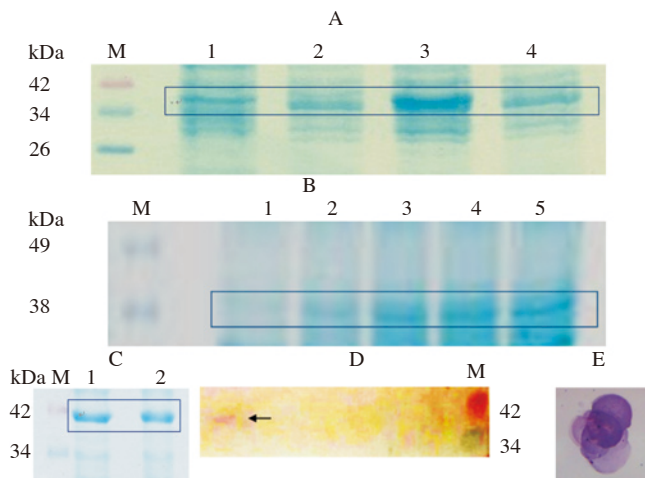


Figure 3. Expression and purification of the HBcAg fusion protein. A: SDS-PAGE of target protein expression under different concentrations of inducer IPTG. Molecular weight marker has been shown in the left lane; Lane 1: Non-induced bacteria lysate; Lane 2: Bacteria lysate from cells with 0.1 mmol/L IPTG induction; Lane 3: Bacteria lysate from cells with 0.3 mmol/L IPTG induction; Lane 4: Bacteria lysate from cells with 0.5 mmol/L IPTG induction; B: SDS-PAGE of target protein expression under different incubation times. Molecular weight marker is shown in the left lane; Lane 1: Bacteria lysate from cells with 1 h incubation time; Lane 2: Bacteria lysate from cells with 2 h incubation time; Lane 3: Bacteria lysate from cells with 3 h incubation time; Lane 4: Bacteria lysate from cells with 4 h incubation time; Lane 5: Bacteria lysate from cells with 5 h incubation time; C: Purification of the fusion protein with chromatography affinity. Molecular weight marker has been shown in the left lane; Lane 1: Dialysis of elution fraction; Lane 2: elution fraction; D: Western blot analysis, the molecular weight of HBcAg fusion protein was about 38.5 kDa; E: Dot blot analysis.

In this research we used *E. coli* BL21 (DE3) as the expression host. It is known that *E. coli* BL21 (DE3) has higher ability to produce specific recombinant protein than other *E. coli* strains[13]. The major problem of the expression system in *E. coli* is the formation of inclusion bodies that make some insoluble protein in the cytoplasm. If a foreign gene is introduced into *E. coli*, the expression of this gene can lead to inclusion body formation. The formations of inclusion bodies are caused by the differences between the microenvironments of the new host and the original source of the gene, and also the unbalanced equilibrium between protein aggregation and solubilization. These factors along with the higher concentration of hydrophobic region in the polypeptide surface will lead to protein instability and aggregation[12].

Inclusion body formation is related to disulfide bonds. Disulfide bonds have many important biological functions. It plays an important role in the folding and stability of the proteins and also as a signal of the redox environment. Mis-pairing of disulfide bond in cysteines will cause mis-folding during protein synthesis and form the inclusion body. The inclusion body formation in recombinant proteins can result in inactive or non-functional proteins[14,15].

In the previous research, HBcAg was expressed in *E. coli*. However, the protein of HBcAg was insoluble after cell disruption[10]. Inclusion body makes the purification process more complex. Denaturing agent such as urea is used to solubilise the inclusion

body and refolding process is used to restore the core particle structure. However, the refolding process may occur randomly which cause low-yield protein[16]. To solve this problem, we used pET32a plasmid that has (His)_{6tag} and TrxA (thioredoxin A) region in the backbone to express the HBcAg in *E. coli*. Thioredoxin (Trx) is a cytoplasmic reductase that can be a fusion partner protein. Trx as fusion partner protein on recombinant protein expression can repair the mis-pairing disulfide bond in *E. coli* cytoplasm[14]. In addition, a high temperature of protein overexpression induction can increase the inclusion body formation. Overexpression induction at 37 °C resulted in very low yield recombinant protein production[17], but induction with temperature below 37 °C resulted in better expression[15]. Based on this knowledge, we used room temperature (28 °C) for protein overexpression induction with IPTG.

The expression of HBcAg protein recombinant in pET32a system resulted in a fusion protein [HBcAg-(His)_{6tag}TrxA]. Protein fusion with Trx A leads to the decreasing of aggregate protein formation by increasing the disulfide bond formation[18]. In previous research, the usage of Trx fusion partner had been successfully applied to express the recombinant protein such as leptin in soluble form. The solubility of foreign protein leptin was increased by using of Trx as a gene partner. The fusion protein that has a high stability and compact Trx structure with the foreign protein can facilitate correct protein folding[19]. Trx utilization as a fusion protein has also been reported that can increase solubility of nerve growth factor protein, fibroblast growth factor 19 protein, and plantaricin F expression in *E. coli*[15,17,20]. Fusion partner protein (His)_{6tag} helped us in the protein purification process. The soluble fusion protein was subjected to Co²⁺ in TALON® Metal Affinity Resin at the (His)_{6tag} region. The HBcAg protein fused with (His)_{6tag} thioredoxin has succeeded to be purified using this method.

The first step of transcription in prokaryotic organism is the binding of RNA polymerase to the promoter region. A strong promoter has a high affinity for RNA polymerase. The T7 promoter in pET system is one of the most frequently used and strong promoter in prokaryotic expression system. Expression of protein under the control of T7 promoter is an effective method for high-level expression of the protein[21]. Expression of plantaricin E and plantaricin F under T7 promoter successfully increased plantaricin production[22].

DNA sequencing showed that HBcAg gene had been inserted in pET32a. Constructed HBcAg protein was fused with (His)_{6tag}-Trx A in pET32a system. This fusion protein had 347 amino acids weighted 38.5 kDa consist of 183 amino acids HBcAg protein (21 kDa) and 164 amino acids fusion protein partner (17.5 kDa). HBcAg protein consists of two domains; they are assembly domain and protamine domain. Assembly domain is composed of amino acid 1–149 that are responsible for the formation of a spherical shell. Protamine domain composed of amino acid 150–183 that are responsible for RNA packaging[23,24].

In this research, the heterologous gene of HBcAg (HBV/B

subgenotype B3) was successfully inserted into pET32a plasmid and expressed in *E. coli* in soluble protein form. Recombinant protein was expressed in fusion form with (His)₆tag and Trx A. Molecular weight of HBcAg fusion protein (HBcAg-his tag TrxA) was about 38.5 kDa.

Conflict of interest statement

We declare that we have no conflict of interest.

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