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Cloning and Expression of 19-kDa Fragment of Merozoite Surface Protein-1 (MSP-119) of Plasmodium Falciparum in Escherichia coli

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Malaria caused by *Plasmodium falciparum* is a disease affecting 300 to 500 million people in tropical countries including Indonesia annually. Out of several ongoing eradication strategies against the disease, vaccine development represents an encouraging approach for improved malaria control globally. The C-terminal 19 kDa fragment of the *P. falciparum* merozoite surface protein 1 ($MSP1_{19}$), a surface protein of merozoite which plays a pivotal role in binding of merozoite to erythrocytes, has been developed as potential vaccines against erytrocytic stages of malaria. *In vitro* studies show that monoclonal and polyclonal antibodies specific to this protein block the entry of merozoite into erythrocytes. The aims of this study were to clone and to express the $MSP1_{19}$ of *P. falciparum* so that the effective vaccine could be produced. Moreover, the availability of the antigen will facilitate the monoclonal and polyclonal antibodies development. For these purposes, genomic DNAs of *P. falciparum* were isolated and were used as a template to amplify a DNA encoding the $MSP1_{19}$. Recombinant plasmids were constructed by insertion of the isolated PCR product into bacterial vectors of pGEMT-Easy for cloning and pET-22b for expression. In this paper, we reported that the gene encoding the $MSP1_{19}$ of *P. falciparum* was successfully amplified from *P. falciparum* genomic DNAs as shown by the 294 base pairs PCR product on agarose gel electrophoresis. Sequencing analysis confirmed that there are no base pair changes in the sequence of the $MSP1_{19}$. Preliminary result on expression of the $MSP1_{19}$ of *P. falciparum* indicated that the gene was successfully produced in *E. coli*.

INTRODUCTION

Malaria is one of the most common infectious diseases and an enormous public health problem. According to the World Malaria Report released by WHO in 2008, there were 247 million malaria cases among 3.3 billion people at risk in 2006 from 109 countries resulting in estimated 1.5 million deaths annually, primarily amongst infants and young children. In 2001, Indonesia health survey estimated that there were 70 million people living in endemic malaria area with 15 million case of clinical malaria, and there were 56.3 million live in mid endemic area (Pardosi, 2005). Roll Back Malaria program was launched by WHO in 1998, with stated goal to halve malaria deaths worldwide by 2010. Unfortunately, one year from the targeted year, malaria is still the most widespread and most serious parasitic disease in the world.

Out of the several current global intervention strategies to combat malaria, vaccination program represents an encouraging approach for improving malaria control globally. Therefore, the development of a safe and effective malaria vaccine is expected to play important and critical role to support the program. Such vaccine development efforts have focused on candidate antigens represented in the pre-erythrocytic, erythrocytic and sexual stages of the parasite.

A protein that is expressed in the merozoite surface during the late stages of erythrocytic schizogony, known as merozoite surface protein-1 (MSP-1), is one of the best characterized antigens of *P. falciparum*. The protein with a molecular mass of approximately ~200 kD also

becomes a leading vaccine candidate antigen for malaria. During invasion of erythrocytes, this molecule undergoes a series of proteolytic cleavages resulting in four major fragments (MSP1–83, MSP1–28, MSP1–38, and MSP1–42) (Holder, 1996). At the time of the schizont rupture and release of merozoite into the plasma, the MSP1–42 carboxy terminal fragment undergoes secondary processing to form a 33-kD product that is shed, whereas a 19-kD fragment ($MSP1_{19}$) remains on the merozoite surface during the invasion of erythrocytes (Blackman *et al.*, 1990).

$MSP1_{19}$, in particular, is interesting in vaccine development since naturally acquired antibodies to $MSP1_{19}$ from *P. falciparum* ($PfMSP1_{19}$) are associated with resistance to clinical malaria in hyperendemic areas (Egan *et al*, (1996). Moreover, according to Angove *et al.* (2003), the C-terminal 19 kDa fragment of the *P. falciparum* merozoite surface protein 1 ($MSP1_{19}$), are being developed as potential vaccines against erytrocytic stages of malaria. Considerable evidence indicates that the two cystein-rich epidermal growth factor (EGF)-like domains included in $MSP1_{19}$ have an essential role in blood-stage growth.

Efforts to develop a malaria vaccine, however, have been thwarted by the complexity of the parasite's life cycle and the ability of the parasite to suppress and to evade the immune responses. Therefore, an alternative approach such as immunotherapy using monoclonal antibodies is needed to improve the control of malaria. Monoclonal antibodies (mAbs) are popular candidates for protection against infection. The long half-life, low toxicity, high affinity, and specificity of mAbs are only a few of the advantages that make them attractive potential therapeutic agents (Casadevall, 1999; Ali, 2006). For the above reason, new therapeutic measures using monoclonal antibodies are needed to treat severe malaria cases.

Passive immunotherapy using monoclonal antibody specific to $MSP1_{19}$ may provide a valuable therapeutic alternative. According to Egan *et al.* (1996), mouse monoclonal antibodies to $MSP1_{19}$ inhibit *in vitro* growth of *P. falciparum*. In addition, antibodies against MSP-1 are protective against human, monkey, and rodent malaria parasites and immunization with MSP-1 affords anti-parasite protection in experimental animals (Braga *et al.*, 2002; Chappel *et al.*, 2006, Cheng *et al.*, 2007).

In an attempt to develop a malaria vaccine candidate and its monoclonal antibodies, cloning and expression of the $MSP1_{19}$ is strongly needed. The purpose of the present study, therefore, was to clone and express the $MSP1_{19}$. The gene encoding the $MSP1_{19}$ of *P. falciparum* was amplified from *P. falciparum* genomic DNAs. Recombinant plasmid having the $MSP1_{19}$ correctly inserted, were sent for sequencing to ensure that there are no base pairs changes was occurred in its sequence.

METHOD

Bacterial strain and Plasmid

E. coli DH5α (Toyobo, Japan) and *E. coli* BL21 Star (DE3) pLysS cells (Invitrogen, Carlsbad, CA) were used for plasmid amplification and for protein expression, respectively, in this research. pGEMT-Easy (Promega, USA) and pET-22b (Novagen, Madison, WI) were used for cloning and for protein expression, respectively.

Blood Collection and Rapid Detection Technique (RDT)

Blood was collected from malaria patients having fever, by finger prick. Immunochromatographic testing was performed directly using Entebe Malaria Kit (Laboratory of Hepatika Mataram) according to the manufacturer's instruction. Blood samples tested positive were then used for DNA isolation using NucleoSpin Blood (Macherey-Nalgen).

PCR amplification and sequencing of a $MSP1_{19}$ Gene

P. falciparum genome was isolated from malaria's blood patient using DNA Isolation Kit (Macherey-Nalgen) according to the manufacturer. A pair of primer that are $MSP1_{19}$F: 5'- CATG

CCATGGCGAACATTTCACAACACCAATGCG-3′ and MSP1$_{19}$R: 5′- CCGCTCGAGAGAGG AACTGCAGAAAATACCAT-3′ were used for MSP1$_{19}$ gene amplification. Underline indicate *Nco*I dan *Xho*I restriction site. Twenty five cycles of PCR were performed as follows: 10 s of denaturation at 94°C, 10 s of annealing at 50°C, and 40 s of elongation at 72°C. After purification from 0.75% agarose gel, the amplified product was ligated with pGEMT-Easy vector (Promega, USA) using T-A cloning technique, and transformed into *E. coli* DH5α. Transformant cells were then spread into LB selective media (50 µg/ml ampicillin) containing X-gal and 1 mM IPTG. The white colonies were used as a template for colony PCR using the same PCR program, and were then continued with electrophoresis on 1.0% agarose gel. Several *E. coli* DH5α transformants bearing plasmid with an insert were isolated using DNA isolation kit (Macherey-Nalgen). To confirm the sequence of the cloned DNA, the recombinant plasmid was sent for sequencing by means of an automated DNA sequencer according to Ali et al., (2006). For expression, the recombinant plasmid was constructed as follows: PCR product digested with *Nco*I and *Xho*I purified from 0.75% agarose gel and cloned into pET-22b (Novagen, Madison, WI). The recombinant plasmid was transformed into *E. coli* BL21 Star (DE3) pLysS.

Expression of MSP1$_{19}$ Gene

The *E. coli* BL21 Star (DE3) pLysS bearing pET-MSP1$_{19}$ was cultured in 500 ml of Luria broth containing ampicillin and chloramphenicol until an OD600 of 0.6 was achieved. The expression of recombinant MSP-119 tagged with histidine residues was achieved by induci with 1 mM IPTG at 30°C for overnight. Induced cultures were harvested and pelleted by centrifugation at 5,000 rpm for 10 min. Following sonication and centrifugation, the supernatant was collected and run on SDS-PAGE with standard procedures (Ali *et al.*, 2005).

RESULTS AND DISCUSSION

Vaccination against *P. falciparum* has the potency to reduce malaria-associated severe morbidity and mortality in areas with the most intense transmission. Some efforts are under way by various groups to test a number of blood-stage vaccine antigens and formulations in both animals and humans. Thus, research on malaria vaccines is currently directed primarily towards the development of vaccines that prevent clinical malaria.

Merozoite surface protein 1 (MSP1) is the most abundant protein on the surface of the invasive blood stage form of malaria parasites and is a leading candidate for a vaccine against *P. falciparum* malaria. Since MSP1$_{19}$ is particularly interesting in vaccine development (Egan *et al.*, 1996), effort to obtained, expressed and characterized the antigen is very crucial. For these purposes, *P. falciparum* genomic DNA were isolated from malaria's blood patients and used as template for *MSP1$_{19}$* amplification. Out of several malaria positive patients based on RDT detection, *MSP1$_{19}$* was amplified only from a few samples. In contrast, more blood samples are detected as positive using PCR. The result indicated that the accuracy of detection using RDT is lower than PCR.

To obtain a genomic DNA fragment containing *MSP1$_{19}$*, we designed the pair primers with *Nco*I and *Xho*I restriction site added as described in material and methods. Amplification of *MSP1$_{19}$* of *P. falciparum* was successfully performed as shown by the by the 294 base pairs PCR product on agarose gel electrophoresis (Fig. 1). Figure 1 also shows the size of amplified products using *P. falciparum* genomic DNA (lane 1) is corresponding to the size of amplified product using P. falciparum 3D7 strain genomic DNA as positive control (lane 2). These results suggest that the band was *MSP1$_{19}$*.

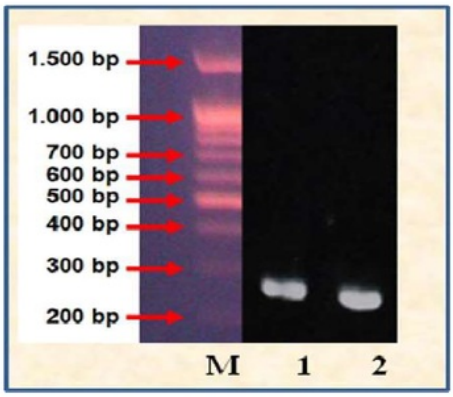

Fig. 1. Electrophoresis results, 1 = PCR product using *P. falciparum* genomic DNAs isolated from malaria's blood patient, 2 = positive control (*P. falciparum* 3D7 strain genomic DNAs, M = Marker λ DNA.

The PCR product obtained after gel purification was ligated with pGEMT-Easy vector and transformed into E. coli DH5α cells. To confirm the availability of insert gene in plasmid bearing by the *E coli*, PCR was performed with standard procedures (Sambrook *et al.*, 1989) using the colony as a template. Results of amplification were shown in Fig. 2. The positive clones were shown in line 1-8; 12-17. All positive bands were in the same size with PCR product of *P. falciparum* 3D7 strain as positive control (lane 10). In addition, there is no band when *E. coli* DH5α cells without insert (negative control) were used as PCR template.

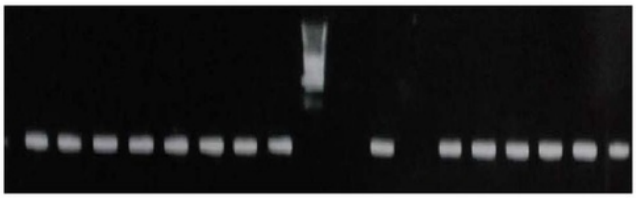

Fig. 2. 1-8, 12-17 = PCR products of *E. coli* DH5α transformants, 9 = PCR product of *E. coli* DH5α without insert (negative control), 10= PCR product of *P. falciparum* 3D7 strain genome (positive control), M = Marker λ DNA.

The results of DNA sequencing of positive clones (Fig. 3) revealed that the sequences of $MSP1_{19}$ obtained from *P. falciparum* genomic DNAs is similar to the sequences of $MSP1_{19}$ in BankGene (Accessed Number DQ907667.1) (Pacheco *et al.*, 2007), indicating that there are no mutation in the targeted fragment.

5' ATG GCG AAC ATT TCA CAA CAA CAA TGC GTA AAA AAA CAA TGT CCA GAA AAT TCT GGA TGT TTC AGA CAT TTA GAT GAA AGA GAA GAA TGT AAA TGT TTA TTA AAT TAC AAA CAA GAA GGT GAT AAA TGT GTT GAA AAT CCA AAT CCT ACT TGT AAC GAA AAT AAT GGT GGA TGT GAT GCA GAT GCC ACA TGT ACC GAA GAA GAT TCA GGT AGC AGC AGA AAG AAA ATC ACA TGT GAA TGT ACT AAA CCT GAT TCT TAT CCA CTT TTC GAT GGT ATT TTC TGC AGT TCC TCT CTC 3'

Fig. 3. Sequencing result of $MSP1_{19}$ obtained from *P. falciparum* genomic DNAs

We then investigated whether the $MSP1_{19}$ could be expressed in *E. coli* to generate the protein. For this purpose, the obtained gene was cloned into pET-22b vector, transformed into *E. coli* BL21 Star (DE3) pLysS, and express in broth media under IPTG induction. SDS-PAGE

analysis in Fig. 4 revealed that the presence of major protein band with 15 kDa in supernatant of overnight IPTG-induced culture (lane 2). Based on the molecular weight, the protein band is corresponding to $MSP1_{19}$. When expression was performed without IPTG induction, the protein band was absent (lane 1). These results indicate that *lac* repressor-*lac* operator DNA interaction is reduced upon binding of the gratuitous inducer, IPTG, so that the $MSP1_{19}$ was expressed successfully.

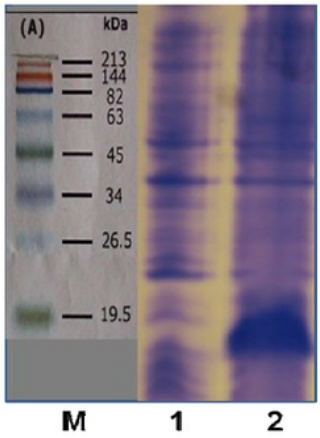

Fig. 4. M = Protein Marker, 1 = *E. coli* BL21 Star (DE3) pLysS bearing pET-$MSP1_{19}$ without IPTG induction, and 2 = *E. coli* BL21 Star (DE3) pLysS bearing pET-$MSP1_{19}$ with overnight 1 mM IPTG induction.

Although the *P. falciparum* MSP-1 gene (*msp1*) is highly polymorphic, the *msp1* region coding for $MSP1_{19}$ is well conserved among parasite isolates (O'donnel *et al.*, 2000). There is accumulating evidence indicating that sera from malaria patients living in highly endemic areas contain antibody against the 19-kDa fragment. Furthermore, the antibody inhibit merozoite invasion into red blood cells. *P. falciparum* occasionally causes severe malaria in children and individuals who have less immunity to the parasite. These indicated that the antibody against the $MSP1_{19}$ would be effective to treat severe malaria.

Since most of the recombinant $MSP1_{19}$ are expressed in the bacterial expression system (Cunha *et al*, 2001), future studies may reveal the level of efficacy and immunogenicity of recombinant $MSP1_{19}$ expressed in several expression systems ie. mammalian expression system (such as yeast, pichia or CHO cells) in order to compare and show that the recombinant proteins have a fully functional as in the *in vivo* system, and mimic the natural infection.

CONCLUSSION

$MSP1_{19}$ of *P. falciparum* was successfully amplified from *P. falciparum* genomic DNAs as shown by the 294 base pairs PCR product on agarose gel electrophoresis. Analysis of recombinant plasmids constructed showed that the gene was correctly inserted into the vector. Sequencing analysis confirmed that there are no base pair changes in the sequence of the $MSP1_{19}$ gene. The $MSP1_{19}$ protein was also successfully expressed in heterologous host *E. coli*.

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