

Basic Medical Research

The production of SPusp45-MSP-1₁₉ gene construct and its recombinant protein in *Lactococcus lactis* to be used as a malaria vaccine

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ABSTRAK

Latar belakang: Merozoite surface protein 1 (MSP-1) adalah protein utama yang digunakan oleh *Plasmodium* selama invasi sel darah merah pada penderita malaria. MSP-1₁₉, salah satu jenis fragmen MSP-1, merupakan utas yang sangat lestari dan berpotensi sebagai kandidat vaksin malaria karena antibodinya mampu menahan invasi pada eritrosit secara *in vitro*. Penelitian ini bertujuan mengonstruksi gen dan mengekspresikan protein MSP-1₁₉ pada *Lactococcus lactis* sebagai kandidat vaksin malaria.

Metode: Gen *usp45-MSP-1₁₉*, yang berasal dari hasil optimasi kodon dan gen sintesis, dimasukkan ke dalam vektor kloning pMAT. Vektor yang mengekspresikan fragmen MSP-1₁₉ termasuk peptida sinyal *usp45* (SPusp45) telah dikonstruksi dengan manipulasi DNA rekombinan menggunakan enzim restriksi. Protein MSP-1₁₉ diekspresikan dengan presipitasi ammonium sulfat 45% dan dimurnikan dengan menggunakan kromatografi filtrasi gel Sephadex-G50. Ekspresi protein dianalisis dengan SDS-PAGE dan dot blot.

Hasil: SPusp45 dilengkapi gen MSP-1₁₉ diamplifikasi dengan menggunakan primer tertentu dan berhasil dimasukkan ke dalam daerah multiple cloning sites (MCS) pada vektor ekspresi pNZ8148 dengan ukuran 3.538 pb sebagai vektor rekombinan. Protein MSP-1₁₉ berhasil diekspresikan ke dalam *Lactococcus lactis* dengan berat molekul 10,45 kDa. Uji dot blot dari 3 jenis perlakuan berbeda menunjukkan hasil positif pada konsentrasi induksi nisin 10 ng/ml.

Kesimpulan: Penelitian ini menunjukkan gen *usp45-MSP-1₁₉* berhasil dimasukkan ke dalam daerah MCS vektor ekspresi pNZ8148 dan Protein MSP-1₁₉ berhasil terekspresi dalam sistem NICE dari sel inang *L. lactis*.

ABSTRACT

Background: Merozoite surface protein 1 (MSP-1) is a major protein used by the *Plasmodium* during red blood cells invasion in malaria. MSP-1₁₉, one of MSP-1 is highly conserved, and it is a potential malaria vaccine candidate because the monoclonal antibodies are capable blocking erythrocyte invasion *in vitro*. The aim of this study was to produce MSP-1₁₉ gene construct and the recombinant protein in *Lactococcus lactis*.

Methods: *Usp45-MSP-1₁₉*, derived from codon optimization and the synthetic gene, was inserted into the pMAT cloning vector. A vector expressing MSP-1₁₉ included *usp45* has been constructed by the manipulation of recombinant DNA using restriction enzymes. The MSP-1₁₉ protein was expressed to 45% ammonium sulfate precipitation and purified using Sephadex-G50 gel filtration chromatography. The expressed protein was characterized by SDS-PAGE and dot blot.

Results: *usp45-MSP-1₁₉* gene was amplified using specific primers and inserted into the multiple cloning sites in the expression vector pNZ8148 with size 3,538 bp as a recombinant vector. The protein of MSP-1₁₉ was successfully expressed in *L. lactis* with molecular weight of 10.45 kDa. The dot blot was tested in 3 different comparisons between the host cells, non-induced cells, and induced cells with 10 ng/ml nisin. The results showed that 10 ng/ml nisin gave a positive reaction as detected by dot blot assay.

Conclusion: This study confirmed that the *usp45-MSP-1₁₉* gene was successfully inserted into the multiple cloning sites of the pNZ8148 expression vector and the MSP-1₁₉ protein expressed in the NICE system of the *L. lactis* host cell.

Keywords: *Lactococcus lactis*, malaria, merozoite surface protein 1, nisin, *usp45-MSP-1₁₉*

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Malaria is one of the most contagious diseases that are still of a public health problem in the world including Indonesia. Malaria occurs in 106 countries. About 3.3 billion people live in endemic areas of malaria, and nearly 80% of malaria cases occur in the eastern part of Indonesia.¹ The prevalence of malaria reflected as annual malaria incidence (AMI) decreased in the period 2000–2004 from 31.09 to 21.2 per 1.000 population. In 2008, it fell to 18.82, and in 2010 it dropped to 12.27 per 1.000 population. Meanwhile, the prevalence of malaria annual parasite incidence (API) in 2000 was 0.81, and in 2004 fell to 0.15 per 1,000 population. In 2006, it increased to 0.19 then in the year 2007–2008 fell into 0.16 per 1,000 population.²

While currently artemisinin has been shown to be effective for malaria treatment, some degree of resistance has been reported to occur worldwide.³ Therefore, a malaria vaccine is likely to be used as a potential tool for malaria control. Merozoite surface protein 1 (MSP-1) is a potential candidate of malaria vaccine because the monoclonal antibodies raised against this protein show its capability to block erythrocyte invasion *in vitro*.⁴ The MSP-1 is a major protein used by the *Plasmodium* during red blood cells invasion.⁵ Merozoite is the smallest cell measuring 1–2 µm in the life cycle of *Plasmodium*.⁶ The MSP-1 contains 2 epidermal growth factors (EGF-like domain) which are important parts during erythrocyte invasion.^{7,8} MSP-1₁₉ gene sequence located on block 17 is highly conserved. Therefore, it is used for the development of malaria vaccine.⁹

Lactococcus lactis has a generally recognized as safe (GRAS) host cell status and is often used in expressing recombinant proteins. *L. lactis* has been characterized as a good microorganism for the industry and capable of secreting recombinant protein into its growth media, so that the resulting product is free of endotoxin. The construction and expression of the MSP-1₁₉ gene in *L. lactis* has been prepared before.⁷ In the study, MSP-1₁₉ native gene construct was made by the fusion of SlpA peptide signals and intracellular protein expression. However, the level of MSP-1₁₉ protein expression is low. Estimated protein MSP-1₁₉ produced are only a few ng per 10⁷ bacterial cells.

In addition, protein isolation is done through cell lysis. Since proteins are still at intracellular level,

protein extraction process becomes more difficult. The fusion of the SlpA peptide signal should be able to bring the MSP-1₁₉ protein out through the cell membrane, so it can be secreted to the extracellular. In this study, the construction and expression of MSP-1₁₉ gene that has undergone codon optimization fused with usp45 signal peptide has been transformed into *L. lactis*. The usp45 signal peptide allows the MSP-1₁₉ protein to be secreted into the extracellular compartment. The usp45 signal peptide is an N-terminal signal peptide capable of optimizing the translocation of precursor proteins from within cells to the outside of the *L. lactis* cell wall.¹⁰ The codon optimization is performed to remove the biased codons that can inhibit the production of recombinant proteins in the prokaryotic system. It is a strategy to improve the production efficiency of recombinant proteins in the prokaryotic system. The purpose of this study was to construct the expression vectors for the MSP-1₁₉ gene and to express MSP-1₁₉ recombinant protein *L. lactis* as a candidate for malaria vaccine.¹⁰

METHODS

Genes, plasmids, bacteria, growth media, and primers used in this study

The material used in this study is the synthetic gene usp45-MSP-1₁₉ (GeneBank: AF165928) which is the result of codon optimization and has been ligated into the plasmid pMA-T. The growth media are *Luria-Bertani* (LB) and M17B bacteria. Plasmid used is pMA-T (cloning vector), and pNZ8148 is used as expression vector. Several vector and bacterial strains can be seen in Table 1, as well as several the primer pairs used in this study: forward ProNisA primer with TTCCCTCGAGGGATCTATGTC sequence (restriction site *Nco*I) and terminator reverse pNZ8148 primer with TGCTTTATCAACTGCTGC sequence (restriction site *Xba*I). *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 100 µg/ml ampicillin (Bio Basic, Canada) at 37°C with agitation. Monoclonal antibody used for dot blot hybridization assay was *Plasmodium falciparum* MSP1 antibody (PEM-1) from Thermo Fisher. MSP-1 is highly conserved among broad *Plasmodium* species. Thus, this antibody could also detect MSP-1 from *P. yoelii*.

Construction of expression vector

The construction of the expression vector began by isolating the pMA-T plasmid. The MSP-1₁₉ gene was obtained by digesting the fragment of interest using restriction enzymes on the *Nco1* and *Xba1* sites, followed by gel purification. Pure DNA fragments of MSP-1₁₉ were ligated into pNZ8148 expression plasmid using T4 DNA ligase enzyme prior to introduction into *E. coli* MC1061. The transformation to *E. coli* MC1061 was performed by the heat shock method.¹¹

Furthermore, pNZ8148-*usp45-MSP-1₁₉* plasmid in *E. coli* MC1061 was isolated and subsequently transformed into *L. lactis*. Competent cells were grown in 0.2% *L. lactis* in 5 ml M17 broth + 0.5% glucose overnight at 30°C. Subsequently, the culture was incubated in 25 ml M17 broth containing 0.5% Glucose, 0.5 M sucrose, and 2.5% glycin overnight at 30°C. The culture was then put into 75 ml of M17 broth containing 0.5% glucose, 2.5% glycine, and 0.5 M sucrose and grown until the culture reached OD600 ~ 0.2–0.3 prior to harvesting the cell using centrifuge. Cells were resuspended with 0.5 M sucrose +10% glycerol. The transformation process was carried out using Gene Pulser BIORAD electroporation at 2,000 V, 25 µF, 260 Ω. The success of the transformation process of the pNZ8148-*usp45-MSP-1₁₉* construct into *L. lactis* was confirmed by amplification of the bacteria colony by polymerase chain reaction (PCR), plasmid isolation followed by restriction enzyme digestion, and sequencing of inserts or plasmid construct.

pNZ8148-*usp45-MSP-1₁₉* plasmid in *L. lactis* was grown further to increase the expression of the recombinant protein.¹² *L. lactis* carrying recombinant plasmid was grown in 5 ml of M17 medium that contained 0.5% glucose. A total of 1.5 ml of *L. lactis* culture was subjected by

centrifugation at 10.000x g for 2 min. The pellet was resuspended with solution I containing 10 mM EDTA (pH 8.0), 25 mM Tris/HCl, 50 mM glucose, and 20 mg/ml lysozyme (Sigma) was added and subsequently incubated for 15 min at 37°C. The mixture was added with 300 µl fresh solution II containing 0.2 M NaOH, 2% SDS and incubated for 3 min at room temperature. As much as 170 µl solution III containing 1.2 M Tris/HCl (pH 7.0) and 2 M NaCl were added to the mixture. The mixture was subsequently homogenized with 500 µl phenol. The upper layer phase was mixed with 600 µl PCI (Phenol Chloroform Isoamyl alcohol) then it was centrifuged. Moreover, 600 µl of isopropanol was added and then subjected to incubation on ice for 15 min. The mixtures were subjected to centrifugation and washed with 70% ethanol. The pellet was dried and dissolved in 20 µl ddH₂O and 5 µl of 1 mg/ml RNase. The results of plasmid isolation were then subjected to *Nco1* and *Xba1* restriction enzymes, PCR of the colonies, and DNA sequencing to confirm the inserts sites. The schematic construction representation of the cloning vector pMAT and expression vector pNZ8148 which was expressed as a fusion protein pNZ8148- *usp45-MSP-1₁₉* can be seen in Figure 1.

Expression and purification of recombinant protein MSP-1₁₉

Expression of MSP-1₁₉ as heterologous protein inside *L. lactis* was conducted with nisin inducible controlled expression (NICE) system. Protein expression was performed by the following methods. The MSP-1₁₉ expression in *L. lactis* was started by inoculating 10% fresh culture into 100 ml M17 broth containing 10 µg/ml chloramphenicol and 0.5% glucose without agitation at 30°C for 10 hours to reach OD600 ~ 0.5. Furthermore, induction was performed using 10 ng/ml nisin and incubated for 5 hours at 30°C. The cells were subjected to centrifugation

Table 1. Bacterial strains and vector used in this study

Strains and vector	Relevant characteristics	Source of reference
Bacterial strains		
<i>Escherichia coli</i> MC1061	Bacterial/host strain for cloning	MoBiTec
<i>Lactococcus lactis</i> NZ3900	Bacterial/host strain for expression	MoBiTec
Vector		
<i>pMA-T</i>	Cloning vector containing ampicillin resistance gene	Invitrogen
<i>pNZ8148</i>	The nisin-induced expression vector containing chloramphenicol resistance gene	MoBiTec

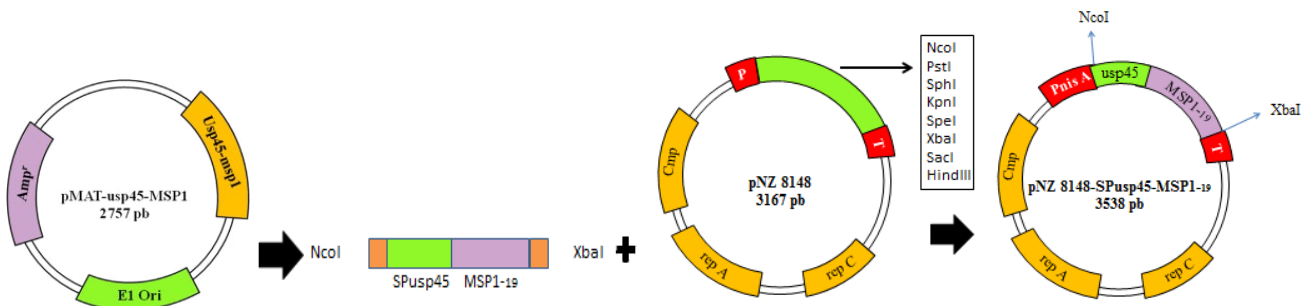


Figure 1. Schematic representation of the pNZ8148-usp45-MSP-1₁₉ expression construct containing the pMAT cloning vector and pNZ8148 expression vector

at 10.000 rpm at 4°C for 30 min. The recombinant protein was subjected to precipitation with 45% ammonium sulfate 27.7 g and subsequently incubated at 4°C for 10 hours. The cells were subjected to centrifugation to form the pellet for further elution step with 50 mM Tris HCl pH 7.4. The pellet suspension was purified by stationary phase adsorption chromatography Sephadex-G50. A total of 1 mL of pellet suspension was incorporated into chromatographic column, and then eluted with 50 mM Tris HCl pH 7.4.^{13,14}

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

The expressed proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE analysis using 10% separation gel and 4% retaining gel to examine the molecular weight of MSP-1₁₉. The recombinant protein was mixed with loading dye buffer (containing 50 mM Tris HCl pH 6.8, 2% SDS, 10% glycerol, 1% β-mercaptoethanol, 12.5 M EDTA, 0.02% bromophenol blue in distilled water) and was subjected to heating at 95°C for 10 min prior to SDS-PAGE gel electrophoresis at 100 V and 20 mA. The gel was stained with silver staining kit (Fermentas Silver Stain Kit).

Determination of total protein concentration

The total protein concentration was quantified with bicinchoninic acid (BCA) kit using standard bovine serum albumine (BSA) curve. The standard concentrations of BSA used were 25 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml, 1,000 µg/ml, 1,500 µg/ml, and 2,000 µg/ml. Working reaction was made by mixing reagents A and B with a ratio of 50:1. The sample and working reaction were added in a microplate 96-well by sample:working reaction ratio of 1:20. Microplate

was incubated at 37°C for 30 minutes, and then the reaction result was read at 540 nm wavelength using enzyme-linked immunosorbent assay (ELISA) reader (Thermo Scientific Multiskan EX Microplate Reader).

Dot blot hybridization

Ten µl of protein samples were spotted on top surface of the membrane prior to blocking with 10% skim milk in 1x tris-buffered saline (TBS) for 1 hour. The membrane was washed 3 times, for 15 min, 5 min, and 5 min with 0.1% TBS-Tween. Furthermore, the membrane was incubated with monoclonal MSP1 antibody for 1 hour, and washed again 3 times with 0.1% TBS-Tween. The IgG mouse alkaline phosphatase conjugates as a secondary antibody was used to incubate the membrane for 1 hour prior followed by the washing step. Staining was performed using nitro blue tetrazolium-5-bromo-4-chloro-3-indolyl-phosphate NBT-BCIP substrate solution. The membrane was immersed in BCIP/NBT solution (500 µl NBT solution, 500 µl BCIP solution, dan 4 ml distilled water) for 1-5 min at room temperature until the color was developed. When the dot blots were detected, the incubation process was stopped by soaking the membrane in distilled water.^{15,16}

RESULTS

The gene used in this study was the synthetic gene *usp45-MSP-1₁₉* that has been ligated into the pMAT plasmid. The first stage of making the expression construct was the isolation of pMAT-*usp45-MSP-1₁₉* plasmid with *Nco1* and *Xba1* restriction enzymes to release the gene/insert from the plasmid. The *usp45-MSP-1₁₉* gene was

then separated from the plasmid using restriction analysis as shown on agarose gel (Figure 2).

Figure 3 describes the next stage was the ligation of the *usp45-MSP-1₁₉* gene into the pNZ8148 expression vector. The 371 bp *usp45-MSP-1₁₉* gene was successfully inserted into the multiple cloning site (MCS) of the pNZ8148 expression vector, forming a 3,538 bp recombinant plasmid. The recombinant vector was confirmed by *Nco1* and *Xba1* restriction enzymes. Ligation of the *usp45-MSP-1₁₉* gene and the pNZ8148 vector was successfully transformed into *E. coli* MC1061.

The pNZ8148-*usp45-MSP-1₁₉* recombinant vector was validated by DNA sequencing to determine whether or not mutations of the inserted *MSP-1₁₉* gene were present. The recombinant plasmid containing pNZ8148-*usp45-MSP-1₁₉* was 3538 bp in size, encoding 124 amino acids, with a starting point of transcription from 47 nucleotides prior to the *usp45-MSP-1₁₉* gene and ended at 559 nucleotides after the *usp45-MSP-1₁₉* gene (Figure 4). The *usp45-MSP-1₁₉* gene was cloned on the *Nco1* site, causing the addition of 2 amino acids (6 nucleotides) during translation.

In this study showed the different concentration of total protein. The highest concentration of total protein was 0.936 ± 0.04 mg/ml for cells that were induced with 10 ng/ml nisin. However, this was not significantly different with the concentration of total non-induced protein (0.549 ± 0.03 mg/ml). The lowest concentration was the vector alone (0.188 ± 0.04 mg/ml).

MSP-1₁₉ molecular weight was 10.45 kDa (Figure 5A) suggesting that the protein was expressed and secreted out of the cell due to addition of the *usp45* signal peptide that was able to recognize the signal recognition particle (SRP). The expression of *MSP-1₁₉* protein was shown by dot blot analysis tested by the *MSP-1₁₉* monoclonal antibody as shown by the purple spot in the membrane (Figure 5B).

DISCUSSION

The expression vector construction in this study was initiated by the ligation of the *usp45-MSP-1₁₉* synthetic gene into the pMAT plasmid. The inserts were verified using *Nco1* and *Xba1*

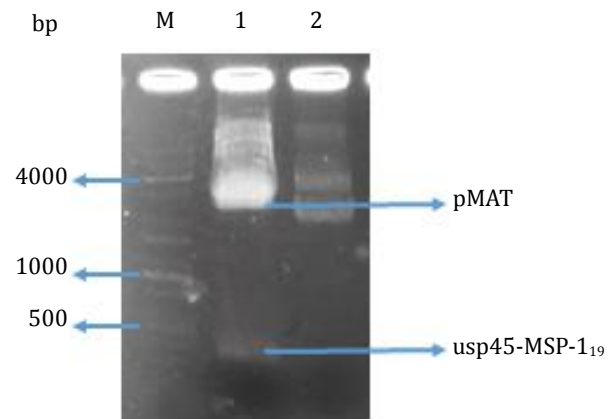


Figure 2. Restriction analysis of pMAT-*usp45-MSP-1₁₉* recombinant plasmid M: 1kb DNA ladder marker, lane 1: digested pMAT plasmid of 2,757 bp, lane 2: undigested *usp45-MSP-1₁₉* of 371 bp

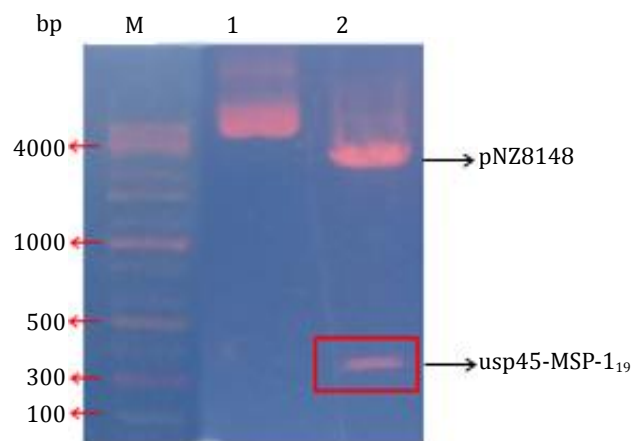


Figure 3. Confirmation of pNZ8148-*usp45-MSP-1₁₉* recombinant protein by restriction enzyme cutting. Lane 1: undigested recombinant plasmid of pNZ8148 containing *usp45-MSP-1₁₉* gene. Lane 2: pNZ8148-*usp45-MSP-1₁₉* recombinant protein cut with *Nco1* and *Xba1* enzymes

restriction enzymes to separate plasmids and genes. The ligation of the *usp45-MSP-1₁₉* gene into the pNZ8148 expression vector was done. The 371 bp *usp45-MSP-1₁₉* gene was successfully inserted into the multiple cloning sites (MCS) in the pNZ8148 expression vector. Thus, it formed a recombinant protein of 3,538 bp.

The recombinant vector pNZ8148-*usp45-MSP-1₁₉* was introduced into *L. lactis* NZ3900 using electroporation technique. This technique was used to insert the foreign DNA into a host of gram-positive bacteria that had the characteristic

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1   CCCCATTTTGAATAGAACATTAACAAATCTAAAACAGTCTTAATTCTATCTTGAGAAAGTATTGG      66

67   TAATAATATTATTGTTCGATAACGCGAGCATAATAAACGGCTCTGATTAAATTTCTGAAGTTTGTTAG      132
                                     -35

133  ATACAATGATTTTCGTTCGAAGGAACTACAAAATAAATTATAAGGAGGCACTCACCATGGCCATGAA      198
1   1                                     RBS                               NcoI
                                     M   A   M   K                               4

199  AAAAAAGATTATCTCAGCTATTTTAATGTCTACAGTGATCTTAAGTGCTGCAGCCCCGTTGTCAGG      264
5   K   K   I   I   S   A   I   L   M   S   T   V   I   L   S   A   A   A   P   L   S   G      26

265  TGTTCACGCCGGTGTGATCCAAAACATGTTTGTGTTGATACACGTGATATTCAAAAAATGCTGG      330
27  V   Y   A   G   V   D   P   K   H   V   C   V   D   T   R   D   I   P   K   N   A   G      48

331  TTGTTTTCGTTATGATAATGGTAATGAAGAATGGCGTTGTTTATTAGTTTCAAAAAAGAAAACAA      396
49  C   F   R   Y   D   N   G   N   E   E   W   R   C   L   L   G   F   K   K   E   N   N      70

397  TACATGTGTTGAAGATAATAATCCAACATGTGATACAAATAATGGTGGTTGTGATACAGCTGCTTC      462
71  T   C   V   E   D   N   N   P   T   C   D   T   N   N   G   G   C   D   T   A   A   S      92

463  ATGTCAAACAGGTGATCGTTCAGGTGAAAATTCAAAAAAGTTATTTGTACATGTAAAGAACCAAC      528
93  C   Q   T   G   D   R   S   G   E   N   S   K   K   V   I   C   T   C   K   E   P   T      114

529  ACCAAATGCTTATTATGAAGGTGTTTTTTGTAATCTAGAGAGCTCAAGCTTTCTTTGAACCAAAA      594
115 P   N   A   Y   Y   E   G   V   F   C   *** XbaI                               124

595  TTAGAAAACCAAGGCTTGAAACGTTCAATTGAAATGGCAATTAACAAATTACAGCACGTGTTGCT      660

661  TTGATTGATAGCCAAAAAGCAGCAGTTGATAAAGCAATTACTGATATTGCTGAAAAATTGTAATTT      726

                                     Terminator
727  ATAAATAAAAATCACCTTTTAGAGGTGGTTTTTTTTATTTATAAATTA                               773

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Figure 4. Sequencing analysis pNZ8148- usp45-MSP-1₁₉ protein recombinant. NisA -35, -10, 1: are the starting points of transcription, RBS is the ribosome binding region (Shine-Dalgarno) and terminator is the end point of transcription. The green color is the restriction site (*NcoI/XbaI*), the blue color is Usp45 signal peptide, the red color is merozoite surface protein 1 (MSP-1₁₉), and the three asterisk is the stop codon

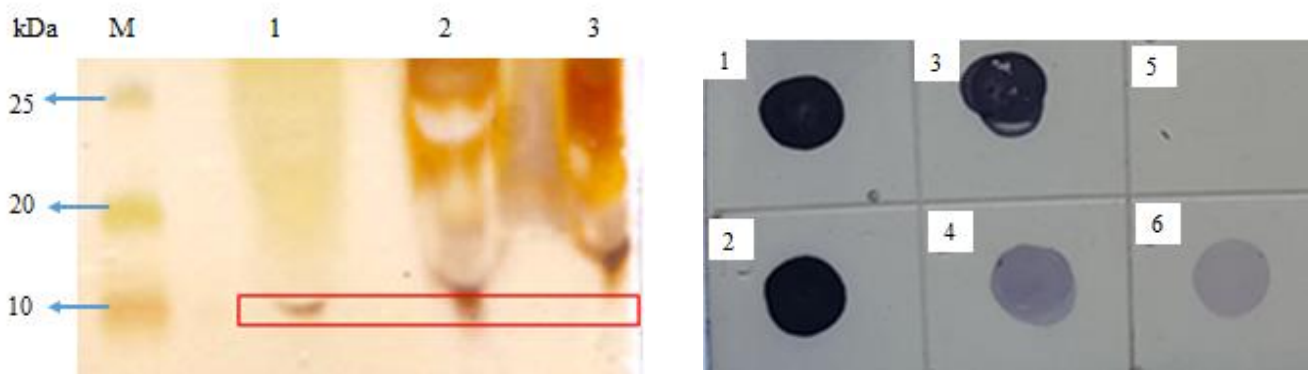


Figure 5. Expression and purification of the MSP-1₁₉ fusion protein. A) SDS-PAGE of target protein expression and purification under different comparisons of induction, non-induced, and the lysed host cell. Molecular weight marker has shown in the left lane; lane 1: induced with 10 ng/ml nisin; lane 2: non-induced; lane 3: *Lactococcus lactis*; B) Dot blot analysis. Boxes 1, 2 and 3 are MSP-1₁₉ dot blots induced with 10 ng/ml nisin; Boxes 4 and 6 are MSP-1₁₉ dot blots non-induced; Box 5 is the dot blot of *Lactococcus lactis*

of a thick cell membrane structure.¹⁷ This electroporation technique applied high electrical voltage passed in a short time. Thus, it formed a pore on the cell membrane that could be inserted with foreign DNA. Selection of transformants using chloramphenicol antibiotics was possible because the vector harbors, the chloramphenicol-resistant genes, was used as selection marker. The addition of sucrose and glycine can help the transformation process to become more efficient.¹⁸

The ligation of *usp45-MSP-1₁₉* gene into pNZ8148 vector which was successfully introduced into *E. coli* host MC1061 was drawn from research by Wu et al.¹² They introduced the vector into *E. coli* first and then to the *L. Lactis*, so that the efficiency of the transformation to *L. lactis* was high. The pNZ8148 expression vectors are known to not have a high vector replication level, but it is balanced by their two starting points of replication, *repA* and *repC*, which are easily recognized by *E. coli* and *L. lactis*, respectively.¹⁹

Referring to Wu et al,¹² the gene inserted into the expression vector was first adapted to the addition of two nucleotide bases of CC after translational starting site fused with *Nco1* restriction sites (CCATGG). This is because the *Nco1* site has already contained the ATG as the starting point of translation leading to mistranslation start site at the *Nco1* site. At the *usp45-MSP-1₁₉* signal peptide gene sequence in the pNZ8148 vector (Figure 4), there was NisA -35, -10, +1 as the transcription beginning of the ribosome-binding region/RBS (Shine-Dalgarno), TAA as the stop codon and it was terminated by a terminator as the ending point of transcription.²⁰

The *usp45-MSP-1₁₉* gene was expressed in the nisin inducible expression (NICE) system. The expression of the *MSP-1₁₉* recombinant protein was performed by the induction of 10 ng/mL nisin. Giving nisin as an inducer from the outside the cells will stimulate the start of transcription at the *nisA* promoter through *nisK* and *nisR* regulon integrated in chromosomal DNA of *L. lactis*.²¹ Possible expression level is controlled by the concentration of nisin given from the outside. The results of this response will form a transcription factor that activates the promoter *nisA*, so that the transcription process by RNA polymerase can be functioning.²²

The presence of target protein bands in non-induced samples is expected due to basal activity causing the *usp45-MSP-1₁₉* gene to be expressed even in the absence of nisin induction. This has been demonstrated in the Mohseni et al²¹ study that NICE expression systems often exhibit basal expression activity (in the absence of induction) when expressed in *Lactobacillus*. In addition to adjusting the dose of nisin, several other ways can be used to increase the gene expression level, such as by substituting an inducible promoter to a constitutive promoter. In accordance to the results of Lages et al,²³ the administration of 10 ng/ml nisin induction in *L. lactis* increased gene expression, resulting in more protein than the non-induced. The concentrations of nisin has compared in the range between 0.5 and 5 ng/ml to induce the expression genes of antibacterial in *L. lactis*, and obtained the result that the highest of antibacterial was produced by *L. lactis* induced with nisin at 5 ng/ml by Mustopa et al.²⁴ Moreover, codon optimization can also be performed. Nisin concentration required for induction is very low, between 0.5–10 ng/ml, while the high concentration of nisin of more than 10 ng/ml used will have negative effect on the host because it can suppress the growth of *L. lactis*.²⁵

As stated in the results section, *usp45* signal peptide was able to recognize signal recognition particle (SRP) indicated by the protein being successfully secreted outside the cell to the growing medium. The SRP will bring the pre-peptide to the SRP receptor present in the membrane, which then will be brought to the protein translocation channel (PTC). The signal peptide opened the PTC, then the mature peptide was removed whereas the *usp45* signal peptide would be cut by the peptidase.

Detection of *MSP-1₁₉* protein was also seen from the dot blot hybridization test which is an immunological test whose function is similar to the western blot to detect the specificity of the reaction between the antigen and the antibody. In the samples induced with 10 ng/ml nisin, the positive results of the dot blot test were observed (Figure 5B). The results showed specific reactions between *MSP-1₁₉* proteins with *MSP1* monoclonal antibodies, seen from color developed on the membrane. Characterization of immunogenicity of primary antibodies tested by dot blot assays

showed the result of 10.45 kDa MSP-1₁₉ antigen was reacted with anti-MSP1 specific antibodies.

In conclusion, this study showed that MSP-1₁₉ protein fused with usp45 as signal peptide has been successfully constructed and expressed inside the GRAS organism in *L. lactis*. This heterologous expression of MSP-1₁₉ protein by GRAS host cell can be expected as the candidate for mucosal malaria vaccine which could induce both mucosal and systemic responses. Although initial test of expressed MSP-1₁₉ with monoclonal antibody *in vitro* has showed a positive result, *in vivo* investigation related to the efficacy of induced antibody should be determined.

Conflicts of Interest

The authors affirm no conflict of interest in this study

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