GST Fusion Assisted Overexpression and Purification of Recombinant Parasite Lactate Dehydrogenase Enzyme in *Escherichia coli*

Ramdhani Haryanti[1], Sulaiman N. Depamede[2], Muhamad Ali[2*]

[1]*Magister Management Program of Animal Husbandry Resources, University of Mataram, Jl. Majapahit No. 62 Mataram, Indonesia, 83125.*

[2]*Lab. Microbiology and Biotechnology, Faculty of Animal Sciences, University of Mataram, Jl. Majapahit No. 62 Mataram, Indonesia, 83125.*

**Corresponding author: ali.molbiotech@gmail.com*

Abstract

Escherichia coli is one of the well-established and most popular organisms for the production of recombinant proteins. However, expression levels and solubility may be issue, since some proteins are generated in low amount and aggregate in inclusion body. Fusion proteins have become essential for the overexpression and solubility improvement of recombinant proteins in *E. coli*. In this study, parasite Lactate dehydrogenase-encoding gene was fused in the C-terminal of glutathione-s-transferase gene and subsequently expressed in *E. coli* BL21. Expression levels and purification results of the fused protein were determined by SDS-PAGE. The SDS-PAGE result shows that the 58 kDa band corresponding to the GST-pLDH protein was successfully overexpressed and purified using GSTrap column. Our results are not only useful for robust production of parasite Lactate dehydrogenase, but also helpful for the enzyme purification.

Keywords: *parasite Lactate Dehydrogenase (pLDH), Glutathione-s-Transferase (GST), Fusion Protein, Escherichia coli, GSTrap column*

Introduction

Parasite Lactate Dehidrogenase (pLDH) is an essential enzyme for plasmodium survival since the parasite lacks a functional tricarboxylic acid cycle (Krebs cycle) during their asexual intraerythrocytic stages. The enzyme regenerates NAD+ from NADH for continued use in glycolysis.Therefore, the asexual stage of of the parasite, residing in the mature red blood cells, depends mainly on anaerobic glucose metabolism (glycolysis) for its ATP requirements. In this condition, glucose uptake is 30-50 folds higher than that of the uninfected erythrocyte.

pLDH is well known diagnostic target owing to presence of unique epitope as compared to human lactate dehidrogenase isoform (Alfiah et al., 2009; Nwazue, 2014). The pLDH-based tests can detect multiple species of malaria (*P. falciparum, P. malariae, P. vivax, P. ovale, and P. knowlesi*). The existence of this enzyme in the patient's blood could be used as an indicator the blood contained *Plasmodium* so the owners are known to suffer malaria. In addition, another property of pLDH is as a target of antimalarial compounds, which could be used to find new drugs for malaria. pLDH crystal structure indicates amino acids near the "active site" that allows the binding of the enzyme with the inhibitor (Berwal et al., 2008 and Ali et al., 2013). The formation of the bond between pLDH with such inhibitors would cause inactivation of pLDH and Plasmodium can not survive.

The use of *E. coli* as a host cell of recombinant protein expression is generally accepted as the best way as it is simple, cheap, and its technology is mature (Hu et al., 2005). Sorensen et al (2004) and Ali et al (2005) reported that the use of the bacteria is very popular because the technology is well established, supported by the tools and

commercially available materials, and required a relatively short time (12 hours). However, one of the major drawbacks associated with the high level expression of recombinant protein in *E. coli* is the inability of many expressed proteins to reach a native conformation and their tendency to accumulate with in refractile aggregates known as inclusion bodies.

According to Rosano and Cecarelli (2014), one of the strategies to increase the expression level of recombinant proteins in *E. coli* is to use fusion technology, including glutathione-S-transferase (GST). Glutathione S-transferase (GST) is a cytosolic enzyme multifunctional group plays an important role in detoxifying electrophilic compounds by conjugation with glutathione (GSH). Glutathione-S-transferase is a multifunctional enzyme complex that plays a role in detoxification of electrophilic xenobiotic compounds (Griscelli et al., 2004).

In this study, pLDH-encoding gene was fused in the C-terminal of glutathione-s-transferase gene and subsequently expressed in *E. coli* BL21 at room temperature. The analysis of SDS-PAGE result shows that the 58 kDa band corresponding to the GST-pLDH protein was successfully overexpressed in large amount and in soluble form. Then, the soluble fraction could be purified using GSTrap column.

Materials and Methods

This study was conducted at the Laboratory of Microbiology and Biotechnology Faculty of Animal Sciences University of Mataram. GST.pLDH-encoding plasmid was transformed into *E. coli* BL21 using heat-shock transformation technique. PCR colonies were performed using a couple of primers which amplify down-stream region of plasmid (GST) and down-stream region of pLDH.

E. coli Bl21 positive clone could be directly used for the next step in the expression of pLDH. The *E.coli* BL21 strain was inoculated into LB medium (5 ml) containing 50 ug / ml ampicillin and cultivated at 37 °C for 12 hours in the presence and absence of inducer (1 mM IPTG). Then, the culture was harvested using centrifugation at 5.000 rpm for 5 min. The obtained pellet was then analyzed for protein expression using SDS-PAGE.

For protein purification, the obtained pellet was diluted using 1x PBS buffer and sonicated. To obtain the soluble fraction, the sample was centrifuged at 12,000 rpm for 15 min. The sonicated supernatant was applied to the GSTrap column (Amersham) and flowthrough was collected for analysis. The column was washed twice using 1x PBS buffer. Bound protein (GST.pLDH) was eluted with 250 mM glutathione. Collected fraction were analyzed by SDS-PAGE.

Result and Discussion

Confirmation of transformant carrying pGEX.pLDH plasmid was performed by PCR method using diluted colony of transformant as a template. *E coli* BL21 colony without plasmid and colony with empty plasmid (pGEX-4T-2) were used as a negative control. Results of the amplification were showed in Fig. 1. As shown in the figure, there is no amplification produced was detected when *E coli* BL21 colony without plasmid used as template (lane K-). However, a smear and small size of PCR products were appeared when *E coli* BL21 colony carrying empty plasmid (pGEX-$T-2) used as an amplification template (lane 1 and 2).

Amplification of GST.pLDH gene using transformants as a template generated about 925 bp PCR product (lane 3-8). The results confirm the presence of recombinant plasmid (pGEX.pLDH) in the assayed transformants. Since the forward primer amplified a short part of down-stream GST gene, the amplification product is bigger than the original

pLDH gene (850 bp). For analysis of recombinant protein expression, the positive clones were cultured in LB medium at 37°C with shaking.

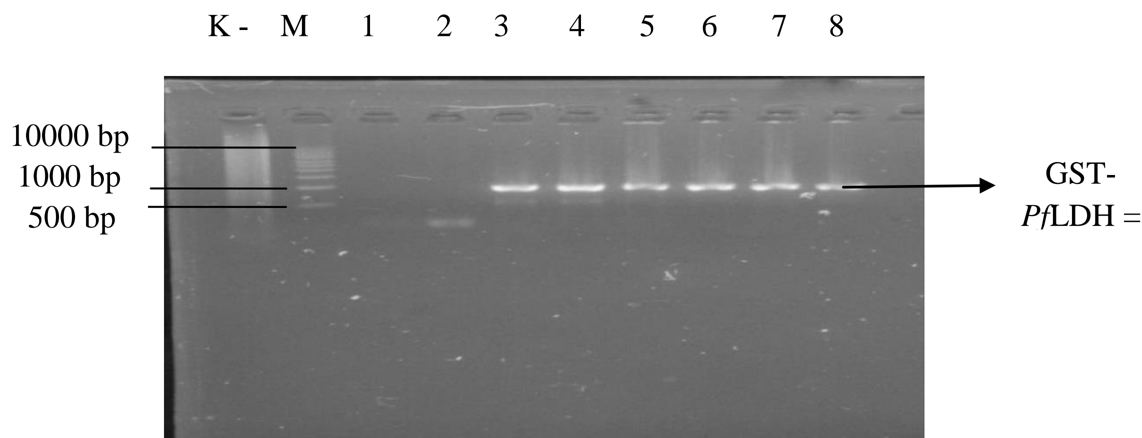

Fig. 1. Result of PCR screening. K– = negative control 1 (*E.coli* without pGEX-*Pf*LDH plasmid); M= Marker (Ladder) 1 kb; 1 – 2 = negative control 2 (*E.coli* carrying empty plasmid pGEX-4T-2); 3 – 8 = transformant carrying pGEX-*Pf*LDH plasmid.

Fig. 2 shows the expression analysis of the recombinant protein in *E. coli* BL21, *E. coli* BL21 carrying no plasmid (lane 1), *E. coli* BL21 carrying pGEX-4T-2 plasmid without IPTG (lane 2), *E. coli* BL21 carrying pGEX-4T-2 plasmid with 1 mM IPTG (lane 3), and *E. coli* BL21 carrying pGEX.pLDH plasmid with 1 mM IPTG (lane 4). The figure shows that the 58 kDa band corresponding to the GST-pLDH band was appeared in the expression of pGEX.pLDH plasmid corresponding to the GST.pLDH. The size of the band was higher than GST (lane 3) after fusion of the protein with pLDH protein.

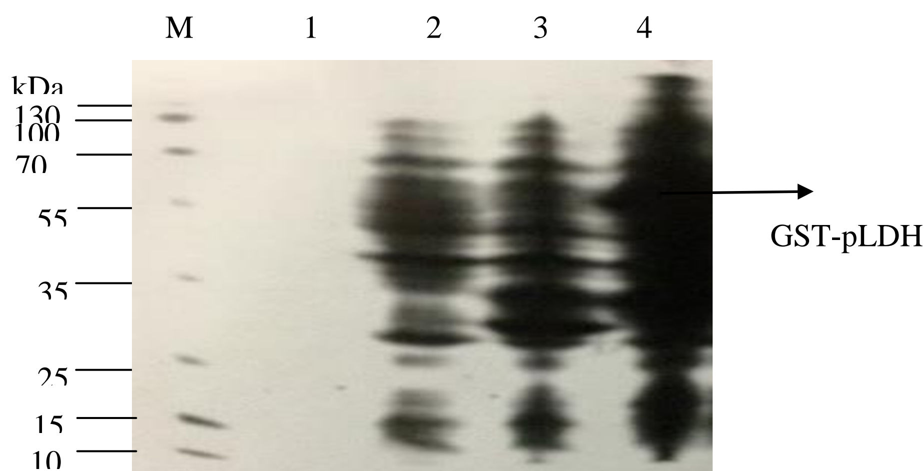

Figure 2. The test results of SDS-PAGE pGEX-pLDH. M = Marker, 1 = *E. coli* BL21 carrying no plasmid, 2 = *E. coli* BL21 carrying pGEX-4T-2 plasmid without IPTG, 3 = *E. coli* BL21 carrying pGEX-4T-2 plasmid with 1 mM IPTG, and 4 = *E. coli* BL21 carrying pGEX.pLDH plasmid with 1 mM IPTG.

Cell lysates containing the soluble fraction of GST-pLDH recombinant protein was purified using GSTrap kit. As shown in the SDS-PAGE analysis results in Fig. 3, the GSTrap-bound GST-fused pLDH protein was purified (Lane 5) comparing to flowthrough

fraction (Lane 3) and washing fraction (Lane 4). Interestingly, most of the GST-pLDH recombinant protein expressed in this research was expressed primarily as soluble form. This results indicated that the use of GST fusion is very suitable to produce high quantity and quality of pLDH precombinant in room temperature.

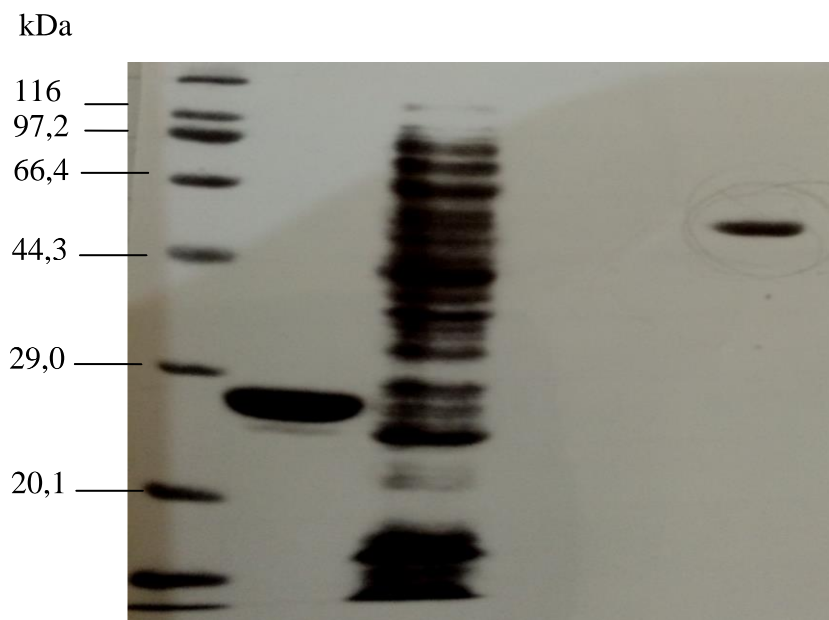

Fig. 3. Purification results of recombinant proteins. M = Marker Protein; 1 = GST; 2 = Flowthrough pGEX-*Pf*LDH; 3 = Washing pGEX-*Pf*LDH, and 4 = Elution pGEX-*Pf*LDH (GST-*Pf*LDH).

Conclusion

In conclusion, we have over expressed the pLDH in *E. coli* using GST fusion and successfully purified using GSTrap column. Our results are not only useful for robust production of parasite Lactate dehydrogenase, but also helpful for the enzyme purification.

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