ABSTRACT

Secretory Leukocyte Protease Inhibitor (SLPI) is an elastase inhibitor that can be used as a wound healing, anti-inflammatory, antibacterial, antifungal and antiretroviral. In the wound healing process, SLPI prevents tissue degradation due to excessive product of neutrophils and suppresses the expression of various cytokines such as IL8 and IL10 which will stimulate tissue damage through inhibition of NFκβ transcription factors. In line with the advances of science and technology including biotechnology, proteins that play an important role are produced by genetic engineering techniques to obtain recombinant proteins and study their molecular characteristics. *S. cerevisiae* is a safe host and contains no lipopolysaccharide pyrogen as in *Eschericia coli*. This host is also capable to produce heterologous proteins doing the post-translation modification and can be secreted to the media. The secreted protein is a better option because of the lack of host protein contamination.

In this study, the construction of the *SLPI* gene was done by (1). fusing with HM1 signal peptides for secretion and (2). co-expression with the *PDI*1 gene to increase recombinant SLPI expression. SLPI is a protein that has eight disulfide bonds. Protein Disulfide Isomerase (PDI) has a role in catalyzing the formation of disulfide bonds. Increasing number of PDI in the cell is expected to assist the folding process of the SLPI as well as increasing the number of secreted SLPIs. The construction of *SLPI* gene co-expression with the *PDI*1 gene was carried out using the pAT425 vector expression which has two multiple cloning sites (MCS). The *SLPI* gene isolated from the Indonesian amniotic membrane. Meanwhile, the plasmid used for the pAT425 co-expression system and the signal peptide used for SLPI secretion comes from the HM1 killer toxin. The purpose of this study is to gain an SLPI secretion system in *S. cerevisiae* hosts and SLPI recombinant proteins which acts as elastase inhibitors.

Steps of the study include: 1) cloning of the *SLPI* and *PDI*1 genes using the pAT425 co-expression vector, 2) SLPI expression and secretion analysis in *S. cerevisiae* hosts, and 3) activity test of in vitro elastase inhibitory. The construction of the *SLPI* and *PDI*1 gene co-expression system was carried out in four stages. Firstly, the *SLPI* gene was fused with the HM1 signal peptide coding gene. Fusion was carried out by inserting the *SLPI* gene into the pYHM1 vector. Amniotic membrane *SLPI* gene–was amplified by PCR using pFSLPI*Sac*I and pRSLPI*Eco*RIprimers.The amplicon and pYHM1 vector were digested using the *Sac*I/ *Eco*RI and transformed into *E. coli* TOP10 after being ligated for 24-48 hours at 4° C. That recombinant plasmid is called pY_SLPI.

Secondly, the fusion of *SLPI* gene and HM1 signal peptide (*hmSLPI*) was subcloned into the pAT425 plasmid on MCS-1. Fusion of the *hmSLPI* gene was isolated from the pY_SLPI recombinant plasmid by PCR using primers pFhmSLPI*Pme*I, pRhmSLPI*Avr*II. Amplicons and pAT425 vectors were ligated for 24-48 hours at 4°C, after each digested with the *Pme*I/*Avr*II. The ligation results were transformed into *E.coli* TOP10. That recombinant plasmid is called pAT_*hmSLPI* and subsequently transformed into *S. cerevisiae* BJ1824 to determine SLPI expression without the addition of *PDI*1 gene.

Third, cloning of the *PDI*1 gene into pAT425 vector in MCS2 by amplifying the *PDI*1 gene from the BYP7700 plasmid. PCR was performed using pFPDI*Mlu*I and pRPDI*Not*I primers. PCR results were digested with *Mlu*I and *Not*I and ligated with pAT425 which had previously been digested with the same restriction enzyme of

*Mlu*I/*Not*I. Ligation results were transformed into *E. coli* TOP10. The recombinant plasmid obtained is calledpAT_*PDI*.

The fourth step was cloning*hmSLPI* gene fusion in the recombinant plasmid of pAT_*PDI*. The procedure was done as in the second step. Yet the plasmid was pAT_*PDI*. The ligation results of the *hmSLPI* and pAT_*PDI* genes, each of which was digested with the PmeI/AvrII enzymes, were transformed into *E.coli* TOP10. This recombinant plasmid was called pAT_*PDI_hmSLPI* and was then sub-cloned into *S. cerevisiae* BJ1824 to find out the SLPI expression.

Analysis of expression and secretion was carried out for 3 days using liquid YPD rich media and liquid YNBD selective media. Yeast cell pellets were separated from culture media by centrifugation. The extracellular protein fraction was obtained by concentrating the culture medium using a 10 kDa MWCO membrane.Meanwhile, the intracellular protein fraction was obtained by breaking the yeast using a glass bead for 10 minutes with 30 seconds on and 30 seconds off. Proteins attached to the cell membrane (associated cells) were also analyzed for expression by dissolving lysis pellets with 0.2 M tris-HCl buffer pH 8. Characterization of recombinant SLPI molecular weight was determined by using SDS-PAGE and Western Blot. SLPI levels were determined using ELISA.

The intracellular and extracellular protein fraction was determined by using the serine protease inhibition activity. The SLPI inhibitory activity test of chymotrypsin-type protease serine was carried out by determining a decrease in the amount of p-nitroaniline (pNA) due to the inhibition of chymotrypsin activity against the specific substrate of N-suc-Ala-Ala-Pro-Phe-ρ-nitroanilide (NPN).

In this study, SLPI secretion vector was obtained without the addition of *PDI*1 gene (pAT_*hmSLPI*) and by adding *PDI*1 gene (pAT_*PDI_hmSLPI*) in *S. cerevisiae* BJ1824. The *SLPI* gene carries HM1 signal peptides for the purpose of secreting out yeast cells. The hmSLPI fusion gene and PDI1 gene were successfully amplified at anealing temperature of 59.3 °C by using pY_*SLPI* and BYP7700 plasmids as a template, respectively. The results of restriction analysis, PCR, and sequencing confirmed that *hmSLPI* fusion gene and *PDI*1 gene were successfully cloned into pAT425 plasmids.

Recombinant plasmid of pAT_*hmSLPI*, pAT_*PDI* and pAT_*PDI_hmSLPI* were successfully subcloned into *S. cerevisiae* BJ1824. The characterization of recombinant SLPI using SDS-PAGE and Western blot indicated that *SLPI* was successfully expressed as a fusion protein with HM1 signal peptide with a size of ~ 16.3 kDa. The concentration of SLPI levels in intracellular fraction without and with the addition of *PDI* produced in YPD rich media were 4.4 ± 0.069 ng/mL and 1.98 ± 0.006 ng/mL, respectively. The concentration of SLPI that were cells associated without and with the addition of PDI were 2.41 ± 0.004 ng/mL and 3.74 ± 0.005 ng/mL while the SLPI level in extracellular fraction without and with the addition of PDI were 0.29 ± 0.017 ng/mL and 0.317± 0.03 ng/mL. Extracellular protein fraction using SDS PAGE showed no SLPI band.

Inhibition percentage of chymotrypsin between SLPI with and without the addition of PDI produced in YPD rich media were 50.19 ± 12.89 and 70.59 ± 8.47 respectively. Meanwhile, the inhibition percentage in YNBD selective media was 21.78 ± 0.87 % and 46.64 ± 2.31%. The addition of gene encoding PDI could increase SLPI expression and SLPI inhibitory activity against chymotrypsin. Inhibitory activity was only confirmed by the extracellular protein fraction. The absence of SLPI band on SDS

PAGE, the presence of inhibitory activity of the extracellular protein fraction and SLPI using ELISA showed that SLPI was successfully secreted from yeast cells. These results indicated that the HM1 signal peptide is capable of secreting SLPI even in small amounts. Most of the expressed SLPIs were in cells or attached to yeast cell membranes. The result of this study have been published on AIP conference proceedings and the Avicenna Journal of Medical Biotechnology.

*Key words : hmSLPI fusion gene, S cerevisiae BJ 1824, PDI*1 gene, co-expression, elastase inhibitor