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Molecular Characterization of ORF *esat* Gene of *M. tuberculosis* Isolated from *E.coli* DE3

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In this study, the open reading frame of a Secretory Antigenic Target (Esat) gene from *M. tuberculosis* in *Escherichia coli* BL21 (DE3) was cloned. Esat gene that was isolated from pulmonary TB patient was cloned into a plasmid vector (pGEM-Teasy) to construct pMB38. The E.coli DE3 clone carrying pMb38 was selected on X-gal medium. The expression of ORF in Esat gene was mediated using pRoExHTc under the control of Tre promoter and *E.coli* DE3 as host. Characterization of clones with recombinant plasmid was cut with restriction enzymes and sequencing of DNA inserts. Our results by BLAST analysis of sequencing showed 100% homology. It is hoped these proteins can then be used as an antigen for serological detection of latent TB in people.

Keywrods: ESAT gene; Clone; *M. tuberculosis; E.coli.*

Tuberculosis (TB) remains a major global health problem. Human TB is the most frequent cause of seath from a single infectious agent, being responsible for about two million people die every year worldwide[1]. About 90% of people who get infected with TB develop a latent TB infection. People who have latent infections do not have TB symptoms and cannot spread the infection to others, but they are at risk of developing an active infection that is both symptomatic and contagious. Latent TB infection was difficult to detect due to the non specific symptom. TST (Tuberculin Skin Test) was the only test used to detect latent TB infection this past 100 years[2] but found to have low spesificity. Furthermore, immunodiagnostic complement test was needed by using host cellular response and antigen identification. The mycobacterial protein ESAT (molecular weight 6 kD), which is also secreted during the early stage of the mycobacterial growth, was encoded by Rv3875 (288bp) and found as immunodominant antigen because it contains epitops recognizable by protective T-cells with high specificity and sensitivity in TB patient serum[3-5]. ESAT increase IFN-^{3} which can be measured by detection kit. Despite the potential of ESAT in detecting latent TB infection, ESAT resulted from Indonesian isolate has not been produced[6]. In order to express and produce it, ESAT encoding gene of *M. tuberculosis* from Indonesian isolate were amplified and cloned. This research will be carried out ligation ORF ESAT into the expression vector pET-32b and the transformation of the host cell *E. coli* BL21 (DE3). Stages of research are important so that ORF ESAT-6 can be expressed into proteins. It is hoped these proteins can then be used as an antigen for serological detection of latent TB in people.

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MATERIALS AND METHODS

Subjects were ORF ESAT-6 size 288 bp generated by PCR with specific primers. Reverse primer sequence: CTC GAG CTA GAA TGC CCC CAT GTT GAC AGT GCC. Forward primer sequence: GGA TCC GAT GAC AGA GCA GCA GTG GAA TTT CGC. Other research materials used are PCR buffer, Taq polymerase enzyme, primers, agarose, TAE buffer, loading buffer, EtBr, 1 kb DNA ladder, GFX kit, pET-32b, Luria Bertani medium, ampicillin, XGAL, IPTG, Qiagen kits, Geneaid kits, BamH1 and XhoI restriction enzymes, *E. coli* BL21.

Ligation of ESAT-6 into the expression vector pET-32b

In this ligation used inserts and vector ratio is 3:1 and 5:1. Components of the reaction with a final volume of 10 mL was treated by mixing the DNA inserts, 25 ng vector pET-32b, 1 mL enzyme T4 DNA ligase (3U / mL) and 1 mL of 10 x ligase buffer. Ligation performed at room temperature for 3 hours.

Transformation of recombinant plasmid pET32b-ESAT-6 into E.coli BL 21 (DE3)

The method used for heat shock transformation is based on Sambrook et al., 1989. Used as a positive control E. coli BL21 competent cells and pET-32b vector without DNA inserts. Negative control is competent cells only. Ligation products, the positive control, and negative controls were then incubated at 37 ° C for 18 hours.

Characterization of recombinant plasmid pET-ESAT-6

Recombinant plasmids were characterized using analysis of migration on agarose gel electrophoresis after restriction enzyme cut XhoI and BamHI. Analysis of the DNA inserts performed by sequencing using T7 primer. Similarities orf ESAT-6 with a bank of data analysis done with BLAST (Basic Local Alignment Search Tool), which uses M. tuberculosis H37Rv as a comparison.

RESULTS AND DISCUSSION

PCR product and Purification

By electrophoresis, a band was revealed which corresponded to the gene with the apparent of 288bp. Purification was done by cutting the gel contain ORF ESAT-6 band as mentioned in producer instruction

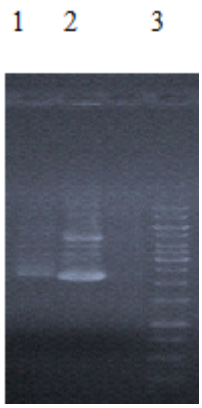
Ligation of ESAT-6 into the expression vector pET-32b

The initial phase was cloned to express the protein ligation ORF ESAT-6 into the expression vector pET-32b resulting recombinant plasmid pET-32b-ESAT-6 as shown in Figure 2. pET-32b plasmid vector without insert DNA (column 2) appeared to have a size shorter than the DNA plasmid inserts ORF ESAT-6 (column 1).

Transformation of pET-32b-ESAT-6 into competent cells of E.coli BL 21 (DE3)

In Figure 3 seemed that the recombinant plasmid pET-32b-ESAT-6 has been successfully

Fig. 1. Purification of PCR product: 1) ORF ESAT-6 288bp; 2) DNA marker



1 = pET-32b-ESAT-6 2 = pET-32b 3 =DNA marker

Fig. 2. Migration analysis of pET32b-ESAT-6 :

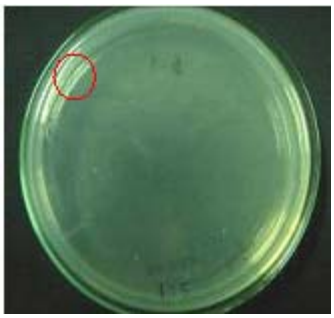


Fig. 3. Recombinant plasmid pET-32b-ESAT-6

1= pET32b=ESAT-6 uncut 2= DNA marker
3 = pET32b=ESAT-6 cut

Fig. 4. Results restriction pET32b-ESAT-6

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>gb|FJ014499.1| Mycobacterium tuberculosis H37Rv 6 kDa early secretory
antigenic
target (esat-6) gene, complete cds
Length=288

 GENE ID: 886209 esxA | 6 kDa early secretory antigenic target ESXA (ESAT-6)
[Mycobacterium tuberculosis H37Rv] (Over 10 PubMed links)

 Score =  436 bits (236),  Expect = 2e-119
 Identities = 236/236 (100%), Gaps = 0/236 (0%)
 Strand=Plus/Plus

Query  1    CGGGTATCGAGGCCGCGGCAAGCGCAATCCAGGGAAATGTCACGTCCATTCATTCCCTCC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  26   CGGGTATCGAGGCCGCGGCAAGCGCAATCCAGGGAAATGTCACGTCCATTCATTCCCTCC  85

Query  61   TTGACGAGGGGAAGCAGTCCCTGACCAAGCTCGCAGCGGCCTGGGGCGGTAGCGGTTCGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  86   TTGACGAGGGGAAGCAGTCCCTGACCAAGCTCGCAGCGGCCTGGGGCGGTAGCGGTTCGG  145

Query  121  AGGCGTACCAGGGTGTCCAGCAAAAATGGGACGCCACGGCTACCGAGCTGAACAACGCGC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  146  AGGCGTACCAGGGTGTCCAGCAAAAATGGGACGCCACGGCTACCGAGCTGAACAACGCGC  205

Query  181  TGCAGAACCTGGCGCGGACGATCAGCGAAGCCGGTCAGGCAATGGCTTCGACCGAA  236
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  206  TGCAGAACCTGGCGCGGACGATCAGCGAAGCCGGTCAGGCAATGGCTTCGACCGAA  261
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Fig. 5. BLAST analysis of the results of sequencing DNA insertion ORF ESAT-6

transformed into E. coli BL21 cells. This is indicated by the presence of three white colonies in LB medium + ampicillin

Characterization of clones' pET-32b-ESAT-6

Cutting the recombinant plasmid pET32b-ESAT-6 with restriction enzymes

Early stages to characterize DNA clones made by cutting inserts with restriction enzymes XhoI and BamHI. In Figure 4 appears not truncated recombinant plasmid (column 1). After being cut by the restriction enzyme, obtained two bands are sized 5900 bp vector DNA and the DNA insert size 288 bp (column 3).

Analysis of DNA inserts ORF ESAT-6 with Sequencing

Characterization of clones is then performed by sequencing. Further analysis of the results of the DNA inserts BLAST homology rate of 100% is obtained.

CONCLUSIONS

1. Ligation ORF ESAT-6 into pET-32b expression vector and transformation into E. coli BL21 (DE3) gained 3 white colonies.
2. Characterization of clones by analyzing the rate of migration after the cut restriction enzymes BamHI and Xho I obtained two bands that ORF ESAT-6 (288 bp) and pET-32b vector (5900 bp). The results of DNA sequencing of inserts with T7 primer and BLAST analysis obtained homology100%.

ACKNOWLEDGEMENTS

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