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Comparison the Concentration of Purification Antigen MTSP11 and MPT63 as Serodiagnostic Active Tuberculosis

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Abstract *— Tuberculosis remains a global concern in many countries because it is a contagious disease and become the second largest killer after HIV / AIDS. In suppressing the number of deaths from TB disease are increasing from year to year we conducted this test target antigen of Mycobacterium tuberculosis that is immunogenic by using molecular biology techniques.*

This study aims to the purification of recombinant proteins MTSP11 and MPT63 measuring the concentration of purified protein as a candidate serodiagnostic active tuberculosis. E. coli cells carrying the recombinant plasmid pQE30 Xa-Rv 3204 and pQE30 Xa-Rv 1926 cultured in LB medium containing ampicillin, then, do cell lysis. Further characterization of proteins and the last stage is the measurement results of protein purification.

The results obtained are MTSP11 protein has a molecular weight of 11 kDa while the MPT63 protein size of 16 kDa. The result of the calculation using the formula showed that the concentration of MTSP11 0.165423045mg / mL and the concentration of MPT63 is 0.155164115 mg / mL, the concentration of purified protein is best found in the last washing results by using the elution buffer.

Keywords *— **Mycobacterium tuberculosis, MTSP11, MPT63, SDS-PAGE.***

I. INTRODUCTION

Tuberculosis (TB) is an infectious disease of the lung caused by *Mycobacterium tuberculosis* (Delogu, 2013). The slow progress in dealing with bacterial infections make the death rate is still relatively high (Liu, 2015).

World Health Organization, Defines the country with high load / high-burden countries (HBC) for TB based on three indicators of TB, TB / HIV and MDR-TB in which 48 countries entered into the list. One country may fall into one or both of the lists, even into the third. Indonesia along with 13 other countries, listed in HBC for all three indicators, meaning that Indonesia has a big problem in dealing with TB disease (WHO, 2017).

Pulmonary TB disease occurs when the immune system decreases. HIV-AIDS sufferers or people with poor nutritional status easier for infected and affected by TB. The percentage of TB patients who know their HIV status was among TB patients recorded an increase from 2009 by 2393 to 7796 in 2017 (Kemenkes RI, 2018). Tuberculosis children have special problems that are different from adults. TB children, the problems faced is the problem of diagnosis, treatment, prevention, and tuberculosis in HIV infection (Rahajoe, *et al*., 2005). During this time the diagnosis, treatment, prevention of tuberculosis in adults is more prioritized than in children, but the child is a higher risk group for immunity and is not fully developed (Walls and Shingadia, 2008).

The difficulty of diagnosing TB is still rising due to a variety of cases that are the resistant and the increasing burden of disease diagnostic tests indicates a need for inexpensive, reliable, easy to use and highly sensitive for TB cases. Utilization of specific *Mycobacterium tuberculosis* antigens and immunogenic as a candidate in the serodiagnostic test could lead to the proper diagnosis and rapid and immediate treatment.

In research Manca, *et al*., 1997 for MPT63 and Malaghini, *et al*., 2011 for MTSP11 discovered that the protein is an immunogenic protein from *Mycobacterium tuberculosis*, Two candidates protein produced by

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Mycobacterium tuberculosis is important because it can interact directly with the host immune system so that the protein can activate the immune response in individuals infected with *Mycobacterium tuberculosis*.

Based on the above, to reduce the number of deaths from TB disease is increasing from year to year we conducted this test target antigens from *Mycobacterium tuberculosis*-reactive serum patients with active tuberculosis by performing the production of recombinant proteins, protein purification and measuring the absorbance value of the results of protein purification.

II. METHOD

Tool

The tools used are the incubator shaker (Heidolph Duomax 1030), centrifugation (Profuge), sonicator, Mini Protean II Bio-Rad, Elektroforator (Bio-rad), autoclave (Hirayama), laminar airflow (Esco Ductless Fume Cabinet), balance (Kern 440-47N), a micropipette (Bio-rad), flask (Pyrex), beaker (Pyrex), Eppendorf tubes (Axygen), Eppendorf tube rack (Biocision), falcon tube (BD), Freezer (GEA), measuring cup (Iwaki Pyrex), McCartney bottles, water bath (Memmert), and spectrophotometer cuvette.

Material

Materials used are clones of Escherichia coli BL21 which carries the recombinant plasmid pQE30 XA-Rv 3204 and pQE30 XA-Rv 1926 LB media (Luria Bertani), ampicillin, lysozyme, lysis buffer, wash buffer, buffer elution, Tris-HCl, Tris HCl, IPTG (isopropyl β-D-1-thiogalactopyranoside), Benzonase, Acrylamide/bis solution (Bio-rad), Sodium dodecyl sulfate (SDS), NaCl, KCl, Na2HPO4, KH2PO4, distilled, Loading buffer, Tris Base (Calbiochem), Ammonium persulfate, Tetramethylethylenediamine, Glisine, protein marker (Thermo Scientific), methanol (Merck), Glacial Acetic Acid, coomassie brilliant blue (Bio-rad), filter tip (Genfollower), Barrier tip (MultiGuard), Phosphate Buffered Saline (PBS) Marker proteins (*Precision Plus Protein ™ Dual Color Standards*), BSA (Bovine Serum Albumin).

2.1 Samples

Samples were taken from white colonies grown as a result of the transformation.

2.2 Culture of Recombinant Clones

Escherichia coli BL21 white colonies that carry the recombinant plasmid pQE30 Xa-Xa Rv pQE30-1926 and Rv 3204 by way were grown in LB medium (Luria Bertani).

2.3 Protein Production

Protein production was done by using sonication. The process by using ultrasonic waves in the frequency range 10 MHz KHz or known by the term ultrasonics.

2.4 Protein Purification

Protein purification using affinity chromatography column of Ni-NTA. Purification of recombinant proteins results then collected in Eppendorf tube to further analyzed by SDS-PAGE.

2.5 Protein Characterization

Characterization of protein by using technology most commonly used is the SDS-PAGE. Separation of high-resolution analysis of protein mixtures is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Staining on electrophoresis results as much as 2x.

2.6 Protein Concentration Calculation

Calculation of protein concentration is calculated by using a spectrophotometer.

III. RESULTS AND DISCUSSION

3.1 Recombinant clones

The study began in October in Lab HUM-RC Wahidin Sudirohusodo Hospital. The clones that carry recombinant plasmids pQE30 Xa-Rv 3204 and pQE30 Xa-Rv 1926 comes from stock clones in an earlier study that examined in the laboratory HUM-RC RSWS. Reculture has done on farmed Luria liquid medium with the addition of *E. coli* bacteria that has brought the target gene and do well the addition of ampicillin. Transformant cells containing the recombinant plasmid will thrive on selection media containing ampicillin, while E. coli itself is sensitive to ampicillin. E. coli containing the recombinant plasmid can not grow on media selection (Mastutik, *et al*, 2015).

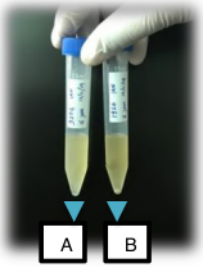

Fig.1: LB Media + Ampicillin + E. coli + IPTG
Description: A = Rv, 1926, B = Rv 3204

The culture media for 18 hours and then added with IPTG which serves to increase the production of recombinant proteins is desirable and will be marked with a thick band on a polyacrylamide gel. IPTG is a compound that has a

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similar structure and function as an inducer lactose gene expression under the control of the promoter.

3.2 Protein Production

Bacterial culture in Eppendorf tubes in centrifuges to separate the supernatant (discarded) and the cell pellet. The addition of PBS buffer on the cell pellet serves as a solvent to assist in maintaining the consistency of the pH of the cells in maintaining the stability of proteins. Pellet cells that have been added to the physically broken PBS using sonication techniques in cold environments to keep the generated heat will not damage the protein that has been out of the bacterial cell.

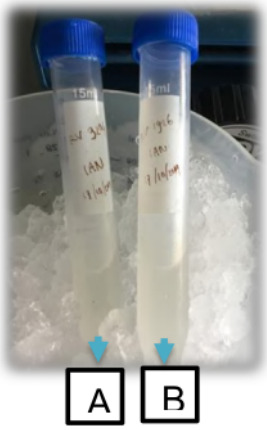

Fig.2. Results of sonication
Description: A = Rv, 1926, B = Rv 3204

3.3 Protein Purification

Sonication proteins purified using Ni-NTA Kit. The principle of purification of this kit is the addition of a buffer differently in the samples NPI-10 as a lysis buffer, NPI-20 as a wash buffer and NPI-500 as the elution buffer. Results obtained protein purification 3 stock code proteins by the addition of buffer. NPI-10 as a lysis buffer is the first washing in the purification of Ni-NTA affinity chromatography. Laundering of NPI-10 is called the Flow-through. NPI-20 as the wash buffer a second washing and the resulting sample in the form of protein, but not the targeted protein. Therefore do last washing with NPI-500 as the elution buffer. Affinity chromatography is used in natural immobilization of ligand by the target particles are then in to the columns. This column which will specifically bind to the desired target protein. In this case, the target protein is MTSP11 and MPT63.

3.4 Protein Characterization

Stock proteins were characterized using the SDS-PAGE method. The principle of the initial denaturation process involves the characterization of the protein components with an anionic detergent that also binds and then makes all the negatively charged proteins and proportional to the mass of the molecule. This step is followed by acrylamide gel electrophoresis through a porous matrix that separates proteins with a very good resolution based on molecular mass. Gel electrophoresis results later colored with a dye solution (staining) which aims to make the protein can bind the dye Coomassie Brilliant Blue. The second staining is done using the destaining solution to eliminate color on a gel that does not contain protein bands that were visible only on the gel that has a single protein thatwould establish a blue ribbon. Protein target is seen is the age of the gel with the code E.

Reading and determination of molecular weight protein purification results seen by marker proteins used. Results characterization weighs 11 kDa protein to protein MTSP11 and 16 kDa for the type of MPT63. The size of the target protein according to research conducted by (Lim, *et al*., 2004 and Siromolot *et al*., 2016).

3.5 Protein Concentration Calculation

The last stage of this study was the measurement of the concentration of each protein sample results from the characterization by using a spectrophotometer. The electromagnetic spectrum is divided into several areas of light. An area will be absorbed by atoms or molecules and the wavelength of light that is absorbed can show the structure of the compounds studied.

The electromagnetic spectrum covers a broad wavelength region of short wave-energy gamma rays high up in the micro wavelength. The standard wavelength used is 500 nm. The main advantage spectrophotometric method is that this method provides a simple way to determine the quantity of the substance that is very small. Besides, the results are accurate, where the numbers recorded by the detector directly read and printed in the form of digital numbers or graphs that have regressed. The results of the calculation of the protein absorbance values presented in the table as below

Table 1. Results of Rv 3204 Spectrophotometer

Code	The absorbance value 500 nm	Result
Ft	0029	1,208
E1	0005	5,000
E2	0008	4,000
W1	0034	1,308
W2	0018	1,500

Table 2. Results of Rv 1926 Spectrophotometer

Code	The absorbance value 500 nm	Result
Ft	0008	1,600
E1	-0006	0750
E2	0013	1,000
W1	0022	1,571
W2	-0003	0429

Measurement of absorbance values that have been completed followed by a dilution of the BSA. BSA is a type of protein that has been used to compare the coating and protein concentration measurements of samples made. Dilution is carried out in stages from 10^{-1} to 10^{-5}.

Table 3. Dilution Spectro BSA

Dilution level	The absorbance value 500 nm
0	0156
0.1	0267
0.2	0:33
0.3	0:38
0.4	0405
0.5	0424

The measurement results in dilution of BSA is used to create a calibration curve equation and determine the best value for protein concentration

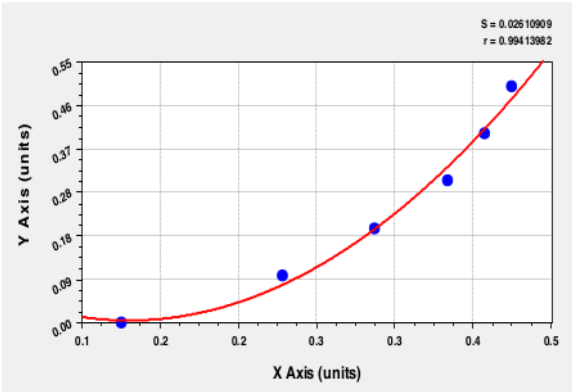

Fig.3: Calibration BSA

Absorbance value at MTSP11 and MPT63 protein compared to BSA absorbance values will result in the concentration of each recombinant protein and is calculated using the formula to the equation

y = a + bx + cx ^ 2 .... (1)

Description: a = 1.83E-01, -2.19E + b = 00, c = 6.80E + 00

Table 5. Results of Recombinant Protein Concentration Rv Rv 3204 and 1926

The sample code	Abs 500 nm	Concentration (mg / mL)
3204 Ft	0,029	0.124620324
W1	0,034	0.115789385
W2	0,018	0.145245182
E1	0,005	0.171741602
E2	0,008	0.165423045
1926 Ft	0,008	0.165423045
W1	0,022	0.137554835
W2	0,003	0.176021973
E1	0,006	0.169621816
E2	0,013	0.155164115

The result of the calculation using the formula shows that for MTSP11 protein, the highest concentration obtained is0.171741602 mg / mL and protein MPT63 with concentration values 0.169621816 mg / mL, high protein concentration which enables a high success rate also in serodiagnostic test.

IV. CONCLUSION

The conclusion of this analysis,

1. proteinMTSP11 has a molecular weight of 11 kDa while the MPT63 protein size of 16 kDa.
2. The highest concentration of the protein is MTSP110.171741602 mg / mL whereas with the MPT63 protein concentration values 0.169621816 mg / mL.

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