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Abstract. Typhoid fever caused by *Salmonella typhi* is an endemic disease in Asia, including Indonesia. Based on data from the World Health Organization (WHO), there are 81% per 100,000 people in Indonesia infected with typhoid. Based on this, development of typhoid detection kit that is simple, fast, specific and sensitive is still needed. One of the developments of detection kits currently available is the antigen and antibody interaction approach. This study aims to obtain comprehensive information about purification of recombinant Fim-C-*S. typhi* proteins as antigens for raw materials for manufacturing typhoid detection kits. This study uses the IMAC method with Ni^{2+} metal, which has advantages in high selectivity and purity. The results of this study report that purification of recombinant Fim-C-*S. typhi* protein inclusion bodies with variations in the binding and washing process at 3, 5, and 7 times gave percent yields correspondingly of 37.84, 39.44, and 38.21% pure protein. Previous studies have also reported variations in binding and washing at 2, 4, and 6 times and gave percent yields respectively were 35.37, 39.11, and 40.49%. Based on the data, we concluded that the variation of 6 times is the best repetition variation to get the largest percent yield so that it can be used as a reference in large-scale purification.

1. Introduction

Typhoid fever or typhus fever is an endemic disease in Indonesia [1,2]. Based on data from the World Health Organization (WHO), revealed that there are 81% of 100,000 people infected with typhoid fever in Indonesia [3]. In addition, it was the results of studies of major hospitals in Indonesia, people who infected typhoid fever increased from year to year with a mortality rate of 0.5 – 6% [4].

For detecting this disease, there is a serological test that is commonly used as a diagnostic tool, namely the widal test. However, the widal test has the disadvantage of low sensitivity and specificity [5,6]. From the results of previous studies, it has successfully developed a new detection prototype kit that uses anti-Fim-C-*S. typhi* antibodies as antigen capture. This prototype correctly identified extract *S. typhi* protein and recombinant Fim-C-*S. typhi* protein in test the sample [7]. The raw material of this prototype is obtained through overexpression with pET-30a(+) system on *E. coli* bacteria. The result of overexpression is purified with Ni-NTA resin, which has a high level of selectivity and purity to obtain recombinant Fim-C-*S. typhi* protein [8,9]. The method used in this purification is Immobilized Metal Affinity Chromatography (IMAC). This method is based on the interaction of Ni^{2+} metal ions, which are immobilized on a matrix with specific- His-tag proteins [10,11]. The target protein that has interacted can be eluted with a low buffer pH, substitute agent, or chelating agent [12].

This research is a continuation of previous studies of purifying Fim-C-*S. typhi* protein to obtain more comprehensive data and modification of standard procedures with the aim of obtaining higher amounts of purified protein. Previous studies have varied the binding and washing process repetitions at 2, 4, and 6 times and variations in the concentration of imidazole. That studies were also obtained that the best imidazole concentration was at 300 mM. Percent yield results obtained respectively in the experiment are 35.37, 39.11, and 40.49% [13]. To obtain more complete percent yield data, this study uses variations of binding and washing at 3, 5 and 7 times with concentration of imidazole 300 mM. Based on these data, the highest percentage yield will be used as a reference for large-scale purification of Fim-C-*S. typhi* protein as a raw material in the development stage of typhoid detection kits.

2. Experimental methods

This study uses the IMAC method with a gravity flow column based on HisPur™ Ni-NTA Resin (Product No. 88221), Thermo Scientific [9]. The binding and washing process used variations 3, 5 and 7 times as a modification of the standard procedure to obtain comprehensive data. Purified proteins are collected in each fraction. After the purification process is complete, the pure Fim-C-*S. typhi* proteins are characterized by SDS-PAGE according to the Bio-Rad procedure (Bulletin 6040) to determine the size of the protein band [14]. In addition, the application of ImageJ is also used to determine the size and thickness of the purified band [15]. The pure Fim-C-*S. typhi* protein concentration was measured with the BCA Kit Thermo Scientific™ (Product No. 23227) so that the yield values from each variation of protein repetition can be known [16].

3. Results and discussion

3.1. Purification and characterization of recombinant Fim-C-S. typhi proteins

Purification begins with equilibrate the Ni-NTA resin column with a Denaturing Equilibrium Buffer of 10× volume of resin to conditioning the resin to be more easily bound to the target protein. Next step, a protein of 1 mL is poured into the column and incubated for 30 minutes so that the protein is bound more optimal to the resin, this process called the binding stage. This stage is repeated with the entry and exit of proteins from the column with predetermined variations. The next step is washing using Denaturing Washing Buffer, which aims to eliminate non-target proteins contained in the column. This washing step uses a predetermined variation of repetition to get more optimal pure protein. The last step is elution using 8× volume resin with Denaturing Elution Buffer to remove the target protein from the column and collected per 1 mL. The purification results characterized by SDS-PAGE shown in **Figure 1**.

Based on **Figure 1 (a) – (c),** the purification results from each variation of binding and washing are 3, 5, and 7 times. In the purification lane of each elution, this indicates that the purification result is successful with only one band of each lane. This shows that there is no protein other than Fim-C-*S. typhi* protein, which is ± 31 kDa in size according to the protein from overexpression [17]. Size ± 31 kDa is obtained using the ImageJ application. The band that appears on the gel is measured its density based on the area of the selected plot. By comparison between the sample area and the standard area, the

relative density can be measured and can be compared for each sample [15]. The greater the relative density value it can be estimated that the protein concentration in the band is higher.

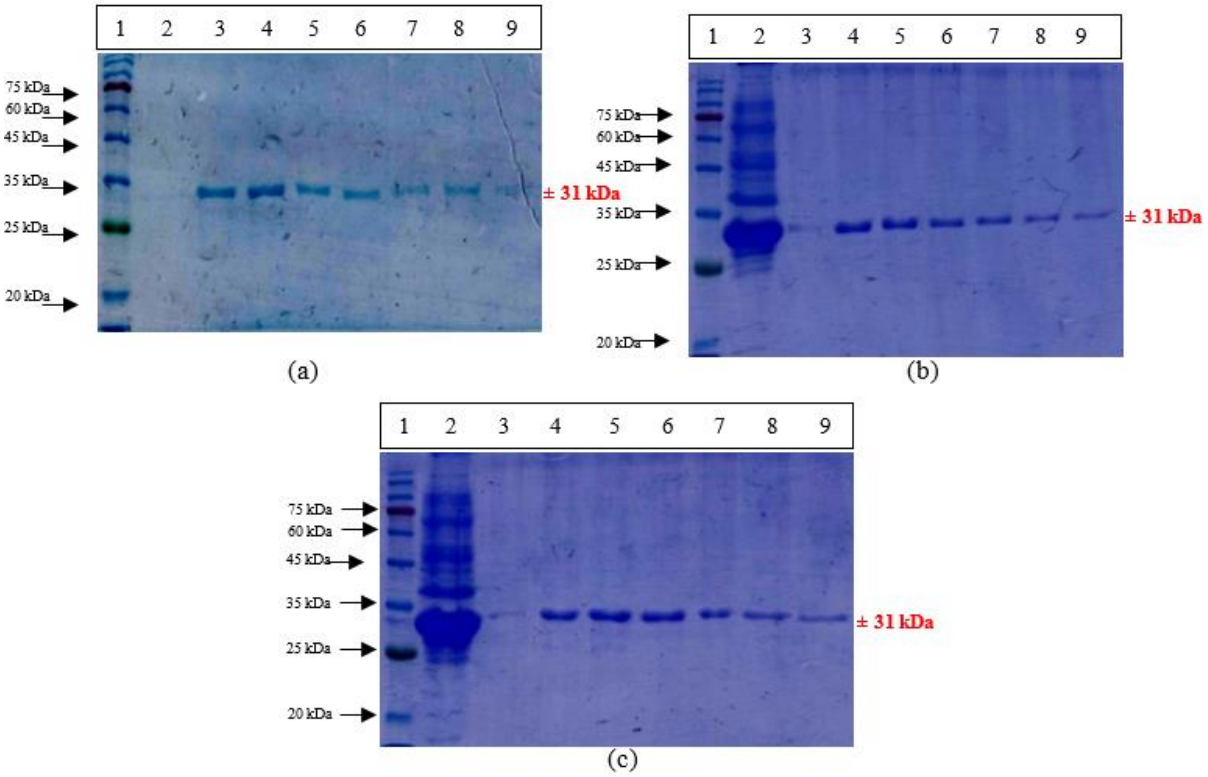

Figure 1. The results of characterization with SDS-PAGE with variations in binding and washing at (a) 3, (b) 5, and (c) 7 times. Lane (a) 1: protein marker SMOBIO 2700, 2 – 9: 15 µL of the purification results for each elution from variations 3×; Lane (b) 1: protein marker SMOBIO 2700, 2: 15 µL of Fim-C-S. typhi protein, 3 – 9: 15 µL of the purification results for each elution from variations 5×; Lane (c) 1: protein marker SMOBIO 2700, 2: 15 µL of Fim-C-S. typhi protein, 3 – 9: 15 µL of the purification results for each elution from variations 7×.

In previous studies, variations in binding and washing were repeated 2, 4, and 6 times. Based on **Figure 2** and **Table 1**, at the 2× to 6× repetition, there was an increase and at 7× repetition decreased. This decrease can occur due to the maximum limit of the ability of Ni-NTA resins to bind to His-tag on the target protein. Thus, the results of purification of Fim-C-*S. typhi* protein with the 6th repetition is the tallest point indicating the highest concentration of pure protein obtained.

Table 1. Relative density of S. typhi Fim-C protein bands.

Band of the 2nd Elution	Relative Density
Variation 2×[a]	0.813
Variation 3×[b]	0.826
Variation 4×[a]	0.927
Variation 5×[b]	0.945
Variation 6×[a]	0.958
Variation 7×[b]	0,875

[a] The result of previous research
[b] The result of this research

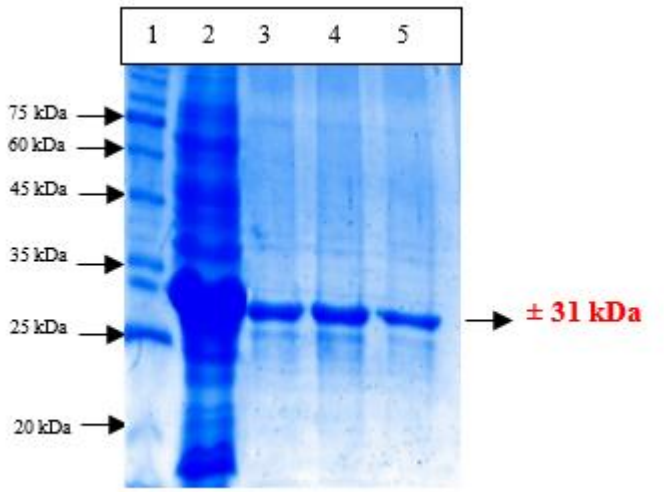

Figure 2. Purification at the 2nd elution of each variation. Lane 1: protein marker SMOBIO 2700; lane 2: protein Fim-C S. typhi; lane 3: 2nd elution of variation 3×; lane 4: 2nd elution of variation 5×; lane 5: 2nd elution of variation 7×.

3.2. Measurement of recombinant Fim-C-S. typhi protein concentration

Measurement of protein concentrations is carried out before, and after purification so that the amount of pure protein yield can be known. The method used is UV-Vis spectrophotometry with the BCA Kit Assay Thermo Scientific™. In the measurement of concentration, a standard curve is made by Bovine Serum Albumin (BSA) with concentrations of 0, 5, 15, 25, 50, 125, 250, and 500 µg/mL. The results of standard curve measurements obtained regression values, y = 1185.5x + 0.1311.

Table 2. The results of the calculation of percent yield from each variation [a].

	Variation 2×[b]	**Variation 3×[c]**	**Variation 4×[b]**	**Variation 5×[c]**	**Variation 6×[b]**	**Variation 7×[c]**
Yield [a]	35.37	37.84	39.11	39.44	40.49	38.21

[a] In percent units (%)

[b] The result of previous research

[c] The result of this research

The regression value obtained is then used to determine the yield value of each elution from each repetition variation. **Table 2** shows the percent yield obtained from the calculation of the overall yield value of each elution. From these data, it can be compared with the results of previous studies to determine the highest percentage yield from the many variations of repetition at the binding and washing stages in the purification process.

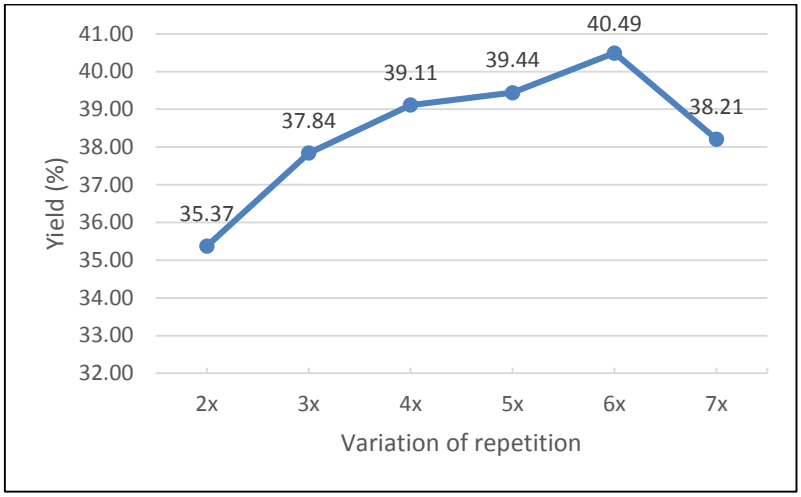

Figure 3. Comparison of yield graphs for each variation of the binding and washing process.

From the graph presented in **Figure 3**, shows the percent yield from the purification results for each variation of the binding and washing process to know which are the highest yield. Based on the graph, it can be determined that the highest percentage yield at 6th times because the binding and washing processes are in optimal condition and have the highest percentage yield. Thus, this variation can be used as a reference in purifying on a large scale.

4. Conclusion

The results of the characterization and measurement of the percent yield from protein purification with widely variations showed that the variation of 6× was the highest. With a yield of 40.49% and relative density band of 0.985, this variation is the most optimal variation in the binding and washing process. Thus, the 6x variation can be used as a reference for the next large-scale purification process for the developed typhoid disease detection kits.

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