Variation in recombinant protein production volume of Fim-C-salmonella typhi as a raw material for typhoid detection kit at laboratory scale

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Abstract. Salmonella typhi is gram-negative bacteria that caused typhoid fever in humans; prevention of the disease is currently through vaccination. The development of disease detection tools is also being carried out so that the detection process is faster and more accurate. In line with the development of typhoid detection devices, prior studies have managed to find factors that influence the production of Fim-C S. typhi protein on a small scale as raw material for typhoid detection kits. The purpose of this research is to apply the results of previous studies in the production of recombinant Fim-C-S. typhi proteins with volume variations of 50mL-300mL, as a foundation for large-scale production. The results of protein production were characterized by Sodium Dodecyl Sulfate Polyacrilamide Gel Electrophoresis (SDS-PAGE) and protein concentration measurements using the Bicinchoninic Acid (BCA) Assay at a wavelength of 562 nm. The results showed an amount of that protein increase along with gradually production volumes, mainly the protein in the form of inclusion bodies. According to the results obtained can be concluded production of the recombinant protein Fim-C-S. typhi at 50-300 mL volume variation on a laboratory scale has been successfully performed with consistent results, which is expected to be basic in production at pilot scale and large scale.

1. Introduction

Salmonella typhi is gram-negative bacteria causes typhoid fever in human [1]. Typhoid fever is a global problem for developing countries [2]. Typhoid fever increased around 10.8 million up to 21.7 million people each year [3]. Typhoid fever could be prevented by vaccines to protect individuals and avoid
transmission [4]. Currently, the diagnose of typhoid fever most commonly used is the widal test [5]. The widal test had low specifications and could be the false-positive results [6]. There is another diagnosis method for typhoid fever; this diagnosis is using Enzyme Link Immunosorbent Assay (ELISA). However, the researchers concluded that the direct ELISA test couldn't be used properly because of the occurrence of false-positive if using monoclonal antibodies as a detection kit [7]. Formerly, both typhoid fever tests had low specificity and sensitivity results. The fast detection kit with high specificity and sensitivity, precise, and accurate is very needed. Recombinant protein is one of the raw materials in a typhoid detection kit in humans.

The advantages of recombinant protein are increased immune response to patient protection, a higher level of purity, more safety and efficiency [8]. The higher stability of gene products and could be effectively on a large-scale [9]. Recombinant protein that could be used as raw material for typhoid fever detection kit is Fimbriae-C-S. typhi recombinant (Fim-C-S. typhi). Factors the production effect of recombinant Fim-C-S. typhi proteins is Isopropyl-β-D-thiogalactopyranoside (IPTG) concentration, host cell count, and overexpression time [10]. The previous research developed a prototype detection kit using anti-Fim-C-S.typhi antibodies by antibody capture. This prototype of can detect correctly the band which has proffered sizing from S. typhi extract protein and recombinant Fim-C-S. typhi protein [11]. The process of developing the detection kit required large amounts of recombinant protein. Then this research was an analysis of variations in the production volume of recombinant Fim-C-S.typhi protein based on factors of production that had been carried out in previous studies as a basis for producing Fim-C-S.typhi recombinant proteins on a large-scale [10].

2. Materials and methods

The steps in this research consisted of Production of recombinant Fim-C-S.typhi protein using the host cell Escherichia coli BL21 (DE3) pLysS, which already contained recombinant plasmid pET-30a-Fim-C Salmonella typhi with volume 50-300 mL [12], the production process based on several factors that are Isopropyl-β-D-thiogalactopyranoside (IPTG) concentration, host cell count, and overexpression time [10]; Measurement concentration of recombinant protein Fim-C Salmonella typhi is using Bicinchoninic Acid (BCA) Thermo Scientific™ Assay Kits [13]; characterization of recombinant protein Fim-C Salmonella typhi with SDS-PAGE [14], and measurement molecular weight of recombinant protein Fim-C using the ImageJ application [15].

3. Results and discussion

3.1. Production of recombinant protein Fim-C-Salmonella typhi

The production of recombinant protein Fim-C Salmonella typhi is carried out in three stages, that are cell culture inoculation, overexpression, and isolation. The overexpression stage of recombinant protein Fim-C using the IPTG inducer, after using the IPTG inducer had an increase in optical density and cell weight values increased with volume. An increase in optical density values before and after adding IPTG was shown in table 1.

| Volume (mL) | The value of OD_{600} before added IPTG 0,5 mM | The value of OD_{600} after added IPTG 0,5mM | Weight cell (gram) |
|-------------|-----------------------------------------------|-----------------------------------------------|-------------------|
| 50          | 0,644                                         | 1,065                                         | 0,393             |
| 100         | 0,675                                         | 1,182                                         | 0,792             |
| 150         | 0,680                                         | 1,412                                         | 2,461             |
| 300         | 0,735                                         | 1,527                                         | 3,402             |

Table 1 shows that adding IPTG inducer could increase the value of optical density and cell weight by the volume of media production; this is related to IPTG binding to the protein lac repressor. The bond causes the repressor to be inactivated and could not bind to the lac operator so that the protein is released
from the lac promoter in the Escherichia coli genome. After the lac repressor is detached and could not bind to the operator, the RNA enzyme polymerization of Escherichia coli is active in express (transcribe and translate) the T7 polymerization RNA gene into the T7 polymerization protein. After the protein polymerase T7 is formed, the protein polymerase T7 will bind to the T7 bacteriophages presents in the recombinant plasmid so that the expression of the target gene could occur and had increased expression [16]. The final stage in the production of recombinant protein Fim-C-Salmonella typhi is isolation. Isolation of recombinant protein Fim-C-Salmonella typhi using native buffer added by the enzyme lysozyme as catalyses lysis, this compound would get extracting soluble protein in the cytoplasm (native protein extract) [17] and denaturing buffer would get aggregates (protein extract inclusion bodies). This protein extracts, which would be used as raw material for typhoid fever detection kits.

3.2. Concentration measurement of recombinant protein Fim-C-Salmonella typhi

Concentration measurement of the extract recombinant protein Fim-C Salmonella typhi was carried out by the spectrophotometric method using the Bicinchoninic Acid (BCA) Thermo ScientificTM Assay Kit. This method is similar to the Lowry method, which relies on the conversion from Cu$^{2+}$ to Cu$^{+}$ in an alkaline atmosphere. Cu$^{+}$ is then detected by BCA reagents, which produce a purple color that can be measured at a wavelength of 562 nm [18]. The reaction to the formation color between proteins and the Thermo ScientificTM BCA Kit Assay is presented in figure 1.

![Figure 1. Purple arrangement.](image)

Formation of purple color occurs because copper chelates with proteins in an alkaline atmosphere using bicinchoninic acid and sodium tartrate present in BCA Reagent A to form light blue to purple produces reduced copper cations. So BCA (cupric sulfate) reacts with Cu$^{+}$ and then an intense purple color is obtained from the chelation of two BCA molecules with one copper ion so that protein could be measured at a wavelength of 562 nm with increasing protein concentration [13]. The result of the standard curve measurement is the equation of the line $y = 0.0005x + 0.039$. Then the concentration of extract recombinant protein native *Salmonella typhi* and extract recombinant protein inclusion bodies *Salmonella typhi* are shown in table 2 and table 3.

### Table 2. The results concentration of protein native.

| Volume (mL) | Weight Cell (gram) | Volume Isolation (mL) | Concentration (µg/mL) | Total Protein (gram) |
|-------------|--------------------|----------------------|-----------------------|----------------------|
| 50          | 0.838              | 1                    | 2167                  | 0.217                |
| 100         | 1.638              | 2                    | 3707                  | 7.413                |
| 150         | 2.461              | 3                    | 6193                  | 12.387               |
| 300         | 3.430              | 6                    | 8989                  | 17.973               |
Table 3. The results concentration of protein inclusion bodies.

| Volume (mL) | Weight Cell (gram) | Isolation volume (mL) | Concentration (µg/mL) | Total Protein (gram) |
|------------|--------------------|-----------------------|-----------------------|---------------------|
| 50         | 0,098              | 1                     | 6047                  | 6,047               |
| 100        | 0,296              | 1,5                   | 8880                  | 13,32               |
| 150        | 0,372              | 2                     | 12253                 | 24,507              |
| 300        | 0,590              | 3                     | 14660                 | 43,98               |

Table 2 and Table 3 showed that the larger volume of protein production could increase cell weight so that the protein extract is also increasing, and the concentration of extract recombinant Fim-C-\textit{S. typhi} native protein and extract recombinant Fim-C-\textit{S. typhi} inclusion bodies is as well increased. In addition to the concentration of extract recombinant Fim-C-\textit{S. typhi} inclusion bodies is greater than extract recombinant native protein. This is following research Singh A, 2005 that the production of recombinant protein in aggregates (inclusion bodies) is a phenomenon that is often found in the production proteins with host \textit{Escherichia coli} due to the following factors: elevate the number of target gene copies, strong promoter system, high inducer concentration, and amino acid sequence of proteins with hydrophobic proteins [19].

3.3. Characterization of extract recombinant Fim-C-\textit{Salmonella typhi} protein

Characterization of extract recombinant Fim-C-\textit{S. typhi} native and extract recombinant inclusion bodies \textit{Salmonella typhi} protein using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) electrophoresis. In previous research, recombinant protein Fim-C \textit{Salmonella typhi} was found at 31kDa [20]. In the path of protein movement, we will get a range of proteins called protein bands based on their molecular weight. The protein in the sample is colourless. It is stained using Coomassie Brilliant Blue R-250 overnight. The results of electrophoretic measurements are presented in figures 2 for volumes 50 and 100 mL and figure 3 for volumes 150 and 300 mL. This research carried out variations in production volumes on a laboratory scale to serve as the basis for large-scale recombinant Fim-C-\textit{S. typhi} protein production.

![Figure 2](image)

**Figure 2.** Result from SDS-PAGE of extract recombinant Fim-C \textit{Salmonella typhi} native protein and extract recombinant Fim-C-\textit{S. typhi} inclusion bodies (IB) at volumes 50 and 100 mL.

In lane 1 showed the 5 µL of Protein Marker (Smobio). In lane 2 showed the 22 µL of extract recombinant Fim-C-\textit{S. typhi} native protein at 50 mL with the amount of protein 20 µg. In lane 3 showed the 22 µL of extract recombinant Fim-C-\textit{S. typhi} protein inclusion bodies at 50 mL with the amount of protein 20 µg. In line 4 showed the 22 µL extracts recombinant Fim-C-\textit{S. typhi} native protein at 100 mL.
with the amount of protein 20 μg. At line 5 showed the 22 μL extracts recombinant Fim-C-\textit{S. typhi} inclusion bodies at 100 mL with 20 μg total protein.

**Figure 3.** Result from SDS-PAGE of extract recombinant Fim-C-\textit{Salmonella typhi} native protein and extract recombinant Fim-C-\textit{S. typhi} inclusion bodies (IB) at volumes 150 and 300 mL.

In lane 1 showed the 5 µL of Protein Marker (Smobio). In lane 2 showed the 22 µL of extract recombinant Fim-C-\textit{Salmonella typhi} native protein at 150 mL with the amount of protein 20 μg. In lane 3 showed the 22 µL of extract recombinant Fim-C-\textit{S. typhi} inclusion bodies protein at 150 mL with the amount of protein 20 μg. In line 4 showed the 22 µL extracts recombinant Fim-C-\textit{S. typhi} native protein at 300 mL with the amount of protein 20 μg. At line 5 showed the 22 µL extracts recombinant Fim-C-\textit{S. typhi} inclusion bodies protein at 300 mL with 20 μg total protein.

The results of electrophoresis of extract recombinant Fim-C-\textit{S. typhi} protein with SDS-PAGE obtained recombinant protein bands at 31 kDa both are in the extract recombinant Fim-C-\textit{S. typhi} native (lines 2 and 4) and as well the extract recombinant Fim-C \textit{S. typhi} inclusion bodies protein (in lanes 3 and 5). The molecular weight of the extract recombinant protein Fim-C-\textit{S. typhi} at 31 kDa was obtained using ImageJ Gel Analysis software. These results indicate that \textit{Salmonella typhi} Fim-C recombinant protein overexpression is mostly in the form of inclusion bodies. The results of the Fim-C-\textit{S. typhi} recombinant protein production will then be purified by the Immobilisation Metal Affinity Coulomb (IMAC) system as a raw material for typhoid detection kits that are being developed.

4. Conclusion

Variations in volume production of recombinant protein of Fim-C- \textit{Salmonella typhi} with a molecular weight of 31 kDa was successfully carried out. Volume variations of 50-300 mL with a concentration of 300 mM IPTG, 150 rpm aeration, and four-hour induction time give consistent results, namely the amount of protein extracts gradually increases with increasing volume production. This result can be used as a basis for large-scale recombinant Fim-C-\textit{S. typhi} protein production so that it can be used as a raw material for the detection of typhoid fever in humans, which is fast, precise, and accurate.

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