Recombinant expression protein of Type 4 Fimbrial gene (ptfA) of *Pasteurella multocida*

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Abstract. The Fimbrial type 4 gene is one of the virulence factor genes associated with bacterial adhesion and colonization factors in *Pasteurella multocida*. The activity of this gene has a surface covering effect on the host it is ridden on. So that the cell surface in the host is difficult to function. *Pasteurella multocida* is a microorganism that attacks the upper respiratory tract, especially in buffalo and cattle, causing infection. The aim of this activity was to analyzed the expression and characterization recombinant ptfA for control and elimination of *Pasteurella multocida*. Gene transformation was carried out using *E. coli*. The induction of gene expression was carried out with IPTG concentrations ranging from 0.1, 0.25, 0.5, 0.75, and 1 mM and incubated at room temperature. The identification analysis was carried out using SDS PAGE showing the 15 KDa gene bands. The 15 kDa recombinant ptfA gene showed the highest expression at a concentration of 0.5 mM of isopropyl thiogalactopyranoside (IPTG).

Keywords: recombinant, ptfA, *Pasteurella multocida*, transformation, expression

1. Introduction
The Fimbrial type 4 gene is a long, fibrous complement protein structure found in Gram-negative microorganisms. This structure is a key structure for pathogenicity and colony on the surface of the host cell (Doughty et al. 2000; Shivachandra et al. 2013) and works in the attachment of bacteria to the surface of the host cell (Hatfaludi et al. 2010). Thus the attachment of the fimbriae structure to the host surface is usually correlated with virulence (Siju et al. 2007).

*Pasteurella multocida* is the main cause of indication for Hemorrhagic septicemia (HS) / Septicamia Epizootica (SE). Generally, due to the virulence of *P. multocida* which causes the main factor of animal disease, it has a big influence on livestock farmers by decreasing economic income. *P. multocida* is a type of Gram-negative pathogenic bacterium that can affect the health of livestock, wild animals, and bird species worldwide by causing a systemic or local infection known as 'pasteurellosis' (Shivachandra et al. 2013). According to Peng et al 2019, the bacterium *Pasteurella multocida* can cause several
diseases in livestock such as cholera in poultry species, hemorrhagic septicemia in ruminant such as cattle and buffalo, progressive atrophic rhinitis and pneumonic pasteurellosis in pigs, and snuffles in rabbits. Furthermore, complex interactions between the host and bacterial virulence (VFs) will affect the pathogenesis of P. multocida (Gharibi et al. 2017).

Septicemia epizootic / hemorrhagic septicemia is a disease that attacks mammals, especially buffalo and cattle. The main cause of this disease is the bacterium Pasteurella multocida. This disease has common clinical signs such as fever, decreased appetite, and stagnant breathing. In Indonesia, this disease is often referred to as "snoring" in buffaloes and cows. This disease is endemic and occurs more frequently in African and Asian countries than in European countries (Karunasree 2016).

The purpose of this activity was to analyze the expression of recombinant ptfA for control and elimination of P. multocida as a cause of hemorrhagic epizootic disease or snoring disease in buffalo and cattle.

2. Material and method

2.1. Transformation gene
A total of 5 μL of pD454-SR-ptfA were added into 100 μL competent cells, then incubated for 30 minutes at -4°C. After that did heat shock was carried out at 42°C for 90 seconds, immediately after that it was put into ice for 10 minutes. Then add 900 μL LBB liquid medium into it and incubate the shaker for 2 hours at 37°C at 150 rpm. Then centrifuged 12,000 g for 1 minute. then the supernatant was discarded. Then the pellet was added to LBB media containing 100 g/mL ampicillin. the final stage was incubated at 37°C for 18 hours.

2.2. Selection of expression the recombinant protein
Optimizing the expression of pD454-SR-ptfA in 15 ml culture media. Cultures of E. coli BL21(DE3) containing the recombinant plasmid were induced at 600 nm optical density (OD600) at 0.6 to 0.8 with the addition of 100 mM isopropyl thiogalactopyranoside (IPTG), then incubated at room temperature overnight. Variation of IPTG concentration was carried out from 0.1 to 1 mM. Whole cell protein samples at different time points were analyzed by 15% SDS-PAGE electrophoresis. The use of negative controls were non-recombinant bacteria and non-induced recombinant clones.

2.3. Varians of IPTG
A Total of 1% (v/v) inoculated culture of E. coli BL21(DE3) Arctic Express carrying pD454-SR-ptfA was grown in media containing 100 g/mL ampicillin. IPTG concentration variations (0.1 mM, 0.25 mM, 0.5 mM, 0.75 mM, 1 mM) at a shaker speed of 100 rpm at room temperature. Cells were harvested after 18 hours. Cell separation was carried out with the supernatant at a speed of 12,000 g by centrifugation. characterization of type 4 fimbrial expression using 15% SDS-PAGE.

3. Result and discussion
The recombinant plasmid pD454-SR-ptfA transformed using E. coli BL21 (DE3) gave a single colony form and spread in LB agar medium (Figure 2). The single colonies formed were cultured to carry out the initial stages of production of the recombinant ptfA protein. According to EWERS et al. 2006. The ptfA gene is a major factor in the ability of bacteria to adhere to the surface of their host epithelial cells.

The initial analysis was carried out with 12% SDS PAGE analysis (Figure 1). It can be seen from 10 colonies (lines 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11) that were induced with a concentration of 1 mM IPTG there was a band size of 15 kDa. The band size obtained is in accordance with the theoretical size of recommended the ptfA protein (Shivachandra et al. 2013).

Furthermore, ptfA protein production is carried out by optimizing the IPTG concentration as an inducer. Figure 3 shows the total protein band of E. coli culture induced using of various IPTG concentrations. The inferred ptfA band has an approximate size of 15 kDa as shown on the SDS-PAGE.
The total ptfA protein induced by 0.5 mM IPTG had a thicker band than those from other IPTG concentrations. However, this information is not sufficient to be used as a reference in determining the optimal IPTG concentration in producing ptfA protein in the periplasmic space. Therefore, further analysis will be carried out to determine the effect of variations in IPTG concentrations on periplasmic protein expression.

Table 1 shows the absorbance of recombinant plasmids induced by IPTG with different concentration variations. It can be seen that the IPTG concentration 0.1 mM has a higher absorbance value than the other IPTG concentrations. The choice of the use of variations in IPTG concentrations is caused because it greatly affects the level of gene transcription. So the use of appropriate IPTG concentrations is one approach to avoid or reduce inclusion bodies. According to Olaofe et al 2018, the addition of IPTG concentration does not affect the formation of biomass during induction in the initial exponential phase. This supports the results obtained from this activity. From Figure 3, it can be seen that the 15 kDa band is in each line except that the thickness is different.

4. Figures
The figure follows the text in the result.

Figure 1. The SDS-PAGE analysis of total proteins induced from the colony. The ptfA band is marked with an arrow (15 kDa). Lane 1, marker protein (Smobio PM 1500); lane 2, transformant colony 1; lane 3, transformant colony 2; lane 4, transformant colony 3; lane 5, transformant colony 4; lane 6, transformant colony 5; lane 7, transformant colony 6; lane 8, transformant colony 7; lane 9, transformant colony 8; lane 10, transformant colony 9; lane 11, transformant colony 10; lane 12, non induced transform

Figure 2. Transformant colonies of pD454-SR-ptfA
Figure 3. The SDS-PAGE analysis of total proteins transformation induced with various IPTG concentrations. The ptfA band is marked with an arrow (15kDa). Lane 1, 1 mM IPTG; lane 2, 0.7 mM IPTG; lane 3, 0.5 mM IPTG; lane 4, 0.25 mM IPTG; lane 5, 0.1 mM IPTG

5. Tables
The table follows the text in the result

| IPTG (mM/mL) | Absorbance value OD$_{600}$ (nM) |
|--------------|----------------------------------|
| 0.1          | 1.028                            |
| 0.25         | 0.892                            |
| 0.5          | 0.955                            |
| 0.7          | 0.911                            |
| 1            | 0.869                            |

6. Conclusion
The analyzed pD454-SR-ptfA plasmid was confirmed at 15 kDa according to the theoretical size. The variation of IPTG did not affect the expression rate of the ptfA gene

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