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# Protein Profile and Hemagglutination Activity of Pilli, an Adhesion Factor Causing Typhoid Fever by *Salmonella typhi*

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**Abstract.** The purposes of this study was to analyze protein profile of pilli member in two *S. typhi* isolates, BA07.4 and KD30.4, and assessing their hemagglutination activity on human red blood cells. Methods: Profile of pillin proteins extracted from two *S. typhi* isolates (BA07.4 and KD30.4) were observed using SDS-PAGE 12% method, hemagglutination activity of the isolated pilli proteins was tested on four erythrocyte of human blood groups ABO. Results showed that SDS-PAGE analysis on pillin proteins of *S. typhi* BA07.4 resulted two major protein subunits sized 87 and 42 kDa along with 14 minor ones. Menwhile, pillin proteins of *S. typhi* KD30.4 isolate showed two major protein subunits sized 87 and 42kDa along with 18 minor protein subunits observed on the SDS-PAGE. From the hemagglutination activity of *S. typhi* BA07.4 on the studied blood groups of A, B, AB, and 0 was 128, 2048, 64, and 2 HA (Hema-Agglutination), respectively. On the other hand, the hemagglutination activity of *S. typhi* KD30.4 on the studied blood groups of A, B, AB, and 0 was 8, 64, 1024, and 0 HA, respectively.

**Keywords:** Protein profile, hemagglutination, pillin protein, *Salmonella typhi,* typhoid fever

## 1. Introduction

Bacterial adhesion to a host cell mucous membrane surface is the beginning of infection which is followed later by bacterial colonization. This happens to several pathogenic bacteria such as *Salmonella typhi* (*S. typhi*) [3, 1, 2]. Pilli plays bacterial adhesion to host cells. This pilli consists of hundreds of pilli protein subunit, called adhesins, which can bind to specific host tissues. Considering this fact, adhesin is therefore a virulence factor owned by pathogenic bacteria such as *S. typhi*, the cause of typhoid fever [2, 8, 10]. Based on the number of pilli protein subunits which make it from several isolates, the *S. typhi* from Kariadi Hospital Semarang, Sarjito Hospital Yogyakarta, and Syaiful Anwar Hospital Malang is highly varied. Some have several hemagglutinin proteins of pilli subunit with the same molecule weight, i.e. 36 and 45 kD [5].

Pilli and fimbriae can stick bacteria to surfaces, but pili are typically longer and fewer in number than fimbriae. Both belong to Gram-negative bacteria, yet they do not exist in all positive Gram bacteria. There are two basic types of pilli: short attachment pili and long conjugation pili [8]. At the end of the shaft is the adhesive tip structure having a shape corresponding to that of specific glycoprotein or glycolipid receptors on a host cell. Bacteria's pilli can agglutinate red blood cells

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(erythrocyte). Pillin protein can agglutinate erythrocyte due to its ability in identifying the receptor that erythrocyte membrane has [4]. Human blood erythrocyte of ABO types has an antigen which are on their surfaces. A blood group has erythrocyte which contains antigen A and Antigen B is on the erythrocyte of B blood group while the erythrocyte of AB blood group contains antigens A and B, and O has no antigen on its erythrocyte surface.

This study aims at analyzing the pilli protein profile using SDS-PAGE method and determining the hemagglutination activity of pilli protein of two *S. typhi* strains on the erythrocyte of ABO human blood groups.

## 2. Materials And Method

### *2.1. Cultivation of S. typhi bacteria*

The bacteria used are *S. typhi* BA07.4 and *S.typhi* KD30.4 strains. The two isolates are from Semarang (Darmawati, 2008). The bacteria cultivation uses biphasic media, namely BHI Agar slants and BHI broth media.

### *2.2. Isolation of pilli protein and analysis of pilli protein profile*

The culture of bacteria in the biphasic media is harvested, centrifuged at 3000 rpm for 20 minutes at 4°C. The pellet is washed and then cut using super vortex mixer. The suspension is then centrifuged at 3000 rpm for 20 minutes at 4°C, and the supernatant is pilli protein. This pilli protein concentrated using 40 % ammonium sulfate. Then, the pilli protein profile is analyzed using SDS-PAGE 12% method.

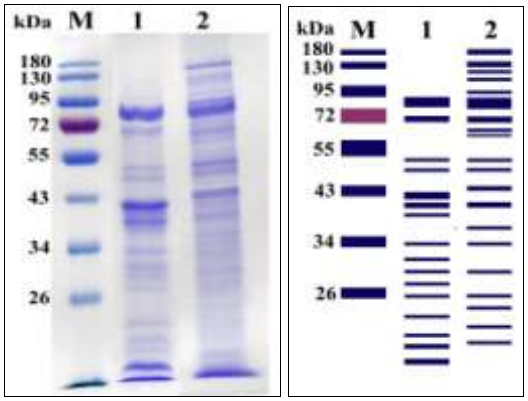
### *2.3. Hemagglutination test*

The hemagglutination test of human blood erythrocyte of A, B, AB and O blood groups is done using Hanne and Finkeltein method (1982). The first to twelfth wells in the microplate are filled with 50 µL PBS pH 7. 50 µL pilli protein of *S. typhi* (100µg/50µL concentration) is added to the first well, then it is homogenized so that the pilli protein concentration in the 1st well becomes 1/2. Next, 50µL is taken from the 1st well and it is added to the 2nd well, then it is homogenized so that the pilli protein concentration in the 2nd well becomes 1/4. This is repeated until the 11th well, hence respectively from the 3rd well (1/8), 4th well (1/16), 5th well (1/32), 6th well (1/64), 7th well (1/128), 8th well (1/256), 9th well (1/512), 10th well (1/1024), and 11th well (1/2048), with the 12th well being the negative control. Furthermore, 50 µL erythrocyte of A human blood group at 1% concentration is added to each well (1st to 12th wells) in the first row. Then, successively in the 2nd, 3rd and 4th rows the same is done to the erythrocyte of B, AB and O human blood groups. The microplate is then shaken slowly using a shaker for 1 minute and then it is incubated at room temperature for 10 minutes and the occurrence of hemagglutination is observed. The hemagglutination titer (HA) is shown by the reverse highest dilution number which still indicates hemagglutination.

## 3. Research Results

### *3.1. Pillin protein profile using SDS-PAGE method*

The pillin protein of two *S. typhi* strains (BA07.4 and KD 30.4), after being isolated, is then analyzed using SDS-PAGE 12% method and colored using Coomassie Brilliant Blue R-250 (CBB). Its protein profile can be seen in Figure 1A, and then visualization of diagrammatic representation is done as shown in Figure 1B. Later, the molecule weight of each pillin protein subunit of the two *S. typhi* strains (BA07.4 and KD 30.4) are analyzed and the result is shown in Table 1.




**A** **B**

Figure 1. Pilli protein profile of *S. typhi* using SDS-PAGE method
(M: Protein Marker; 1: *S. typhi* BA07.4; 2: *S. typhi* KD30.4)

The obtained result indicates that the pillin protein of *S. typhi* BA07.4 strain is composed of 16 protein subunits, consisting of two major protein subunits of 87 and 42 kDa sizes, and 14 minor protein subunits of 22.5-75 kDa sizes. Meanwhile, the pillin protein of *S. typhi* KD30.4 strain is composed of 20 protein subunits, consisting of two major protein subunits of 87 and 45 kDa sizes, and 18 minor protein subunits of 21-180 kDa sizes (Table 1). The major protein subunits from SDS-PAGE as shown by the ribbon thickness and colored by CBB solution seem thicker than the colored minor protein subunit ribbons.

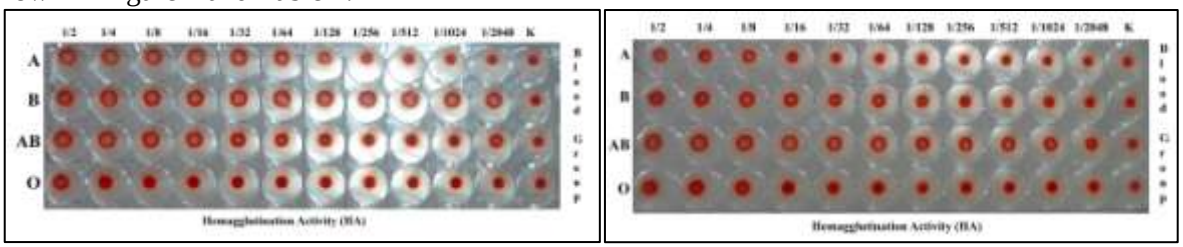
Table 1. Pilli protein profile of *S. typhi* BA07.4 and *S. typhi* KD 30.4 based on SDS-PAGE method

| Subunit | kDa Subunit | BA 07.4 | KD 30.4 |
|---|---|---|---|
| 1. | 180.0 | - | + |
| 2. | 130.0 | - | + |
| 3. | 121.0 | - | + |
| 4. | 112.0 | - | + |
| 5. | 95.0 | - | + |
| 6. | **87.0** | + | + |
| 7. | 70.5 | + | + |
| 8. | 66.0 | - | + |
| 9. | 63.0 | - | + |
| 10. | 52.0 | + | + |
| 11. | 49.5 | + | + |
| 12. | **45.0** | - | + |
| 13. | 42.0 | + | - |
| 14. | 41.0 | + | + |
| 15. | 39.0 | + | - |
| 16. | 37.0 | - | + |
| 17. | 34.5 | + | + |
| 18. | 31.5 | + | - |
| 19. | 29.5 | + | + |
| 20. | 27.0 | + | - |
| 21. | 25.5 | + | + |
| 22. | 24.0 | - | + |
| 23. | 22.5 | + | - |
| 24. | 21.0 | - | + |

| | | | |
|---|---|---|---|
| 25. | 19.0 | - | + |
| 26. | 18.5 | + | - |
| 27. | 16.0 | + | - |

*3.2. Pillin protein hemagglutination test*

The pillin protein hemagglutination test of the two *S. typhi* strains (BA07.4 and KD 30.4) which are isolated from positive Widal patient against the erythrocyte of A, B, AB and O human blood groups is shown in Figure 2 and Table 2.




A B

Figure 2. Hemagglutination activity in ABO human blood groups
(A) of *S. typhi* BA07.4 (A: 128HA; B: 2048HA; AB: 64HA; O: 2HA) and
(B) *S. typhi* KD30.4 (A: 8HA; B: 64HA; AB: 2048HA; O: 2HA)

From the test of hemagglutination activity of 100µg pillin protein of *S. typhi* BA07.4, it is found that the highest hemagglutinin protein titers against the erythrocyte of A, B, AB and O human blood groups are respectively 128 HA, 2048 HA, 64 HA and 2 HA. Meanwhile, in the same test against *S. typhi* KD30.4, it is found that the highest hemagglutinin protein titers against the erythrocyte of A, B, AB and O human blood groups are respectively 8 HA, 64 HA, 2048 HA and 2 HA (Table 2).

Table 2. Hemagglutination activity of pilli protein of *S. typhi* BA07.4 and *S. typhi* KD 30.4 against the erythrocyte of A, B, AB and O human blood groups

| Blood group | Hemagglutination titer (HA) | |
|---|---|---|
| | BA07.4 | KD30.4 |
| A | 128 | 8 |
| B | 2048 | 64 |
| AB | 64 | 2048 |
| O | 2 | 2 |

## 4. Discussion

The number of pilli protein subunits of *S. typhi* BA07.4 strain (16 subunits) and *S. typhi* KD30.4 strain (20 subunits) shows that there is a variation in the number of pilli subunits composing these two strains of bacteria. Likewise, a variation of molecule weight of protein subunits composing them is also found. This research result confirms Darmawati et al (2012) who find that the pilli protein profile of 26 *S. typhi* strains from Central Java, East Java, Bandung, Bogor, Jakarta and Yogyakarta has highly varied number of pilli protein subunits (7-17 subunits). It also confirms Kundera et al. (2014), who suggest that the pilli protein of *S. typhi* isolates from Denpasar, Bandung, Malang and Palu is composed of 8-14 pilli subunits. Furthermore, it confirms Busch et al. ( 2015) who state that the pilli protein is composed of hundreds of pilli subunits which can do adhesion to the host cell surface. This is the beginning of infection, constituting one of virulence factors and the component capable of forming biofilm. In turn, this is very important for human health since pillin protein plays the role as an adhesin which will specifically bind to the surface of host cell. Pillin protein is coded by

*pil*S gene, which is classified into *pil* operon [12]. The varied protein profiles of *S. typhi* are the expression of equally varied genes. This is an indication of genetic diversity in *S. typhi* deriving from different isolates. This confirms the research conducted by Darmawati et al. (2013).

The pilli proteins of *S. typhi* BA07.4 and *S. typhi* KD30.4 can aggluniate the erythrocyte of A, B, AB and O human blood groups because they are capable of identifying the receptor on the erythrocyte surface. The different agglutination activities against the erythrocyte of A, B, AB and O human blood groups are a result of the receptors on the surface of erythrocyte of these different blood groups. Blood type A's erythrocyte surface has N-Asetil-D-Galactosamine carbohydrate, blood type B's has D-Galactosa, and blood type O's has L-fucosa. Pillin protein which has hemagglution activity is hemagglutinin protein [6]. Hemaglutinin protein is lectin which is able to agglutinate the erythrocyte of humans with ABO blood type. Lectin belongs to some organisms including microorganisme (bacteria), which has the function in the infection occurrence processes. Lectin interaction with carbohydrate on the surface of humans' erythrocyte specifically happens and is reversible resulting in agglutination [13, 9].

## 5. Conclusion

Results showed that SDS-PAGE analysis on pilli proteins of *S. typhi* BA07.4 resulted two major protein subunits sized 87 and 42 kDa along with 14 minor ones. Menwhile, pilli proteins of *S. typhi* KD30.4 isolate showed two major protein subunits sized 87 and 42kDa along with 18 minor protein subunits observed on the SDS-PAGE. From the hemagglutination activity of *S. typhi* BA07.4 on the studied blood groups of A, B, AB, and 0 was 128, 2048, 64, and 2 HA (Hema-Agglutination), respectively. On the other hand, the hemagglutination activity of *S. typhi* KD30.4 on the studied blood groups of A, B, AB, and 0 was 8, 64, 1024, and 0 HA, respectively.

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