

Full Length Research Paper

Haemagglutination of *Shigella dysenteriae* subunit pili protein with anti-haemagglutination of *S. dysenteriae* subunit pili protein as a molecule adhesion in mouse enterocytes

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Shigellosis is a global human health problem. Unfortunately, there has not been any effective vaccine available worldwide. Molecule adhesion of bacteria can be used as a component of vaccine as it facilitates attachment to a cell surface structure in which the adhesion molecule is located in the pili. The purpose of this study was to clarify whether the protein subunit pili *Shigella dysenteriae* has a molecule adhesin. The purification of pili was done by isolating them using a pilus bacterium cutter. Purification of protein subunit pili resulting from SDS-PAGE was obtained by an electro-elution method. Adherent assays for mice enterocyte were used by conducting in a dose dependent manner and by doing an immuno-cytochemistry. The purified pili proteins with MW 49.8 kDa showed a haemagglutinin towards mouse erythrocytes. The pili proteins with MW 7.9 kDa showed an anti-haemagglutinin if added to a haemagglutinin pili proteins with MW 49.8 kDa and can prevent haemagglutination. Furthermore, pretreatment of the enterocytes purified with MW 49.8 and 7.9 kDa pili proteins, the adherence of *S. dysenteriae* to mouse enterocytes was inhibited. Immuno-cytochemistry showed that haemagglutinin protein with MW 49.8 kDa and anti-haemagglutinin protein with MW 7.9 kDa adhered to mouse erythrocyte. These results suggest that haemagglutinin protein with MW 49.8 kDa and anti-haemagglutinin protein with MW 7.9 kDa of *S. dysenteriae* pili proteins are adhesive proteins involved in the *S. dysenteriae* initial adherence mechanisms for the enterocytes.

Key words: *Shigella dysenteriae*, protein subunit pili, haemagglutinin, molecule adhesion.

INTRODUCTION

Diarrheal diseases remain a major cause of morbidity and mortality in all age groups in impoverished areas of

South East Asia (Agtini et al., 2007; Kosek et al., 2003; Herwana et al., 2010). Shigellosis is a type of diarrhoea

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due to *Shigella* spp. The annual number of *Shigella* episodes throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries (with 1.1 million deaths) and 1.5 million in industrialized countries (Winickoff et al., 1999).

Shigellosis accounts for 70% of morbidity cases, 60% of which cause mortality mostly amongst children under 5 years of age. In Indonesia, diarrhea is the third leading cause of overall morbidity and the leading cause of infant mortality (Nazir et al., 1985). In a study conducted from 1997 to 1999, *Shigella* spp. was found to be the most frequent isolated organism from diarrhea patients in a community setting in the slums of Jakarta, the capital of Indonesia (Oyofa et al., 2002a, b).

In order to reduce the number of cases of diarrhea, it is critical to develop a vaccine for Shigellosis. Unfortunately, there has been no report on the development of adhesion molecule base of Shigellosis vaccine so far (Levine et al., 2007; Kweon et al., 2008). One of the molecule adhesins containing vaccine is a pertussis vaccine. The pertussis vaccine contains pertactin, the *Bordetella pertussis* adhesin molecule which has a molecular weight of 65 kDa (Poolman and Hallander, 2007).

Infection is initiated by ingestion of shigellae (usually via fecal-oral contamination) (Hale and Keusch, 1996). *Shigella* organisms can survive transit through the stomach since they are less susceptible to acid than other bacteria; for this reason, as few as 10 to 100 organisms can cause disease (Bennish, 1991). There are some major steps before it acquires its pathogenicity. The initial step is to attach to enterocyte (Todar, 2012). Then, the bacteria can enter the enterocyte or colonize on the surface of the enterocyte. During this step, the bacteria produces enterotoxin shiga toxin and verotoxin – which is also produced by *Escherichia coli* which causes diarrhea (Leelaporn et al., 2003).

The adherence of *Vibrio cholerae* *El Tor* into enterocyte uses fimbriae or pili (Ehara et al., 1987). This study aimed at clarifying whether pili *S. dysenteriae* has a role in the adhesion process into the enterocyte of mice.

MATERIALS AND METHODES

Subject

The bacteria used for this research is *S. dysenteriae* in which no process of typing was obtained from Surabaya Referral Laboratory, East Java Indonesia.

Culture conditions and isolation of pili protein

Our previous study with a slight modification was done for pili isolation (Sumarno et al., 2012). The pili were harvested and collected from cultures of bacteria that grew on every bottle of TCG medium that had been incubated. This medium contained 0.02% thioproline; 0.3% NaHCO₃, 0.1% mono sodium 1-glutamate, 1% bactotryptone; 0.2% yeast extract, 0.5% NaCl, 2% bacto agar and 1 mM β amino-ethyl ether-N,N,N',-tetra acid (EGTA). Centrifuged pellets were taken and resuspended with PBS pH 7.4. The bacterial suspension

was then sheared by using pili bacteria cutter. The cutting was performed using the speed specifications for 6 cycles; respectively (from 1 - 6): 5,000 rpm for 30 s; 5,000 rpm for 1 min; 5000 rpm for 2 min; 10,000 rpm for 1 min; 10,000 rpm for 2 min; and lastly 10,000 rpm for 2 min. After each cycle, the sample was centrifuged with a speed of 6,000 rpm for 30 min at 4°C. The supernatants containing pili pieces were transferred into an Eppendorf. The pellets were then re-suspended with PBS pH 7.4 and continued to the next cycle of pili cutter. After the last cycle, all the supernatants-containing pili proteins were centrifuged at 12,000 rpm for 15 min at 4°C. The supernatants were transferred (containing fractions of pili) to Eppendorf and stored at -20°C.

Sodium dodecyl sulfat polyacrylamide electrophoresis (SDS-PAGE)

Obtaining the weight of molecules is mostly done by using SDS-PAGE (Laemli, 1977). Protein sample was heated at 100°C for 5 min in a buffer solution containing 5 mM Tris pH 6.8: 5% 2-mercapto ethanol, 2.5 w/v sodium dodecyl sulfate, 10% v/v glycerol tracking gel 4%. The applied voltage electric current is 120 mV. The color protein, coomassie brilliant blue was used along with sigma standard low range molecular marker. After the calculation of molecular weight of the proteins, multiplications for protein of interest were done in six gels SDS-PAGE product.

Pili protein purification

Purification of protein HA pilus refers to the method of electro-elusion (Agustina et al., 2012). Results of pili collection were run for electrophoresis by using SDS-PAGE. The result of gel electrophoresis gave us the characterization of pili proteins. Bands of interest were cut perpendicularly so that each piece contained one protein band. The cut bands were collected and inserted into a piece of membrane tape which was filled with an electrophoresis running buffer. The membrane was put in a horizontal electrophoresis apparatus, taking 90 min with 120 mV. Following this, the membrane tape was dialyzed with PBS pH 7.4 fluid buffer for 28 h with the replacement of the buffer 4 times in between.

The haemagglutination assay

Haemagglutination assay was done based on Hanne and Findkelstein (1982) method. Duplo dilution of the sample was made of several concentrations on microplate having 96 wells with V bottom hole where each well had the volume of 100 µl. Each well was added with a suspension of red blood of bulb-c 50 ml with concentrations of 0.5% volume shaken using a rotator plate for 1 min. Subsequently, the plate was placed at room temperature for 1 h. The titer was determined by observing the agglutination of red blood on the lowest dilution. The tested samples were the whole *S. dysenteriae* cell, and pili protein of interest that has been extracted through the first up to sixth cycle of pili cutter and purified. Red bloods were taken from healthy Balb/c mice.

Mouse enterocytes preparation

Balb/c enterocytes were prepared by the Weiser (1973) method. Briefly, the small intestine was excised from a mouse. The intestine was slit open and cleaned from any mucus and excreta with PBS containing 1.0 mM dithiothreitol (DTT) at 4°C. The intestinal tissue was placed in a solution (pH 7.3) containing 1.5 mM KCl, 9.6 mM NaCl, 27.0 mM sodium citrate, 8.0 mM KH₂PO₄ and 5.6 mM Na₂HPO₄ and incubated at 37°C for 15 min with gentle shaking

resulting in supernatant. The supernatant rich with tissue was taken, transferred into PBS containing 1.5 mM EDTA and 0.5 mM DTT and incubated for a further 15 min at 37°C with vigorous shaking. Following the process, the centrifugations at 1,500 rpm for 5 min took place and this process was repeated three or more times until the clean supernatant was obtained. Furthermore, the supernatant was removed, and the sediment was added with PBS.

The isolated enterocytes were collected by centrifugation at 1500 rpm for 5 min and suspended in PBS containing 1% bovine serum albumin (BSA) with concentration of approximately 10^6 /ml. A number of enterocytes were counted with a hemocytometer. Enterocytes were kept at 4°C until they were used in the adherence assays.

Assay of adherence to mouse enterocytes

Strains of *S. dysenteriae* grown in BHI broth for 24 h at 37°C were harvested and suspended in PBS containing 1% BSA with concentration of approximately 10^8 /ml. One hundred micro liter of the bacterial suspension was mixed with 100 ml of a suspension of 10^6 mouse enterocytes per ml in PBS containing 1% BSA. The mixture was allowed to incubate at 37°C for 30 min with gentle shaking, and then non-adherent bacteria were removed by repeating the washing with PBS containing 1% BSA. The enterocytes were collected by centrifugation at 1500 rpm for 2 min, suspended in 300 μ l of PBS.

Twenty micro liters of the sample suspension was extracted and put on a glass slide to form a smear. Smear was stained by Gram and adhesion index was calculated by microscopic observation.

Assay of inhibition adherence to mouse enterocytes

Strains of *S. dysenteriae* grown in BHI broth for 24 h at 37°C were harvested and suspended in PBS containing 1% BSA with concentration of approximately 10^8 /ml. 100 μ l of the bacterial suspension was mixed with 100 ml of a suspension of 10^6 mouse enterocytes/ml and 100 μ l of purified pili protein in PBS containing 1% BSA. The mixture was allowed to incubate at 37°C for 30 min with gentle shaking, and then non adherent bacteria were removed by repeated washing with PBS containing 1% BSA in the centrifugation process. The enterocytes were collected by centrifugation at 1,500 rpm for 2 min and suspended in 300 μ l of PBS.

Twenty μ l of the sample suspension was extracted and put into a glass slide to form a smear. Smear was stained by Gram and adhesion index was calculated by microscopic observation through dose-dependent interaction (Nagayama et al., 1995).

Gram staining

Staining was done to see the big picture and the description of enterocytes morphology and *S. dysenteriae* adhesion on enterocyte cells. Slides were covered by using crystal violet for 20 s and rinsed with water. Gram's iodine was applied for 1 min and followed by washing with 95% ethyl alcohol down the slides. Before and after covering with safranin for 20 s, slides were rinsed by using water. Once the slides were dry, observation was done under a microscope of 1000x magnification.

Isolation of antibody anti protein HA

Protein HA of *S. dysenteriae* subunit pili MW 49.8 kDa was used as an antigen. Balb/c was immunized with the 100 μ g antigen which was emulsified with Complete Freud's Ajuvant in 100 μ l PBS through intra peritoneal route. Moreover, another similar antigen was

emulsified with Incomplete Freud's Ajuvant which was given three times in every week as booster injection. The anti body produced was then isolated from the mouse blood serum (Harlow and Lane, 1988).

Immunocytochemistry to detect molecule adhesion

The concentration of erythrocytes was adjusted until the amount was sufficient to examine under a microscope. Eppendorf tube containing sufficient concentration of the erythrocytes was added with 500 μ l sample of *S. dysenteriae* subunit pili protein anti HA MW 7.9 kDa. Then Eppendorf tube was placed in a shaker water bath with the temperature of 37°C for one hour. To wash the protein which did not adhere to erythrocytes, the sample tube was centrifuged at 1000 rpm, temperature 4°C for 15 min with PBS for three times. The final volume of Eppendorf was adjusted to 500 μ l. 20 μ l sample from Eppendorf was placed on glass to be dried in room temperature.

Methanol solution was used to fix the sample on the glass object twice. Every 5 min, the sample was washed with H₂O for 3 times. Then H₂O₂ 3% was dropped on the surface of glass object and was incubated for 10 min in room temperature. After that, the sample was washed with PBS pH 7.4. To block nonspecific protein, the NGS 1% was added to the solution for one hour. Blocking solution was taken by using the filter paper.

The sample was given primary antibodies (serum rich of anti HA protein) and was incubated at room temperature over night. After incubation period has finished, the sample was cleaned up by PBS pH 7.4 every 5 min for three times. After that, a secondary antibody labeled alkali phosphatase was dropped on it and the mixture was incubated at room temperature for 60 min. The sample was washed with pH 7.4, three times every 5 min and then SA-HRP was dropped on it and incubated for 40 min. In the next washing process, the PBS pH 7.4 was used to wash the mixture every 5 min for three times. It was floated with DAB for 3 min, and washed again every 5 min with H₂O three times. Methylene green was used as the counter stain. It was then washed with tap water and after that it was dried at room temperature and was ready for examination (Sakanaka et al., 1988).

RESULTS

Identification of pili *S. dysenteriae* proteins

SDS-PAGE was used to identify the proteins in *S. dysenteriae* pili. The profile of the result showed that the cutting of pili subjected to SDS-PAGE was similar to our previous study (the data is not shown).

The result of profile of pili proteins from the 1st (Sp1) to 5th cycle is depicted in Figure 1. Using this result, molecular weight (MW) of the bands was calculated by using linear regression. Bands ranged from 7.9 to 117.7 kDa, the most intense bands weighed 7.9, 11.2, 27.3, 49.8, 85 and 117.7 kDa (Figure 2A). In relation to the production of proteins of interest (weighed at 7.9, 11.2, 27.3, 49.8 and 85 kDa), there was a consistency and complete separation seen throughout the SDS-PAGE. Figure 1B shows the result of isolation by doing electroelution of protein subunit pili with MW 49.8 (P2) and 7.9 (P5) kDa *S. dysenteriae*.

For the subsequent experiments, pili proteins from the

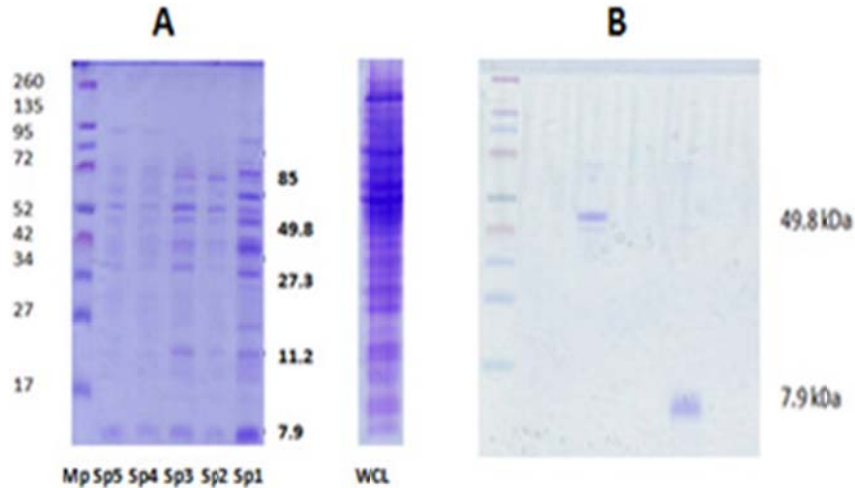


Figure 1. A: Profile of pili proteins from the 1st cycle (Sp1) to 5th used SDS-PAGE. B: The result of purification protein subunit pili with MW 49.8 and 7.9 kDa *S. dysenteriae*.

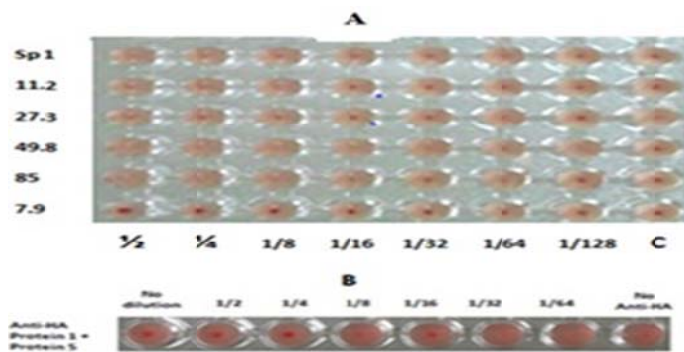


Figure 2. A: HA assay results of protein 1 and 5. B: Anti HA assay (anti-HA) for protein 5 by using protein 1.

1st cycle (Sp1) were used as the main source for HA. First HA assay was done by using the supernatant of pili protein from the first through the fifth cycles (Sp1 – Sp5) (data is not shown). The results showed that all of them were HA positive (able to bind to the erythrocytes). Isolation of protein subunit 85 (P1), 49.8 (P2), 27.3 (P3), 11.9 (P4) and 7.9 (P5) kDa was done by electro-elution and tested separately for their agglutination property. The interesting result observed was that P5 was not able to bind the erythrocytes, forming sediment at the bottom of the plate with dilution of 1/128. The sedimentation rate was faster than control (erythrocytes without protein pili). However, P1 was positive for HA, meaning that the protein was able to bind the erythrocytes, stopping it from forming sediments at the bottom of the plate.

To demonstrate directly that P1 and P5 have the capacity to adhere to mouse enterocytes, the inhibitory effects of the purified P1 and P5 prepared from pili fraction were examined by incorporating these preparations into

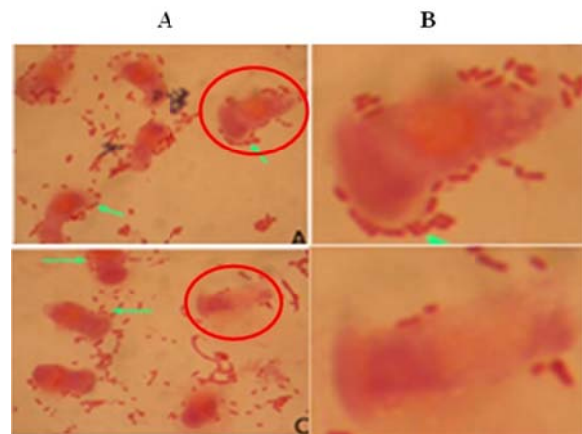


Figure 3. Attachment of *S. dysenteriae* in dose dependent manner of protein HA. 4A dose of protein 1 was 12.5 ug; 4B dose of protein 1 was 50 ug; → Attachment of *S. dysenteriae*.

the test of number bacterial attachment and amount of protein concentration. The pretreatment of mouse enterocytes with the purified P1 and P5 to mask the receptor involved in the adherence of bacteria resulted in an inhibition of further adherence of *S. dysenteriae* to mice enterocytes ($P < 0.05$) (Figure 3). The ability found in P1 and P5 is adhesins therefore using the principle antigen antibody in a dose dependent test was performed.

Using P1 and P5 with concentrations of 100, 50, 25, 12.5 and 0 μg , we found that at 100 μg the adhesion index is significantly inhibited ($P < 0.01$) as compared to 0 μg or control. As the concentration is decreased to 200 μg , the inhibition for both proteins further decreased though no significance was found as compared to 400 μg .

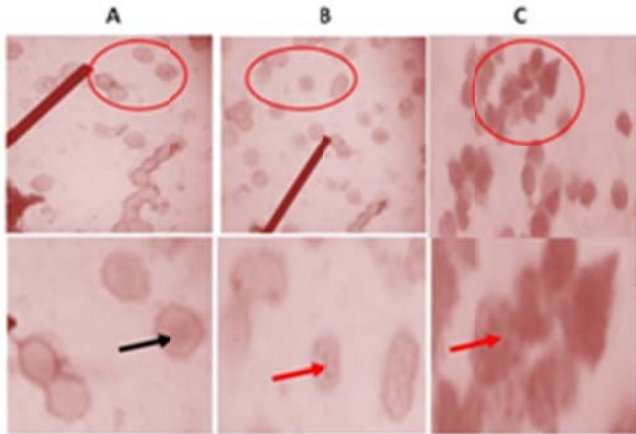


Figure 4. The attachment of P1 and P5 *S. dysenteriae* on the surface of erythrocyte can be seen by using immunocytochemistry method with antibody of anti-protein P1. A: The erythrocyte was taken from C (control); B: The erythrocyte was taken from P5 (1/2 dilution), no agglutination; C: The erythrocyte was taken from P1 (1/2 dilution), with agglutination; \blackrightarrow no dots on the surface of erythrocytes (protein 7.9 kDa is not present); $\color{red}\blackrightarrow$ dots on the surface of erythrocytes (protein 7.9 and 49.8 kDa is present).

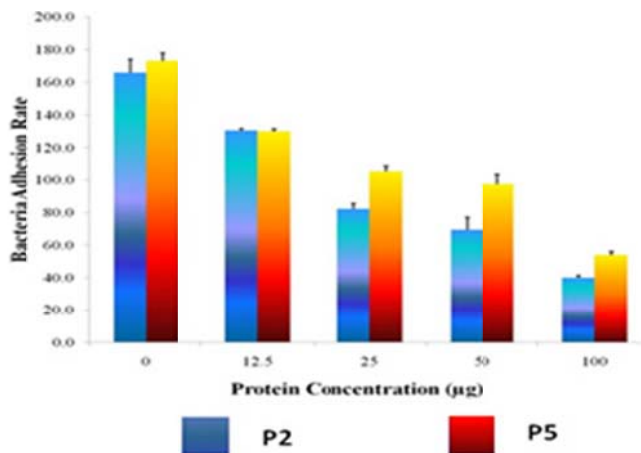


Figure 5. Inhibition of adherence of *S. dysenteriae* to mouse enterocytes by pre-treatment of the purified protein 1 and 5 ± SEM. At 100 µg, bacterial adherence rate significantly decreased as compared to 0 µg for both P2 and P5.

At 100 µg, the adhesion index rate decreased further reaching its maximum capability in inhibition and at this concentration, the difference was significant as compared to all other concentrations for both P1 and P5 ($P < 0.05$).

Further decrease in bacterial concentration (50 µg) afterwards did not have any effect on the adhesion index, in fact the rate increased, leveling up gradually to the value of 0 µg adhesion rate for both P1 and P5. This rate was going up as the concentrations decreased until it reached its peak at 0 µg (Figure 5).

The adhesion rate at 100 µg for both proteins was significantly different as compared to other bacteria concentrations ($P < 0.01$). At 50 µg, the adhesion rate increased and from here onwards as the concentration decreased the adhesion rate increased until it reached its peak at 0 µg or control.

Anti-HA assay was performed to display that P5 has the capacity to inhibit the adherence of protein 1 to mice erythrocyte. P2 that showed negative HA at dilution 1/64 was used in the entire well (HA titer 1/32). P2 with dilution 1/16 was used and were put on entire wells. After that, P5 at dilution of 1 up to 1/64 were added to entire wells. The result shown in Figure 2B and P5 has anti-HA (not able to bind to the enterocytes) with the titer 1/16 (no dot at the dilution 1/32).

To show that P5 and P1 *S. dysenteriae* were attached to erythrocyte was confirmed by immunocytochemistry as seen in Figure 4.

DISCUSSION

Bacteria are able to resist the cleansing action of solutes to colonize the epithelial tissues and infect the underlying tissues through attachment to mucous surfaces (Gibson, 1973). The tissue and cell specific adherence of bacteria are key aspects for the bacterium-host cell interaction. Association between bacteria and mucous surfaces does not occur randomly but specifically for each species (Beachey, 1981). The results from studies clearly confirm that the attachment of *Enterobacteriaceae* strains with a given set up of adhesion varies with different target cells, that is, depending on the availability of receptors. For a given target cell, on the other hand, bacterial binding will occur only if the appropriate adhesion is present on the bacterial surface. Many of the adhesion involved in the adherence of the bacterium towards host cell enables the bacteria to agglutinate erythrocytes of different species (Beachey, 1981).

To the best of our knowledge, no study have ever looked specifically into the pili components that act as HA and adhesion. Previously, it was shown that *S. dysenteriae* of different strains can cause HA and some of the strains were unable to agglutinate the erythrocytes due to the growth condition that causes them to have almost no HA pili expression (Qadri et al., 1989). However, none has tried to find out the protein part of pili that acts as HA and anti HA adhesion of their protein profiling.

Prior to the profiling of the pili protein using SDS-PAGE, isolation of the protein pili is necessarily done using the pili bacterial cutter. Based on the profiling, we observed that the 1st to 5th cycle of pili cutter is enough to obtain almost all the pili on the bacterium (Figure 1A). The supernatant of the 1st cycle is used for subsequent test as it gives a strong and clear band and contains abundant amount of *S. dysenteriae* pili.

Many *Enterobacteriaceae* can cause agglutination of erythrocytes, but previous investigations have not proven

Table 1. Average adherence rate of *S. dysenteriae* after dose-dependent manner of pretreatment of P1 and P5.

Protein concentration (μg)	Protein	Average	Standard Deviation	Standard Error Mean
100	Protein1	39.8	5.3	1.7 ^A
	Protein5	54.0	6.5	2.1 ¹
50	Protein1	69.4	24.8	7.8 ^B
	Protein5	97.4	19.7	6.2 ²
25	Protein1	82.3	10.9	3.4 ^B
	Protein5	105.1	11.2	3.5 ²
12.5	Protein1	130.5	4.4	1.4 ^C
	Protein5	130.1	4.9	1.5 ³
6.125	Protein1	166.0	25.4	8.0 ^D
	Protein5	173.0	15.2	4.8 ⁴

Letter ^{A-D} and ¹⁻⁴ indicate significant difference ($P < 0.05$), whereas the same letter or number are no significant different.

which components of the *Shigella* bacteria are responsible. We used a strain of *S. dysenteriae* which causes HA in mice enterocyte cells. Pili were purified from these bacteria by shearing them from the bacteria. These pili are purified by electro-elution of SDS-PAGE (Figure 1B).

We tested the complete pili proteins (un-purified) for its agglutination properties and some of them did not have any agglutination properties (data is not shown). This is due to the presence of other proteins on the pili that can bind towards each enterocyte but without HA and can inhibit the HA proteins subunit pili to exert their effect (Figure 2A). However, when the band is purified individually (P1), it exhibits HA properties which by linear regression method has an MW of 49.8 kDa. This was the protein that caused the agglutination of the erythrocytes. The use of the purified pili confirmed that HA is caused by the P1 alone. Although the mode of such agglutination is unclear, it is likely that a specific recognition binding event occurs as found in lectin binding of sugar molecules since saccharides with only a limited range of configurations inhibit binding. Such saccharides are presumed to resemble or in some cases are identical to residues available for binding of pili on the mammalian cell membrane (Sharon, 1987). It is concluded that the purification of pili P1 by electro-elution that causes HA of erythrocytes by binding to such sugar molecules on the erythrocyte surface should be clarified. Meanwhile P5, with MW of 7.9 kDa is a protein that enables a bigger and faster formation of erythrocyte sediments as compared to control (Figure 2A). This protein can attach to enterocyte and without agglutination, it is assumed that P5 indeed inhibits HA properties to P1 (competitive reaction) (Figure 2B).

The results of this study strongly suggest that the pili are responsible also for the attachment to epithelial cells of *S. dysenteriae* tested in this study. The ability of *S. dysenteriae* to adhere on mice enterocytes is correlated to the presence of pili on the bacteria. Therefore, a dose dependent manner of the adhesion assay was performed

(Figure 3). At concentration of 100 μg , the protein 1 (results from electro-elution) pre-treated mouse enterocytes showed a significant decrease of bacterial adherence as compared to the control (0 μg) ($P < 0.05$). As the concentration of protein 1 decreased we expected the adhesion rate of the bacterium would slowly increase until it reached its peak at 0 μg . At 50 μg , adhesion rate of the bacterium increased and at 25 μg , the rate leveled up gradually towards the 0 μg adhesion rate. As the concentration further decreased to 12.5 μg , the adhesion rate increased closely to the adhesion rate of those with 0 μg . Finally, at 0 μg , the rate reached its peak as the bacteria had their full adhesion towards the enterocytes. These results were also observed in P5 in dose dependent manner. At 100 μg , pre-treated enterocytes with P5 had its lowest bacterial adhesion rate which was significantly different as compared to 50, 25, 12.5 and 0 μg . Further decrease in the concentration of P5 pre-treated enterocytes did not lower the adhesion rate of the bacterium but rather increased it slowly until it reached its peak at 0 μg .

S. dysenteriae attachment towards mice enterocytes was significantly inhibited by P1 and P5 ($P < 0.05$). This result strongly suggested that P1 and P5 are adhesions, while P5 is anti HA (no erythrocyte agglutination) and can inhibit agglutination of P1 subunit pili *S. dysenteriae* (Table 1 and Figure 5). However, this result alone is insufficient to claim that they are indeed adhesions.

P5 protein anti HA subunit pili with MW 7.9 kDa to protein HA *S. dysenteriae* is a molecule adhesion to erythrocyte improved by immunocytochemistry method (Figure 4).

Recently, Mitra et al. (2012) have found that hemagglutinating activity is directly correlated with colonization ability of *Shigellae* in suckling mouse model.

Maybe we can make clarification of the profile of *Shigella* sero group haemagglutinating activity protein subunit pili with MW of 49.8 kDa and anti- haemagglutination

protein subunit pili with MW of 7.9 kDa.

Acellular pertussis vaccines currently available has one or more different components pertussis toxin, filamentous haemagglutinin (FHA), 69 kDa protein (also know a pertactin/adhesion molecule), fimbrial-2 and fimbrial-3 antigens in different concentrations, and with different adsorption to different adjuvants (WHO, 2009).

Thus, the haemagglutinin protein with MW 49.8 kDa and anti-haemagglutinin protein with MW 7.9 kDa of pili *S. dysenteriae* proteins are adhesive proteins HA bacterial pili that can serves as component of Shigellosis vaccine and useful model system for the early determination of mechanism of bacterial pili attachment to cell membranes of the host cell.

Conclusion

Based on this study, it can be concluded that subunit pili proteins of *S. dysenteriae* have two kinds of protein. They are P1 with MW 49.8 kDa which has haemagglutinin capacity towards mice erythrocytes, and P5 with MW 7.9 kDa which has anti-haemagglutinin capacity towards P1 with MW 49.8 kDa, making both of them adhesion molecules in enterocyte. This finding, by far, suggests that these two proteins are prime candidates for vaccine development of *S. dysenteriae*. This research was approved by the ethical committee of Medical Faculty, University of Brawijaya, Malang, Indonesia.

Conflict of interests

The authors did not declare any conflict of interest.

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