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The Role of Protein Pili 95 KDa *Shigella dysenteriae* as Protein Adhesin in Balb/c Mice Enterocytes

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Shigella dysenteriae is an obligate pathogen that usually found in clinical specimen from shigelosis patients and causes gastrointestinal tract infections with high morbidity and mortality. The pathogenic mechanism of the bacteria is not fully elucidated especially its potential activity of the pili as hemaglutinin and adhesion molecule. The aim of this study is to predict the molecule weight of pili and OMP from *S. dysenteriae*. The research stages included identification of *S. dysenteriae* isolation of OMP *S. dysenteriae*, sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), hemagglutination test, isolation of enterocyte of BALB/c mice and and adhesion test. The study showed that the molecule weight protein of *S. dysenteriae* were 155 kDa, 124 kDa, 95 kDa, 78 kDa, and 32 kDa. The protein pili *S. dysenteriae* with a molecular weight of 95 kDa functions as an adhesin. Changes in pili protein concentration of 95 kDa molecular weight *S. dysenteriae* coated on the enterocyte of mice Balb/c strain, had a significant effect on the adhesion index. The protein pili *S. dysenteriae* with a molecular weight of 95 kDa functions as an adhesin.

The Role of Protein Pili 95 KDa Shigella dysenteriae as Protein Adhesin in Balb/c Mice Enterocytes

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Abstract

Shigella dysenteriae is an obligate pathogen that usually found in clinical specimen from *shigelosis* patients and causes gastrointestinal tract infections with high morbidity and mortality. The pathogenic mechanism of the bacteria is not fully elucidated especially its potential activity of the pili as hemaglutinin and adhesion molecule. The aim of this study is to predict the molecule weight of pili and OMP from S. dysenteriae. The research stages included identification of *S. dysenteriae* isolation of OMP *S .dysenteriae,* sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), hemagglutination test, isolation of enterocyte of BALB/c mice and and adhesion test. The study showed that the molecule weight protein of *S. dysenteriae* were 155 kDa, 124 kDa, 95 kDa, 78 kDa, and 32 kDa. The protein pili *S. dysenteriae* with a molecular weight of 95 kDa functions as an adhesin. Changes in pili protein concentration of 95 kDa molecular weight *S. dysenteriae* coated on the enterocyte of mice Balb/c strain, had a significant effect on the adhesion index. The protein pili *S. dysenteriae* with a molecular weight of 95 kDa functions as an adhesin.
Keywords: S. dysenteriae, Pili Protein, Adhesion Index

Introduction

Diarrhea is still an important public health problem as it is a major contributor to child morbidity and mortality rates in various country including Indonesia. As a tropical country, commonly, the etiologic agent of child diarrhea in Indonesia is Shigella The most common groups of Shigella that infect human are S. dysentriae and S. felxneri, while S. sonnei is more common in subtropical areas. The incubation period of Shigella;s diarrhea is aroud 24-48 hours and the duration of illness is more than 7 days. These bacteria can invade intestinal epithelial cells by inducing the bacterial cell surface with a specific protein compound (1–3)

The pathogenesis of bacterial infection involves several stages. It is starting with adhesion to the surface of the host cell, invasion, and spread locally or systemically. The ability of bacteria to adhere to host cells is mediated by adhesion molecules present in bacteria and host cell receptors. Bacterial adhesion molecules can be located on the pili or on the outer membrane protein (OMP). The attachment of bacteria to this host cell is specific. This specificity is related to the availability of suitable receptors and this determines which parts of bacteria will infect (4,5).

The molecular weight of adhesion proteins varies for each bacteria (Mufida, 2007). The characteristics of adhesion molecules are known by their ability to coagulate red blood cells (hemagglutination). In preliminary research, the hemagglutinin protein Shigella dysentriae has been identified and the results obtained that Shigella dysentriae has several hemagglutinin pili proteins with molecular weights of 135 kDa, 95 kDa, 42 kDa, 23 kDa. This study aims to prove that pili protein with a molecular weight of 95 kDa is an adhesion protein of Shigella dysentriae.

Material and Methods

Shigella dysentriae Culture
↓
Isolation of Shigella dysentriae Pili Protein
↓
Sodium Dodecyl Sulfate Polyacrilamide Gel Electroforesis (SDS-PAGE) Method
↓
Pili Shigella dysentriae Hemagglutinin Protein Purification
↓
Hemagglutinin Test
↓
Intestinal Epithelium Isolation of Balb/c Mice
↓
Adhesion Inhibition Test
↓
Data Analysis

Results

After identifying S. dysenteriae bacteria, then the bacteria were cultured on biphasic media, TCG-BHI. After 48 hours the bacteria were harvested and pili isolation was carried out. Furthermore, SDS-PAGE was carried out to predict the molecular weight of proteins with the results shown in Figure 1.

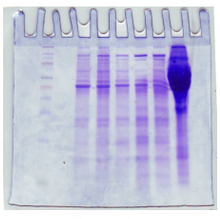

Figure 1.

Protein profiles on SDS-PAGE from several S.dysenteriae pili isolates obtained several proteins. This study used the results of pili isolation with a protein molecular weight of 95 kDa, which was then carried out by electroelution and dialysis, in order to obtain a protein solution. The results of electroelution and pili protein dialysis were then carried out by hemagglutination and adhesion tests on mice erythrocytes with the results as shown in Table 1 and Figure 2.

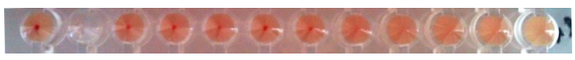

Figure 2. The results of the 95 kDa pili protein hemagglutination test in mice erythrocytes with stratified dilutions.

Protein Weight	Dilution 1x	2x	3x	4x	5x	6x	7x	8x	9x	10x
95 kDa	+	+	+	+	+	-	-	-	-	-

Table 1. The results of the 95 kDa S. dysenteriae pili protein hemagglutination test using mouse erythrocytes.

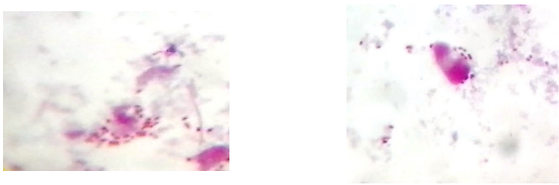

Figure 3 S. dysenteriae attached to enterocyte.

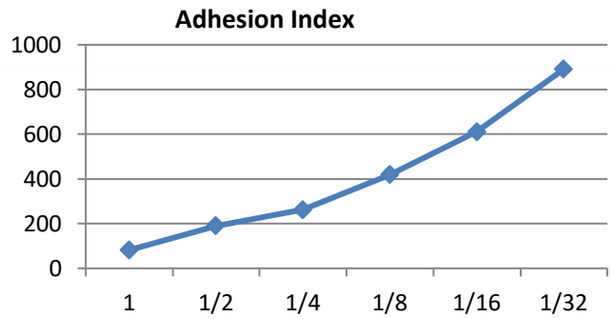

Figure 4 Adhesion index of S. dysenteriae attached to enterocyte.

Discussion

The tips of pili are known to function to mediate the attachment of bacteria to molecules on the surface of the host cell. Host cell receptors are generally glycoprotein or glycolipid carbohydrate residues. The binding of the pili to the target host cell is quite specific. This specificity is important because the availability of suitable receptors often determines which part of the body the bacteria is infected with. The specific binding between the pili tip and the host cell carbohydrates is mediated by a special structure consisting of several different proteins known as adhesins. The introduction of the adhesive portion by the host cell creates a selective and sensitive interaction with the host. This interaction between adhesive and host cells reflects the specificity of the host cell and microbial tropism in the tissue.

The role of pili protein 95 kDa S. dysenteriae as adhesin protein in enterocytes of balb / c mice was indicated by the amount of bacterial attachment to 100 enterocytes of balb / c mice. The amount of bacterial attachment to 100 enterocytes of mice is called the adhesion index. The results of adhesion inhibition test have shown that the higher the pili protein concentration of S. dysenteriae 95 kDa which is coated on enterocytes, the less the number of S. dysenteriae adhering to the enterocytes of Balb / c mice. This could occur because the enterocyte surface receptors of Balb / c mice were already filled with 95 kDa pili protein S. dysenteriae. Thus adhesion can be prevented and the pathogenesis process does not continue. This result is in accordance with the research conducted on Myocbacterium tuberculose, mirabilis, Klebsiella pneumoniae, Streptococcus pneumoniae and Shigella flexner, Acinobacter baummanii.

95 kDa pili protein S. dysenteriae is able to mediate the occurrence of adhesion, this mechanism shows the interaction S. dysenteriae 95 kDa between pili protein piti strong host cells. Adhesion factors provide innovative targets and new therapeutic opportunities and new strategies to control and prevent Shigella infection.

Conclusion

- The protein pili S. dysenteriae with a molecular weight of 95 kDa functions as an adhesin.
- Changes in pili protein concentration of 95 kDa molecular weight S. dysenteriae coated on the intestinal epithelium of mice balb / c strain, had a significant effect on the adhesion index.

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CERTIFICATE OF PARTICIPATION

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Lessons from the Pandemic: Reshaping Medicine and Healthcare

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This is to certify that

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Contributed as a

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