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Molecular Weight Identification of Shigella Dysenteriae Adhesion Molecule Receptors in Mice Enterocyte

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ABSTRACT: *The ability bacteria to adhere to specific host receptor cells is mediated by bacterial specific adhesion component(s). Previous study found that there were some receptors of Vibrio cholera adhesion molecules in mice enterocytes with some different molecular weights. Until now, the receptor of Shigella dysenteriae adhesion molecule is still unknown. The purpose of this research was to find out the molecular weight of Shigella dysenteriae adhesion molecule receptor at mice enterocyte by measuring its ability to block adhesion process. Detection and estimating of S. dysenteriae adhesion protein and its receptor at mice enterocyte was done using SDS page electrophoresis continued by electroelution. Hemagglutinin test and its ability to block adhesion process of suspected receptor proteins were proven by measuring their dose responses and adhesion indexes with S. dysenteriae bacteria in some dilutions, those were 1/500, 1/1000. 1/2000, 1/4000, 1/8000, and 16.000 respectively. Regression analysis found that the molecular weight of Shigella dysentriae adhesion molecule receptor at mice enterocyte is 11.65 kDa ($p<0.05$).*

KEYWORDS : *About five key words in alphabetical order, separated by comma*

I. INTRODUCTION

Shigellosis is an infectious disease caused by a group of bacteria called *Shigella* The symptoms can range from mild watery diarrhea to severe inflammatory bacillary dysentery, characterized by abdominal cramps, fever, and bloody and mucus stools (1,2). S*higellosis*. is endemic in temperate and tropical climates. It particularly common and causes recurrent problems in settings where hygiene is poor. Every year, about 14,000 cases of shigellosis are reported in the United States, and worldwide it is estimated to cause 80–165 million cases of disease and 600,000 deaths annually. Children, especially under five years old, are the most likely to get shigellosis. (1,2,3).The pathogenesis of shigellosis is very complex. In general there are some stages in the pathogenesis of bacterial infections, such as shigellosis, cholera and other intestinal bacteria. It begins with the attachment of bacteria onto intestinal epithelial cells (enterocytes) using certain protein(s) which called cellular adhesion molecule(s) CAM(s). Basically, a bacterium may express several adhesion molecules, each with a specificity for a distinct receptor molecule on the epitehlal cell surface (2,4). A previous study reported that protein sub unit pili with molecular weight 49.8 kDa of *Shigella dysentriae* was a hemagglutinin protein and served as an adhesion molecule (4). Other study found some protein receptors of *Vibrio cholerae* 01M04V on rat enterocytes. Their molecular weights were 62 kDa, 12,7 kDa and 10 kDa respectively (5). Up to now, there is still no report of molecular weight of protein receptor of *S. dysentriae* adhesin molecule.The purpose of this research was to find out the molecular weight of *Shigella dysenteriae* adhesion molecule receptor at mice enterocyte by testing its ability to block adhesion process.

II. MATERIALS AND METHOD

Culturing of Shigella dysenteriae

The media used for culturing the *Shigella* bacteria were McConkey and SSA selective media. In order to augment the bacterial growth, the TCG media then was used (6)

Sodium Dodecyl Sulfate Polyacrylamid Gel Electrophorosis (SDS PAGE)

Molecular weights were monitored with standard SDS-PAGE Laemmli method, using *bromophenol blue* as tracer dye, 12.5% mini slab and 4% tracking gel. Coomassie brilliant blue was used for gel staining and pre stained protein ladder was used as the protein marker. The electrical voltage used was 120 mV. (7).

Isolation of mice enterocyte

The research had been approved by Ethical Committee of Health Research Faculty of Medicine University of Brawijaya.

Isolation of mice enterocyte was performed according to Weisler as used by Nagayama [8]. Two Balb/c mice 12 weeks old were used as enterocyte donor. They were killed using chloroform. the abdomen was opened by scissor and the intestine was taken out and separated from the omentum. Five pieces of 5 cm intestinal cut each were collected from each mouse, and each pieces of cut intestine were opened longitudinally, the lumen of intestine were washed with PBS pH 7.4 solution by putting into solution of 1,5 mM KCl; 9.6 mM Na Cl; 2.7 mM Na-citrate; 8 mM KH2PO4 and 5,6 mM Na2HPO4, pH 7.3. It was then incubated in water incubator at 37°C and for 30 minutes. The solution were changed with PBS pH 7.4, EDTA 1.5 mM and dithio-threitol 0.5 mM pH 7.4. The intestinal cuts in the solution were oscillated for 20 minutes at 37°C and then washed with PBS pH 7.4 and centrifuged of 1000 rpm for 5 minutes, at 4°C. After being washed with PBS the intestine cut was resuspended with PBS. The result were shaken slowly, then the white suspension rich in enterocytes was separated into eppendorf (9).

Isolation of extracellular matrix protein of mice intestinal epithelial cells

The method used was same as the isolation of *V. cholerae* O1OMP M094V using N-Octyl-ß-D-glucopyranoside (NOG) with 0.05% concentration (9) which modified with 1% CHAPS. Determination of the protein profile of intestinal epithelial cells was performed by electrophoresis using standard SDS-PAGE (5).

Detection of S. dysenteriae adhesin protein and its receptor protein in mice intestine epithelial cells.

The method used in this research was same as t previous study (10). The bacteria were cultured until the concentration achieved 10^8 bacteria/ml. Furthermore, as much as 1 ml of bacterial suspension washed with a solution of PBS pH 7.4 and then centrifuged at 6000 rpm for 5 minutes at 4°C. The precipitate was suspended into suspension of extra-cellular matrix protein of mice enterocytes with 30 µg per ml concentration. Furthermore the suspension was incubated in water bath at 37° C with shaking at medium speed for 30 minutes, then centrifuged at 6000 rpm for 5 min at 4°C. The precipitate was separated from supernatant and the supernatant then electrophorized with SDS-PAGE, after 1 ml of sediment part was suspended with PBS at pH 7.4 The suspension of S. *dysenteriae* and mice extracelluler protein matrix were used as control, and the next step was to determine which proteins acting as bacterial adhesion protein and receptor respectively (10).

Electroelution.

Purification of protein from extracelllular matrix was performed by the electroelution method, according to the previous research (5). The desired protein band was inserted into the dialysis membrane, and the elution of protein band was done horizontally. Results of the elution was dialyzed for 48 hours.

Identification test of adhesion receptor molecule using dose respons method.

Shigella dysenteriae were breeded in BHI broth and suspended PBS until the concentration reached 10^8/ml. As much as 100µl of bacterial suspension were mixed with 100µl mucous protein, which was expected as receptor, and isolated in PBS which contain 1% BSA. It was then mixed with 100 ml of 10^6 mice enterocyte suspension per ml. The mixture was incubated in 37^{0}C for 30 minutes, and shaked gently. The bacterias which were not adhere would be discarded by repeated laudering. The enterocyte were gathered with 1500 rpm centrifugation for two minutes and suspended in 300 µL of PBS. A 20 µL of extract suspension was smeared on glass patchslide, and stained with Gram staining. Adhesion index was calculated under microscopic observation (11).

Gram staining

Staining was performed to describe the morphology of enterocytes and adhesion of *S.dysenteriae* on enterocytes. Slides were protected with crystal violet for one minute and rinsed with water. Iodine were applied for one minute followed by washing with 96% ethanol to the slides. Before and after covering with safranin for 30 seconds, slides were rinsed with water The slides were observed under light microscope at 1000x magnification.

III. RESULTS

The result of SDS-PAGE from *Shigella dysenteriae* whole cell, *Shigella dysenteriae* was coated with mice enterocyte extracellular matrix protein (figure1).

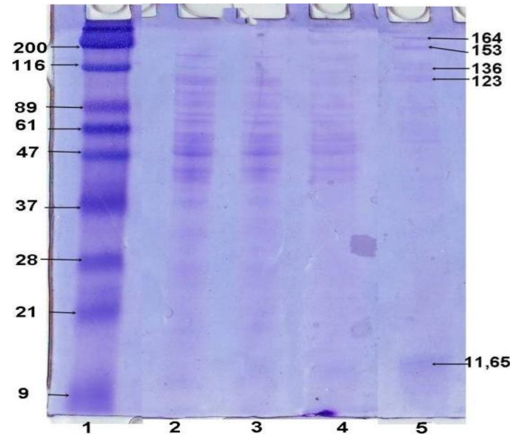
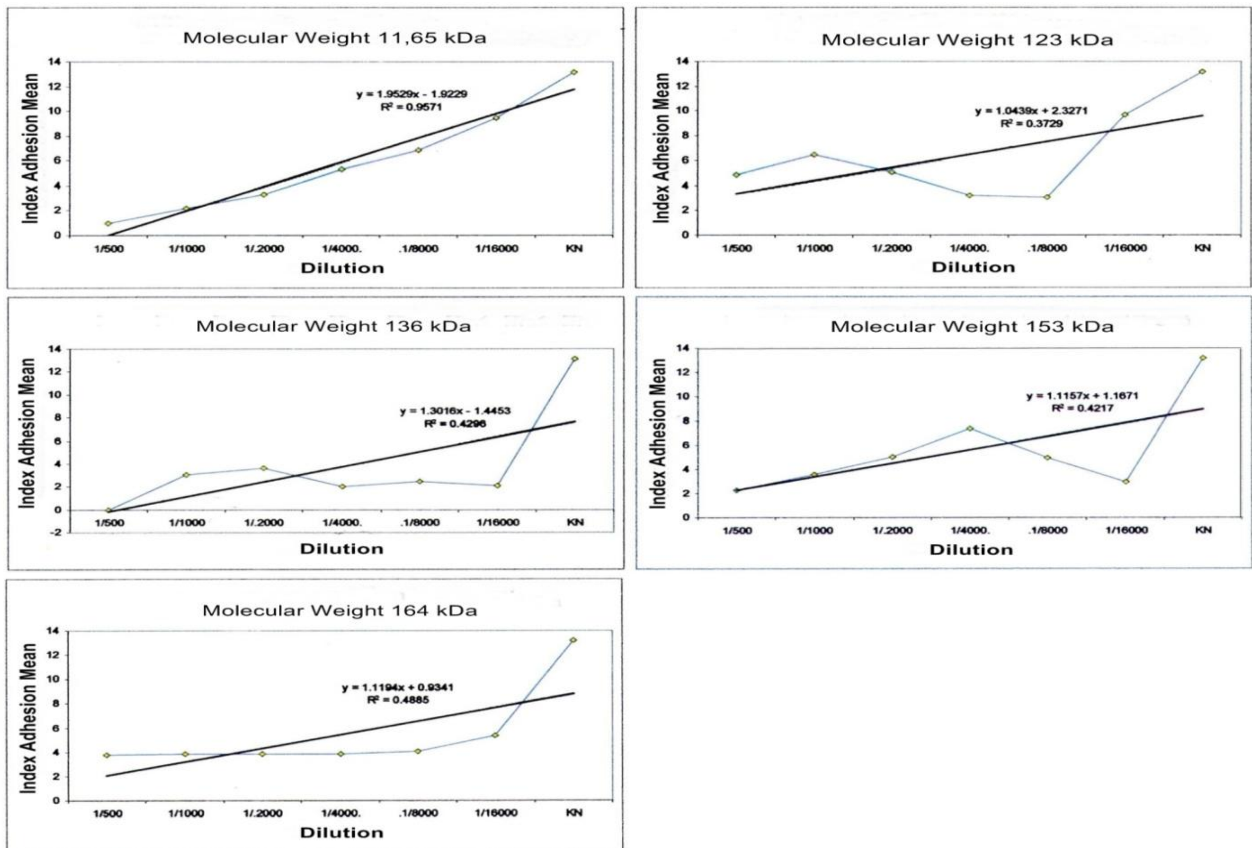


Figure 1 : Estimated molecular weight of *Shigelladysenteriae* protein receptor profile in mice enterocytes using SDS-PAGE

1stline : Tracer protein (Marker)
2ndline : *Shigella dysenteriae Whole cell*
3rdline : Extracellular enterocyte matrix supernatan
4thlilne : Extracellular enterocyte matrix supernatanwhich exposed with *Shigella dysenteriae* whole cell
5thline : Extracellular enterocyte matrix

On the SDS PAGE electrophoresis we found that there were five proteins with different molecular weight which suspected to be the receptor(s), those were 11.65 kDa, 123 kDa, 136 kDa, 153 kDa and 164 kDa respectively. The identification test of receptor protein of adhession molecule using dose respons method (Figure 2) showed that the protein with molecular weight of 11.65 kDa was the most consistent in giving response to the increasingdose, and the most likely to be the receptor (r=0.97, Regression analysis)



more number of bacteria attached to enterocyte

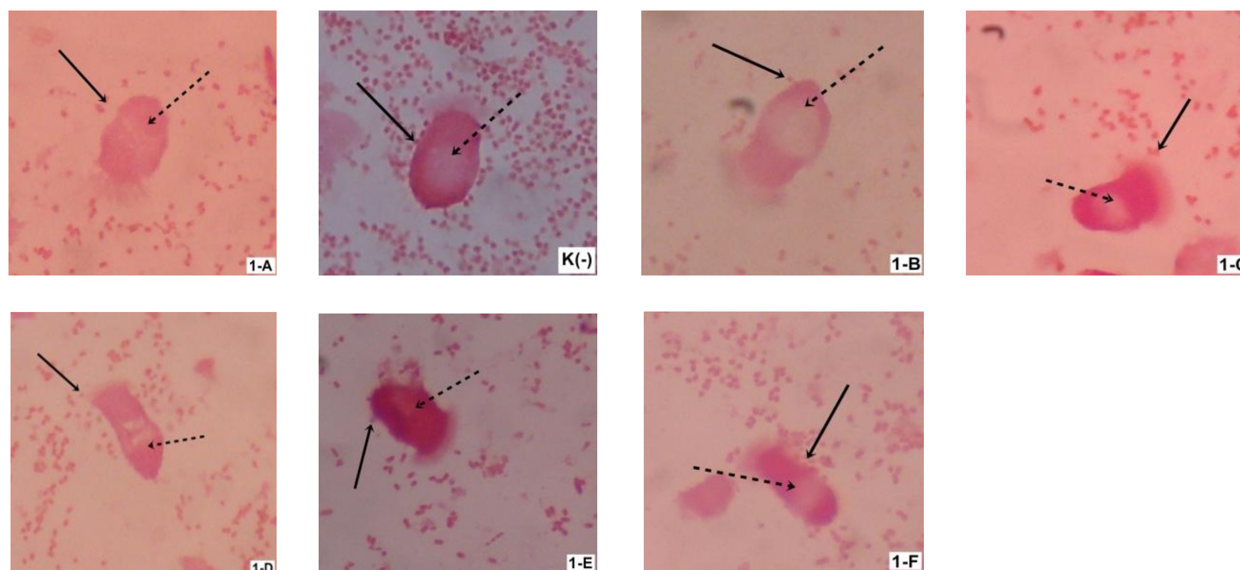


Figure 2: The dose response test of 11.65 kDa *Shigella dysenteriae* receptor protein to attach with enterocyte K(-) :negative control,. Mice enterocyte coated by mice epithelial protein membrane with dilutions of 1/500 (1-A), 1/1000 (1-B), 1/2000 (1-C), 1/4000 (1-D), 1/8000 (1-E), 1/16.000 (1-F),
- -> enterocyte; →*Shigella dysenteriae*..

IV. DISCUSSION

Enterocyte protein isolation was done based on the former success of mice enterocyte cell isolation research (12). The isolation method is done the some way like research which used NOG 0,5%, and modified by using carp 1% to get the mice intestine epithelial extracellular matrix (12), continued with SDS-PAGE. The experiment results can be seen in figure 1, in seventh protein strip, with molecule weights are 11,65 kDa,123 kDa,136 kDa,153 kDa,164 kDa.Receptor molecule weight from those experiment can be estimated with doing such research, by modification to find hemaglutinin *V* protein molecule weight*, cholerae* T79-6 (13). It was found that protein strip from mice enterocyte extracellular matrix is "move" to the bacteria cell suspension, and vice versa. The protein strip which move, was originated from enterocyte extracellular protein matrix which weight is 11,65 kDa. While there is no movement in protein which weights were 123 kDa,136 kDa,153 kDa,164 kDa. The protein that derived from bacteria suspension which move and not prominent to intestine cell protein molecule suspension. The estimation of the mice receptor protein molecule weight in this research is 11,65 kDa. Receptor protein was a protein which can hamper bacterial adhesion to the receptor cell if the protein is attach to bacteria. It is still needed further research with isolation and purification to assure the estimation of the protein. This pure protein isolation can be one with electroelution (5). The next step was adherance test, like this experiment. Figure 2 and 4 has shown that protein with 11,65 kDa in weight was estimated as *Shigella dysenteriae*receptor. Based on ANOVAtest, the significance value is 0.000 ($p<0,05$), so Ho is rejected, it was inferred that there is a difference between the amount of adhesion index in enterocyte protein molecule which weight is 11.65 kDa, based on dilution groups.

Cell surface receptor is an adherance molecule which is responsible to the intercell interaction, or interaction between cells and extracellular matrix. This adherance molecules are classified into four main types: *integrins*, *cadherin*, *Ig.superfamily* and *selectins* .Receptor molecule types which already known as pathogen bacterias receptors are *carbohydrate residues* (extracellular matrix, *integrins*) for gram negative bacteria, RGD (*glicin-arginin-aspartic acid*), *binding integrins* for *Bordetella pertussis*, *fibronectin collagen* for *Yersinia,*and *fibronectin* and *lamelin* for *Mycobacterium* (14). Sperandio did an indirect research to determine protein receptor types by using Elisa method (15). This method is relatively difficult compare with this research method. The receptor molecules which act as the estimated antigen at Elisa method are: fibronectin, lamilin, collagen, and peptide which has 3 types of RGD amino acid sequences. It is inffered from the research that fibronectin, lamilin, and Dari hasil penelitian ini didapatkan bahwa *fibronectin, laminin* andpeptide which has 3 types of RGD amino acid sequences make bond with Omp *V. cholerae* O1 protein, which weight is 38 kDa, while there is no bond to collagen is found(12).It is still needed further research to find wether the same result will be found in mice epithelial cell or another epithelial cell, including human epithelial cell, if exposed *Shigella dysenteriae*.

V. CONCLUSION

Based on the SDS-PAGE, adhesion index test result, and ANOVA test, it is shown that protein which molecule weight is 11,65 kDa from mice enterocyte extracelluar matrix is *Shigella dysentriae* receptor. While mice enterocyte extracellular matrix which weight are 123 kDa, 136 kDa,153 kDa,164 kDa, are not *Shigella dysenterie* receptors.The ability of 11, 65 kDa protein receptor as an enterocyte blocker in *Shigella Dysenteriae* adhesion proccess is highly affected by its concentration.

VI. SUGGESTION

It is need further research to assure that protein 11, 65 kDa is a protein receptor, such as antibody which contain antiprotein receptor dan do molecular characterization of mice enterocyte protein receptor to *Shigella dysenteriae*

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