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Peptide Sequence of Pili Subunit Protein 49,8 kDa *Shigella flexneri* as Antigenic Epitope for Shigellosis Vaccine Development

Shigellosis Aşı Geliştirme için Pili Alt Birim Proteini 49,8 kDa Antijenik Epitopu, *Shigella flexneri* Peptit Dizisi

Pili Protein *Shigella flexneri* for Shigellosis
Shigellosis için Pili Protein *Shigella flexneri*

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ABSTRACT
Objectives: This study aims to investigate the amino acid sequence and identify antigenic epitopes of 49,8 kDa pili protein *Shigella flexneri*, which will be used as candidates for the Shigellosis vaccine.
Materials and Methods: Our study is prospectively descriptive laboratory. We used bacterial isolate *Shigella flexneri*. Pili isolation using pili cutter and SDS-PAGE electrophoresis. Analysis of amino acid sequences using the LC-MS/MS method in the proteomic laboratory. The target epitope antigenicity analysis was tested using Kolaskar and Tongaonkar Antigenicity software. Bepired Linear Epitope Prediction software is used for epitope mapping. Visualization of proteins and molecular docking used PymOL software. Peptides and antibodies were hemagglutinations test and immune response tested using the dot blot method.
Results: LC-MS/MS analysis results from the mascot server shows that the 49,8 kDa pili protein is *Shigella flexneri* similar to the flagellin protein of *Shigella flexneri* 1235-66 (ID I6H2T2). The results of antigenicity analysis and epitope mapping showed that areas of

protein that have the most potential and antigenic epitopes are regions 98-111 and 263-290 with the sequence of amino acid sequences QSSTGTNSQSDLDS (Q-S) and DTTITKAETKTVTKNQVVDTPVTTDAAK (D-K). The results of the molecular docking interaction test between the peptide and the B cell receptor have a low binding energy. Peptide Q-S and peptide D-K antigens are hemagglutinin molecules because it can agglutinate erythrocytes. The immune response between peptide antigens and anti-peptide antibodies can react based on color gradations in the dotblot method.
Conclusion: The amino acid sequences QSSTGTNSQSDLDS and DTTITKAETKTVTKNQVVDTPVTTDAAK are potentially antigenic epitopes. These peptides can be used to develop as candidates for Shigellosis vaccine.

Key words: *Shigella flexneri*, pili protein, antigenic, epitope

ÖZ
Amaç: Bu çalışma, amino asit dizisini incelemeyi ve Shigellosis aşı adayı olarak kullanılacak olan Pili Alt Birim Proteini 49,8 kDa Antijenik Epitopu *Shigella flexneri'*yi belirlemeyi amaçlamaktadır.
Gereç ve Yöntemler: Bu çalışma, prospektif olarak tanımlayıcı bir laboratuvardır. Bakteri izolatı Shigella flexneri kullandık. Pili izolasyonu pili kesici ve SDS-PAGE elektroforezi kullanmaktadır. Amino asit dizilerinin analizi proteomik laboratuvar ile LC-MS/MS yöntemi kullanmaktadır. Hedef epitop antijenite analizi, Kolaskar ve Tongaonkar Antigenity yazılımı kullanılarak test edilmektedir. Epitop haritalama için Bepired Linear Epitope Prediction yazılımı kullanılmaktadır. Proteinlerin görselleştirilmesi ve moleküler yerleştirmesi PymOL yazılımı.kullanmaktadır. Peptitler ve antikorlar hemaglütinasyon testine dahildir ve bağışıklık tepkisi the dot blot yontemi kullanarak test edilmiştir.
Bulgular: Mascot sunucusundan alınan LC-MS/MS analiz sonuçları, Shigella flexneri 49,8 kDa pili proteini, Shigella flexneri 1235-66(ID I6H2T2) flagellin proteine benzer olduğunu gösterir. Antijenisite analizi ve epitop haritalama sonuçları, en çok potansiyelli ve antijenik epitoplara sahip protein alanlar, QSSTGTNSQSLDDS (Q-S) ve DTITKAETKTVTKNQVVDTPVTTDAAK (D-K) amino asit dizileri ile 98-111 ve 263-290 bölgeler olduğunu göstermiştir. Peptit ve B hücresi reseptörü arasındaki moleküler kenetlenme etkileşimi testinin sonuçları, düşük bir bağlanma enerjisi göstermektedir. Peptit Q-S ve peptit D-K antijenleri, eritrositleri aglutine edebildikleri için hemaglutinin molekülleridir. Peptid antijenleri ve anti-peptid antikorları arasındaki bağışıklık tepkisi, the dot blod yöntemindeki renk geçişlerine dayalı olarak reaksiyon gösterir.
Sonuç: QSSTGTNSQSLDDS ve DTTITKAETKTVTKNQVVVTPVTTDAAK amino asit dizileri potansiyel olarak antijenik epitoplardır. Bu peptitler, Shigellosis aşısı adayları olarak geliştirilebilir.

Anahtar kelimeler: *Shigella flexneri*, pili proteini, antijenik, epitop.

INTRODUCTION

Shigellosis is an acute intestinal infection. The symptoms can range from mild diarrhea to severe inflammation. The characterization of bacillary dysentery is stomach cramps, fever, bloody stools, and mucus, especially in toddlers.[1,2] Infection occurs globally, and in all people of all ages, endemic diseases occur in children aged 1-4 years, especially those living in low income and income middle areas.[3]
Research conducted by Sumarno et al.[4] shows that in Pili *Shigella dysenteriae* contains a molecular weight hemagglutinin protein of 49,8 kilodalton (kDa) adhesin protein. Besides, there are other proteins found 7,9 kDa subunit protein, which is an anti-hemagglutinin. Both

of these proteins are adhesin molecules in mice enterocytes. The results also founded that in *Shigella flexneri, Shigella sonnei* and *Shigella boydii* also found pili proteins with molecular weights of 49,8 kDa and 7,9 kDa.[5]
The type and function of the protein of *Shigella flexneri* (*S. flexneri*) 49,8 kDa still unknown. We have to the analysis of amino acid sequences of the 49,8 kDa pili protein type *Shigella flexneri*. Several studies have carried out secondary analysis and identification of epitope in adhesin proteins as vaccine candidates. Pore et al.[6] conducted research of amino acid sequences on 34 kDa protein *Shigella flexneri* before recombinant. The results show that the 34 kDa protein is an OMPA protein from *Shigella flexneri*. Sharma et al.[7] performed an analysis of modeling prediction of epitopes on OMPs protein *Shigella flexneri* 2a. Research with 3D structural modeling has also carried out with a 38 kDa protein model, which is the OmpC *Shigella flexneri* 3a protein.[8] After knowing the expectation of the type of protein and the epitope, it is easier to do *cloning* to produce recombinant proteins. The purpose of this study was to determine the amino acid sequence and identify antigenic epitopes from BM 49,8 kDa pili protein *S. flexneri,* which we would like to use as candidates for the Shigellosis vaccine.

MATERIALS AND METHODS

Shigella flexneri bacteria
Our study is a descriptive study conducted in the laboratory for the identification and exploration of the 49.8 kDa *Shigella flexneri* pili protein epitope which is an adhesive molecule and has potential as a shigellosis vaccine candidate. Bacteria used in this study were *Shigella flexneri*. Bacteria cultured *Mac Conkey, brain-heart broth* (BHI) and TCG medium.[9] The results of bacterial collection on TCG media were collected and then shaved used a *pili cutter.* The isolated pili were then electrophorized to monitor the weight molecular of 49,8 kDa protein.
Animal and Antigenic Peptides
We used 10 male mice (Mus musculus) Balb/C 6-8 weeks old. *Mus musculus* was obtained in the Experimental Animal Laboratory, Faculty of Medicine, Universitas Brawijaya, Indonesia. We use antigenic peptide was chemically produced. We purchased the antigenic peptides through the Apical Scientific Sdn. Bhd (Malaysia) in the form of synthetic peptides.
Amino acid sequence, Antigenicity identification and epitope mapping of 49,8 kDa pili protein Shigella flexneri
We processed an amino acid sequence used *the in-gel digestion method* and analyzed *Mass Spectrometry* (LC-MS/MS). The amino acid analysis from MASCOT SERVER (https://sysbio-mascot.wehi.edu.au/mascot). Analysis of the identified antigenic protein carried out used the approach *in silico* bioinformatics with the *Kolaskar and Tongaonkar Antigenicity* software (http://www.iedb.org) with values threshold (*threshold value*) 1.0.[10] *Epitope Mapping* using *Linear Epitope Prediction Bepired* with a threshold value *(entry)* 0.35 of IEDB. The structure of proteins with epitope regions visualized by *software Pyre* and *PyMOL.*[11,12] Visualization of 3D structures resulting from molecular docking between peptides and B cell receptors (BCR) (PDB ID: 5IFH) used PyMol software (https://pymol.org/2/).

Production of Serum Antibody Pili protein epitope 49.8 kDa Shigella flexneri
We used 5 mice per epitope for antibody production. The dose for immunization of each epitope is 50 µg in a volume of 100 µL. The epitope of pili protein 49.8 kDa *Shigella flexneri* was emulsified with Complete Freud's Adjuvant (CFA), and then 100 uL was injected intraperitoneally. Weekly boosts were performed using antigens emulsified with Incomplete Freud's Adjuvant (IFA) at the same dose. The blood was taken from the heart one week after

the last booster. The blood was placed in a sterile tube and centrifuged at 10,000 rpm for five minutes. Then the serum was collected for further examination.

Hemagglutination test

We used two epitopes in the form of synthetic peptides to test for hemaagglutination, namely QSSTGTNSQSDLDS (Q-S) and DTTITKAETKTVTKNQVVDTPVTTDAAK (D-K). As well as serum antibodies from synthetic peptides that have been produced in mice. Samples IgG diluted half a series in a well-contained microplate V with a volume of 50 µl each in their wells a dilution solution used in PBS pH 7,4. Furthermore, in each of the wells, a synthetic peptide antigen of 50 µl. Then we incubated in a water bath by shaking 60 times a minute at 370C for half an hour. After our incubation period completed in each well, we added 50 ml of mice's blood cells to a concentration of 0.5%. We read the results of the agglutination inhibition reaction after incubation at room temperature for 1 hour. As a negative control, there is a hemagglutination inhibition reaction used pre-serum.[5,13]

Dot blot test

We used also two epitopes in the form of synthetic peptides to test for dot blot, namely QSSTGTNSQSDLDS (Q-S) and DTTITKAETKTVTKNQVVDTPVTTDAAK (D-K), as used in the hemagglutination test. Dot blot test with immersed the nitrocellulose membranes in sterile H2O for 30 minutes. the membrane, dripped with 50 µl antigen (synthetic peptide), is incubated overnight at 40C. The membrane with primary antibodies was 50 µl, set for 2 hours at room temperature. Secondary antibody added with 1: 500 dilution in FFB solution, incubated at room temperature for 1 hour. Chromogen substrate was added and incubated at room temperature for 30 minutes. We stopped the reaction by adding H2O-positive. Quality results are seen based on color gradations.[13]

This research has obtained a statement letter from the ethics commission of Universitas Brawijaya with letter number: 1192-KEP-UB.

RESULTS

Characterization of pili 48,9 kDa protein of Shigella flexneri

SDS-PAGE identified *Shigella flexneri* pili protein. Pili protein profiles were generated from the first to third cut of pili proteins, as shown in (Figure 1).

The analysis amino acid sequence of the protein of 49,8 kDa pili Shigella flexneri

Analysis *Mascot server* showed that 49,8 kDa protein *Shigella flexneri* have homology with *flagellin* protein belonging to *Shigella flexneri* 1235-66 (ID I6H2T2), with a query coverage of 18% and a molecular weight of 51,75 kDa (Table 1).

Antigenicity analysis and epitope mapping

Analysis results from antigenicity of protein did with the *Kolaskar and Tongaonkar antigenic software* (Figure 2A and Table 2). Analysis of *epitope mapping* used *The Bepired Linear Epitope Prediction software* shows that some regions have epitopes shown in the yellow area in Figure 2B. Some of these epitopes have high scores as potential antigenic epitopes and areas adhesin molecules in the regions 98-111 and 263-290 with amino acid sequence QSSTGTNSQSDLDS and DTTITKAETKTVTKNQVVDTPVTTDAAK (Table 3; *highlight* yellow).

Modeling and visualization flagellin proteins

Results of modeling proteins structure with antigenic regions and areas epitope visualized by *Pyrx* and *PyMO.* Based on the visualization results, areas that have potential epitopes are in the order of 98-111 and 263-290. Known areas with antigenic potential at positions 276-283 are potential epitopes in region 263-296 (Figure 2C. wire; red and yellow).

Docking molecular visualization and interaction
Two peptides which were considered as potential epitope were predicted for binding interaction between peptide antigen and B cell receptor. Molecular docking simulation was performed by interacting BCR-peptide (Figure 3).

Antigenic Peptides
The result of insilico analysis of 49.8 kDa protein similar to flagellin protein, we selected two epitopes that were considered as potential epitopes. The characteristics of these epitopes are presented in Table 4.

Hemagglutination assay of epitope pili subunit protein
For the antigens' determining ability to agglutinate erythrocyte cells, we used the hemagglutination test (Figure 4A). The results show the function of the anti-hemagglutination test. Antibodies can determine in inhibiting antigens' ability to agglutinate erythrocytes (Figure 4B).

Immune response test using the dot blot method
The dot-blot method results showed that the most effective immune response to peptide Q-S antigen-antibody occurred at 1/500 and 10 ng dilutions (Figure 5A). The most significant result of the immune response to peptide D-K antigen-antibody occurred at 1/1000 and 1µg dilutions (Figure 5B).

DISCUSSION
The results of the study using a bioinformatics approach to identify antigens in several serotypes of *Shigella* spp. Shows the results of the identification of many peptides in Shigella bacteria that are immunogenic.[14] Bioinformatics serves to design vaccine candidates and can also be used to analyze the mechanism of bacterial resistance to drugs.[15]
The profile band of the *Shigella flexneri* clearly shown that it has a molecular protein weight of 49,8 kDa as adhesion protein. Indicated that the hemagglutinin and an adhesion protein in *Shigella dysenteriae* and *Shigella flexneri.* have a molecular weight of 49,8 kDa.[4,5] Adhesins are proteins that can attach to cells receptors. This protein also can clump the enterocyte cells.[9]
Pili protein 49,8 kDa of *Shigella flexneri* analyzed with LC-MS/MS an in-gel digestion approach. Analysis *Mascot server* showed that 49,8 kDa protein *Shigella flexneri* has homology with *flagellin* protein belonging to *Shigella flexneri* 1235-66 (ID I6H2T2), with a query coverage of 18% and a molecular weight of 51,75 kDa. Accession number I6H2T2 which is the ID of the uniprot database. we use database uniport because our study is about proteomics. However, the data is the same as the protein in the NCBI database with Accession number EIQ75074.1 (Table 1). *Flagellin* is a structural component that helps motility bacterial. This ability helps bacteria to avoid the immune system and avoid harmful components in the host.[16,17] As a virulent factor for gram-negative pathogenic bacteria, Flagellin is responsible for several functions such as movement, adhesion, and invasion.[18] Shigella (flash) flagella has similarities to the flagellin *Escherichia coli*, *Salmonella* spp., and *Proteus mirabilis*. The results of the study indicate that Shigella is capable of forming flagella.[19] Shigella bacteria showed four out of 12 strains of *S. boydii* have fliC gene as protein-coding flagellin similar to *S. flexneri*.[20]
The analysis of protein antigenicity *in silico* with the *Kolaskar* and *Tongaonkar antigenicity software* on flagellin proteins show that the protein is very immunogenic because it has peptide regions that have potential antigenic. These results follow Utami et al.[9] research,

which states that the 49,8 kDa *Shigella flexneri* pili protein is an adhesin protein that can increase the intestinal immune response mucosa. Results of analysis *epitope mapping* in *silico* used the *Bepired Linear Epitope Prediction* software showed that the protein *flagellin* epitope there are some regions. The immune system can recognize those epitopes following the yellow area in Figure 2B. After scoring, some epitopes are identified based on antigenic epitopes two, and the most potential is in the regions of 98-111 and 263-290 with the sequences QSSTGTNSQSDLDS (Q-S) and DTTITKAETKTVTKNQVVDTPVTTDAAK (D-K). A study has successfully identified the IpaC protein parts and the IpaD protein *Shigella flexneri* 2a, which are epitopes of that protein.[21,22] 3D structural modeling can predict the presence of antigenic peptides or epitopes from the OMP protein *Shigella flexneri* 2a and *Shigella flexneri* 3a.[7,8]

Based on analysis of visualization used Pymol software shows the model of the structure of the flagellin protein with antigenic regions peptides in the amino acid sequence DTTITKAETKTVTKNQVVDTPVTTDAAK appear to have the same area: the antigenic region and the epitope location. The peptide is considered a potential epitope. Further analysis used software for adhesin prediction shows that the peptide QSSTGTNSQSDLDS is an adhesive region. These results provide the hypothesis that receptors will recognize the peptide on the surface of the host cell. On the surface of the host cell are specific proteins called receptors. The bacterial adhesive can be glycoprotein or lipoprotein found in fimbriae or pili.[23]

We performed docking analysis between peptides with B cell receptors or BCRs. Molecular docking aims to determine vaccine candidate peptides that have low binding energy. The docking results show that pep_1 and pep_2 have a low average binding energy, this allows the initiation of a biological response, namely activation of the B cell receptor (BCR) capable of triggering an immune response in B cells to produce specific antibodies. The PatchDock and FireDock programs are significantly faster and perform slightly better than other programs because they can overcome protein flexibility. Docking applications can be used for polypharmacology prediction, drug use, fishing targets and profiling.[24,25,26]

We ordered the antigenic peptides from the insilico analysis in the form of synthetic peptides. We use peptides with purity crude because this is a preliminary study to prove our peptides as potential ingredients for vaccine candidates. So that later we can use a higher purity if our current results are promising. The amino acid sequences we use are soluble in water, making them easy to dissolve in solvents such as PBS (Table 4). We injected peptides into experimental animals for the production of serum antibodies. Our immunizations were administered intraperitoneally using Complete Freud's Adjuvant (CFA) and Incomplete Freud's Adjuvant (IFA) to facilitate peptides to dissolve in the blood.

Our hemagglutination analyzes use two antigenic peptides and serum antibodies from both. Antigenic peptides to test for hemaagglutination, namely QSSTGTNSQSDLDS (Q-S) and DTTITKAETKTVTKNQVVDTPVTTDAAK (D-K). The results of the hemagglutination test showed a difference in erythrocyte agglutination. We can observe from Q-S peptides and D-K peptides. The Q-S peptide antigen is capable of agglutination at 1/4 titer. Meanwhile, the D-K peptide showed agglutination at 1/32 titer. These results prove that the Q-S and D-K peptide antigen can bind to erythrocyte cells or known as hemagglutinin molecules. The peptide antigen used is the epitope of *Shigella flexneri* bacteria's pili protein with a molecular weight of 49,8 kDa which is an adhesive protein.[9] The anti-hemagglutinin test carried out used serum Q-S peptide antibodies against Q-S peptides, and D-K peptide antibodies against D-K peptides. The Q-S peptide antibody can inhibit Q-S peptide antigen starting at 1/128 dilution. While the D-K peptide antibody was able to inhibit the D-K peptide antigen starting from 1/16 dilution. Sediment that occurs at the bottom of the well shows antihemagglutination results.[13]

Process of immunoblotting analysis used dot blot analysis.[27] The dot blot test also uses the same two antigenic peptides and antibodies as in the hemagglutination test. Antigen-antibody reaction of Q-S peptide and D-K peptide antibodies by reacting to Q-S peptides and D-K utilized, the dot blot method. The purplish-blue marked positive dot blot test results between Q-S peptide antibody with Q-S peptide and D-K peptide antibody with D-K peptide. The results of the research used the dot blot method in this study are following the results of previous studies, namely that the synthesis of peptide A-K antigen from the 49,8 kDa *Shigella flexneri* pili protein can carry out an immune response with its serum antibodies.[13] The reaction of the peptide with the antibody will cause a color gradation of the dot blot results and quantitatively use Corel photo paint.[28] Our study did not adhesion test between antigen with enterocyte cells as a confirmation method to prove an antigen is an adhesion molecule, as did Milliana et al.[29] who proved that the 28 kDa OMP protein of *S. flexneri* is an adhesion protein.

CONCLUSION

Pili protein 49,8 kDa has potential antigenic epitopes, namely QSSTGTNSQSDLDS and DTTITKAETKTVTKNQVVDTPVTTDAAK peptide. Both peptides are hemagglutinin molecules.

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Table 1. Results of amino acid analysis of the 49,8 kDa protein pili using the LC-MS/MS method on mascot server that has homology with the *flagellin* protein of *Shigella flexneri*

Accession Number (Uniprot/NCBI)	**Protein**	**Query coverage (%)**	**MW (Da)**	**Subcellular location**
I6H2T2/ EIQ75074.1	*Flagellin* [*Shigella flexneri* 1235-66]	18	51755	Secreted

MW: Molecular weight, Da: Dalton

Table 2. Analysis of antigenicity *flagellin* protein used *Kolaskar* and *Tongaonkar* software

Protein Identification	**Accession number**	**Start**	**End**	**Peptide**	**Length**
Flagellin [*Shigella flexneri* 1235-66]	I6H2T2	23	31	SSLSSAIER	9
		92	100	VRELAVQSS	9
		109	115	LDSIQAE	7
		134	141	GVKVLAKD	8
		168	178	LGLDSLSVQDS	11
		182	189	TATVVGAG	8
		225	231	GQHYVNI	7
		255	261	GAVVIGA	7
		276	283	KNQVVDTP	8
		289	296	AKALVDAG	8
		325	336	ALKVDDKYYAAD	12
		344	350	AKTVAYT	7
		356	364	SKEAAVQFG	9
		372	388	IATVGGKQYLASSVKDH	17
		405	422	ESPLAKIDAALAKVADLR	18
		424	429	DLGAVQ	6
		469	482	NILQQAGTSVLAQA	14

Table 3. Identification of epitopes *Shigella flexneri's* flagellin protein used *Bepired Linear Epitope Prediction* software

Protein Identification	**Accession number**	**Start**	**End**	**Peptide**	**Length**
Flagellin [*Shigella flexneri*	I6H2T2	232	257	TDSTSTDPGKGNGMYKATIDPDTGAV	26

1235-66]				
	98	111	QSSTGTNSQSDLDS	14
	175	224	VQDSYKTTATVVGAGTYKDGVTITAPT QGEIDAAVGGTAGEGKATVEFKD	50
	39	49	NSAKDDAAGQA	11
	347	358	VAYTDDKGVSKE	12
	296	305	GVTGATDTNT	10
	263	290	DTTITKAETKTVTKNQVVDTPVTTDAAK	28
	149	155	GANDGET	7
	232	257	TDSTSTDPGKGNGMYKATIDPDTGAV	26
	63	84	QASRNANDGISIAQTTEGSLSE	22
	400	409	ATAKTESPLA	10
	481	487	QANQTTQ	7
	451	465	SRIEDADYATEVSNM	15
	313	321	EDKNGKVID	9
	331	343	KYYAADYKDGKIT	13

Table 4. The characteristics of antigenic peptides determined by in silico analysis

Name	Sequence of Peptide (Epitope)	Length	Formula	Purity	Solubility
Peptide1	QSSTGTNSQSDLDS (Q-S)	14	$C_{53}H_{87}N_{17}O_{29}$	Crude	Soluble in water
Peptide2	DTTITKAETKTVTKNQVVDTPVTTDAAK (D-K)	28	$C_{126}H_{218}N_{34}O_{48}$	Crude	Soluble in water

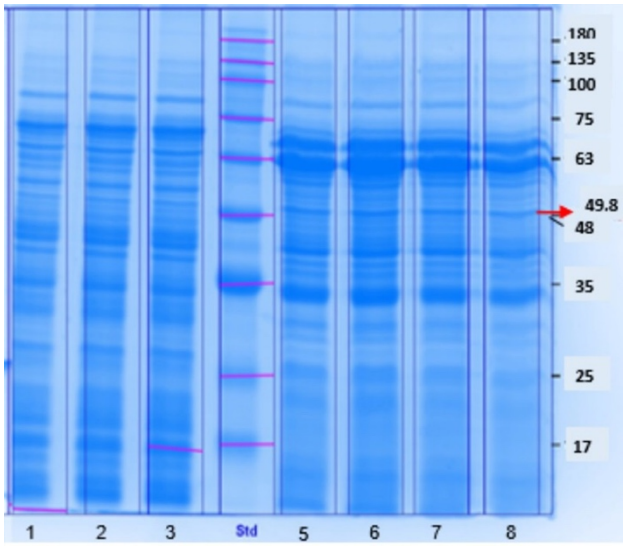


Figure 1. Electrophoresis results in a molecular weight of 49,8 kDa pili protein *Shigella flexneri.* Pili protein profiles have various molecular weights. Column 1,2,3 results from the 3rd pillar cut; 5.6 results from the 2nd pili cut; and 7.8 results from pili first cut

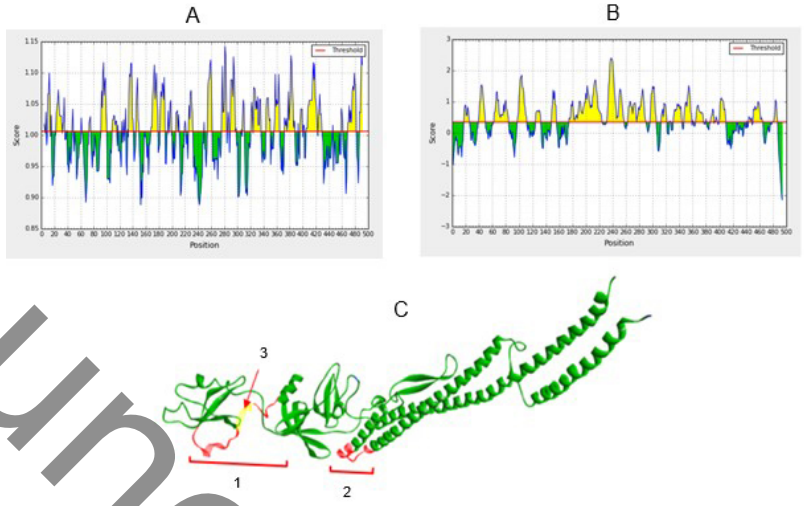
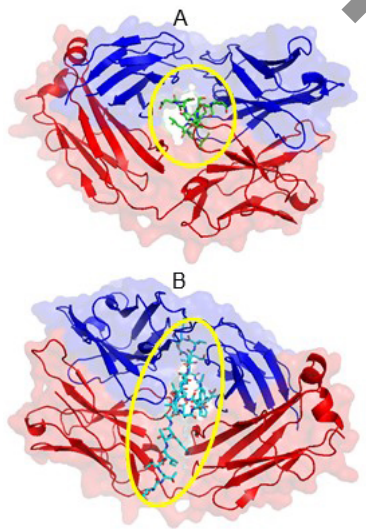


Figure 2. Analysis graph prediction of antigenicity, epitope mapping and visualization of the *Shigella flexneri* flagellin protein. A. Antigenicity analysis (the yellow graph shows the lively antigenic areas, green shows antigenic negativity); B. Epitope mapping analysis (The yellow graph shows areas with potential epitopes, green indicates negative potential epitopes); C.Visualization of protein structures are characterized by (ribbon; green) with antigenic regions (line; yellow) and epitopes (wire; red). The potential epitope shows in areas C1 (98-111), C2 (263-290) (wire; red), and antigenic C3 (276-283) (line; yellow)



C

Peptide	Global Energy	Van der Waals		Hydrogen Bond
		Attractive	Repulsive	
Pep_1	-33,23	-23,66	2,84	-2,94
Pep_2	-42,43	-38,02	18,58	-3,18

Figure 3. Visualization of the 3D structure of the molecular docking analysis used PyMol software (https://pymol.org/2/). A. Pep_1 vs BCR; B. Pep_2 vs BCR; C. Value of binding energy peptide and BCR. The yellow circle indicates the location of the peptide when it binds to BCR.
Pep_1: QSSTGTNSQSDLDS, Pep_2: DTTITKAETKTVTKNQVVDTPVTTDAAK

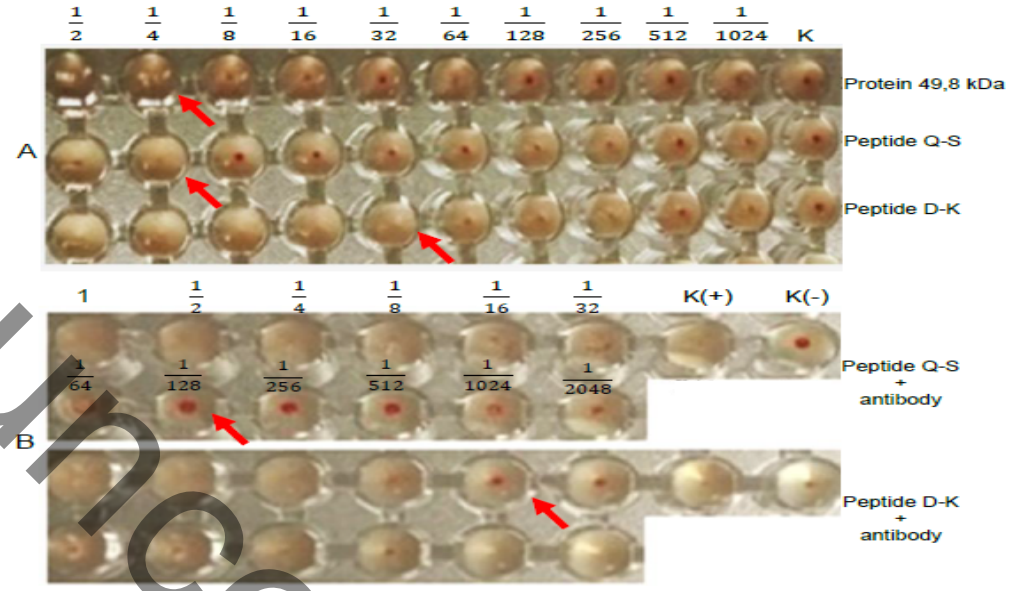


Figure 4. The results of hemagglutination and hemagglutination inhibition examination. A. The dilution used for the hemagglutination test of 49,8 kDa pili protein, Q-S peptides and D-K peptides are 1/2 - 1/1.024 (positive agglutination is indicated by a red arrow); B. The dilution used for the antihemagglutination test for Q-S peptides and D-K peptides is 1 - 1/2.048 (positive antiagglutination is indicated by a red arrow)
Q-S: QSSTGTNSQSDLDS, D-K: DTTITKAETKTVTKNQVVDTPVTTDAAK, K: Control

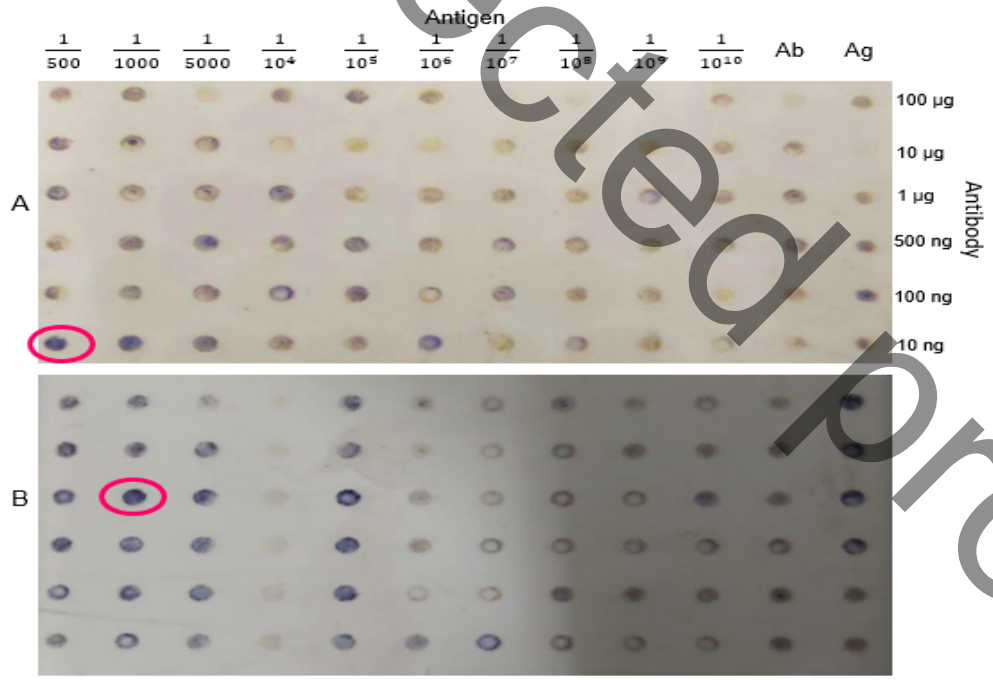


Figure 5. The results of the immune response antigen and antibody used the dot blot method. A. Q-S peptide; B. D-K peptide. More purplish blue indicates the stronger the immune response (red ring)