THE IMMUNOGENICITY OF FLAGELLIN PROTEIN OF SALMONELLA TYPHI OF SEMARANG AND SALATIGA ISOLATES

by Sri Darmawati

Submission date: 07-Oct-2021 08:54PM (UTC+0700)
Submission ID: 1667751727
File name: CONFERENCE_INTERNASIONAL_FLAGELLIN_PROTEIN.pdf (320.13K)
Word count: 5052
Character count: 25759

THE IMMUNOGENICITY OF FLAGELLIN PROTEIN OF SALMONELLA TYPHI OF SEMARANG AND SALATIGA ISOLATES

Sri Darmawati[1*], Aprilia Indra Kartika[2]
ciciekdarma@unimus.ac.id

[1]*Microbiology Laboratory, Health Analyst Study Program, Nursing and Health Science Faculty, Muhammadiyah University of Semarang, Central Java Indonesia*
[2]*Molecular Biology Laboratory, Health Analyst Study Program, Nursing and Health Science Faculty, Muhammadiyah University of Semarang, Central Java Indonesia*

ABSTRACT

Background*; Salmonella typhi (S.typhi) is the cause of typhoid fever infection. Its pathogenicity is influenced by the adhesion ability of S.typhi's flagella to the host cells. Each flagellum's adhesion ability is varied, influenced by the flagellin protein type and its interaction with the host.*
Objectives; *at finding out the immunogenicity of flagellin protein of S. typhi of Semarang and Salatiga isolates.*
Methods; *The samples are flagellin protein coded by fliC 1500bp gene (Semarang isolate) and fliC 1262 gene (Salatiga isolate), each is used as an antigent for the immunization of 8-weeks-old male Balb C mice.*
Results*; The research indicate that there are different sequences of flagellin protein-coding fliC gene between Semarang and Salatiga isolates and these influence the flagellin protein types expressed. From NO content testing, it is found that the generated phagocytosis activity of macrophages, and antibody level and type are higher in mice induced with S.typhi's flagellin from Semarang isolate than the one with S.typhi's flagellin from Salatiga isolate.*
Conclusions; *The immunogenicity level of S.typhi's flagellin protein from Semarang isolate is stronger than Salatiga isolate.*

Keywords: *Salmonella typhi, flageLlin, Typhoid fever, Immunogenecity.*

INTRODUCTION

Bacterium Salmonella typhi (S. typhi) is the cause of typhoid fever infection. Typhoid fever still continue to be a public health problem in the world, particularly in developing countries, including Indonesia (Vollaard et al., 2005). It's pathogenicity is highly dependent on a number of virulence factors, such as ability to adhere to host cells, flagella, enzyme, toxin, bioactive factors, which will facilitate the bacterium to adhere to small intestinal mucosa, invade, multiply and spread into lymphoid tissue up to blood circulation and circulate throughout the body to liver, bone marrow, spleen, gallbladder and Peyer's patch(Alexan, Mohamed and Ibrahim, 2009). The flagella that a bacterium owns will facilitate it to get into a cell or get out of the cell, since this flagella plays a role in bacterial motility (Hatta, Andi R. Sultan, et al., 2011).

Bacterium S. typhi is distinguished into 2 serotypes based on the flagellum antigen expressed. Both antigens also have different antigenicities (Bishop et al., 2012). Salmonella typhi of H1 serotype expresses H1(fliC) flagellin gene which is in the chromosome, i.e. Hd antigen, and H2 serotype expresses H2(fljBz66) flagellin gene which is in the linear plasmid, i.e. Hj antigen (Bishop et al. 2012; Scott & Simon 1982). fljBz66 gene is a fliC gene which experiences deletion in its central parts at 261bp, i.e. the antigenically determinant part (Hatta, Andi R Sultan, et al. 2011; Frankel et al. 1989). Antigen Hd is found in S. typhi throughout the world, meanwhile the antigen Hj can only be found in isolates from Indonesia (Frankel et al., 1989). Most Salmonella have these two genes, yet only one gene is expressed at one time (Sabir et al. 2014; Frankel et al. 1989).

Bacterium S. typhi of Salatiga isolate (SLT-1) has (fliC) a flagellum gene of 1262bp, and S. Typhi's fliC gene of Semarang isolate (BA07.4) is 1458bp in size, indicating different protein profile (Darmawati, Santosa and Prastiyanto, Muhammad Evy, 2015). The flagellum consisting of many protein subunits is immunogenic, i.e. capable of causing adaptive immune response, hence it can trigger the occurence of anti flagellin antibody (Alexan, Mohamed and Ibrahim, 2009). Flagellin will also induce iNOS (inducible NO synthase) gene

transcription by activating the iNOS promoter, which eventually might stimulate macrophages to produce NO. Flagellin protein can also increase the level of antimicrobial substance such as lysozim enzyme which plays a role in lysing bacterial cell walls and increase the phagocytosis activity of dendritic and macrophage cells (Sano et al., 2007). The different length of flagellin-coding gene DNA and different flagellin protein profile in S. typhi bacteria of Salatiga isolate (SLT-1) and S. typhi's fliC gene of Semarang isolate (BA07.4) are the background of this research to distinguish their immunogenicity levels in hosts. Therefore, this research aims at distunguishing the immunogenicity of flagellin protein of two S. typhi isolates which have different flagellum gene sizes (Salatiga and Semarang isolates).

METHODS

Bacterial culture

The bacterial culture is 2 strains of *S. typhi* (BA07.4 Semarang town isolate, SLT-1 Salatiga town isolate), which are the results of isolation of blood culture of Widal positive patients, which is then identified using API20E and API 50CHBE media (Darmawati, Sembiring and Asmara, 2011).

Isolation of Potein Flagellin and SDS-PAGE

The isuolation of flagellin protein uses modified method of Alexan et al. (2009). The flagellin protein is obtained by growing a bacterial colony from Mac Conkey medium in 50mL liquid BHI medium, incubated at 37˚C for 48 hours with agitation which is used as a starter. The starter is then put into 500 mL BHI mediyum, incubated at 37˚C for 48 hours with agitation. Furthermore, the bacterial culture is centrifuged at 4˚C, at 3000 rpm for 20 minutes. The pellet is suspended using 5mL physiological solution until it becomes a thick suspension, then the suspension acidity is made up to pH 2 by adding 1M HCl to it, stirred for 30 minutes at room temperature, centrifuged at 3000 rpm for 30 minutes. The supernatant containing flagellin protein is then added with 1M NaOH to make the pH 7.2.

The flagellin protein is then measured for its concentration using Biorat Assay reagent and for its absorbance at λ595nm using spectrophotometer visible, separated using SDS-PAGE method (12%) to see its protein profile, with coloring 0.1% *Coomassie Brilliant Blue R-250*.

Mice Immunization

30 8-weeks-old BalbC mice are kept under hygienic condition. 10 of these mice are immunized using physiological salt and act as the control, and 20 mice are immunized using flagellin protein (10 are immunized using flagellin antigen *S. typhi* BA 07.4, and 10 other using flagellin antigen *S. typhi* SLT-1) subcutaneously. Each of Balb C mice is immunized 4 times, the first one using 10μg/100μl PBS1X emulsified in *Freund adjuvant complete* (1:1) v/v, *booster* is made on 7th, 15th, 22nd days since the first day upon immunization, at the same doses yet in *Freund adjuvant in-complete* subcutaneously. The blood sample is taken from conjunctiva in the 30th day upon immunization, the serum is separated, ready to be analyzed for its antibody titer using Elisa method, then to figure out the antibody specificty, immunoblotting is used.

Isolation of macrophage and measuring the phagocytosis activity of macrophages

The isolation of peritoneal macrophage from mice is done 15 days after the first immunization. The method of isolating macrophage: 5mL cold, sterile medium RPMI is provided in 6mL syringe. The killed mouse is laid on their back, then sprayed with alcohol 70% on the skin surface of its stomach. The fur is tidied up by fondling it using hand towards the tail. The skin on the stomach is lifted using a tweezer, scissored and separated between its skin and meat, so that the meat on the stomach is visible. The medium RPMI is injected to the peritoneal caviry on the right and left stomach, then the stomach is massaged using fingers and left for 3 minutes to release the macrophages into RPMI medium. The macrophage suspension is taken back (aspired) using syringe carefully (to prevent the organs from being punctured). Afterwards, the macrophage suspension is immediately stored at 4˚ C, then it is centrifuged at 2000rpm, for 10 minutes at 4˚ C. The supernatant is disposed of, and the pellet is the macrophage. To this pellet 1mL complete grower medium (RPMI containing 10% FBS) is added, then it is resuspended and calculated for its density using hemocytometer.

Macrophage cells are prepared in RPMI complete medium whose density is $2.5X10^6$/mL. The macrophage cell suspension is then cultured at a well plate 24 (6x4) which has been given a round slip cover, put into each well at 200μL ($5x10^5$ sel) macrophage. Then, it is incubated in an incubator with CO_2 5%, at 37^0C for 30 minutes, and added

with RPMI complete medium at 800 μL so that the macrophage suspension volume in each well becomes 1 mL. The culture is incubated back in the incubator with CO_2 5%, at 37^{0}C for 2 hours, then the medium is disposed of, added with 1 mL complete media to each well and incubated back for 24 hours. The cultured macrophage is then washed 2 times using RPMI medium.

A latex suspension is prepared at 2.5X10^{7}latex/mL density, then it is put into PBS of 200μL (5x10^6) in each well. Afterwards, it is incubated in an incubator with CO2 for 1 hours, washed with PBS 3 times. It is then dried at room temperature, fixated with absolute methanol for 30 seconds. The methanol is then disposed of, and wound. Next, painting is done by adding Giemsa 20% for 20 minutes. It is then washed with distilled water until it is clean, then wound at room temperature. The slip cover is lifted from the well and examined using a microscope to calculate the phagocytosis activity by the macrophages.

Measuring NO

The Nitric Oxid (NO) level is examined using this method: the macrophage cells from the isolation of mice are counted using hemocytometer, and its density is made to be 1x10^{6}sel/mL, by adding RPMI complete grower medium. This cell suspension is then cultured in a microplate of 12x8 wells (96 wells). Each well contains 200μL (2x10^5 cells), 2 repetitions or duplo are made, incubated in an incubator with CO_2 5%, at 37^{0}C for 24 hours. The cultured macrophages are taken, the standard solution and supernatant from the culture are pipetted, inserted into microplate of 96 (12x8) wells at 100μL / well, and made duplo.

Griess A and B mixture is prepared at 1:1 ratio, added into microplate 96 at 100μL/well, and a reaction will occur between NO and Griess A and B mixture as purple color appears. It is then read using Elisa Reader at 550nm wavelength, waited for 15 minutes and the reading is repeated. The NO content is examined indirectly since its half-life in the tissue is very short, i.e. using Griess reaction.

Immunoblotting

For detecting the flagellin protein's immunogenicity and antibody specificity produced, Immunoblotting method is used. The flagellin protein samples are separated using 12% SDS-PAGE. After the protein tapes within polyacrylamide gel are obtained, this protein is then transferred to the nitrocellulose membrane using *semi dry blot apparatus* (Bio-Rad) at 500 mA for 30-60 minutes. The method of transferring the protein from polyacrylamide gel to nitrocellulose paper in sequence from the bottom is: 1) Thick watman paper, 2) One piece of thin watman paper, 3) Nitrocellulose paper, 4) Polyacrylamide gel, 5) One piece of thin watman paper, 6) One piece of thick watman paper (every time a piece of watman/nitrocellulose paper is placed, it is first wetted using buffer transfer and laid slowly to prevent any bubble from occuring).

The nitrocellulose membrane is taken after the transfer process is done, its marker is cut, and separated alone. The nitrocellulose membrane (without marker) is incubated in blocking solution, incubated overnight. Afterwards, the membrane is washed the next day using 0.05% TBS Tween for 3 x 10 minutes. Policlonal antibody is added (1:100 dilution using incubation buffer pH 7.2), incubated at room temperature, shaken for 1 hours. It is then washed using 0.05% TBS Tween for 3 x 10 minutes. 1:4000 IgG anti mouse Al-Phosphatase conjugate is added to 1% BSA in a buffer and incubated with pH 7.2. Next, it is washed using 0.05% TBS Tween for 3 x 10 minutes. Substrate (60μL NBT, 30 μL BCIP for 20mL in substrate buffer) is added. The protein band is waited to occur, the reaction is halted using sterile double-distilled water. Then it is wound.

RESULTS AND DISCUSSION

1. Flagellin Gene

The PCR results of *fli*C flagellin gene are obtained using primaries LPW 1856 and LPW 1857, which are then electrophoresized using 1% agarose as shown in Figure 1. The results indicate that the band size in PCR results of *fli*C gene in *S. typhi* strain of SLT.1 Salatiga isolate is equal to 1260 bp. It looks different from the band of *S. Typhi* BA07.4 strain which is equal to 1500bp. This incident matches the result of research by Lau *et al*. (2005) and Baker *et al*. (2007) which finds that *fli*C flagellin gene which has a size equal to 1260 bp codes the *fli*C H1-J gene from *S. typhi* serovar H1-j. This bacterium is less motile and less invasive than *S. typhi* serovar H1-d.

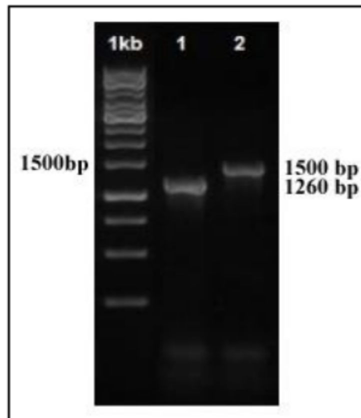

Figure 1. PCR of *fli*C gene, Marker DNA 1kb, 1) *fli*C of *S. typhi* SLT-1, 2) *fli*C of *S. typhi* BA07.

2. Flagellin protein profile

The flagellin protein of 2 strains of *S. Typhi*, i.e. BA07.4 Semarang isolate and SLT-1 Salatiga isolate strains is then separated using SDS-PAGE 12% as shown in Figure 2. The SDS-PAGE results regarding flagellin protein show that the flagellin of both *S. typhi* BA07.4 and *S. typhi* SLT-1 strains consists of three major protein subunits as shown in Table 1.

Table 1. Profile of flagellin protein of *S.typhi* BA07.4 and SLT-1 strains using SDS-PAGE 12%

Subunit	*S. typhi* BA07.4 (kDa)	*S. typhi*SLT-1 (kDa)
1	64	55
2	42	42
3	20	20

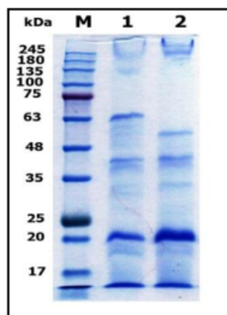

Figure 2. SDS-PAGE of flagellin protein of *S. typhi*, M) Protein marker, 1) *S. typhi* BA 07.4, 2) *S. typhi* SLT-1

3. Nitric Oxid (NO)

The Nitric Oxid (NO) level is examined in the macrophage of mice which have been immunized with the total flagellin protein of *S. typhi* BA07.4 and *S. typhi* SLT-1 (Table 2).

Table 2. NO level of macrophage supernatant of mice which have been immunized 2 weeks ago with flagelliin protein of *S. typhi* BA07.4 and *S. typhi* SLT-1

No.	Sample	NO Level
1.	*S. typhi* BA 07.4/H1-d	0.9296 ± 0.8529
2.	*S. typhi* SLT-1/ H1-j	0.5760 ± 0.6709
3.	Control	0.0623 ± 0.0556

The NO level produced by the macrophage of mice immunized with flagellin of *S. typhi* BA07.4 is higher than the one produced by those immunized with flagellin of *S. typhi* SLT-1. Likewise, it is also higher than the control.

4. Phagocytosis activity of macrophages

The phagocytosis activity of macrophages of mice which have been immunized 2 weeks ago with flagellin protein of *S. typhi* BA07.4 and *S. typhi* SLT-1 to latex is shown in Table 3. On average, the latex phagocytized by macrophages of mice immunized with flagellin of *S. typhi* BA07.4 and the phagocytocyte index are higher than the one obtained in macrophages of mice immunized with flagellin of *S. typhi* SLT-1 and control. The latex phagocytized by macrophages seems inside the cytoplasm of macrophage cells, as shown in Figure 3.

Table 3. Phagocytosis activity of macrophages of mice mice which have been immunized 2 weeks ago with flagellin protein of *S. typhi* BA07.4 and *S. typhi* SLT-1 to latex

No.	Sample	Average Phagocytized Latex	Average phagocyte index
1.	*S. typhi* BA 07.4/H1-d	1.61 ± 0.2945	0.87 ± 0.3063
2.	*S. typhi* SLT-1/ H1-j	1.58 ± 0.1628	0.82 ± 0.2135
3.	Control	0.46 ± 0.0764	0.46 ± 0.0764

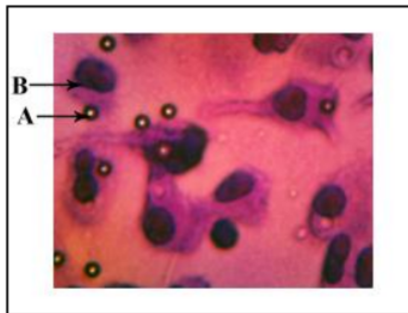

Figure 3. Phagocytosis activity of macrophages of mice immunized 2 weeks ago with flagelliin protein of *S. typhi* (A). Macrophage cells, (B). Phagocytized latex

5. Specificity of anti flagellin

Table 5. Results of Elisa of Anti flagellin of *S. typhi* BA07.4 and *S. typhi* SLT-1

No.	Antibody	Flagellin antigen	
		S. typhi BA07.4	*S. typhi SLT-1*
1.	Anti flagellin of *S. typhi* BA07.4	2.764±0.02995	1.380±0.6110
2.	Anti flagellin of *S. typhi* SLT-1	0.822±0.3370	1.008±0.3745
3.	Control	0.353±0.0553	0.349±0.0489

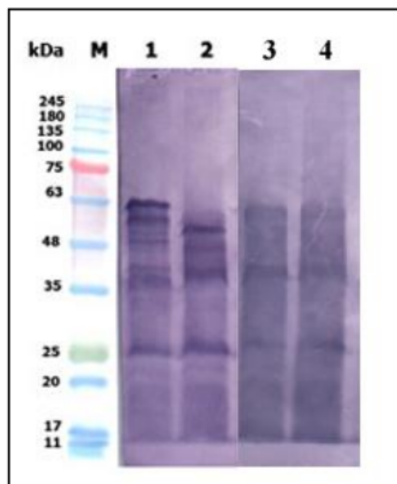

Figure 4. Immunoblotting of anti flagellin of *S. typhi*: M) Protein marker, 1). Anti flagellin of BA07.4 with flagellin BA07.4, 2) Anti BA07.4 with SLT-1, 3) Anti flagellin of SLT-1 with flagellin BA07.4, 4) Anti flagellin SLT-1 with flagellin SLT-1

DISCUSSION

Flagellin is a protein which builds flagella expressed from flagellin-coding gene. Each flagellin-coding gene has different expressions, thus it generates difference in flagellar antigen, referred to as phase 1 (H1) and phase 2 (H2) flagellins, also known as FliC and FljB flagellins. FliC has a structure of flagellar filament subunits, contributes to pathogenicity and activation of inflammation response in host cells. Flagellin (FliC) plays a role in bacterial movement, and it is the ideal candidate for natural immune system introduction (Bameri *et al*., 2018). The PCR results of fliC gene from *S. typhi* of different isolates show different DNA band lengths. From the PCR results, it is found that the fliC gene in S. typhi of SLT.1 Salatiga isolate is 1260 bp long. Meanwhile, the flagellin gene in S.typhi shows that its DNA band is around 1260 bp long, and there is a deletion of 261 bp in fliC gene. This gene points to H1-j antigen and it belong sto phase 1 flagellar antigen (Lau *et al*., 2005). From the PCR results, it is found that the fliC gene in S.typhi BA07.4 strain has a DNA band of 1500bp long. The flagellin-coding fliC gene has a sequence of heterogeneity existing between alleles where the alleles coding the same antigen are homologous. It is possible that the flagellin genes can be developed as a specific target in its molecular difference in flagellar antigen types (Mcquiston *et al*., 2004).

The different flagellin proteins can cause different immunogenic responses since fliC is identified as a Pathogen-associated molecular pattern (PAMP) which can be bound to Toll-like receptor 5 (TLR5) in the host cells. FliC has a hypervariable antigenic molecule and a conserved domain involved in TLR5-dependent systemic, mucosal pro inflammatory, and adjuvant. FliC is a strong adjuvant which has some effects on the immune system through mucosal routes (Bameri *et al*., 2018). The different DNA band lengths of fliC gene in *S,typhi* SLT.1 Salatiga isolate and S.typhi BA07.4 Semarang isolate strains allow the expression of different flagellin proteins. The next test is profiling flagellin protein using protein electrophoresis with SDS PAGE method.

The resulting flagellin protein protein shows a difference between *S. typhi* SLT.1 Salatiga isolate and *S. typhi* BA07.4 Semarang isolate strains. *S.typhi* SLT-1 has protein bands of 55 kDa, 42 kDa, and 20 kDa and *S. typhi* BA07.4 has a specific protein profile of 64 kDa, 42 kDa, and 20 kDa. Flagellin protein can code acid induced outer membrane protein (AIP)(Jindal, Tewari and Rishi, 2011). Flagellin protein has a high potential of stimulating macrophages to improve inflammatory mediator such as malondialdehyde (MDA) and nitrite (Jindal *et al*., 2012). For the next test, to discover whether the difference in molecule weights of flagellin-forming protein subunits can influence the amount of Nitric Oxid (NO), a NO concentration test is conducted. The flagellin protein with high virulence level can highly induce inflammatory mediator (Jindal *et al*., 2012).

From the results of NO test, the NO levels produced by macrophages of mice immunized with flagellin of *S. typhi* BA07.4 is higher than the ones immunized with flagellin of *S. typhi* SLT-1. Likewise, it is also higher than the control. Nitric oxide is an important signal molecule to regulate the tissue in a physiological process. When the amount of nitric oxide is too high, it has the potential to be toxic and to result in chronic inflammation (Bogdan, 2001). Reactive nitrogen intermediate (RNI) such as nitrites is known to be the final product in labile nitric oxide and its quantification can serve as an indicator for NO generation. (Chanana et al 2007).

The increase in level of nitrite as an effect of the existence of FliC in both *S.typhi* BA07.4 and SLT-1 occurs as an attribute of TNF-alpha which is known and has the potential for immune-stimulating activity in iNOS and then increase the level of NO (Pacher, Beckman and Liaudet, 2018); (Liaudent et al 2003, Zeng et al 2003). Flagellin can induce the expression of several inflammatory mediators including TNF-alpha and NO.

On average, the latex phagocytized by macrophages of mice immunized with flagellin of *S. typhi* BA07.4 has higher results than what is obtained in the macrophages of mice immunized with flagellin of *S. typhi* SLT-1 and control. The virulence factors of *S.typhi* is associated with the response of inflammatory cells in intestinal mucosa (Valdez, 2009). *S.typhi*'s ability to adhere to intestinal cell mucosa is determined by the type of flagellin proteins. The flagellin of *S. typhi* BAO7.4 has higher virulence ability than *S. typhi* SLT-1. The virulence factors of *S typhi* are known as OMPs which have the ability to induce inflammation reaction for release of cytokines (Chanana, Ray and Rishi, 2007). Flagellin protein can induce inflammation response by increasing the amount of pro-inflammatory mediator such as MDA and nitrite.

The anti flagellin of *S. typhi* BA07.6 in mice induced with *S. typhi* BA07.4 is higher than the anti

flagellin of *S typhi* BA07.6 in mice induced with *S.typhi* SLT-1. The anti flagellin of *S.typhi* SLT-1 in mice induced with *S. typhi* BA07.4 is lower than the ones induced with *S. typhi* SLT-1. However, the formed antibody is higher when it is induced using *S. typhi* BA07.4. Yet, each antibody's concentration will be linear with the antigen type used for induction. Based on the results of research on the type of flagellin in *S. tiphy* of Semarang and Salatiga isolate, it is found that the immunogenicity of Semarang isolate strain *S. typhi* BA07.4 is higher. This can be seen from the level of NO which is a mediator expressed by inflammatory cell, additionally the phagocytic activity macrophages in BA07.4 flagellin-induced mice is higher, and antibody produced by the mice induced with BA07.4 is higher. The different virulence ability of *S. typhi* presented by flagellin can affect the increase in celular natural immune system level in the form of macrophages and the components mediated by inflammatory immune cells in the form of NO. The diversity of sequences of DNA flagellin protein-coding fliC gene determines the protein types expressed and has specific antibody to each antigen in one *S. typhi* species of different isolates.

CONCLUSION

The research indicate that there are different sequences of flagellin protein-coding *fliC* gene between Semarang and Salatiga isolates and these influence the flagellin protein types expressed. From NO content testing, it is found that the generated phagocytosis activity of macrophages, and antibody level and type are higher in mice induced with *S.typhi*'s flagellin from Semarang isolate than the one with *S.typhi's* flagellin from Salatiga isolate. The immunogenicity of flagellin protein of Semarang isolate *S.typhi* is higher than Salatiga isolate.

ACKNOWLEDGMENT

In this occasion, we would like to extend our gratitude to to the Director General of Research and Development Reinforcement of the Ministry of Research, Technology and Higher Education through L2Dikti of Central Java whose institution has funded this research under the Agreement Letter for PTUPT Implementation, Number: 021/L6/AK/SP2H/PENELITIAN/2019, dated 28 March 2019.

REFERENCES

Alexan, A. F., Mohamed, S. H. and Ibrahim, A. M. (2009) 'Immune Response Elicited in Mice after Immunization with Flagellin from Salmonella enterica Serovar Enteritidis', *Global Veterinaria*, 3(6), pp. 465–471.

Baker, S., Hardy, J., Sanderson, KE., Quail, M., Goodhead, I., Kingsley, RA., Parkhil, J., Stocker, B., Dougan, *G.*. (2007) 'A Novel Linear Plasmid Mediates Flagellar Variation in Salmonella Typhi', *PLoS Pathogens*, 3(5), pp. 0605–0610. doi: 10.1371/journal.ppat.0030059.

Bameri, Z., Asadi Karam, MR., Habibi, M., Oloomi, M., Ehsani, P., Bouzari, S. (2018) 'In silico Studies for Comparison of FliCs from *Salmonella typhimurium, Pseudomonas aeruginosa*, and *Escherichia coli* and their Implication as an Adjuvant', 4(2).

Bishop, C., Honisch, C., Goldman, T., Mosko, M., Keng, S., Arnold, C., and Gharbia, S. (2012) 'Combined genomarkers approach to Salmonella characterization reveals that nucleotide sequence differences in the phase 1 flagellin gene fliC are markers for variation within serotypes', *Journal of Medical Microbiology*, 61(PART 11), pp. 1517–1524. doi: 10.1099/jmm.0.047431-0.

Bogdan, C. (2001) 'Nitric oxide and the immune response', 2(10), pp. 907–916.

Chanana, V., Ray, P. and Rishi, D. B. (2007) 'Reactive nitrogen intermediates and monokines induce caspase- 3 mediated macrophage apoptosis by anaerobically stressed Salmonella typhi', pp. 368–374. doi: 10.1111/j.1365-2249.2007.03503.x.

Darmawati, S., Santosa, B. and Prastiyanto, Muhammad Evy, R. S. (2015) 'Molecular Characterization And Hemagglutination Activities of Flagellin Protein of Salmonella typhi', in, pp. 325–330.

Darmawati, S., Sembiring, L. and Asmara, W. (2011) 'Klasifikasi Numerik-fenetik *Salmonella typhi* Asal Jawa Tengah dan Daerah Istimewa Yogyakarta Berdasarkan Hasil Karakterisasi Fenotipik Pendahuluan Metode Penelitian', *BIOTA Atmadjaya Yogyakarta*, 16(1), pp. 128–132.

Frankel, G., Newton, S.M.C., Schoolnik, GK., Stocker, B.A.D. (1989) 'characterization of the H1-j gene of Salmonella typhi', *EMBO Journal*, 8(1), pp. 3149–3152.

Hatta, M., Sultan, A. R., *et al*. (2011) 'New flagellin gene for Salmonella enterica serovar typhi from

the East Indonesian archipelago', *American Journal of Tropical Medicine and Hygiene*, 84(3), pp. 429–434. doi: 10.4269/ajtmh.2011.10-0605.

Hatta, M., Sultan, A. R., Sultan, A.R., Pastoor, R., and Smits, H. (2011) 'New Flagellin Gene for Salmonella enterica serovar Typhi from the East Indonesian Archipelago', *AM. J. Trop. Med. Hyg*, 84(3), pp. 429–434. doi: 10.4269/ajtmh.2011.10-0605.

Jindal, G., Tewari, R., Gautam, A., Pandey, S.K. and Rishi, P. (2012) 'Immunological characterization of recombinant Salmonella enterica serovar Typhi FliC protein expressed in *Escherichia coli*', pp. 1–9.

Jindal, G., Tewari, R. and Rishi, P. (2011) 'Acid Induced Outer Membrane Phenotype in Salmonella typhi -a Gene Product of fliC', 3(1), pp. 23–30.

Lau, S. K. P., Woo, P.C.Y., Chan, C.Y.F., Woo, WL., Woo, G.K.S. and Yuen, KY. (2005) 'Typhoid Fever Associated with Acute Appendicitis Caused by an H1-j Strain of Salmonella enteric a Serotype Typhi Typhoid Fever Associated with Acute Appendicitis Caused by an H1-j Strain of Salmonella enteric a Serotype Typhi', *Journal of Clinical Microbiology*, 43(3), pp. 1470–1472. doi: 10.1128/JCM.43.3.1470.

Liaudet, L.; Szabo, C.; Evgenov, OV.; Murthy, KG.; Pacher, P.; Virag, L.; Mabley, JG.; Marton, A.; Soriano, FG.; Kirov, MY.; Bjertnaes, LJ.; Salzman, AL. Flagellin from Gram negative bacteria is a potent mediator for acute pulmonary inflammation in sepsis. Shock 2003, 19, 131-137.

Mcquiston, J. R., Parrenas, R., Ortiz-Rivera, M., Gheesling, L., Brenner, F. and Fields, P.I. (2004) 'Sequencing and Comparative Analysis of Flagellin Genes fliC , fljB , and flpA from Salmonella', 42(5), pp. 1923–1932. doi: 10.1128/JCM.42.5.1923.

Pacher, L., Beckman, J. S. and Liaudet, L. (2018) 'Nitric Oxide and Peroxynitrite in Health and Disease', pp. 315–424. doi: 10.1152/physrev.00029.2006.

Sabir, M., Dwiyanti, R., Febrianty, A., Tandirogang, N., Yasir, Y., Amir, M., Sultan, A.R., Purnamasari, N.I., Primaguna, M.R., Muhammad, M., Hatta, M. (2014) 'Relationships between Flagellin Genes Variants of *Salmonella enterica* Serovar Typhi and Severity of Illness from Acute and Carriers State of Typhoid Fever', *American Journal of Infectious Diseases and Microbiology*, 2(4), pp. 74–80. doi: 10.12691/ajidm-2-4-1.

Sano, G., Takada, Y., Goto, S., Maruyama, K., Shindo, Y., Oka, K., Matsui, H. and Matsuo, K. (2007) 'Flagella Facilitate Escape of Salmonella from Oncotic Macrophages, *J. Bacteriology*, 189(2), pp. 8224–8232. doi: 10.1128/JB.00898-07.

Scott, N. and Simon, M. I. (1982) 'Genetic analysis of the mechanism of the Salmonella phase variation site-specific recombination system', 188, pp. 313–321.

Valdez, Y., Ferreira, R.B., Finlay, B.B.(2009) Molecular mechanisms of Salmonella virulence and host resistance. Curr Top Microbiol Immunol 337:93-127

Vollaard, A. M., Soegianto, A., Suwandhi, W., A.G.H. van, A.H., Leo G, V., Charles, S., Jaap T., V.D. (2005) 'Identification of typhoid fever and paratyphoid fever cases at presentation in outpatient clinics in Jakarta , Indonesia', *Tropical Medicine and Hygiene*, 99, pp. 440–450. doi: 10.1016/j.trstmh.2004.09.012.

Zeng,H., Calson,A.Q., Guo, Y., Yu, Y., Hyams, L.S.C., Madara, J.L., Gewirtz, A,T.,Neish, A.S.(2003). Flagellin is the major proinflammatory determinant of enteropathogenic Salmonella. J Immunol 171:3668-3674

THE IMMUNOGENICITY OF FLAGELLIN PROTEIN OF SALMONELLA TYPHI OF SEMARANG AND SALATIGA ISOLATES

ORIGINALITY REPORT

6%	4%	3%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

#	Source	%
1	ibbj.org Internet Source	1 %
2	www.ncbi.nlm.nih.gov Internet Source	<1 %
3	open.library.ubc.ca Internet Source	<1 %
4	scholarship.shu.edu Internet Source	<1 %
5	Remigy, H., D. Fotiadis, G. Hauska, B. Wolpensinger, S. A. Müller, A. Engel, and G. Tsiotis. "Evidence for the association of three FMO subunits per reaction center of Chlorobium Tepidum by scanning transmission electron microscopy", Photosynthesis Mechanisms and Effects, 1998. Publication	<1 %
6	Submitted to Universitas Jenderal Soedirman Student Paper	<1 %

#	Source	Match
7	Aditya Rahman Ernanto, Junita Rensa Palimbongan, Anjar Richardo Manufandu, Sri Darmawati. "Identification of Salmonella typhi contamination by amplification fliC gene in grass-jelly from traditional markets and minimarket in Semarang city", Jurnal Teknologi Laboratorium, 2020 Publication	<1 %
8	theses.gla.ac.uk Internet Source	<1 %
9	www.alliedacademies.org Internet Source	<1 %
10	Macarena A. Varas, Sebastián Riquelme-Barrios, Camila Valenzuela, Andrés E. Marcoleta et al. "Inorganic Polyphosphate Is Essential for Salmonella Typhimurium Virulence and Survival in Dictyostelium discoideum", Frontiers in Cellular and Infection Microbiology, 2018 Publication	<1 %
11	panafrican-med-journal.com Internet Source	<1 %
12	mafiadoc.com Internet Source	<1 %
13	mts.intechopen.com Internet Source	<1 %

14	Logan Grimes, Allie Doyle, Aaron L. Miller, Richard B. Pyles, Gabor Olah, Csaba Szabo, Sarah Hoskins, Tonyia Eaves-Pyles. "Intraluminal Flagellin Differentially Contributes to Gut Dysbiosis and Systemic Inflammation following Burn Injury", PLOS ONE, 2016 Publication	<1%
15	Louis-Philippe Bergeron-Sandoval, Aurélie Girard, François Ouellet, Denis Archambault, Fathey Sarhan. "Production of Human Rotavirus and Salmonella Antigens in Plants and Elicitation of fljB-Specific Humoral Responses in Mice", Molecular Biotechnology, 2010 Publication	<1%
16	P. Petrov. "Occurrence and characterization of Salmonella enterica subspecies enterica serovar 9,12:l,v:- strains from Bulgaria, Denmark, and the United States", European Journal of Clinical Microbiology & Infectious Diseases, 05/2009 Publication	<1%
17	R Belas. "Expression of multiple flagellin-encoding genes of Proteus mirabilis.", Journal of Bacteriology, 1994 Publication	<1%

jcm.asm.org

18	Internet Source	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		