

Identification and Characterization of Antigenic 36 Kda Outer Membrane Protein (OMP) of *Salmonella entericaserovar Typhi* (*S.typhi*) from Makassar, South Sulawesi, Indonesia

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Received January 20, 2015; Revised March 12, 2015; Accepted March 17, 2015

Abstract Typhoid fever caused by *Salmonella entericaserovar Typhi* (*S. typhi*), still remains a public health concern. A simple, rapid and early diagnostic test has been a long felt need for clinicians. Adh036 protein, discovered from Malang, Indonesia, has a candidate vaccine potential for Typhoid Fever. This study aims towards identification and characterization of 36 kda Outer Membrane Protein (OMP) from Makassar, South Sulawesi, Indonesia as a generalization protein test. Isolated bacteria were cultured in MacConkey medium and Brain Heart Infusion Broth (BHIB), and then harvested by using sarcosyl and sonication methods. Then, crude protein was purified by dialysis method using fractional ammonium sulfate precipitation and the protein contents were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophores (SDS-PAGE) for identification it's molecular weight. The results, from five purified proteins, only three fractions show band at 36 kda, were 0-20%, 20-40%, and 40-60%.

Keywords: Outer Membrane Protein (OMP), *Salmonella entericaserovar Typhi* (*S. typhi*), Purification, SDS-PAGE

Cite This Article: Cut Muthiadin, Rosdiana Natsir, Rosana Agus, Muhammad Nasrum, Ressa Dwiyaniti, Muhammad Sabir, Yadi Yasir, and Mochammad Hatta, "Identification and Characterization of Antigenic 36 Kda Outer Membrane Protein (OMP) of *Salmonella entericaserovar Typhi* (*S.typhi*) from Makassar, South Sulawesi, Indonesia." *American Journal of Biomedical Research*, vol. 3, no. 1 (2015): 9-12. doi: 10.12691/ajbr-3-1-3.

1. Introduction

Typhoid fever remains an important public health priority, particularly in developing countries, with an estimated 16 million new cases annually and 600.000 death [1]. It is caused by *Salmonella enterica* serovar Typhi (*S. typhi*), which replicates within the cells of reticuloendothelial system [2]. The emergence of multidrug-resistant strains of *Salmonella* with increased virulence, communicability and survivability leading to increased morbidity and mortality has further complicated its management. Currently available vaccines for typhoid fever have less-than-desired efficacy and certain unacceptable side effects, making it pertinent to search for new immunogen suitable for vaccine formulation [3,4]. The outer membrane proteins (OMPs) of *Salmonella* have been considered possible candidates for conferring protection against typhoid. Over the past year, several

Salmonella OMPs have been investigated as potential vaccine candidates, virulence factors, and diagnostic antigen and the molecular structure and function of OMPs and their respective genes have been studied. However, only a small number of OMPs have so far been characterized [5,6].

In order to control the disease typhoid fever, there are currently three vaccines that Ty21a, ViCPS (Vi capsular polysaccharide) and Inactivated typhoid vaccine [7]. However, the efficacy of both the vaccines (Ty21a and ViCPS) was approximately 65-70% [8]. Inactivated typhoid vaccine provides only moderate protection with effectiveness of 51-67%. Therefore, further development in typhoid fever vaccine is required.

A previous studies using *Salmonella typhi* isolates from Malang reported that *Salmonella typhi* has adhesion protein related to Outer Membrane Protein (OMP) with a molecular weight of about 36 kDa and named AdhO36. The study also reported that the oral immunization of AdhO36 proteins provide meaningful protection by

inhibiting the attachment of *Salmonella typhi* to the intestines of mice [9]. Furthermore t AdhO36 was found to be immunogenic and capable of stimulating both humoral mucosal and systemic immunity. In the protein AdhO36 was also able to stimulate cellular immune responses [10]. because of its ability to stimulate the immune response and protection against bacterial adhesion, AdhO36 was investigated as a potential vaccine candidates.

A generic vaccine can be used widely for various isolates of *Salmonella typhi* from all over the world, particularly in Indonesia, because the bacteria easily spread through contaminated water and food, accompanied with high human mobility. Thus the need further investigation of this protein as a generic vaccine candidate is required. The aims of this study is to identify the existence of 36 kDa OMP of *S. typhi* particularly from Makassar region. These results will help identify a highly effective vaccine against *Salmonella typhi* infection that can be used widely in Indonesia.

2. Material and Methods

2.1. Bacterial Culture

5 mL of blood was taken aseptically and then inserted into the BACTEC transport medium and incubated at 37°C for 24-48 hours. These cultures were then isolated on selective solidified medium (MacConkey) incubated at 37°C for 18-24 hours. Identification of *S. Typhi* was carried out by its characteristic colony growth. To further biochemical tests and microscopis test (Gram's Staining) were performed to confirm the presence of *S. Typhi* [11,12].

2.2. Extraction of Outer Membrane Protein (OMP) *S. typhi*

Salmonella OMP were individually extracted by following protocol described by S. Kim et al (2006) [13] Briefly, 6-8 ose culture of *S.typhi*was added to BHIB 1 ml of medium and incubated at 37°C for 24 hours. Furthermore, the cell cultures were centrifuged at 15,000 g for 20 min at 4°C. The pellets then added with 10 mmol 1-1 Tris-HCl (pH 8) and sonicated on ice using a sonicator for 4 times for 5 s [14]. Furthermore centrifuged again at 15,000 g for 1 h at 4°C. Returned pellets were separated and added with 10 ml of 10 mmol 1-1 Tris-HCl (pH 8) and sarcosyl to reach a final concentration of 1.5% (v / v). After allowed to stand at room temperature for 20 min, the membranes were collected by centrifugation at 15,000 g again for 90 min at 4°C.

2.3. Dialysis and Protein Estimation

Ammonium sulfate fractionation of the membrane was performed (0-20%, 20-40%, 40-60%, 60-80%, 80-100%). Filtrate was collected and dialyzed using selofanmembran [15]. Then stirrer against distilled water (H₂O) with three

changes buffer over period of 48 hours. Protein content was determined by Lowry Methods [16].

2.4. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophores (SDS-PAGE)

Polyacrylamide gel electrophoresis, using 10% acrylamide gels, was carried out using the method of Laemmli (1970) [17]. Proteins were stained with Coomassiebriliant blue (Bio-Rad).

2.5. Serology Assay

The *S. typhi*Dri Dot assay consists of an agglutination card containing a dot of dried, antigen-activated latex placed on the surface in the center of the card. The card was placed in a sealed moisture resistant sachet for optimal protection of the reagent during transportation and storage. The latex has a blue colour and the assay was simply performed by placing a 10 µl drop of serum next to the blue dot. The serum and the latex was then mixed using a plastic spatula provided with the card. After the latex is homogenously dispersed, the fluid is swirled by rotating the card in a near horizontal position to further mix the serum and the latex. Agglutination is readable within 30 seconds and is revealed by particulation of the latex suspension [18].

3. Results and Discussions

3.1. Identification *S. typhi* and Extraction OMP

The existence of colony grown on MacConkey differential medium from blood samples of patients suspected of typhoid infection provided initial evidence of typhoid existence. This was then examined with Gram's Staining and identified as Gram's-negative (Figure 1).

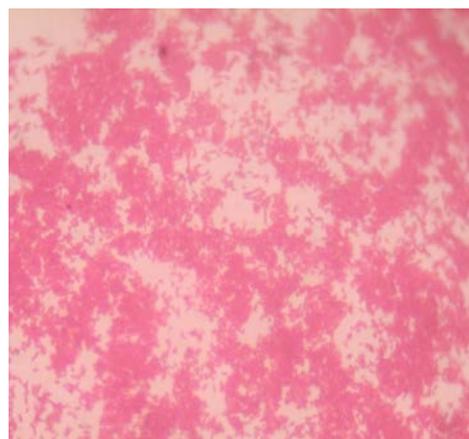


Figure 1. Gram's Staining. Shown bacilli, red's color, identified as Gram-negative of *SalmonellaentericaserovarTyphi* (*S. typhi*),

Further biochemical tests, TSIA, SIM, Citrate, MR-VP, and carbohydrate: glucose, lactose, sucrose, mannitol (see Table 1). confirmed the isolates were indeed *S. typhi*.

Table 1. Biochemical test of *Salmonella entericaserovar Typhi* (*S. typhi*),

TSIA				SIM			MR	VP	Urea	Citrate	Carbohydrate			
Slant	Butt	H ₂ S	Gas	Indol	H ₂ S	Motility					G	S	L	M
Alkali	Acid	+	-	-	+	+	+	-	-	-	+	-	-	+

3.2. Extraction and Purification OMP's *S. typhi*

OMPs were extracted by Ojanen methods, using sarcosyl and harvested by sonication and centrifugation. The extraction products of OMP's from *S. typhi* in this study resulted in a pale yellow liquid containing crude OMP proteins and was further analyzed by SDS PAGE. This is consistent with reports by Prasilia [19] that the OMP protein resulting from the extraction after sonication yields yellowish liquid.

Outer Membrane Protein (OMP) is one of the layers of the cell membrane which is part of the major antigens associated with nonspecific endotoxin activity especially in Gram-negative bacteria. Outer Membrane Protein (OMP) *S. typhi* is part of the cell membrane which is located outside the cytoplasmic membrane and peptidoglycan layer that limits the cells to the surrounding environment [20] The use of SDS PAGE method in this study, in addition to a common method in the analysis of proteins to see the bands of protein samples and determination of molecular weight, was also to see the degree of purity of the protein antigens OMP *S. typhi*.

Sarkosyl is commonly used in the purification of OMPs of Gram-negative bacteria. Hobbs (2008) used sarcosyl for Outer Membrane Protein resulting pure samples with little contaminants [21]. This is consistent with other studies [22,23] which compared extraction techniques and detergent sarcosyl and noted that this is the best detergent suitable for OMP extraction which also reduces the enzymatic reactions that can damage OMP structure.

Its has been purified OMP's *S. typhi* by dialysis method, results purified protein with protein content below (Table 2). Dialysis process occurs by osmosis principle and was performed at low temperatures so that the protein was not damaged and remained in stable condition [24]. Dialysis aims to eliminate the salt molecules and ions, which also settles with other proteins that affect enzyme activity. Using cellophane dialysis membrane with a molecular cut-off of <10 kDa. The buffer solution used in the outer membrane has a lower concentration ion concentration than in the cellophane bag. Thus, small molecules such as salts or other ions will pass through the pores of the membrane until the concentrations inside and outside the sac cellophane achieve the same or a balance [25].

Table 2. Protein Content of *Salmonella entericaserovar Typhi* (*S. typhi*),

No	Fraction	Protein contents (mg/mL)
	Protein	
1	Crude	6,878
2	0 - 20%	0,294
3	20 - 40%	1,641
4	40 - 60%	0,965
5	60 - 80%	0,223
6	80 - 100%	0,168

In this study, measurement of protein content of the samples was determined by the Lowry method using bovine serum albumin (BSA) as standard [10]. The results showed that the protein content of the crude extract was 6.878 mg / mL (Table 2). Table 2 shows that the concentration of protein in each fraction is different. This indicates that the protein precipitated from each fraction is different proteins. The protein is precipitated by differences in solubility in water, the higher solubility in

water, the more ammonium sulfate is added. The highest protein concentration was found in fractions 20-40% in the amount of 1.641 mg / mL. These data suggested that the fraction of 20-40% is a type of protein that is low solubility in water. High concentrations were still found in the 40-60% that is equal to 0.965 mg / ml. The highest protein concentration was found in the crude extract (6.878 mg / ml)..

3.3. Identification Molecular Weight By Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophores (SDS-PAGE)

To determine the molecular weight of the protein, SDS PAGE analysis was done for the crude membrane fraction before precipitation and the 5 precipitation fractions (Figure 2).

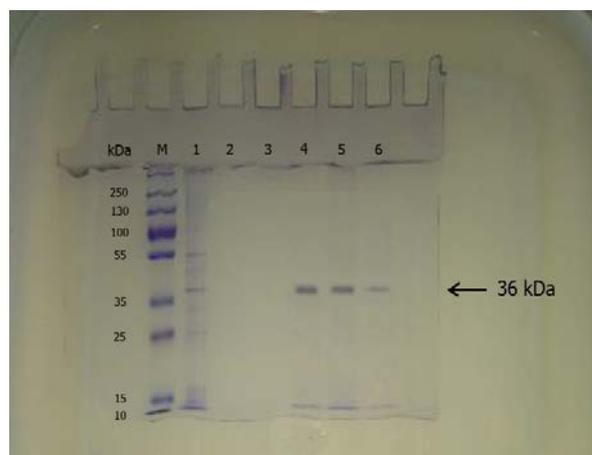


Figure 2. SDS PAGE of OMP of *Salmonella entericaserovar Typhi* (*S. typhi*), M: Marker, 1: crude, 2: 80-100%, 3:60-80%, 4: 40-60%, 5: 20-40%, 6:0-20%

Outer Membrane Protein (OMP) is one of the layers of the cell membrane which is part of the major antigens associated with nonspecific endotoxin activity especially in Gram-negative bacteria [26]. Outer Membrane Protein (OMP) *S. typhi* is part of the cell membrane which is located outside the cytoplasmic membrane and peptidoglycan layer that limits the cells to the surrounding environment [27]. The use of SDS PAGE method in this study, in addition to a common method in the analysis of proteins to see the bands of protein samples and determination of molecular weight, also to see the degree of purity of the protein antigens OMP *S. typhi* of the sample itself.

In this study, Figure 2. for crude fraction, the results of running SDS PAGE of OMP *S. typhi* showed major bands at 25 kDa, 35 kDa, 55 kDa,. Upon purification, the 36 kDa protein was enriched in, 40-60%, 20-40%, 0-20%, fractions. This is consistent with research that has been conducted by Dini, Agustina (2010). OMP identified molecular weight in 4 areas namely 2 of Malang, 8 of Yogyakarta, Surabaya 5, and 4 of Bandung, Indonesia obtained results all show band 36 kDa, except 1 isolates from Surabaya and three isolates of Bandung, Indonesia [28].

Good vaccine is a vaccine that can be used widely for various isolates of *Salmonella typhi* from all over the world, especially in Indonesia, because as it is known that *Salmonella typhi* has spread to many regions mainly due

to the nature of the easy spread through contaminated water and food were also accompanied with high human mobility, thus need further investigation as a generalization of the test vaccine made from the protein. The results of this study are expected to be the first step to finding typhoid fever vaccine has high efficacy and effectiveness in inhibiting *Salmonella typhi* infection and can be used widely in Indonesia, at least in Makassar city.

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