Sequence Homology and Epitope Prediction of 37 kDa Outer Membrane Protein H(ompH) Gene of Pasteurella Multocida Type B Isolate from Nusa Tenggara Timur (NTT)

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Sequence Homology and Epitope Prediction of 37 kDa Outer Membrane Protein H(ompH) Gene of Pasteurella Multocida Type B Isolate from Nusa Tenggara Timur (NTT)

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Abstract

Haemorrhagic Septicaemia (HS) in Indonesia known as Ngorok disease is a fatal acute septicemia disease in cattle and buffalo caused by *Pasteurella multocida*. Outer membrane Protein H (OmpH) is one of the major proteins in *P. multocida* envelope has been purified and characterized as porin immunodominant. The aim of this study is to find out the homology of nucleotide sequence of 37 kDa OmpH gene of *P. multocida* of local isolate from NTT with vaccine strain (Katha) and 5 referen isolates and predict the epitope of 37 kDa OmpH gene. Detection of 37 kDa OmpH gene was done using PCR. Homology analysis of nucleotide and amino acid sequences was performed on the BLAST program at NCBI. Epitope prediction of 37 kDa OmpH genes *P. multocida* from NTT and vaccine strains was performed using the online programme B Cell Epitope Prediction Tools. The results showed that 37 kDa OmpH gene of *P. multocida* from NTT has 99% homology to vaccine strain (Katha) and isolates from China, Iran, India, Mesir, and 89% homology to isolate from USA and 37 kDa OmpH gene of *P. multocida* local isolate from NTT were likely to be immunogen candidates based on B cell epitope that had 11 epitopes. These findings indicate that *P. multocida* local isolate from NTT has a chance as immunogen candidate, which can be developed as a vaccine candidate and diagnostic kit for HS.

Keywords: *Haemorrhagic septicaemia; P. multocidatype B; outer membrane protein H(omp H) gene; PCR; sequence; epitope.*

Introduction

Hemorrhagic Septicemia caused by *Pasteurella multocida*is a fatal acute septicemia disease in cattle and buffalo.Inperspective of economic, it be[illegible]esthe most important bacterial disease in Southeast Asia including Indonesia, Philippines, Thailand, Malaysia, Middle East and South Africa[1].

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In Indonesia, HS outbreak still occurs although vaccination has been done once a year using vaccine made from Katha strain. The most recent case is the sudden death of 24 livestock in Kupang district, NTT in December 2016. The results of laboratory tests at the Balai Besar Veteriner Denpasar, confirmed the death of these livestock due to HS [2].

P. multocidastrains belong tosero group B and E are the one w[illegible] cause HS.The complex interactions between host factors and bacterial virulence factors, such as lipopolysaccharide (LPS), capsule, protein outer me[illegible]rane (omp), and putative hemolysin has an effect on the pathogenesis of the disease[3].

Outer membrane Protein H (OmpH) is one of the main proteins in P. multocida envelopes that has been

purified and marked as immunodominantporin. This porin is highly immunogenic, showing epitopes on the surface of bacteria [4]. Study by Tan et al.[5]using mice polyclonal antiserum in immunoblotting studies showed that omp with a molecular weight of 37 kDa was reactive to all local isolates and ATCC strains. In the same study, recombinant 37 kDa OmpH can protect mice and be able to stimulate high antibody titers.

The new vaccination approach based on the rational design of B cell and T cells epitopes (epitope-based vaccines) promises a great induction of immune responses, and can handle more effective pathogens with genetic variation[6]. Identification of epitopes suitable for diagnostic use or prophylactic intervention is an important prerequisite for epitope-based vaccines and diagnostics.

The purpose of this study is to find out the homology of nucleotide sequence of 37 kDa OmpH gene of P. multocida of local isolate from NTT with vaccine strain (Katha strain) and 5 referen isolates and predict epitope of 37 kDa OmpH gene.

Material and Method

Sample: P. multocida isolate from NTT (Nusa Tenggara Timur) were obtained from Balai Besar Veteriner Denpasar, Bali. Previously, this isolate had been tested for its capsular type. Haemorrhagic Septicaemia vaccine from Pusat Veterinaria Farma (Pusvetma) Surabaya was used as a vaccine strain (Katha) sample. Reference isolates from GenBank were used as a comparison with accession numbers,namely: HM582886.1 (China), CP017961.1 (Iran), FN908433.1 (India), KY436382.1 (Egypt), CP015562.1 (USA).

Reculture and Identification of P. multocida: P. multocida isolate was re-cultured in Blood Agar media and incubated for 24-28 hours at 37° C. After that, the morphology was seen using Gram examination and its growth on MacConkey Agar (MCA). Furthermore, the examination using biochemical test, namely: TSIA test, SIM test, SCA test, Urease test, and Sugar test [7].

DNA Extraction: Total genomic DNA was extracted with QIAamp® DNA Mini kit according to standard protocol Qiagen[8].Briefly, bacteria that have been cultured on Nutrient Agar (NA) at 37°C overnight, were inserted into tube. After addition of bacteria/ vaccine incubated at 60 °C for 30 minutes. The 200µL AL buffer is added to the tube, then vortex. The mixture of the ingredients in the tube is transferred to the spin column, then adds 200 µL of 96% ethanol. After that, centrifuged at 8,000 rpm for 1 minute and washed using AW1 500µL. Then centrifuged back at 8.000rpm for 1 minute and washed again using AW2 as much as 500µL. After that centrifuged at 13,000 rpm for 3 minutes and the bottom of the spin colomn removed. After that, it was centrifuged again for 1 minute at 13,000 rpm and transferred to an empty sterile tube. After that added 50µL AE Buffer and incubated at room temperature for 1 minute. Then centrifuged at 8,000 rpm for 1 minute

PCR assay: Amplification of the ompH gene was performed using both ompH-F and ompH-R primer in 20µl PCR mixture. The primers used was developed from the primers used by Tan et al.[5]. Primer ompH-F forward 5'-CAGCAACAGTTTACAATCAAGACGGTAC-3 'and omp H-R reverse 3'-GAAGTGTACGCGTAAA CC-5' with gene target 946 bp. The thermal cycling conditions are: 1 cycle at 95ºC for 5 minutes, 40 cycles at 94ºC for 30 sc, 50ºC for 1 minute, and 72ºC for 1.5 min; 1 cycle at 72ºC for 10 minutes. This PCR profile is repeated three more times to ensure specific PCR products. The PCR product was analyzed by electrophoresis in 1% agarose gel.

Sequencing: Purification of the PCR product was carried out with QIA quick spin columnt from Qiagen. Addition of Big Dye Terminator 1.1 cycle version as much as 1.6 µl and 5x sequencing buffer as much as 6.4 µl, then added H_20 ad libitum. Then the mixture was put into the PCR machine with a pre denaturation program of 96°C for 3 minutes. 25 cycles for denaturation 96°C for 10 seconds, annealing 50°C for 5 seconds, extension temperature 60°C for 4 minutes. Final extension 4°C. After that, 3M NaOAC 2 µl (± 1/10 x vol) and 95% ethanol 50 µl (± 2 x Vol) were added then vortexed and incubated in ice for 10 minutes. Then centrifuged 14,000 rpm for 20 minutes at 4°C. Then the ethanol solution is slowly taken, after that pellet is added 200µl 70%-80% ethanol, then centrifuged 14,000 rpm for 5 minutes at 4°C. Then a 70% ethanol solution is slowly taken, then the resulting pellets are dried in the vaccum pump for 10 minutes, and stored at-20°C[9].

Homology Analysis and Epitope Prediction: Sequencing results were processed using Bioedit program. Homology analysis was carried out using the BLAST program atNCBI (National Center for Biotechnology Information). Homology analysis of sequence of 37 kDa OmpH of NTT local isolate

compared with vaccine and nucleotide sequence data of 5 isolates from other countries accessed from GenBank. The nucleotide sequences of the 37 kDa OmpH were then translated into amino acid sequences using the online DNA to Protein Translation program. Epitope prediction was carried out through the B Cell Epitope Prediction Tools online program.

Nucleotide Sequence Accesion Number: The DNA sequence of the 37 kDa OmpH gene of *P. multocida* isolate from NTT and vaccine were submitted to GenBank and assigned the accession number MK183754 and MK205393.

Result and Discussion

Recultureand Re-identification of Pasteurella multocida: Reculturedon Blood Agar showed the colonies were transparent, grayish, shiny, and were found to be non haemolytic. No growth was observed on Mac Conkey Agar medium. The colonies were then confirmed by biochemical test, while it fermented glucose, fructose, mannitol and sucrose, but did not fermented maltose and lactose, the TSIA was obtained in alkaline slat and butt, yielding no gas and H_2S. The colonies were positive for indole test and no reaction was seen with Simon Citrate Agar and Urease medium.

The ompH gene was successfully amplified using OmpH-F and OmpH-R primers. The PCR product was obtained with the same bandsize of 946 bp of both samples (Fig. 1).

Study by Kanaiyalal [10] on the OmpH gene *P. multocida* HS case obtained 1kb PCR products. Whereas Tan et al.[5] whose primerswas used in this study 37 kDa OmpH gene was obtained 980 bp.

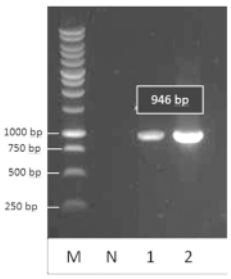

Figure 1. PCR detection of ompH gene ofP. multocida. Lane M: 1kb molecular ladder (Vivantis); Nnegative contro P .multocida from vaccine; 2local isolate of P. multocida

The sequencing results of *P. multocida* isolate from NTT was 894 bp while for *P.multocida* from vaccine was 893 bp. In the analysis of nucleotides sequence of the 37 kDa OmpH gene *P. multocida*, the total length of the nucleotides that are parallel to the gap and insertion is 909 nucleotides. Table 1. shows the results of homology analysis.

The results showed that 37 kDa OmpH gene*P. multocida* from NTT isolate was relatively more similar to isolates from the continents of Asia and the Middle East. Nucleotide sequences of 37 kDa OmpH *P. multocida* isolates NTT and vaccines showed high homological levels of *P. multocida* OmpH from other countries.

The results of the protein sequences of the 37 kDaOmpH*P. multocida* NTT isolates were 298 amino acids while the vaccines were 297 bp amino acids. Kanaiyalal[10] obtained 296 amino acids of OmpH protein sequencein cattle and buffaloes. Multiple sequence alignment from amino acid sequences showed that 37 kDa Omp H*P. multocida* from several countries shows high homology with little variation in amino acid composition in several parts. Luo *et al.*[11]aligned the OmpH sequence of 15 *P. multocida* serotypes and found that this protein was very conserved (72-100% identity), which supports the findings of this study.

Results of analysis of amino acid sequence OmpH P. multocida P-52 by Singh et al. [12] obtain the greatest variation limited to two discrete regions that are associated with the hydrophilic domain, i.e. amino acids to 82-102 and 223-240. According to Sellyei et al. [13] these two discrete regions encode large external loops that might interact with the host's immune system. This variable area can work as a strain-specific epitope that takes an important role in serotype specific immune responses [12].

Epitope prediction was done using Kolaskar and Tongaonkar Antigenicity scale. The results of the prediction of B cell epitope on 37 kDa OmpH were found 11 epitopes in NTT isolate and vaccine, with 9 identical epitopes, namely: GLSALAYAEL, DVHVKRLYAGF, DVGVSDYTYFLG, GAYVFSA, GFVVAGL, SQKYVTVA, ALEVGLN, KVYTDL, and SIILGAGYKLHKQVETF (Table 2 and Table 3).

The ability of epitope to induce an immune response is determined by the log score, the greater the log score, the better the ability of epitope to induce immunity. The linear epitop prediction results showed that 37 kDa *P.*

multocida OmpH local isolates NTT and vaccine strains were immunogenous and had almost the same ability to induce humoral responses, with the best ability, namely epitope with the largest log score of 1,266.

Epitope identification and analysis can show that proteins are responsible for triggering the body's immune respon and proteins with high immunogenic properties can be used for the development of peptide-based vaccines from Indonesian local isolates. In the diversity of epitopes in pathogens, it is important to note that not all epitopes, even those that appear dominant, are equally capable of producing antibody production. Particularly for linear epitope B cells there are several reasons, including, the accuracy of epitope prediction results is only 75% and most B cell epitopes (approximately 90%) are conformational, some studies have found that linear B epitope cells produce antibodies that do not cross reacting with original antigens and linear B epitope cells does not always give rise to memory cells so that to know the ability of the epitope to protect exactly need continuous research [14][15].

Table 1. Homological Value of Nucleotide Sequencesof OmpH P.multocida NTT Isolate and Vaccine

Reference Isolate (GenBank)	NTT isolate	Vaccine
Pasteurella multocida outer membrane protein H (OmpH) gene, complete cds. Accession HM582886.1. Cina	99%	99%
Pasteurella multocida strain Razi_ Pm0001, complete genome. Accesion CP017961.1. Iran	99%	99%
Pasteurella multocida partial OmpH gene for outer membrane protein H, strain P 52. Accession FN908433.1. India	99%	99%
Pasteurella multocida strain PM/ VSVRI/1962 outer membrane protein (OmpH) gene, partial cds. Accession KY436382.1. Egypt	99%	99%
Pasteurella multocida strain USDA-ARS-USMARC-59910 chromosome, complete genome. Accession CP015562.1. USA	89%	89%
Vaccine Katha strain	99%	100%

Table 2. Prediction of Cell B Epitope from Sequence of Amino Acid OmpH P. multocida NTT Isolate

Cell B Epitope Prediction (Kolaskar and Tongaonkar Antigenicity Method)				
No.	**Amino Acid Position**	**Peptide**	**Length**	**Log Score**
1.	4-10	SVRLILK	7	0,877
2.	37-46	GLSALAYAEL	10	1,027
3.	69-79	DVHVKRLYAGF	11	1,073
4.	98-109	DVGVSDYTYFLG	12	1,102
5.	136-142	GAYVFSA	7	0,876
6.	154-160	GFVVAGL	7	0,885
7.	177-184	SQKYVTVA	8	0,941
8.	197-212	ELSYAGLALGVDYAQS	16	1,206
9.	223-229	ALEVGLN	7	0,868
10.	237-242	KVYTDL	6	0,804
11.	258-274	SIILGAGYKLHKQVETF	17	1,226
	Total	**11**		

Table 3. Prediction of Cell B Epitope from Sequence of Amino Acid OmpHP. multocida Vaccine strain (Katha).

Cell B Epitope Prediction (Kolaskar and Tongaonkar Antigenicity Method)				
No.	**Posisi Asam Amino**	**Peptide**	**Lenght**	**Log Score**
1.	4-12	NGSVRLILK	9	0,985
2.	39-48	GLSALAYAEL	10	1,027
3.	71-81	DVHVKRLYAGF	11	1,073
4.	100-111	DVGVSDYTYFLG	12	1,102
5.	138-144	GAYVFSA	7	0,876
6.	156-162	GFVVAGL	7	0,885

7.	179-185	SQKYVTVA	8	0,941
8.	200-212	YAGLALGVDYAQS	13	1,113
9.	223-229	ALEVGLN	7	0,868
10.	237-242	KVYTDL	6	0,804
11.	258-274	SIILGAGYKLHKQVETF	17	1,226
	Total	**11**		

Conclusion

From this study it can be concluded that *P.multocida* local isolate from NTT has a high homology in nucleotide sequence and gene of 37 kDa OmpH *P. multocida* local isolate from NTT can be potential as immunogenic candidates that can be used as vaccine candidates and diagnostic kits.

Ethical Approval: Not required.

Conflict of Interest: None declared.

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