Detection of 37 kDa Outer Membrane Protein H (ompH) gene of Pasteurella multocida Type B Local Isolate From Nusa Tenggara Timur (NTT)

by Hani Plumeriastuti

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Detection of 37 kDa *Outer Membrane Protein H (ompH)* gene of *Pasteurella multocida* Type B Local Isolate From Nusa Tenggara Timur (NTT)

Firdausy Kurnia Maulana[a], Didik Handijatno[b*], Hani Plumeriastuti[c]

[a]*Magister Student on Infectiouse Disease and Veterinary Public Health Study Program, Veterinary Medicine Faculty, Airlangga University, Indonesia*

[b]*Department of Microbiology and Mycology, Veterinary Medicine Faculty, Airlangga University, Indonesia*

[c]*Department of Veterinary Pathology, Veterinary Medicine Faculty, Airlangga University, Indonesia*

[*]*Corresponding author: Didik Handijatno*

[b]*Department of Microbiology and Mycology, Veterinary Medicine Faculty, Airlangga University Surabaya – Indonesia*

Kampus-C, Mulyorejo. Surabaya-60115. INDONESIA

didik_fkhunair@yahoo.com

Abstract

Haemorrhagic Septicaemia (HS), in Indonesia known as Ngorok disease is a fatal acute septicemia disease in cattle and buffalo caused by *Pasteurella multocida*. Omp has a number of important roles for bacterial cells, such as uptake of nutrients, transporter for molecules in and out of cells and interactions with the host and environment. Outer membrane Protein H (OmpH) in *P. multocida* is one of the major proteins in the envelope which has been purified and characterized as porin immunodominant. The purpose in of this study was to detect the 37d kDa outer membrane protein H (ompH) gene from *P. multocida* local isolate from Nusa Tenggara Timur (NTT) and compare it with vaccine strain. Detection of 37 kDa ompH of P. multocida was done using PCR. Sample used in this study were *P. multocida* local isolate from NTT and vaccine strain (Katha). Isolation and identification were performed using bacteriological culture and biochemically characterization. Results from PCR showed a 37 kDa ompH gene on *P. multocida* local isolate and vaccine strain is 946 bp.

Keywords: haemorrhagic septicaemia; P. multocida type B; outer membrane protein H (ompH) gene; PCR

1. Introduction

Haemorrhagic Septicaemia (HS), in Indonesia known as Ngorok disease is a fatal acute septicemia disease in cattle and buffalo caused by *Pasteurella multocida*. Hemorrhagic Septicemia in an economic perspective become the most important bacterial disease in Middle East, South Africa, Southeast Asia including, Thailand, Philippines, Malaysia and Indonesia (Jabeen *et. al.*, 2013).

Haemorrhagic septicemia caused by infection of *P. multocida* strains belonging to serogroup B and E. *P multocida* is a coccobacillus, Gram negative, non motile bacteria, producing toxin, and found as part of the commensal microflora in the upper respiratory tract of domestic and wild animals (Hotchkiss *et al*., 2010).

The complex interactions between host factors and bacterial virulence factors, such as lipopolysaccharide (LPS), capsule, putative hemolysin, and outer membrane protein (omp) has an effect on the pathogenesis of the disease (Joshi et al., 2013). Omp has a number of important roles for bacterial, such as nutrient uptake, transport of molecules in and out of cells and interactions with the environment and host (Hatfaludi et al., 2010).

Outer membrane Protein H (OmpH) in *P. multocida* is one of the major proteins in the envelope which has been purified and characterized as immunodominant porin. Research has been conducted on the prospect of using OmpH as a subunit vaccine in its original and recombinant form. Immunoblotting studies by Tomer *et al*. (2002) was identified that 37 kDa polypeptide was the most antigenic in the omp profile of all serotype B:2 isolates, which was applied a P-52 whole-cell hyperimmune anti-P. multocida strain made on rabbits and buffalo. Further research conducted by Tan *et. al*. (2010) showed that 37 kDa ompH was more reactive to polyclonal antiserum of mice and recombinant ability of 37 kDa ompH in protecting mice against *P. multocida* approximately 100% which was immunized intraperitoneally and 80% which was immunized subcutaneously and intraperitoneally. Therefore, OmpH is one of the most important bacterial antigens and promising candidates for the development of the vaccine and the diagnostic kit of HS.

The problem of HS disease in Indonesia is still commonly endemic, although vaccination has been done once a year using the HS vaccine made from the Katha strain. DNA Genomic analysis of *P.multocida* by Supar and Arianti (2007) showed that isolate of P. *multocida* from cattle and buffalo that sufferd HS in Indonesia, DNA profile was different from Katha vaccine strain from Burma and different to isolate / strain referens from other region

The purpose of this study was to detect the 37 kDa outer membrane protein H (ompH) of P. *multocida* local isolate from NTT and compare it with vaccine strain.

2. Material and methods

2.1 Sample

Sample of *P. multocida* isolate originated from NTT (Nusa Tenggara Timur) were obtained from Balai Besar Veteriner Denpasar, Bali. Sample was cultured on Blood Agar medium by streak and then incubated for 24-48 hours at 37° C in incubator. Previously, this isolate had been tested for its capsular type. Haemorrhagic Septicaemia vaccine (*P. multocida* Katha strain) from Pusat Veterinaria Farma (Pusvetma) Surabaya was used as a vaccine strain sample.

2.2 Re-Identification of Pasteurella multocida

Inoculation on MacConkey Agar medium was performed to determine the growth properties of *P. multocida*. Which is *P. multocida* do not grow on Mac Conkey media. Furthermore, the examination using biochemical test, namely: TSIA test, SIM test, SCA test, Urease test, and Sugar test (Fajrin,2016).

2.3 DNA Extraction

Total genomic DNA of *P. multocida* was extracted with QIAamp® DNA Mini kit according to standard protocol (Qiagen, 2016). 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

2.4 Detection of ompH gene by 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*. (2010). Primer ompH-F forward 5'-CAGCAACAGTTTACAATCAAGACGGTAC -3 'and ompH-R reverse 3'-GAAGTGTACGCGTAAACC -5' with gene target 946 bp. The thermal cycling conditions are: 1 cycle at 95°C for 5 minutes; 40 cycles at 94°C for 30 sc, 50°C for 1 minute, and 72°C for 1.5 minutes; 1

cycle at 72ºC for 10 minutes. This PCR profile is repeated three more times to ensure specific PCR products. The electrophoresis of PCR product was analyzed in 1% agarose gel.

3. RESULT

3.1 Reculture and Re-identification of Pasteurella multocida

The result from recultured of *P. multocida* on Blood Agar incubated 37 ° C for 24-48 hours as shown in (Fig. 1.) Colonies of clear shiny bacterial bacteria, does not hemolyzed red blood cell and does not growing on Mac Conkey Agar medium can be said suspect *P. multocida*. The suspect colonies of *P. multocida* were then confirmed by biochemical test, the TSIA was obtained in alkaline slat and butt, yielding no gas and H_2S. In the Indol test the pink ring appeared on the surface after Kovach's reagent was added. In Urease Test this bacteria does not produce urease enzymes so it can not change the color of Urease media. In the Simon Citrate Agar Test this bacteria can not use citrates as carbohydrates so as not to change the color of the media Simon Citrate Agar.

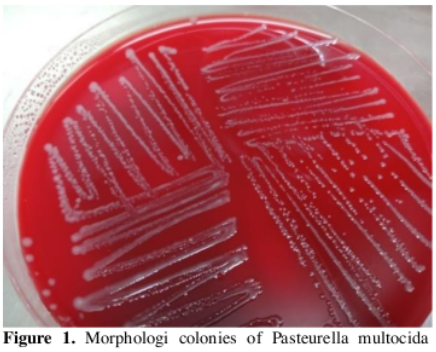

Figure 1. Morphologi colonies of Pasteurella multocida on Blood Agar media

3.2 Detection of ompH by PCR assay

The ompH gene was successfully amplified from local isolate *P. multocida* and vaccine. The PCR product was obtained with the same band size of 946 bp of both samples (Fig. 2).

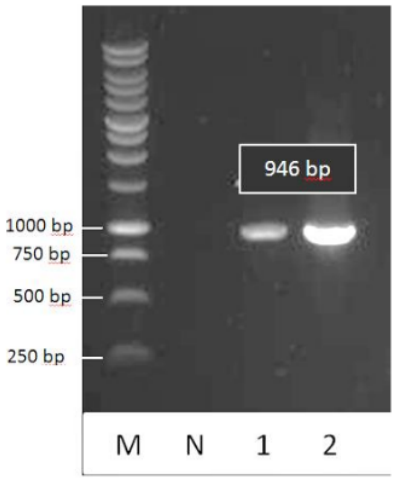

Figure 1. PCR detection of ompH gene of *P.multocida*. Lane M: 1kb molecular ladder (Vivantis); N negative control; 1 *P .multocida* from vaccine; 2 local isolate of *P. multocida*

4. DISCUSSION

In Indonesia, Haemorrhagic septicemia disease is still commonly endemic, although vaccination has been done once a year using vaccine made from Katha strains. (Ghani *et al*., 2016). Omps are exposed to the outside of the bacterial cell and are the first line of contact between the bacterium and environment. Omps has numbers of important role, such as acting as adhesion factors in virulence, channels for nutrients uptake, siderophore receptors and enzymes (Rollauer e*t al*., 2015). Identification of ompH as one of the virulence factors is helpful for the determination of pathogenesis mechanisms and development of prevention and control measures of the disease such as the establishment of an efficient vaccine (Furian *et al*., 2013).

OmpH or porin H of *P. multocida*, is the major outer membrane protein in the envelope. Research has been conducted in purification and characterization this protein and because its structure and functional related to the superfamily of porins of Gram negative bacteria, this protein classified as Porin (Ganguly *et al*., 2015). In native conformation, porin H is a homotrimer, stable in sodium dodecyl sulfate (SDS) at room temperature, and is dissociated into monomers upon boiling. The molecular masses of ompH range between 34 and 42 kDa depending on the serotype of bacteria and

the electrophoretic system used for analysis (Lou *et al.,* 1997)

In an earlier study, 37 kDa ompH were identified as major immunodominant proteins and has been shown to be provide immunity, hence primers used in this study are specific for 37 kDa omph genes. In this study the ompH gene of P.multocida local isolate and vaccine strain was detected using PCR. PCR product obtained result of 946 bp for both samples. Research by singh et al. (2011) in isolate P. multocida P-52 (vaccine strain for HS), the ompH gene was obtained approximately 1.2 kb. While on research by Tan et al. (2010) which were the primer developed in this study 37 kDa ompH gene was obtained for 980 bp. The difference from the primer use in each study had an effect on the result of PCR of the ompH gene itself.

In ompH gene detection in this study, P. multocida local isolate and vaccine strains showed the same band. But it is not known whether they have the same DNA sequence.

From the results obtained in this study, it is necessary to further investigate the prediction of epitope genes encoded by 37 kDa ompH for development in a vaccine consistent with the incidence of Haemorrhagic septicemia in Indonesia

Conclusion

In this study, 37 kDa ompH gene from local *P. multocida* isolate and vaccine strain (katha) was obtained with PCR yield of 946 bp

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References

1. Fajrin, F.A. 2016. Deteksi Gen Penyandi Kapsular *Pasteurella multocida* tipe A Pada Fowl Cholera Dengan Teknik Polymerase Chain Reaction [skripsi]. Fakultas Kedokteran Hewan. Universitas Airlangga
2. Furian, T.Q., Borges, K.A., Pillati, R.M., Almeida, C., Nascimento, V.P., Salle, C.T.P., and Moraes, H.L. 2014. Identification of The capsule Type of Pasteurella Multocida Isolates from Cases of Fowl Cholera by Multiplex PCR and Comparison with Phenotypic Methods. Brazilian Journal of Poultry Science; 31-36
3. Gangul, B., K. Tewari, R. Singh. 2015. Homology modeling, functional annotation and comparative genomics of outer membrane protein H of Pasteurella multocida. Journal of Theoretical Biology. 386 (2015):18–24
4. Ghani, R.A., N. A. Khattak, M. Saqlain, M. J. Asad, A. Khanum, S. M. S. Naqvi, G. K. Raja. 2016. Comparison of the Common Immunogenic Protein Components of Pasteurella multocida Serotypes B:2 and B: 3,4. Kafkas Univ Vet Fak Derg. 22 (4): 485-491
5. Hatfaludi, T. 2009. Membrane proteins of Pasteurella multocida: roles in immunity & disease [A thesis] Department of Microbiology Faculty of Medicine, Nursing and Health Sciences Monash University Clayton, VIC 3800, Australia.
6. Hotchkiss, E.J., M.P. Dagleish, K. Willoughby, I.J. McKendrick, J. Finlayson, R.N. Zadoks, Prevalence of Pasteurella multocida and other respiratory pathogens in thenasal tract of Scottish calves, Vet. Rec. 167 (2010) 555e560, http://dx.doi.org/10.1136/vr.c4827.
7. Jabeen, A., M. Khattak, S. Munir, Q. Jamal, M.Hussain. 2013. Antibiotic Susceptibility and Molecular Analysis of Bacterial Pathogen Pasteurella Multocida Isolated from Cattle. Journal of Applied Pharmaceutical Science. 3 (04): 106-110
8. Joshi, S., K. Tewari, R. Singh. 2013. Comparative immunogenicity and protective effi cacy of different preparations of outer membrane proteins of Pasteurella multocida (B:2) in a mouse model. Veterinarski Arhiv 83 (6): 665-676.
9. Luo, Y., J. R. Glisson, M. W. Jackwood, R. E. W. Hancock, M. Bains, I. N. Cheng, C. Wang. 1997. Cloning And Characterization Of The Major Outer Membrane Protein Gene (Omph) Of Pasteurella Multocida X-73. J. Bacteriol. 179: 7856-7864.
10. Rollauer S.E., M.A. Sooreshjani, N. Noinaj, S.K. Buchanan. 2015 Outer membrane protein biogenesis in Gram-negative bacteria. Phil. Trans. R. Soc. B 370: 20150023.
11. Singh, R., K. Tewari, N. Packiriswamy, S. Marla, dan V. D. P. Rao. 2011. Molecular characterization and computational analysis of the major outer membrane protein outer membrane protein (ompH) gene of Pasteurella multocidaPasteurella multocida P52. Veterinarski Arhiv. 81 (2): 211-222
12. Singh, R., K. Tewari, N. Packiriswamy, S. Marla, dan V. D. P. Rao. 2011. Molecular characterization and computational analysis of the major outer membrane protein outer membrane protein (ompH) gene of Pasteurella multocidaPasteurella multocida P52. Veterinarski Arhiv. 81 (2): 211-222
13. Tan, H.Y., N.H. Nagoor, dan S.D. Sekaran. 2010. Cloning, expression and protective capacity of 37 kDa outer membrane protein gene (ompH) of Pasteurella multocida serotype B:2. Tropical Biomedicine 27(3): 430–441
14. Tomer, P., G.C Chaturvedi, Minakshi, P. Malik, dan D.P. Monga. 2002. Comparative analysis of the outer membrane protein profiles of isolates of the Pasteurella multocida (B:2) associated with haemorrhagic septicaemia. Veterinary Research Communications 26: 513– 522.Paterson, D.L., dan R. A. Bonomo. 2005.Extended-spectrumlactamases: a clinical update. Clin Microbiol Reviews 18(4).
15. Qiagen. 2016. QIAamp® DNA Mini and Blood Mini Handbook 5-ed. https://www.qiagen.com/ [28 Januari 2018]
16. Supar dan Ariyanti, T. 2007. Karakterisasi Molekuler Pasteurella multocida: Kaitannya Dengan Epidemiologi dan Pengembangan Vaksin Isolat Lokal. Wartazoa . 17(4):147-155

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