Outer Membrane Protein (OMP) Profiles of *Brucella abortus* Local Isolate by SDS-PAGE Procedure

Tri Handayani [1*], Dadang Priyoatmojo[1], Afi Candra Trinugraha [1]

[1]*Department of Agriculture, Research and Technology Center for Isotopes and Radiation Application, National Agency for Research and Innovation (BRIN) Jakarta, Indonesia*
[*]*Corresponding author. Email: trihanda1980@gmail.com*

ABSTRACT
Brucella abortus is a Gram-negative, facultative intracellular bacteria of the genus Brucella that common cause of brucellosis in cattle. The Outer Membrane Protein (OMP) is an essential organelle of Gram-negative bacteria to fulfil several some many tasks and is crucial as a protective antigen. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a universal method for molecular weight separation and analysis of proteins. This study aimed to determine the profile Outer Membrane Protein (OMP) band composition of *Brucella abortus* local isolate. The *B. abortus* isolate is a collection of Department of Agriculture, Research and Technology Center for Isotopes and Radiation Application-BRIN), isolated from the hygroma fluid in the case field. N - Lauroylsarcosine (Sarkosyl) methods used to extract outer membrane bacteria. OMP *B. abortus* was measured protein molecular weight by SDS-PAGE and standard molecular weight of 10 – 250 kDa. This study showed that the profile Outer membrane Proteins (OMP) *Brucella abortus* local isolate had eight bands there were 16.7-kDa, 18-kDa, 26.6-kDa, 33-kDa, 42-kDa, 55-kDa, 72-kDa, and 91.3-kD.

Keywords: *Brucella abortus local isolate, OMP, SDS-PAGE.*

1. INTRODUCTION

Brucellosis is a zoonotic bacterial disease with a significant economic impact, also known as Bang's Disease [1][2]. Brucellosis is caused by various bacteria from the Brucella family, which infect several animals, including cattle (*B. abortus*), pigs (*B. suis*), sheep (*B. ovis*), goats (*B. melitensis*), dog (*B. canis*), and rodents (B. neotomes). This classification is mainly based on pathogenicity and host preference [3][4]. In Indonesia, Brucellosis is one of the diseases that government's focus [5], the disease is zoonotic and causes losses in the livestock world [6], characterized by abortion in livestock or reproductive failures such as abortion, especially in the third trimester of pregnancy, retained placenta, orchitis, and infertility [6]. Tulu *et al.* [7] abortion in brucellosis is usually around the 7^{th} month. In Indonesia, the economic loss caused by Brucellosis in large ruminants is estimated at IDR 3.6 trillion per year or 1.8% of the total value of livestock assets in Indonesia [8].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is a common method used to determine the molecular weight of a protein [9]. The principle of SDS-PAGE is proteins that are separated by electrophoresis through a gel matrix and an electric flow, proteins with smaller molecules migrate faster while larger molecules are trapped in gel due to slower movement. Another influence on the rate of migration protein is based on the structure and charge of the protein. SDS-PAGE gel is divided into two parts, its separating gel and stacking gel [10].

Brucella cell membrane consists of three layers, the innermost layer is the cytoplasmic membrane, the intermediate layer is the peripheral cytoplasmic membrane, and the outermost layer is the outer membrane (OM). The outer membrane binds to the peptidoglycan layer makes a cell wall containing lipopolysaccharide (LPS), proteins and a phospholipid layer [11]. Outer membrane protein (OMP) is the main structure of the cell wall of Gram-negative bacteria, which contains LPS as virulence factor of *Brucella sp.*,

conserved and strong immunogen [4]. Therefore immunogen for cell-mediated immune induction against intracellular pathogens can be used for diagnostic antigens and protective antigens in vaccine development [12]. Cassataro *et al.* [13] OMP as a subunit vaccine candidate was reported in various animal species.

Vaccination of animals is usually carried out using live attenuated strains, such as *B. abortus S19*, *B. melitensis Rev.1*, and *B. abortus RB51* [3]. Live attenuated vaccines have some lacks in animals, such as residual virulence, abortion, and interference with serology [14]. This study aimed to determine the profile Outer Membrane Protein (OMP) band composition of *Brucella abortus* local isolate.

2. MATERIALS AND METHODS

2.1. Bacterial strains, media, and growth conditions

Brucella abortus B4 was used in this study. The bacteria are a collection of Research and Technology Center for Isotopes and Radiation Application, National Agency for Research and Innovation (BRIN) Jakarta-Indonesia from the hygroma fluid cow in case field and have been identified biochemically. The stock culture was maintained at -80 °C in sheep's blood or lyophilization. *B. abortus B4* was cultured in Tryptic soy agar (TSA) media at 37°C with 5% CO_2 for 3 days [15][16].

2.1. Extraction of Outer Membrane Protein (OMP) using N-lauroylsarcosine

OMP extraction was carried out based on the method used by Muthiadin [17] with modifications. The culture suspension was harvested and centrifuged at 15.000 rpm for 20 minutes at 4°C. The pellet was re-suspended in 1 ml 10 mmol Tris-HCl, pH 8, and homogenized by a vortex. Cells bacteria were sonicated with a Branson brand sonicator 4 times among 5 seconds with a break and the chamber was added ice. After that, centrifugation was carried out at 15.000 rpm for 1 hour at 4 °C. The pellets were added 0.5 ml 10 mmol Tris HCl and 0.5 ml 10% N-laurylsarcosine (sarkosyl) and then homogenized with a vortex, left for 20 minutes at room temperature. The sarkosyl-treated membranes were ultracentrifuge at 15.000 rpm for 90 minutes at 4 °C. Following the final centrifugation, the membrane was added PBS for SDS PAGE analysis.

3.1. Profil Protein Outer Membran SDS-PAGE

OMP *B. abortus* was measured protein molecular weight (MW) by SDS-PAGE and standard molecular weight of 10 – 250 kDa. The calculation is done by measuring the total tracking distance from the stacking gel to the separating gel, followed by measuring the tracking distance from the stacking gel to each protein band formed. The molecular weight of each protein was determined using standard curves made from standard markers and calculated using Microsoft Excel software[18].

3. RESULTS AND DISCUSSION

The whole-cell protein profiles of the *B. abortus* were determined using 12% (w/v) separating and 4% (w/v) stacking gels of SDS-PAGE. Eight protein bands could be resolved to range from below 92 kDa to 16.7 kDa as determined by visual assessment of their approximate molecular weight.

The culture of *B. abortus* on Tryptic Soy Agar as shown in Figure 1, bacteria showed small colonies, round and cream colored. Microscopic observation of Gram stain showed red color with short rods (coccobacillus) (Figure 2). This shows that *B. abortus* is not resistant to alcohol, and loses the primary stain then the safranin dye can penetrate the cell wall, giving it a red color. According to Quinn *et al.* [16], Gram staining *B. abortus* was coccobacillus and red color. It is known that the cell wall structure of Gram-negative bacteria is surrounded by thin peptidoglycan, which is surrounded by an outer membrane containing lipopolysaccharide [2][19].

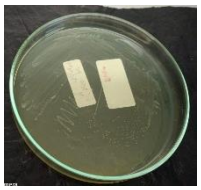

Figure 1 Colony growth of *B. abortus* on Tryptic Soy Agar media

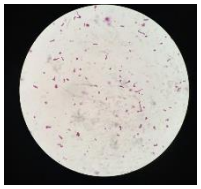

Figure 2 Gram-negative *B. abortus* imaging; short rods (coccobacillus);10x100

In the study, a sonicator was carried out to break intermolecular bonds or damage *B. abortus* bacterial cells, the process of converting electrical signals into mechanical vibrations. Sodium N-laurylsarcosine (sarkosyl) is useful to dissolve in the inner membrane while the outer membrane is resistant to sarcosine dissolution. OMP extraction in Gram-negative bacteria using the sarkosyl method has been carried out by other

studies, its *E. coli* [20], *Pasteurella multocida type B-2* [21].

The results of SDS PAGE with Coomassie Brilliant Blue staining presented in Figure 3, that protein bands of OMP *B. abortus* have appeared on lanes 1 and 2. The SDS-PAGE gel showed the presence of 8 protein bands of *B. abortus* OMP, they are 16.7 kDa, 18 kDa, 26.6 kDa, 33 kDa, 42.5 kDa, 55 kDa, 72 kDa, and 91.3 kDa. The protein band from this study differs from the study by Siadat *et al*. [22] which reported band OMP of *B. abortus* S99 is 36-38 kDa then classified as porins. This difference might be related to variations in bacterial strains, OMP extraction methods, buffer composition, and gel permeability, thus affecting the size of the protein that will be separated. The protein results obtained from this study is closely related with Santos *et al*. [23] which classified OMP into three groups, group 1 is proteins with MW of 88 – 94 kDa; group 2 is proteins with a MW of 35-39 kDa, and group 3 is proteins with MW of 25 -31 kDa. In another study, categorizes OMP based on molecular weight with group 1 approximate 94 kDa, group 2 with MW of 41- 43kDa, and group 3 approximate MW of 28-30 kDa, also band of 14 kDa referred to as lysozyme [24]. Based on these, molecular weight *B. abortus* B4 can be grouped into groups 1, 2, and 3, besides that some proteins show bands with low molecular weight are 16.7 kDa and 18 kDa. The low molecular weight in Brucella sp. has been reported by Tibor *et al*. [25] which defined lipoproteins consists of Omp10, Omp16, and Omp19.

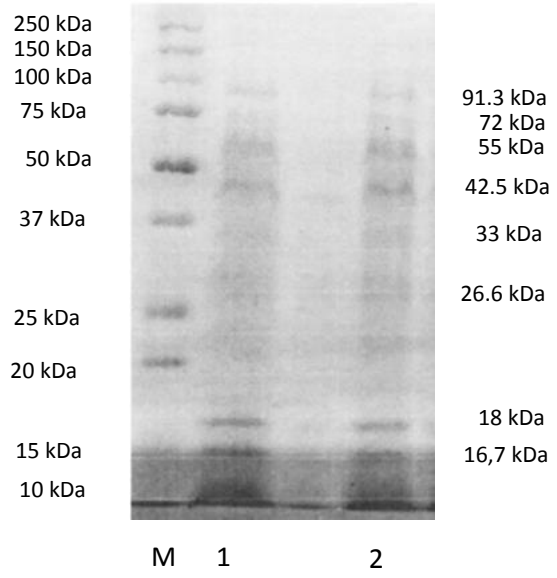

Figure 3 SDS-PAGE analysis of the Outer membrane protein profiles of *B. abortus* local isolates. M = protein marker as standards on the left; Lane 1- 2 = extract OMP *B. abortus* B4

4. CONCLUSION

This study demonstrates that the OMP profile of local isolates of *Brucella abortus* B4 using SDS-PAGE obtained 8 band patterns were 16.7 kDa, 18 kDa, 26.6 kDa, 33 kDa, 42.5 kDa, 55 kDa, 72 kDa, and 91.3 kDa. Our results suggest that *Brucella abortus* B4 is closely related to groups 1, 2, and 3.

AUTHORS' CONTRIBUTIONS

Tri Handayani designed the experiment, collected the data, and wrote the manuscript. Dadang Priyoatmojo and Afi Candra Trinugraha performed data analysis and reviewed the manuscript.

ACKNOWLEDGMENTS

The authors thank to Teguh Wahyono for guidance and all personnel in the laboratory for the support of this research. This research was funded by DIPA budget.

REFERENCES

[1] E. Moreno, "Retrospective and prospective perspectives on zoonotic brucellosis," *Front Microbiol*, vol. 5, no. May, pp. 1–18, 2014, doi: 10.3389/fmicb.2014.00213.

[2] The Center for Food security and Public Health [CFSPH], "Brucellosis : Brucella abortus Brucella abortus," *Brucellosis: Brucella abortus*. Iowa State University, pp. 1–12, 2018.

[3] Office International des Epizooties [OIE], "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: Chapter 3.1.4. Brucellosis (Brucella abortus, B. melitensis and B. suis) (infection with B. abortus, B. melitensis and B.suis)." 2021, [Online]. Available: https://www.oie.int/en/what-we-do/standards/codes-and-manuals/terrestrial-manual-online-access/.

[4] P. G. Cardoso, G. C. Macedo, V. Azevedo, and S. C. Oliveira, "Brucella spp noncanonical LPS: Structure, biosynthesis, and interaction with host immune system," *Microb. Cell Fact.*, vol. 5, no. February 2006, 2006, doi: 10.1186/1475-2859-5-13.

[5] Keputusan Menteri Pertanian Nomor 4026/Kpts./Ot.140/3/2013, "Penetapan Jenis Penyakit Hewan Menular Strategis(PHMS)." 2013.

[6] S. Ryu, R. J. S. Magalhães, and B. C. Chun, "The impact of expanded brucellosis surveillance in beef cattle on human brucellosis in Korea : an interrupted time- series analysis," *BMC Infect. Dis.*, vol. 19, pp. 1–7, 2019, doi: 10.1186/s12879-019-3825-6.

[7] D. Tulu, B. Deresa, F. Begna, and A. Gojam, "Review of common causes of abortion in dairy cattle in Ethiopia," *J. Vet. Med. Anim. Heal. Ethiop.*, vol. 10, no. January, pp. 1–13, 2018, doi: 10.5897/JVMAH2017.0639.

[8] C. Basri and B. Sumiarto, "The Estimation of Economic Losses Caused by Brucellosis in Livestock Population in Indonesia," *J. Vet.*, vol. 18, no. 4, pp. 547–556, 2017, doi: 10.19087/jveteriner.2017.18.4.547.

[9] A. Pavlova, E. Dyudeeva, M. Kupryushkin, N. Amirkhanov, D. Pyshnyi, and I. Pyshnaya, "SDS-PAGE Procedure : Application for Characterization of New Entirely Uncharged Nucleic Acids Analogues Short Communication SDS-PAGE procedure : Application for characterization of new entirely uncharged," *Electrophoresis*, 2017, doi: 10.1002/elps.201700415.

[10] R. Sharma and Y. Rajput, "SDS-PAGE – Principle and Applications Rajan." ICAR-National Dairy Reseach Institute, India, pp. 36–40, 2015.

[11] W. Y. Zheng, Y. Wang, Z. C. Zhang, and F. Yan, "Immunological characteristics of outer membrane protein omp31 of goat Brucella and its monoclonal antibody," *Genet. Mol. Res.*, vol. 14, no. 4, pp. 11965–11974, 2015, doi: 10.4238/2015.October.5.10.

[12] J. M. C. Carpio and C. Mingala, "Outer Membrane Proteins: Its Role in Brucella Virulence and Immunogenicity," *Inter J Vet Sci*, vol. 7, no. 1, pp. 33–37, 2018.

[13] J. Cassataro *et al.*, "A recombinant subunit vaccine based on the insertion of 27 amino acids from Omp31 to the N-terminus of BLS induced a similar degree of protection against B. ovis than Rev.1 vaccination," *Vaccine*, vol. 25, no. 22, pp. 4437–4446, 2007, doi: 10.1016/j.vaccine.2007.03.028.

[14] G. Wareth, M. W. Pletz, H. Neubauer, and J. Murugaiyan, "Proteomics of Brucella : Technologies and Their Applications for Basic Research and Medical Microbiology," *Microorganism*, vol. 8, no. 766, 2020, doi: 10.3390/microorganisms8050766.

[15] G. Alton, L. Jones, and D. Pietz, *Laboratory techniques in brucellosis*, 2nd ed. World Health Organization & Food and Agriculture Organization of the United Nations, 1975.

[16] P. Quinn, B. Markey, M. Carter, W. Donnelly, and F. Leonard, *Veterinary Microbiology and Microbial Disease*. Black-well publishing, 2002.

[17] C. Muthiadin, "Purifikasi Antigen Outer Membrane Protein (OMP) Dari Isolat Salmonella enterica serovar Typhi," *Pros. Semin. Nas. Mikrobiol. Kesehat. dan Lingkung.*, no. ISBN 978-602-72245-0-6, pp. 106–114, 2015.

[18] Bio-Rad Laboratories. Inc, "Molecular Weight Estimation." Bulletin 6210, pp. 1–2, [Online]. Available: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6210.pdf.

[19] S. K. Khurana *et al.*, "Bovine brucellosis – a comprehensive review," *Vet. Q.*, vol. 41, no. 1, pp. 61–88, 2021, doi: 10.1080/01652176.2020.1868616.

[20] B. Dehghani, M. Mottamedifar, H. Khoshkhram-Roodmajani, A. Hassanzadeh, K. Zomorrodian, and A. Rahimi, "SDS-PAGE Analysis of the Outer Membrane Proteins of Uropathogenic Escherichia coli Isolated from Patients in Different Wards of," *Iran J Med Sci*, vol. 41, no. 5, pp. 399–405, 2016.

[21] I. Altaf, A. Khalid, J. Nazir, W. Shahzad, and R. Bashir, "Outer membrane proteins and lipopolysaccharides mediated antibody response against bovine Pasteurella multocida type B-2," *Malays.J.Microbiol*, vol. 12, no. 5, pp. 370–375, 2016.

[22] S. D. Siadat, M. R. Aghasadeghi, M. Sadat, and A. Moshiri, "Biological and immunological characteristics of Brucella abortus S99 major outer membrane proteins Biological and immunological characteristics of Brucella abortus S99 major outer membrane proteins," *Jundishapur J Microbiol*, vol. 4, no. 1, pp. 29–36, 2011.

[23] J. M. Santos, D. R. Verstreate, V. Y. Perera, and A. J. Winter, "Outer Membrane Proteins from Rough Strains of Four Brucella Species," *Infect Immun*, vol. 46, no. 1, pp. 188–194, 1984.

[24] D. Verstreate, M. Creasy, N. Caveney, C. Baldwin, M. Blab, and A. Winter, "Outer Membrane Proteins of Brucella abortus : Isolation and Characterization," *Infect. Immun.*, vol. 35, no. 3, pp. 979–989, 1982.

[25] B. Tibor, A. Decelle and J. Letesson, "Outer Membrane Proteins Omp10 , Omp16 , and Omp19 of Brucella spp . Are Lipoproteins," *Infect. Immun.*, vol. 67, no. 9, pp. 4960–4962, 1999.