Jurnal Kedokteran Hewan March 2018, 12(1):7-9
P-ISSN : 1978-225X; E-ISSN : 2502-5600 DOI: https://doi.org/ 10.21157/j.ked.hewan.v12i1.8091

CHARACTERIZATION OF VirB4 PROTEIN OF LOCAL ISOLATE *Brucella abortus* WITH WESTERN BLOTTING TECHNIQUE

Ratih Novita Praja[1]*, Didik Handijatno[1], Setiawan Koesdarto[2], and Aditya Yudhana[2]
[1]Department of Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia
*Corresponding author: ratihnovitapraja@fkh.unair.ac.id

ABSTRACT

This research aimed to characterize VirB4 protein of local isolate *Brucella abortus* with Western blotting method. The result showed that there were four protein bands with molecular weights of 64.61, 59.25, 21.63, and 16.70 kDa by triggering a reaction between the whole *Brucella abortus* and anti-*Brucella abortus* serum. The results also revealed that there was only one protein band with a molecular weight of 59.25 kDa triggering a reaction between the whole *Brucella abortus* and anti-VirB *Brucella abortus* serum. Finally, it can be concluded that VirB4 protein can affect the virulence factor of *Brucella abortus*, successfully characterized with the appearance of one band with a molecular weight of 59.25 kDa by using Western blotting method.

Key words: *Brucella abortus*, SDS-PAGE, VirB4, Western blotting

ABSTRAK

Penelitian ini bertujuan mengkarakterisasi protein VirB4 B. abortus isolate local dengan metode Western blotting. Hasil karakterisasi protein Brucella abortus isolat lokal dengan teknik Western blotting yang mereaksikan whole Brucella abortus dengan serum anti-Brucella abortus terdapat 4 pita protein dengan berat molekul 64,61; 59,25; 21,63; 16,70 kDa sedangkan yang mereaksikan whole Brucella abortus dengan serum anti-VirB Brucella abortus terdapat 1 pita protein dengan berat molekul 59,25 kDa. Kesimpulan penelitian ini adalah terdapat protein yang memengaruhi faktor virulensi B. abortus yaitu VirB4 dan berhasil terkarakterisasi melalui metode Western blotting ditandai dengan munculnya 1 pita dengan berat molekul 59,25 kDa.

Kata kunci: Brucella abortus, SDS-PAGE, VirB4, Western blotting

INTRODUCTION

Brucellosis is a disease caused by pathogenic bacteria belong to the genus Brucella. This disease can infect some mammals, such as cows, goats, and pigs. This disease even can be transmitted to humans. Consequently, the disease can threaten farming since it can cause miscarriage. Brucellosis cases in cattle are generally caused by *Brucella abortus* (*B. abortus*) bacteria (Agasthya *et al*., 2007). Brucellosis can lead to Brucellosis can lead to reproductive disorders in male such as orchitis and epididymitis. Additionally, *B. abortus* infection in bullcan trigger a wider contamination of brucellosis since Brucella bacteria can be found in the semen (Macedo *et al*., 2011).

Brucellosis is also known as undulant fever since its fever symptoms appear with temperatures varying and recurring in infected individuals not only animals but also human. According to that statement, brucellosis is also an important zoonotic disease. However, Brucella infection in human does not cause miscarriage, but only causes clinical symptoms such as intermittent fever, headache, myalgia, malaise, pain, and indigestion. Moreover, Brucella also include in intracellular bacteria which has specific virulent factors when infect the host cell (Megid *et al*., 2010).

Brucella can survive in a long period in the host cell despite the limitations of nutrients and oxygen (Moreno and Gorvel, 2004). Brucella entering the host cells then directly move to vacuolar pathways in phagocytic cells to prevent endocytosis and inhibit phagosome-lysosome unification. Next, Brucella transit in cells via Brucella containing vacuole (BCV) that interacts with the membrane of the endoplasmic reticulum (ER) to trigger bacteria to conduct processes of maturation and intracellular replication, thus allowing them to multiply themselves (Pitt *et al*., 1992). Brucella also express unique virulence characteristics with certain determinants of virulence, one of which is VirB protein (Pizarro *et al*., 1998). In general, VirB can be considered as a factor triggering Brucella to survive by affecting expression of other genes and modifying the signaling pathway of the host cells (Nijskens *et al*., 2008).

In addition, type IV secretion system (T4SS) is a combination of 12 proteins, namely VirB1, VirB2, VirB3, VirB4, VirB5, VirB6, VirB7, VirB8, VirB9, VirB10, VirB11, and VirD4. VirB4, VirB11, and VirD4 can interact with each other as ATPase. This is essential for the assembly of T4SS and the transport of effector proteins (Cellini *et al*., 2005). Cellular interactions occur between T4SS and the endoplasmic reticulum has an important role in secreting certain factors that can inhibit phagocyte system responsible for the bacterial elimination.

For those reasons, this research aimed to provide data on characterization of VirB4 protein of local isolate of *B. abortus* using Western Blotting method. Results of this research are expected to be used as a reference for the manufacture of diagnostic kits as well as new vaccine candidates for Brucellosis disease in Indonesia.

MATERIALS AND METHODS

This research used four male New Zealand rabbits aged 8 months. *B. abortus* isolates obtained from the

Laboratory of Bacteriology and Mycology of the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. Characterization of VirB4 protein of *B. abortus* was performed using Western blotting method as followed. *Brucella abortus* was grown in the Brucella agar medium (BAM), and identified by Gram staining and biochemical examinations using urease test, citric test, sulfide indole motility (SIM) test, and triple sugar iron agar (TSIA) test. The bacteria were then cultured in tryptic soy broth media and subjected to centrifugation at 75 rpm for 36 hours for cell pellets collection. Cell pellets were resuspended in 5 mL of 1x phosphate buffered saline (PBS), and sonicated at an amplitude speed of 30% for 1 minute with an interval of 15 seconds. The sonication process was repeated 30 times at a temperature of 4° C. The products were spun at 12,000 rpm for 10 minutes. Supernatants obtained were separated from the pellets using polyacrylamide gel electrophoresis technique (Ali *et al*., 2006).

The VirB4 protein (59.25 kDa) *B. abortus* (Celli *et al*., 2003) was isolated from the gel and dialyzed to remove undesirable components. Next, VirB4 protein solution, 0.25 mL, was subcutaneously injected into rabbits for triggering antibody formation process. The injection was carried out by adding Complete Freund's Adjuvant (CFA) with a ratio of 1:1 in the first immunization and Incomplete Freund's Adjuvant (IFA) in repeated immunization (booster). The booster was performed twice with an interval of two weeks. Two weeks after the last booster, whole blood was collected from auricular veins and centrifuged at 2000 rpm for 10 minutes for serum collection. Anti-VirB4 antibody produced was used for protein identification using Western blot technique (Munir, 2010). The results was analyzed based on molecular weight by using regulation method conducted by dividing the distance (cm) of protein bands from separator gel limits with the tracking dye distance (cm) from the upper limit of separator gel (Rand and Laing, 2011).

Molecular weight (MW) calculation was performed using a regression equation between retardation factor (rf) and log MW on marker. The rf value was obtained by dividing the distance (between preparation gel and a ribbon-shaped on the marker) with a length of gel. Results of the regression analysis using statistical product and service solution (SPSS) for Windows 20 between rf value and log BM (Da) on the markers were in cubic form, equal to a equation of $y = 2.561 – 3.847x + 5.445x^2 – 3.099x^3$. This equation then was used for calculating protein BM in the samples, with y = log BM (Da) and the x value of rf.

RESULTS AND DISCUSSION

The identification of local isolate *B. abortus* grown on BAM showed round, smooth, and shiny yellow honey-like bacterial colonies. These colonies were identified by using conventional bacteriological examination (Gram staining) and by performing some biochemical tests. Results of bacteriological examination indicated that *B. abortus* isolates belonged to Gram negative bacteria form clustered or in pairs, not encapsulated coccobacillus.

The biochemical tests performed showed that the bacteria were urease positive (a yellow to reddish color changes of culture media), citrate and indole negative (unchanged test medium). Moreover, results of TSIA test showed that butt and slant were alkaline marked with both red color on the top and bottom of the media without gas formation. Catalase test performed showed positive result as marked with bubbles of air formation. Overall, by referring to bacterial properties (Sangari *et*

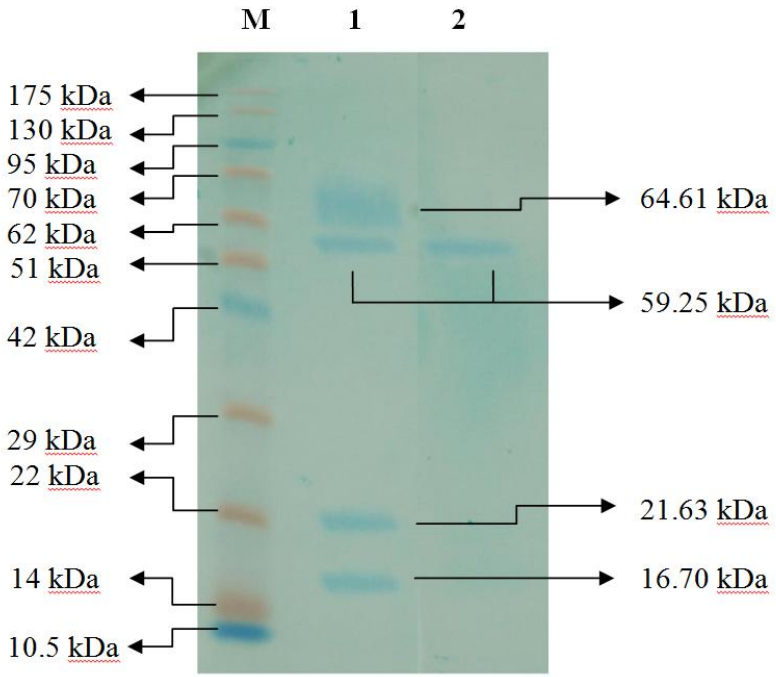

Figure 1. Results of the characterization of VirB4 protein using Western blotting (M= Marker; 1= A reaction of the whole *Brucella abortus* and anti-*Brucella abortus* serum, 2= A reaction of the whole *Brucella abortus* and anti-VirB4 *Brucella abortus* serum)

al., 2000), the results of both bacteriological and biochemical examinations confirmed the isolated bacteria were *B. abortus.*

Furthermore, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique was used to determine the molecular weight of the unknown proteins (Grabski and Burgess, 2007). There were 10 protein bands of *B. abortus* identified namely 158.93, 110.89, 99.93, 70.60, 64.61, 59.25, 45.32, 42.35, 21.63, and 16.70 kDa.

Results of the characterization of VirB4 protein of local isolate *B. abortus* using Western blotting technique showed that there were four protein bands with molecular weights of 64.61, 59.25, 21.63, and 16.70 kDa triggering a reaction between the whole *B. abortus* and anti-*B. abortus* serum. The results also revealed that there was only one protein band with a molecular weight of 59.25 kDa triggering a reaction between the whole *B. abortus* and anti-VirB *B. Abortus* serum. Those findings were in line with the previous research using polymerase chain reaction (PCR) method showing the gene of VirB4 protein of *B. abortus* obtained was 1600 bp, then converted into kDa. Consequently, a molecular weight of VirB4 protein of *B. abortus* was estimated at 59.25 kDa. GenScript Converter Software used to calculate the convertion, and the result is 270 bp equal to 10 kDa so 1600 bp equal to 59.25 kDa.

VirB4 protein has been detected with molecular weight 59.25 kDa and this result indicates that virulent factor can play role when Brucella infect the host cell. With the present of VirB4 protein, Brucella entering the host cells then directly move to vacuolar pathways in phagocytic cells by preventing endocytosis and inhibiting phagosome-lysosome unification. Brucella transit in cells via BCV, then BCV interact with the membrane of the endoplasmic reticulum (ER) to trigger bacteria to conduct processes of maturation and intracellular replication, as a result, the bacteria can multiply themselves (Pitt *et al*., 1992). Brucella can also express unique virulence characteristics with certain determinants of virulence, one of which is VirB4 protein. Based on previous study, VirB4 protein found inside T4SS of intracellular bacteria. In all living organisms, such as bacteria, secretion system mediates part of macromolecules crossing cell membranes. Therefore, the secretion system is very essential for virulence and life sustainability of bacteria (Pizarro *et al*., 1998).

T4SS is a combination of the 12 proteins, namely from VirB1 to VirB11 as well as VirD4. T4SS usually has three special ATPases forming a secretion energy center. Those three ATPases are constituent proteins of the secretion system, namely VirD4, VirB11, and VirB4. Those three proteins are also important for the secretion system of Gram-negative bacteria. As a result, those proteins tend to form a great ATPase complex providing energy for substrate transportation from the cytoplasm through translocation mechanism. VirB11 even can directly interact with VirB4 and VirD4, involving domain nearby the center region of the two proteins inside or near the inner membrane (Taminiau *et al*., 2002).

CONCLUSION

It can be concluded that the 59.25 kDa VirB4 protein of local isolate *B. abortus* could be successfully characterized using Western blotting method.

REFERENCES

Agasthya, A.S., S. Isloor, and K. Prabhudas. 2007. Brucellosis in high risk group individual. **Ind. J. Med. Microbiol.** 25(1):28-31.

Ali, B.A., T.H. Huang, H.H. Salem, and Q.D. Xie. 2006. Influence ot thermal cycler day to day reproducibility of random amplified polymorphic DNA fingerprint. **Biotechnology.** (5):324-329.

Celli, J., C. de Chastellier, D.M. Franchini, J. Pizarro-Cerda, and E. Moreno. 2003. Brucella evades macrophage killing via VirB-dependent sustained interactions with the endoplasmic reticulum. **J. Exp. Med.** 198:545-556.

Cellini, J., S.P. Salcedo, and J.P. Gorvel. 2005. From the cover: Brucella coopts the small GTPase Sar1 for intracellular replication. **Proceedings Nat. Acad. Sci.** 102:1673-1678.

Macedo, G.C., D.M. Magnani, N.B. Carvalho, and O.B. Romero. 2011. Central role of MyD88-dependent dendritic cell maturation and proinflammatory cytokine production to control *Brucella abortus* infection. **J. Immunol.** 180:1080-1087.

Megid, J., L.A. Mathias, and C.A. Robles. 2010. Clinical manifestations of brucellosis in domestic animal and human. **Open Vet. Sci**. 4:119-126.

Moreno, E. and J.P. Gorvel. 2004. Invasion, intracellular trafficking and replication of Brucella organisms in professional and nonprofessional phagocytes. **Bioscience Horizon**. 1:287-312.

Munir, R., M. Afzal, M. Hussain, S. M. S. Naqvi, and A. Khanum. 2010. Outer membrane proteins of *Brucella abortus* vaccinal and field strains and their immune response in buffaloes. **Pak. Vet. J.** 30(2):110-114.

Nijskens, C., R. Copin, X. De Bolle, and J.J. Letesson. 2008. Intracellular rescuing of a *B. melitensis* 16 M VirB mutant by co-infection with a wild type strain. **Microb. Pathog.** 45:134-141.

Grabski, A.C. and R.R. Burgess. 2007. Preparation of Protein Samples for SDS-Polyacrylamide Gel Electrophoresis: Procedures and Tips. Laboratory for Cancer Research. University of Wisconsin. Madison. **http://teachline.ls.huji.ac.il/72682/Booklets/NOVAGEN_Prepare_Sample_PAGE_SDS.**

Pitt, A., L.S. Mayorga, P.D. Stahl, and A.L. Schwartz. 1992. Alterations in the protein composition of maturing phagosomes. **J. Clin. Invest.** 90:1978-1983.

Pizarro-Cerda, J., S. Meresse, R.G. Parton, G. van der Goot, and A. Sola-Landa. 1998. *Brucella abortus* transits through the autophagic pathway and replicates in the endoplasmic reticulum of nonprofessional phagocytes. **Infect. Immun.** 66:5711-5724.

Rand, N.D. and M.D. Laing. 2011. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of crude extracted insecticidal crystal proteins of *Bacillus thuringiensis* and *Brevibacullus laterosporus.* **Afr. J. Biotechnol.** 10(66):15094-15099.

Sangari, F.J., J. Aguero, and J.M. Garcia-Lobo. 2000. The genes for erythritol catabolism are organized as an inducible operon in *Brucella abortus*. **Microbiology.** (146):487-495.

Taminiau, B., M. Daykin, S. Swift, M.L. Boschiroli, and A. Tibor. 2002. Identification of a quorum-sensing signal molecule in the facultative intracellular pathogen *Brucella melitensis*. **Infect. Immun.** 70:3004-3011.