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by Wiwiek Tyasningsih

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THE CHARACTERIZATION OF GENE ENCODING SURFACE PROTEIN-31(BCSP-31) *Brucella abortus*FIELD ISOLATE BY PCR TECHNIQUE

Wiwiek Tyasningsih[1], Didik H.[2], Fedik A. Rantam[3], Aulanni'am[4]

[1,2,3] *Microbiology Department, Faculty of Veterinary Medicine, Airlangga University, (Indonesia)*
[4]*BiochemistryLaboratory, Faculty of Life Sciences, Brawijaya University, (Indonesia)*

ABSTRACT

Brucellosis is a major bacterial zoonosis of global importance. The causative organisms are Gram-negative facultative intracellular pathogens, that may affect a range of different mammals including man, cattle, sheep, goats, swine, rodents and marine mammals. The disease primarily affects the reproductive system with concomitant loss in productivity of animals affected. Human infection is characteristically recurrent febrile as known undulant fever. The development of an effective subunit vaccine against brucellosis is a research interest. The protein antigenic of cell envelope Brucella sp. have been extensively characterized as potential immunogenic and protective antigens. The aim of the study was to characterize the gene encoding surface protein-31Brucella abortus (BCSP-31) field isolate as the basic for the molecular diagnosis of the disease Brucellosis in animals. The method used in this study is the isolation and reidentification of Brucella abortus bacteria based on biochemical tests and genomics with Polymerase Chain Reaction (PCR); the result of the PCR was then performed and analyzed sequensing by Blast homology. The data were derived from field isolates of Brucella abortus. The results showed that the gene Surface Protein-31 Brucella abortus (BCSP-31)field isolat had a size of 224 bp, and the homology analysis on Gene Bank the field isolate Brucella abortusshowed that the homology is 97% with the others strainsB. abortus in the world including B. abortus S19.

Key words: Gen Surface Protein-31(BCSP-31), B. abortus field isolate, PCR Sequence.

I. INTRODUCTION

Brucellosis is still one of the most common bacterial zoonosis which already spread out all over the world. The disease in cattle is caused *Brucella abortus*, Gram-negative coccoid and may cause some reproductive disorders such as abortus, sterility and calf death [1,2]. In human, brucellosis can cause recurrent febrile known as *undulant fever*.

The incidence of Brucellosis tends to increase as well as the distribution, this happens due to the frequency of the stock's mutation, so that can threaten the growth of stock raisings especially the cattles [3].Most standard serological test such as serum agglutination test (SAT), complement fixation (CFT) and Enzyme linked Immunosorbent assays (ELISA) use whole cell preparations [4]

The control effectivity of Brucellosis depends on the accuracy of the disease diagnosis and PCR is the most proper diagnosis technique now, and several PCR based assays have been developed, these are mainly genes specific PCR assays targeted to genes such as the *BCSP-31, Omp 2a and Omp 2b*([5].In Indonesia, as a gold standard to do the Brucellosis diagnosis is CFT test, because not all animal health laboratories are equipped with PCR tools. CFT test takes a long time, requires the accuracy in calculating the amount of antibody titer, and frequently gives false positive results. During this time, whole cells *B. abortus* S19 are being used in the serological test and making it is quite difficult to distinguish the antigen reactivity with antibodies from the vaccine and infection.

According to the background above, it is necessary to find the diagnosis technique that can be done fastly and accurately by using the protein marker which comes from the surface protein 31 *Brucella abortus*field isoltes (BCSP-31) as the source in preparing the diagnostic kit for the Brucellosis disease.

II. MATERIALS AND METHODS

The sample of this research is *Brucella abortus*field isolate which obtained from Indonesia Research Center for Veterinary Maros South Sulawesi.

2.1 Culture and reidentification of *Brucella abortus* field isolate

Culture and reidentification of *Brucella abortus* field isolates wich had been previously described by Alton (1988). The samples are being cultured in the Brucella Medium Base (*Oxoid cat no. CM0169*) which addedBrucella Selective Suplement (*Oxoid cat no.* SR0083A), 10% horse serum (*Oxoid cat no. SR004SC*), incubated at 37^0C under aerobic conditions in the presence of 5% CO_2. The colony which grows in the media then being identified with microscopic examination, catalase test, urease test, oxidase test, production of hydrogen sulfide (H_2S) and dye.

2.2 Characterization of Gene Surface Protein-31 *Brucella abortus* field isolate by PCR.

The characterization of gene Surface Protein-31 *Brucella abortus*(BCSP-31) by Polymerase Chain Reaction (PCR)begins with DNA extraction of *Brucella abortus* culture using DNAzol (Merck), take 2 µl the DNAzol to the microtube and added with bacteria taken with a tip, let it stands for the whole 15 minutes. The DNA extrated was used as a template in amplification by using PCR. The primers used were primer BCSP-31 *Brucella abortus* Forward 5' – TGG CTC GGT TGC CAA TAT CAA – 3' and Reverse 5' – CGC GCT TGC CTT TCA GGT CTG – 3' (*Invitrogen*). The reaction volume was set up in 50 μl containing 10x PCR buffer 2 μl, $MgCl_2$ (25 mM) 2 μl, dNTPs (10 mM) 0,5μl, 50 pmol Forward Primer and Reverse Primer each 0,5 μl, DNA polymerase (2,5 U/μl) 0,5 μl, DNA template 2 μl and add distilated water to final volume of 50 μl. Amplification of the DNA in 35 cycles with an initial denaturation step of 4 minutes at 94 ^{0}C. The next 34 cycles were performed

with 1 minute denaturaturation at 94 ^{0}C, annealing at 60^{0}C for 1 minute and extension at 72 ^{0}C for 10 minutes and hold at 4 ^{0}C.

2.3 Detection of PCR Product

The amplified PCR product were separated using agarose gel Electrophoresis and analyzed by 1,0 (w/v) % agarose gel in 1X TBE buffer. 5 μl of the PCR products were mixed with 2 μl loading dye and loaded into the gel. 5 μl of 1 kb DNA marker was used as standard and the DNA was electrophoresis at 80V for 90 minutes. The gel was stained with GelRed^{TM}Nucleic Acid Gel Stain (Biotium, US) before the bands were visualized under an ultraviolet light transilluminator.

2.4 Gel Extraction and Purification of PCR Product

The DNA PCR product was extracted and purified according to the protocol described by Qiaquick Gel Extraction Kit (Qiagen, Germany). The DNA fragment was exised from the agarose gel and finally get to a tube of DNA elution and stored in -20^{0}C until futher used.

2.5 DNA Sequencing

The PCR product was sequence by using Automated Sequencer (ABI PRISM 3100 Genetic Analyzer). The purpose of sequencing in this research is to identify the DNA sequence in the gene Surface Protein-31 of *Brucella abortus* field isolate (BCSP-31).

III. RESULTS

In this research we were approached by Indonesia Research Center for Veterinary Maros South Sulawesi, the isolate *Brucella abortus* field isolate was culture and reidentificationwich had been previously described by [6]. In selective Brucella Agar which added 10% bovine serum in the presence of 5% CO_2, the growth colonies are in the honey creamy color, rounded in shape, smooth, slippery and glossy surface in a small size, as has been reported [6,7]that in the growth media, Brucella colonies shaped like a drop of honey, round, smooth, convex and slippery surface, glossy and translucent with diameter of 1-2 mm. Furthermore, in the microscopic examination using Gram stain, it can be seen that the bacteria are Gram negative and coccoid shape. According [7,8] in the Gram stainthat *Brucella abortus* are coccoid shape and red color. The reidentification is *Brucella abortus* because the resluts biochemical identification are catalase test positive, oxidase test positive,urease positive, cytrate positive, production of hydrogen sulphide (H_2S), and dye thionine and basic fuchsin sensitivity [6,8].

ThePCR result of gene Surface Protein-31 (BCSP-31) *Brucella abortus* field isolateshowed a band appropriate amplicon 224 bp (Fig. 1) as reported before [9,10] by Asif *et al*., (2009) and Al-Mariri *et al*., (2010) that the gene Surface Protein BCSP-31 *Brucella abortus* has 224 bp nucleotide length.

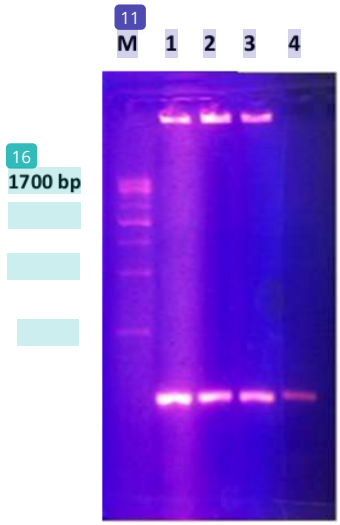

Figure 1. The Electrophoresis results of PCR BCSP-31 *Brucella abortus*

M= Marker; 1,2,3=*Brucellosis abortus* field isolates;4 = positive control

The sequencing result of gene Surface Protein-31 (BCSP-31) *Brucella abortus* field isolate shows the nucleotide sequence as follows:

TCTTGCACATCACTTCGGGCGATACAGGACTCCGGCCTTTACGCAGTCAGACGTTGCCTATTGGGC
CTATAACGGCACCGGCCTTTATGATGGCAAGGGCAAGGTGGAAGATTTGCGCCTTCTGGCGACGCT
TTACCCGGAAACGATCCATATCGTTGCGCGTAAGGATGCAAACATCAAATCGGTCGCAGACCTGA
AAGGCAA

The homology analysis then be done by using the data in Gene Bank using the BLAST methods, the result is that the gene Surface Protein-31 *Brucella abortus* field isolate shows the homology for about 97% with some other strain *Brucella abortus* provided in the Gene Bank, including the *Brucella abortus* S19 (Table 1).

Table 1: *Brucella abortus* sequences that produce higest significant alignments with the BCSP-31 gene of the *B. abortus* field isolate using nucleotide BLAST.

No.	Accession No.	Description	Query Coverage	Max. Identify
1.	CP 003176.1	*Brucella abortus* A13334 chromosome 1	93%	97%
2.	HO 132292.1	*Brucella abortus* KOL-79 BCSP31	93%	97%
3.	O 132291.1	*Brucella abortus* AHM-900 BCSP31	97%	97%

4.	GO 167387.1	*Brucella abortus* BMA 2008 BCSP31	97%	97%
5.	CP 000887.1	*Brucella abortus* S19 chromosome1	97%	97%

IV. CONCLUSION

The Gene Surface Protein-31 (BCSP-31) *Brucella abortus* field isolate can be characterized has nucleotide lenght of 224 bp with level of homology 97%.

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