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Characterization and Production of Polyclonal Antibody Anti Excretory Secretory Protein of *Blastocystis* sp

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Abstract

This study aims to produce and characterize polyclonal antibody anti excretory secretory (ES) protein of *Blastocystis* sp. ES protein profile was analyzed using SDS-PAGE and used to immunize 2 rabbits. Rabbit's serum were analyzed using indirect ELISA and Western Blot. The result showed that molecular weight of ES protein of *Blastocystis* sp was 40 and 50 kDa and the protein was immunogenic. Both ES protein and antibody anti ES of *Blastocystis* sp can be promoted as diagnostic kit.

Key words : *Blastocystis* sp, Excretory Secretory Protein, Polyclonal Antibody.

Blastocystis sp is a protozoan parasite that widely prevalent in many countries and it causes gastrointestinal symptoms such as diarrhea, nausea, vomiting, abdominal pain, irritable bowel syndrome, and urticaria (Ajjampur *and* Tan., 2016). This parasite can infect human (Roberts *et al.,* 2014) and various animals like amphibian, reptiles, bird, and mammals (Alfellani *et al.*, 2013).

Excretory secretory (ES) protein of parasites have an important role as virulence factor, it can affect and control host immune system during infection and it can be used as a biomarker to detect the presence of parasite and status of the infection in infectious disease (Gomez *et al.,* 2015). This study aims to characterize and produce polyclonal antibody anti ES protein of *Blastocystis* sp.

Materials and Methods

Blastocystis sp was isolated from dunges of beef cattle in Bangkalan, Madura. The sample was cultured using yeast extract media (Mohammed *et al.,* 2015). The growing culture was confirmed with PCR examination using primers b11400 FORC (5`-GGA ATC CTC TTA GAG GGA CAC TAT ACA T-3`) and b11710 REVC (5`-TTA CTA AAA TCC AAA GTG TTC ATC GGA C-3) from Badparva *et al* (2014).

Blastocystis sp was harvested from media and centrifuged with 10.000 rpm for 10 minutes. Pellets was washed 3 times with PBS and

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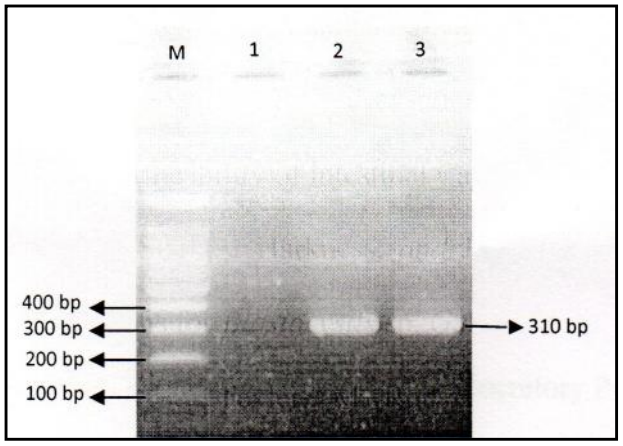

Fig 1. PCR Product of *Blastocystis* sp. , M = marker (Promega); 1 = Negative control; 2,3 = positive culure

incubated in 37°C overnight. The supernatant was as ES protein. The profile of ES protein was analyzed with SDS-Page and visualized with a digital camera.

Two male New Zealand rabbits were immunized with 250 µg of ES protein. Immunization was done 4 times. The serum was collected before and 7 days after immunization Antibody production was verified by indirect ELISA based on value of optical density (OD) and Western Blot. The OD value between groups was analyzed using one-way ANOVA.

Results and Discussion

Blastocystis sp from feces of cattle was cultured with yeast extract media, after 3 days, the organism reproduced and reached a number $5x10^9$/ml. Based on PCR, the sample showed positive result with band in 310 bp (Fig 1) as described in Badparva *et al* (*loc cit*).

SDS PAGE of ES protein of *Blastocystis* sp showed that there were two protein bands with molecular weight; 40 kDa and 50 kDa (Fig 2). Fadl *et al* (2016) identified 22 proteins from whole proteins of *Blastocystis*, which ranged from 12 kDa to 200 kDa, include 40 kDa and 50 kDa. According to Nagel *et al*. (*2015*), the 50 kDa is Matrix metalloproteinase-9 (MMP-9), member of zinc-binding matrix metalloproteinases (MMP) which degrades extracellular matrix including gellatin (gelatinase B), collagen and elastin, which explained that MMP-9 exist as pro-enzyme around 92 kDa with an active form of around 84 kDa and turncuted around 50 kDa and it has important role in tissue invasion and has corelation with immune system, including potentiation of interleukin (IL)-8. Nourrisson *et al*. (2016) reported that rocombinant legumain had a size ~40 kDa. Legumain is a lysosomal protease that localizes on cell surface, cytoplasm, and central vacuole, this protein has

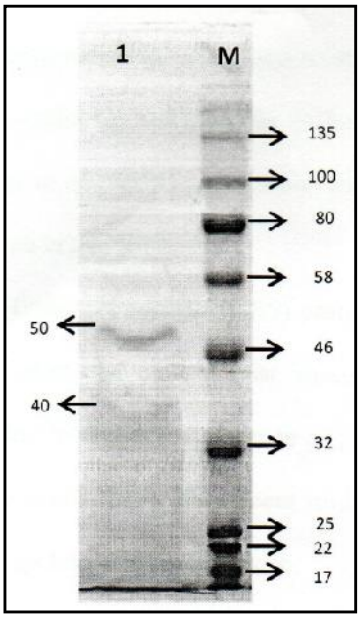

Fig 2. Profile of excretory secretory protein of *Blastocystis* sp in SDS-PAGE, M = marker; 1 = ES protein of *Blastocystis* sp

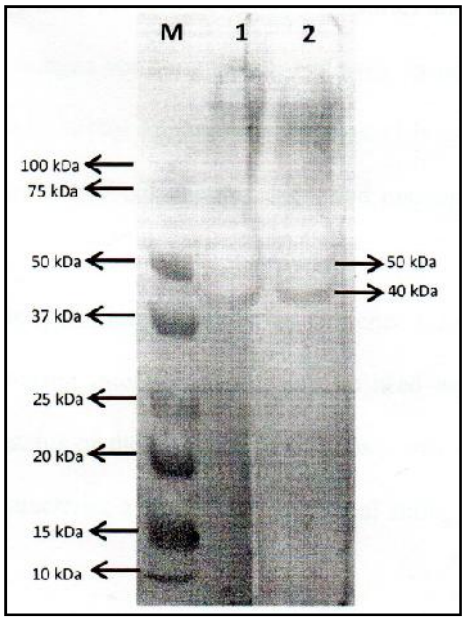

Fig 3. Immunobloting of exretory secretory protein of *Blastocystis* sp with antibody anti ES, M = marker; 1,2 = antigen with Rabbit`s Serum 1;2

several functions such as mediated pro-survival function for surface molecule and inflamation reaction in host cell (Wu *et al.,* 2009).

Antibody in rabbit's serum increased significantly after immunization with ES protein. Before immunization the OD was $0.0415^{c} \pm 0.0007$, 7 days after 3rd and 4th immunization, respectively the OD were, $1.6217^{b} \pm 0.2216$, and $2.2232^{a} \pm 0.0300$ respectively. It indicated that ES protein of *Blastocystis* sp was immunogenic.

In blotting product, antibody was reacted with 40 kDa and 50 kDa proteins (Fig 3). Indirectly that these proteins were immunogenic. Nagel *et al* (*loc cit*) also reported that 50 kDa protein reacted with blastocystosis patient serum and 50 kDa protein was found in *Blastocytis* sp subtype 1, 2, 3, 4, 5, 7, and 8.

Summary

Molecular weight of ES protein of *Blastocystis* sp is 40 kDa and 50 kDa and both of protein are immunogenic. ES protein of *Blastocystis* sp can be promoted as kit diagnostic to detect serum blastocytosis patient and antibody anti ES as kit diagnostic to detect coproantigen.

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