Oral Presentation (MP-10)

Subcloning Gene Encoding Rophtry 1 (ROP1) *Toxoplasma gondii* WTA Isolate

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasite which could infected all organism, and built a vacuola parasitoporus for multiplicity their self in host [1]. Toxoplasmosis is the one of zoonotic diseases which could infected animal and human and involved that two organism to their life [2].

Toxoplasmosis in animal is difficult to held, it cause involved the environment. Oosit could sporulated in the water, it made fish, walrus and other mamalian infected by *T. gondii*. Bat could be a vector of *T. gondii* if they bite cattle where in it bloods contain with tachyzoites [3]. Toxoplasmosis in ranch such as cattle, pork, sheep, goat and poultry focused on reproduction health that impact economics system and causes congenital disease. This condition can impact for fulfill of prime seed and good meat for human consumption [4].

The invasion of *T. gondii* to host cell could cause immunology reaction like cytokine secretors such as IL-12, IFN-γ, TNF-α and T cells such as CD4+ and CD8+. It involved *surface antigen/*SAG protein and *excretory-secretory antigen/*ESA protein [5].

Rophtry-1 protein has functioned as a penetration factor and it has 66 kDa molecular of weight. Cloning gene of rophtry-1 *T. gondii* RH isolate have done and result on vaccination using recombinant plasmid rop1, improving activity of *natural kill cells*, cell and T proliferation. Recombinant antigen of ROP1 also can use for toxoplasma diagnosis [6,7].

The aim of these studies was to sub-clone the gene encoding ROP-1 protein *T. gondii* WTA isolate to pET-32a(+) and produced a recombinant plasmid of ROP-1 in *E. coli* BL-21(DE3). This recombinant plasmid will be used to produce a recombinant protein which will be able to perform preliminary studies on its stability on detect *T. gondii* specific antibodies.

MATERIALS AND METHODS

Toxoplasma gondii ROP1 gene sequence was accessed via the World Wide Web (GenBank accession no. M71274.1 and AF350261.1) and has 99% and 98% homology to both. This research was using qualitative and descriptive design studies.

Genomic DNA and recombinant plasmid PWTA-R1 for polymerase chain reaction was obtained by phenol/chloroform extraction, and ethanol precipitation. We designed and ordered a set of primer to amplify only the sequence coding for the part of ROP1 protein with *Eco*R I and *Hin*d III restriction site at 5' end of 3 forward and reverse primers, respectively (R1F4 5`-CGTGAC*GAATTC*TGCACTGAC-3`) and (R1R45`-ATCTGC*AAGCTT*GATCACCGT-3`).

The DNA of rophty-1 protein was collected from plasmid pWTA-R1 790µg and adding 1µg restriction enzymes of *Eco*R I and incubated for 2 hours. Others from PCR reaction contain of 790µg and 162,5µg, pWTA-R1 and Tachyzoites of *T. gondii* WTA isolate, respectively. Add 20pmol of forward and reverse primers, and dH2O up to 25µl. Polymerase chain reaction amplification was carried out with 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. Polymerase chain reaction was incubated at 94°C and 72°C for 5 min before and after the PCR cycling, respectively.

The DNA of rophtry-1 protein from pWTA-R1 and tachyzoites WTA isolate were electrophoresis on 1% agarose gel and the DNA band were sliced under long-wave ultraviolet (UV) light, recovered by electro elution, ligated into pET32a(+) vector (Novagen). Recombinant plasmid pET32a(+)/R1 was transformed into BL21 (Invitrogen) competen cells. Bacterial colonies containing recombinant plasmids were screening and confirmation by PCR analysis.

Escherichia coli strain BL21 (Invitrogen) was transformed using recombinant plasmids and grown in Luria Bertani (LB) medium supplemented with 25 mg/ml ampicillin at 37°C overnight. Two hundred and fifty culture flasks

containing 50 ml of LB containing 25 mg/ml ampicillin were inoculated by 10 ml samples of the overnight cultures. The cultures were grown at 37°C with vigorous shaking until OD600= 0.5 – 1.0. Induced cells were pelleted by 3000 rpm centrifugation 4°C for 10 min.

RESULT AND DISCUSSION

Extracted pWTA-R1 was subjected to a digestion by *Eco*R I and amplified in 35 cycles by PCR. Figure 1 and 2 shows that 1% agarose gel containing a ±1440 bp band as a the digestion product and the PCR product [8], it was sub-cloned into pET32a(+) expression vector. Polymerase chain reaction product of pWTAR1 amplified with R1F4 and R1R4 are continued to digest with *Eco*R I and *Hin*d III before do ligation into pET32a(+).

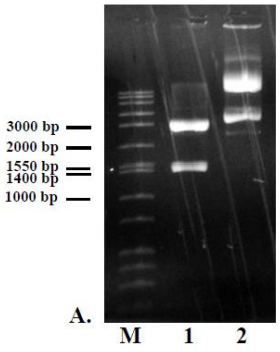

Fig 1: Restriction analysis of ROP1 gene on pWTA-R1 digested by *Eco*R I. Lane: M: DNA marker; lane 1: DNA ROP-1 ±1533 bp; lane 2: pWTA-R1.

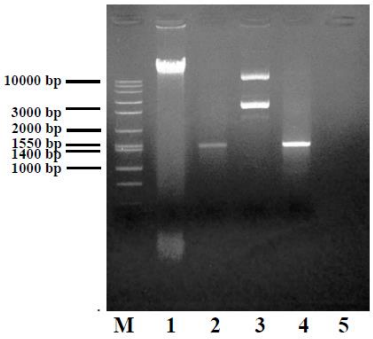

Fig 2: PCR analysis of pWTA-R1 dan DNA tachyzoites WTA isolate with R1F4/R1R4 primers. Lane M: DNA marker; lane 1: tachyzoite DNA WTA isolate; lane 2: PCR product (R1F4/R1R4). tachyzoite DNA WTA isolate; lane 3: pWTA-R1; lane 4: PCR product (R1F4/R1R4) pWTA-R1; lane 5: negatif control (no template)

Transforming of *Escherichia coli* strain BL21 grows as white colonies (Fig. 3). Collected of recombinant plasmid with alkali lysis methods and screening by PCR. The integrity of the DNA plasmid was checked by agarose gel electrophoresis after PCR with R1F1, R1F4, R1R2 and R1R4 forward and reverse primer (Fig. 4). It showed that the insert succeed cloned into pET32a(+).

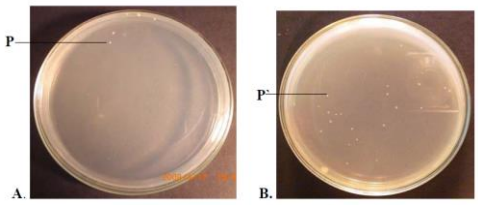

Fig 3: Transforman white colonies of pET-32a(+) in *E. coli* BL-21(DE3). **A.** Transforman pET-32a(+)/gen rop-1 (pWTA-R1 digested) ligation. P: White colonies (M1) {pET-32a(+)/R1 in *E. coli* BL-21(DE3)}. **B.** Transforman pET-32a(+)/gen rop-1 (pWTA-R1 amplified) ligation. P`. negatif white colonies (M2) {pET-32a(+) in *E. coli* BL21(DE3)}.

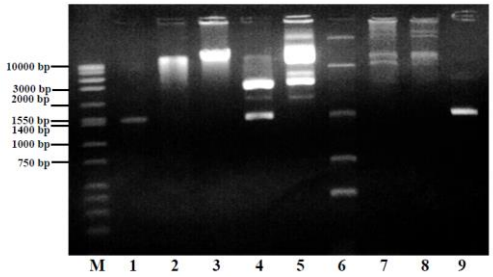

Fig 4: PCR product analysis white colonies plasmids M1, pWTA-R1 dan pET-32a(+). Lane M: DNA marker; lane 1: pET-32a(+)/R1 amplified with R1F1 and R1R2 primers (ROP-1 DNA ± 1513 bp); lane 2: pET-32a(+)/R1 digest with *Eco*R I; lane 3: *uncut* pET-32a(+)/R1; lane 4: pWTA-R1 digest with *Eco*R I (ROP-1 DNA ± 1533 bp); lane 5: *uncut* pWTA-R1; lane 6: pET-32a(+) amplified with R1F1 and R1R2 primers; lane 7: pET-32a(+) digest with *Eco*R I; lane 8: *uncut* pET-32a(+); lane 9: elution ROP-1 DNA (ROP-1 DNA ± 1533 bp).

CONCLUSION

The conclusion of this research is produced a sub-cloned M1 (pET-32a(+)/R1) that contain a rop-1 gene insert with molecular length is ± 1510 bp and ± 2105 bp, amplified with R1F1/R1R2 and T7 promoter [pET-32a(+)]/R1R2 respectively. A ± 750 bp band of PCR product of M2 plasmids shows if the plasmid does not contain the rop-1 gene.

ACKNOWLEDGMENTS

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