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Expression of Complete Rhoptry Protein 1 (ROP1) Gene of *Toxoplasma gondii* in Eukaryotic Cell

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ABSTRACT

T. gondii producing proteins are strong antigens that can start strong immune reactions and one of these kinds of antigens is rhoptry protein 1 (ROP1) that is discharged from rhoptry cell-organ. These entire attribute for ROP1 makes it a competitor for protein vaccine and recombining vaccine against toxoplasmosis. A main objective of the current study was the Cloning and expression ROP1 of *Toxoplasma gondii* in a cloning vector for later studies. In the present work, genomic DNA of *Toxoplasma gondii* was removed and used for amplifying of ROP1 gene as a template. Then PCR product was cloned into the EcoR1 and BamH1 sites of cloning vector, pUET1 and transformed into *Escherichia coli* BL21 plysS strain and sub cloned from pTROP1 into the HindIII and EcoRI sites of the pcDNA3 to produce recombining eukaryotic declaration vector pcROP1. The cloned ROP1 was verified by PCR, limitation enzymes (HindIII and BglI) digestion and nucleotide sequencing. A fragment about 757 bp was separated fore more nucleotide sequence analysis of the ROP1 cloned in pUET1 vector revealed high homology (96%) with RH strain Gene Bank Accession No. M71274. This expressed ROP1 to make recombinant vaccine against toxoplasmosis will be useful in the future study.

Key words: Cloning, sequencing, *Toxoplasma gondii*, ROP1

INTRODUCTION

Toxoplasma gondii is the within a cell protozoan parasite reliable for animal and human toxoplasmosis. In immune capable human being, state of being disease with this parasite generally is clinically without symptoms, but it may bring about serious complicated state in immunodeficient human being and in pregnant woman (Bhopale, 2003). In immunodefecient treatment, chronic state of being disease with *T. gondii* can make active again and product encephalitis, which is often fatal (McCabe and Remington, 1988). In fact, *T. gondii* is one of the major of opportunism pathogens in HIV-disease treatment (McCabe and Remington, 1988). First *T. gondii* state of being disease of a mother during pregnancy can lead to abortion, neonatal malformations or other deficiency which seem during child development (Remington and Krahenbuhl, 1982; Wong and Remington, 1994). Natural state of being disease with *T. gondii* generally leads to a state of long lasting non-barren defensive immunity (Suzuki and Remington, 1988; Gazzinelli *et al*., 1991). This defense is T-cell-intervene and includes both $CD4^+$ and $CD8^+$ t-cells (Suzuki and Remington, 1988; Gazzinelli *et al*., 1991). Therefore, organism with recombined genes

vaccines and protein vaccines against toxoplasmosis should be based on parasite antigens which cause this t-cell-intervene defensive immunity. Like other single-celled organisms *T. gondii* is self-controlled of different antigens, which are immunogenic and are structural parts or metabolism results of parasite. Bhopale (2003) argued that the most important kinds of toxoplasma antigens are exterior tachyzoite antigens and discharged antigens. Nowadays there is interest in the somatic antigens also known as discharged antigens or exoantigens. *T. gondii* producing proteins are strong antigens that can start strong immune reactions. One of these kinds of antigens is rhoptry protein 1 (ROP1) that is discharged from rhoptry cell-organ. Rhoptry proteins are a variety of secretory antigens that secreted from rhoptry organelle.

ROP1 is one of the most important meddlers in cell-organ and PVM mixing (Saffer *et al*., 1992). The timing of the freedom of these molecules, as well as their targeting to the host cell exterior or parasitophorous vacuole, suggests this role in invasion for Rop1. Foremore, bring about fate transfer from host cells mitochondria and ER to PVM and so rhoptry protein 1 (ROP1) has been associated with a molecular activity that can enhances the invasion *in vitro*.

All of these traits for ROP1 make it a nominee for protein vaccine and organism with recombined genes vaccine against toxoplasmosis (Saavedra *et al*., 1991). These entire attribute for ROP1 makes it a competitor for protein vaccine and recombining vaccine against toxoplasmosis (Saavedra and Herion, 1991; Saavedra *et al*., 1991). Martin *et al*. (2004) stated categorical ROP1 is used as a competitor for evaluation of resistance response against disease and is used as a competitor to make an identification kit for toxoplasmosis. A main objective of the current study was the Cloning and expression ROP1 of *Toxoplasma gondii* in a cloning vector for later studies.

MATERIALS AND METHODS

Primer design: In the first two primers (for and back) was designed for rop1: The nucleotide sequences of the *T. gondii* gene encoding ROP1 antigen was obtained from the GenBank database (http://www.ncbi.com) (accession No. M71274).

Primers list are:

- **Forward:** 5'GTGCCAGATCTAGCGTCGCATTCTCATTCG3'
- **Reverse:** 5'CCAAAGCTTTTGCGATCCATCATCCTGCTCTG3'

The primers contained the BglII and HindIII recognition sequences (underlined) to facilitate cloning.

Animals: Six to eight weeks old female BALB/c mice were purchased and maintained under pathogen-free conditions in the experimental animal facility at the Centre of Laboratory Animals, Sanandaj Azad University.

Preparation of *T. gondii* tachyzoites: Tachyzoites from the *T. gondii* RH strain a highly virulent strain for mice were used in this study to isolate genomic DNA, which was used as the template for amplification of rop1 gene by the use of a standard PCR amplification protocol. The RH strain tachyzoites were harvested from the peritoneal fluids of mice that had been intraperitoneally infected 3-4 days earlier and maintained by serialintraperitoneal passage in Kunming mice. The tachyzoites were collected from the peritoneal fluids. About

5×107 *T. gondii* tachyzoites were focused by centrifugation, washed with Phosphate Buffer Saline (PBS), then lysed in lysis buffer (0.1 M Tris-HCl pH 8.0) and then consider with proteinase K (100 μg mL^{-1}) at 55°C for 2 h (Kimbita *et al*., 2001). The lysate was then added to an equal volume of phenol/chloroform (25:25) to remove proteins. This mixture was centrifuged at 13000 rpm for 15 min and an equal volume of chloroform was added to the supernatant was mixed with 1/10 volume of 3 M sodium acetate and two volumes of 100% ethanol to trigger DNA by centrifugation at 13000 rpm for 10 min. The DNA pellet was washed with 70% ethanol, dissolved in sterile distilled water and stored at -20°C until use (Sambrook *et al*., 1989). DNA extraction products were notice in 0.8% agarose gel and photographed.

PCR amplification: The entire ROP1 reading frame (nucleotides 252 to 1188) was obtained by PCR using the following primers:

- Sense primer (5'-GTGCCAGATCTAGCGTCGCATTCTCATTCG-3') and antisense (5'-CCAAAGCTTTTGCGATCCATCATCCTGCTCTG-3')

Cloning of ROP1: The purified PCR products were digested with both BglII and HindIII and inserted the BglII and HindIII sites of pUET1 cloning vector. The ligation product was used to transform accomplished *Escherichia coli* BL21 plyS.

According to the protocol (Sambrook *et al*., 2001), the ligation product was transformed in competent cells. These cells recovered in Luria-Bertani (LB) broth medium free antibiotic by incubated at 37°C for 2 h and were plated onto LB agar plates. Content of plates were ampicillin 100 mg mL^{-1}, IPTG 200 mg mL^{-1} and X-Gal 20 mg mL^{-1}.

These plates were incubated at 37°C for an overnight to screening blue and white colonies. The selected blue and white colony passage in LB broth or LB agar and incubated at 37°C for an overnight. The plasmid was extracted according to the protocol. The cloned ROP1 was verified by PCR, limitation enzymes (HindIII and BglI) digestion and nucleotide sequencing.

Expression plasmid constructions and *in vitro* transient transfection of CHO cells: The coding area for the *T. gondii* ROP1 was sub cloned from pTROP1 into the HindIII and EcoRI sites of the pcDNA3 (Invitrogen, USA) to produce recombining eukaryotic declaration vector pcROP1. These were used to transfected CHO line cells. CHO cells were grown to 60-70% confluence at 37°C and 5% CO2 in 35 mm wells in Dulbecco's modified Eagle's medium (DMEM, Gibco) each including 100 mL^{-1} penicillin and streptomycin and 10% fetal calf serum (FCS). Cells were washed in a serum-free medium and the transfection was discharged with a transfection kit (Genejuice Transfection Kit, Novagene, USA) according to instructions of the manufacturer.

RESULTS AND DISCUSSION

The ROP1 gene was amplified by PCR using extracted genomic DNA from RH strain of *Toxoplasma gondii* as template. Figure 1 shows that DNA fragment PCR amplified was a band at about 757 bp similar to the expected *T. gondii* ROP1 gene size. The PCR product has ligated successfully into plasmid pUET1 cloning vector and then has transformed in *Escherichia coli* BL21 plysS strain and then was compare of pUET1 and pT-ROP1 plasmids by electrophoresis (Fig. 2). The extracted pUET1 and pT-ROP1 plasmids were digested with HindIII and BglI restriction

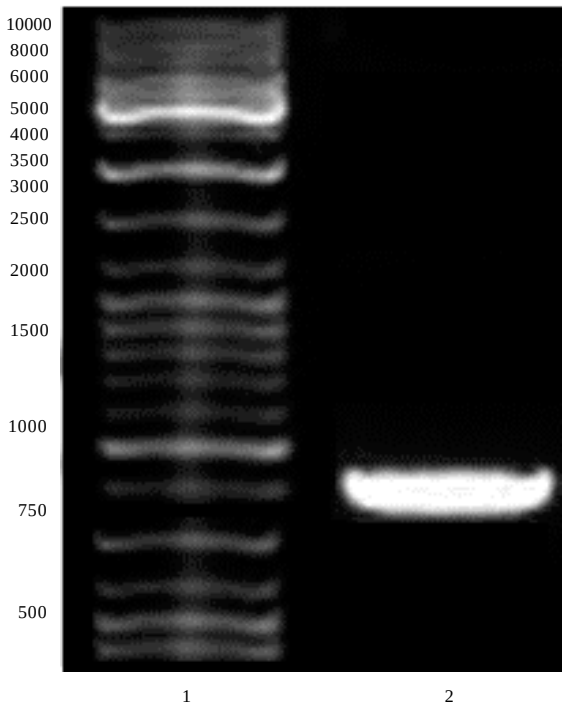

Fig. 1: Electrophoresis of PCR product for ROP1 fragments of *Toxoplasma gondii* amplification, Lane 1: ROP1 gene (757 bp), Lane 2: Ladder

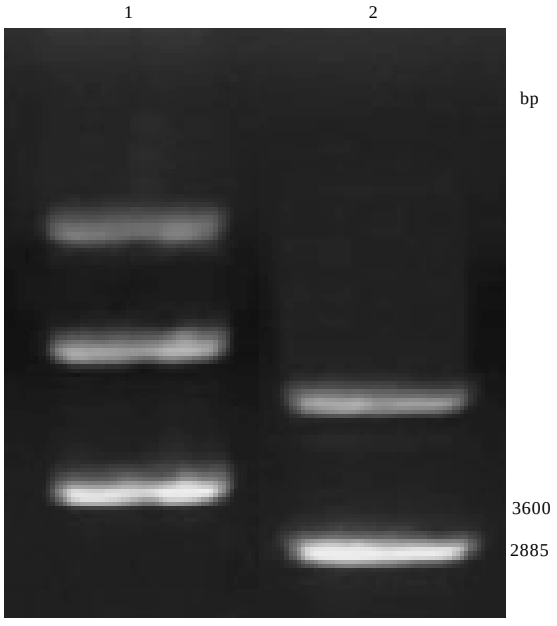

Fig. 2: Electrophoresis result of pUET1 and pT-ROP1 plasmids, Lane 1: Plasmid extracted from pT-ROP1 and Lane 2: Plasmid extracted from pUET1

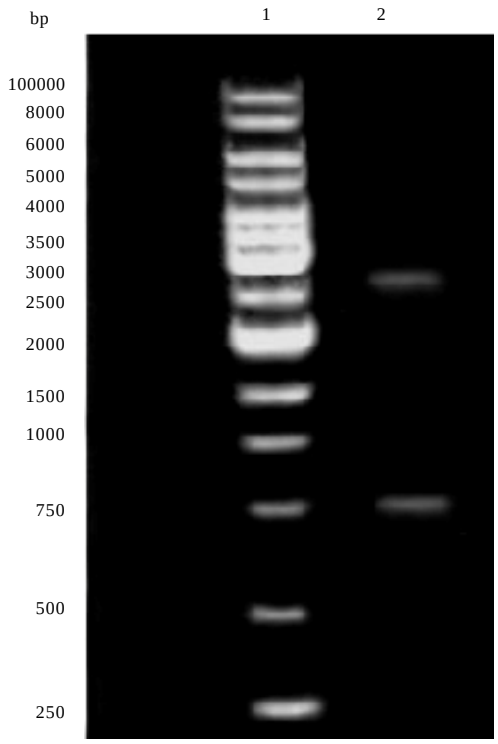

Fig. 3: Digestion of extracted pT-ROP1 after ransformation: Lane1: Ladder 1kb, Lanes 2: (ROP1 757 bp, pUET12876 bp)

enzymes. Figure 3 shows results of electrophoresis from white colonies, two bands where ROP1 fragment placed on 757 bp and pUET1 on 2876 bp, thus the ROP1 gene has cloned into pUET1 plasmid. Nucleotide sequence analysis of the ROP1 cloned in pUET1 vector revealed high homology (96%) with RH strain Gene Bank Accession No. M71274. The digested pT-ROP1was cloned into eukaryotic declaration plasmid pcDNA3 by using the restriction enzymes HindIII and EcoRI (Fig. 4). In this study, the declaration of the entire gene which encodes ROP1 of *T. gondii* in CHO cells was described. A number of researchers have already successfully used this protein to detect patients with toxoplasma (Saavedra *et al*., 1991; Herion *et al*., 1993; Perkins, 1992; Saffer *et al*., 1992; Beckers *et al*., 1994; Wei *et al*., 2006; Leyva *et al*., 2001). Gamma interferon-producing cells (CD 4^+ and CD 8^+ T) were kill germs in this disease. Due to reactivation to the pathogenic form, live vaccine is dangerous and is not suitable for human; therefore, the use of recombining vaccineand declaration of genes arises as a useful way for the development of a vaccine for humans (Martin *et al*., 2004; Fachado *et al*., 2003).

In this study was describe the declaration of the entire gene which encodes ROP1 of *T. gondii* in *Escherichia coli* BL21 plyScells and verified it by electrophoresis, PCR amplification and restriction digestion. Also, was cloned the entire ROP1 gene into a eukaryotic declaration plasmid pcDNA3.

Previous studies have shown that protection with ROP1 peptides, proteins or as recombining vaccine can generate a broad range of resistant responses. These are capable of decreasing

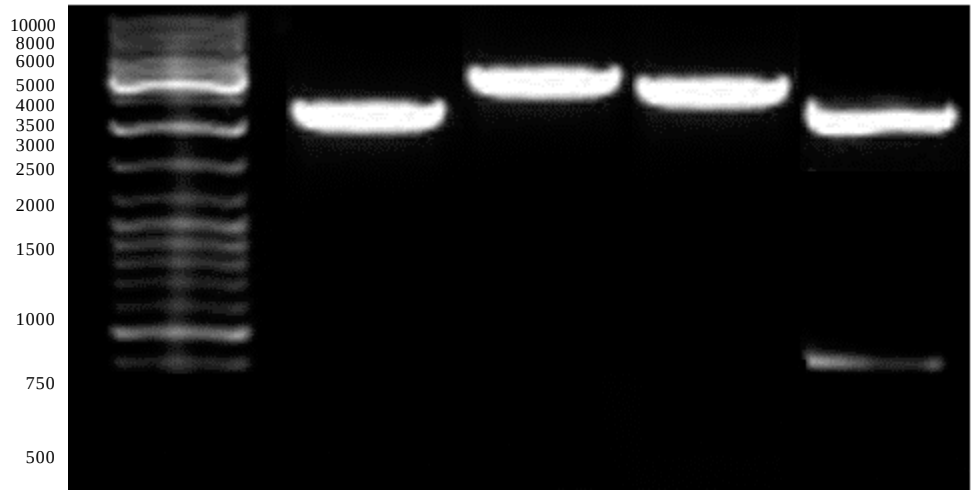

Fig. 4: Detection of pcDNA3-ROP1 by enzyme Digestion: Lane 1: Ladder 1 kb, 2: pcROP1, 3: pc-ROP1 after digestion EcoRI enzyme, 4: pc-ROP1 after digestion with HindIII and 5: pcROP1 after digestion with each other enzymes (ROP1~760 bp)

physically of animals acutely infected with *T. gondii* and reducing the level of tissue cysts in the brain of infected animals; but still required to increase their efficacy. Develop a recombining vaccine, focuses on the declaration of entire rhoptry protein 1 (ROP1) gene in a eukaryotic plasmid.

Results showed that successfully cloned the entire ROP1 gene into declaration plasmid pcDNA3. Control of a gene and cloning in a plasmid that leads to the secretion of the protein can modify the type and strength of the resistant response. Thus, recombining declaration plasmid (that includes a complete ROP1 gene) will be useful to make vaccine against toxoplasmosis.

CONCLUSION

In the future it is hope to use this expressed ROP1 to make protein vaccine and recombinant vaccine against toxoplasmosis as well as a diagnostic kit for toxoplasmosis.

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