African Journal of Biotechnology Vol. 10(40), pp. 7746-7750, 1 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2580
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Cloning and expression of *Toxoplasma gondii* tachyzoite P22 protein

Maryam Arabpour [1], Mojgan Bandehpour[2,3], Maryam Niyyati[1], Seyyed Hossein Abdollahi[4*], Amaneh Koochaki[2,3] and Bahram Kazemi[2,3]*

[1]Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[3]Biotechnology Department, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[4]Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.

Accepted 27 May, 2011

Delay in diagnosis of *Toxoplasma gondii* infection in pregnant women who have been infected during the first trimester of gestation can lead to death of her fetus. Serological tests based on recombinant proteins are the main diagnosis methods for the detection of anti *Toxoplasma* antibody in serum samples. The aim of this study was to clone and express a gene encoding a P22 protein of *T. gondii* tachyzoite as using antigen for ELISA serology method. DNA was extracted from *T. gondii* (RH-strain) tachyzoites and PCR reaction was done using corresponding primers. The PCR product was purified, ligated to PTZ57R plasmid via T/A cloning method and subcloned into SacI and BamHI digested pET32a expression vector. Recombinant plasmid was transformed in *E. coli* (Bl21 DE3) and induced by 1 mM IPTG and analyzed by 12% SDS-PAGE. Expressd protein was purified by affinity chromatography and confirmed by western blot analysis. We successfully cloned and expressed *T. gondii* P22 protein.

Key words: *Toxoplasma gondii,* cloning, recombinant P22.

INTRODUCTION

Toxoplasma gondii is an obligatory protozoan parasite with a complicated life cycle. Sexual reproduction of this parasite develops in intestine of felines while asexual reproduction occurs in the tissue of mammals and birds (Frankel et al., 1970).

The main transmission routes of *T. gondii* is by ingestion of oocyst via contaminated foods, poorly cooked or raw meat of livestock and birds or via intrauterine transmission (Miller et al., 1972). It is important to mention that immunocompromised patients and pregnant women who become infected during gestation are the two important groups for developing an acute and severe disease (Guerina, 1994; Luft and Remington, 1992; Cohen, 1970). The later can lead to congenital toxoplasmosis with many adverse effects on fetus and neonates. It should be mentioned that the severity of the disease depends on the duration of acquired infection in pregnant women. In fact, if the infection develops in the first trimester, toxoplasmosis can lead to irreversible complications such as hydrocephaly, microcephaly, encephalitis and retinochoroiditis. *Toxoplasma* encephalitis can lead to brain calcification. Besides, vision loss, blindness and mental problems may also occur in this stage (Weiss and Dubey, 2009). Diagnosis of high risk people in the start of pregnancy should be performed using serological tests. Additionally, accurate treatment of pregnant women can decrease the risk of fetus infection up to 50% (Flori, 2009).

Indeed, accurate and rapid diagnosis of the infection in women who have been infected during gestation is of utmost importance in the outcome of the disease. Detection of specific antibodies using serological tests are one of the main diagnosis approaches. However, whole tachyzoites grown in mice serves as the antigen in most of the commercial kits, but they have cross reaction by other microorganism.

*Corresponding author. E-mail: habdolahi34@yahoo.com, bahram_14@yahoo.com, kazemi@sbmu.ac.ir. Tel: +98391 5234003, +98 21 22439956.

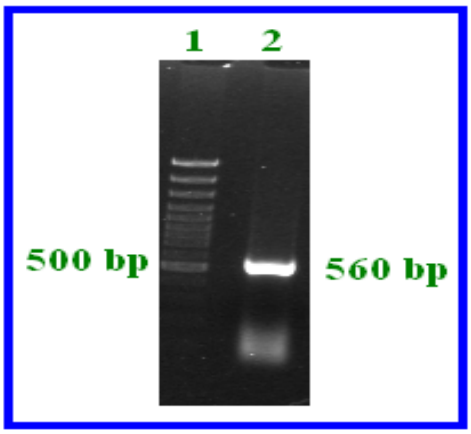

Figure 1. 2% agarose gel electrophoresis (PCR product). Lane 1: 100 bp DNA ladder marker; lane 2: PCR product of P22 gene.

In recent years, attentions have focused on purified and recombinant surface molecules of tachyzoites and bradyzoites for developing a better serological test. Researches have been conducted for developing recombinant form of these proteins for diagnosis purposes. Different antigens of tachyzoite and bradyzoite cover the surface of parasite. It is interesting that some of these antigens are specific to tachyzoite form including P30 surface antigen 1 (SAG-1), P22 (SAG_2) and P35, while other proteins present on both tachyzoite and bradyzoite such as P23 and P43 (SAG3) (Manger et al., 1998; Lekutis et al., 2001; Burg et al., 1988; Prince et al., 1990).

The main goal of this study was to clone and express a gene encoding a P22 protein of *T. gondii* tachyzoite form.

MATERIALS AND METHODS

Parasite

T. gondii tachyzoites (RH-strain) were harvested from the peritoneal fluid of infected mice. The fluid was then centrifuged and washed for three times using phosphate buffered saline.

Genomic DNA extraction and PCR-reaction

DNA lyses buffer were added to the compact pellet. *Toxoplasma* total genomic DNA was extracted as previously described (Zia-Ali et al., 2005). Primer designing were performed on the basis of nucleotide sequence of a P22 gene (accession number: AF249698). It should be mentioned that $5'$ end of forward and reverse primers implicated with Sac1 and BamH1 restriction sites, respectively. The nucleotide sequences of primers were as following:

P22 F 5`- GAG CTC ATG AGT TTC TCA AAG ACC AC 3`
P22 R 5` - GGA TCC TTA CAC AAA CGT GAT CAA CAA-3`

PCR reaction was set up in a 50 µL volume containing 1.25 U Taq DNA polymerase, 100 ng DNA, 1.5mM MgCl2, 0.1mM dNTP, 40 pmol of forward and reverse primers , 1X PCR-buffer. PCR reaction was performed in theromocycler (Primus) through 35 cycles at 94°C for 30 sec (denaturation), 60°C for 30 sec (annealing) and 72°C for 30 sec (extension) followed by last extension time of 10 min (72°C).

Cloning and transformation

PCR-product (560 bp) was purified using a low melting point (LMP) agarose gel as described previously (Gaastra and Jorgensen, 1984) and ligated to PTZ57R plasmid via T/A cloning method. Competent cells were prepared according to Hanahan (1983) method. Reaction was spread on LB agar plate containing 50 µg/mL ampicillin, 2 mM IPTG and 20 mg/ml X-gal. White colonies were selected as containing recombinant plasmids (Bothwell et al, 1990), mass-cultured and plasmid extraction was performed using miniprep alkaline method (Feliciello and Chinali, 1993). Electrophoresis on 0.8% agarose gel was done (Boffey, 1984) and recombination was confirmed using restriction analysis using SacI and BamHI restriction enzymes.

Sub-cloning and gene expression

The P22 gene was subcloned into SacI and BamHI digested pET32a expression vector and transformed in *E. coli (*Bl21 DE3). Recombinant plasmids were selected and transformed into bacterial cell (Bl21 DE3). Bacterial colony containing recombinant plasmid was mass-cultured in YT medium (1.2% bacto trypton, 2.4% yeast extract, 0.04% glycerol, 1% M9 salts) (M9 salts contain: 6.4% $Na_2H_2O_4$- $7H_2O$, 1.5% KH_2PO_4, 0.025% NaCl, 0.05% NH_4Cl) (Kazemi et al, 2008). The culture was induced by 1 mM IPTG when OD_{600} = 0.5 to 0.7 and sampling were done before and after induction and analyzed by 12% SDS-PAGE (Smith, 1984). Western blot was done as described previously (Shewry and Fido, 1998).

Purification of recombinant protein

Purification was performed as described previously (Spiro et al., 1997) with some modifications. Briefly, the induced bacterial cells were cultured in YT medium and pelleted using centrifugation at 6500 rpm. The pellet was resuspended in 50 ml equilibration buffer (50 mM Tris, 0.5 M NaCl) containing a cocktail of protease inhibitors and frozen at -20°C.

Pellet was sonicated (2X 30 sec), centrifuged (2000 rpm) for 15 min and then resuspended in cold buffer containing 6 M urea. Incubation was performed on ice for an hour. Non soluble materials were decanted using centrifuge at12000 rpm for 20 min.

The recombinant protein was purified by affinity chromatography, based on its *N*-terminal His_6 tag using Ni-NTA His-bind resin. The cell lysate was applied to the column at a flow rate of 15 drops/min and allowed to bind. The bound protein was eluted with elution buffer (4 mM urea, 50 mM Tris, 0.5 M NaCl, and 1 mM imidazole).

RESULTS

PCR results

Primers were designed according to a gene encoding P22 (560bp). PCR reaction was done and the band were detected on 2% agarose gel (Figure 1).

Transformation

PCR-product was purified using low melting point

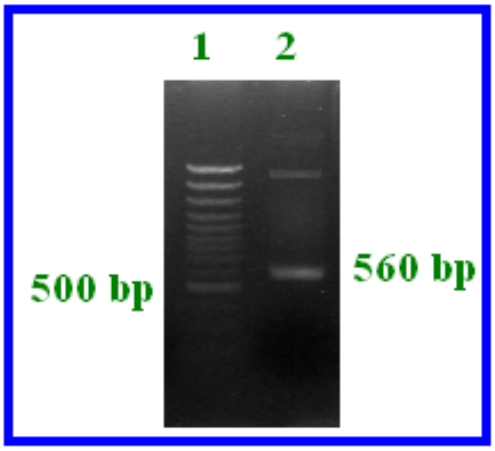

Figure 2. The 1.5% agarose gel electrophoresis. Lane 1: 100 bp DNA ladder marker; lane 2: SacI and BamHI digested recombinant PTZ57R.

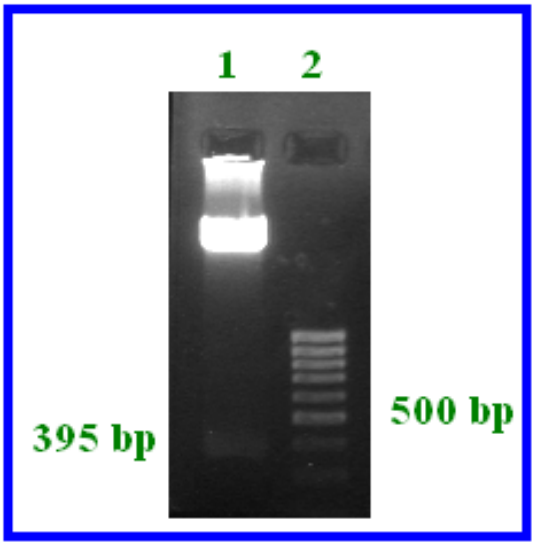

Figure 3. Gel electrophoresis of restriction analysis of recombinant plasmid. Lane 1: Xho 1digested plasmid using; lane 2: 100bp DNA ladder.

agarose gel and cloned into pTZ57R T vector. Recombinant plasmids (pTZ P22) were then transformed into *E. coli*. After incubation at 37°C for 16 h, colony screening were done using alpha-complementation test. White colonies (containing recombinant plasmids) were selected for further analysis.

Recombinant plasmid was extracted and digested by SacI and BamHI (Figure 2) and released fragment (P22 gene) was subcloned into SacI and BamHI digested pET32a expression vector. Recombination was confirmed by restriction analysis using XhoI enzyme.

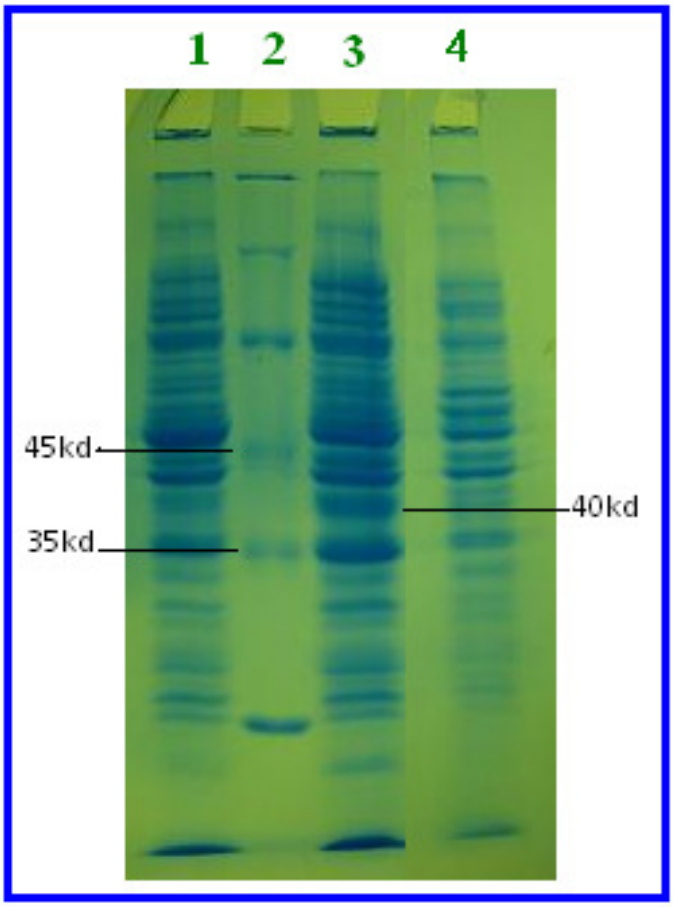

Figure 4. SDS-PAGE analysis of bacterial cells. Lane 1: Induced bacterial cells containing PET32a; lane 2: Protein size marker; lane 3: Bacterial cells containing recombinant PET32a 4 h after induction; lane 4: Induced bacterial cells.

Restriction analysis

XhoI enzyme has a restriction site on P22 gene at position 237 and a restriction site on pET32a at position 158. If recombinant plasmid digested by XhoI, a 395 bp DNA fragment will released (Figure 3) and non-recombinant plasmid will linear when digested by XhoI. Recombinant plasmid was purified, sequenced and deposited to GenBank at accession number GU174755.

Protein expression

The recombinant plasmid was transformed in to *E. coli* Bl21 (DE3) strain. Plasmid promoter was induced by IPTG. Before and after induction, bacterial samples were lysed and electrophoresed on 12% SDS-PAGE. Gel was stained by coomassie brilliant blue G_{250}. Figure 4 shows protein band approximately 40 KDa (including Trx fusion protein and P22) in induced cells compared with noninduced cells.

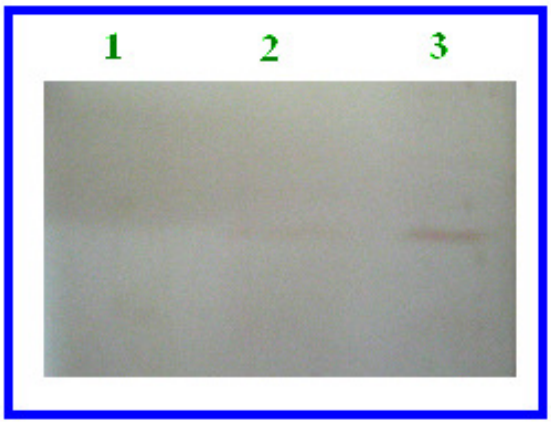

Figure 5. Western blot analysis of recombinant P22 by His tag monoclonal antibody. Lane 1: Bacterial cell lysates; lane 2: Bacterial cells containing PET32a; lane 3: Bacterial cells containing recombinant PET32a.

Purification of recombinant protein

Bacteria lysate were sonicated and subjected to purification using affinity chromatography. SDS-PAGE was performed by two separate gel and the protein bands were transferred to nitrocellulose membrane using western blotting device. The membrane were exposed to UV cross linker and detected by His Tag mono clonal antibody (Figure 5).

DISCUSSION

T. gondii is an intracellular coccidian parasite which can invade to almost every nucleated cells of intermediate host (Sibley, 1995). Delay in diagnosis of *Toxoplasma* infection in pregnant women who have been infected during the first trimester of gestation can lead to abortion. To date, serological tests are the main diagnosis methods for the detection of anti *Toxoplasma* antibody in serum samples (Aubert et al., 2000). However, whole tachyzoite proteins are used as an antigen in ELISA and IFA based tests. Since tachyzoites contains a wide variety of antigens may cross react by antiserum against other microorganisms. Additionally, presence of the non-specific materials in the tachyzoite solution is one of the main drawbacks to these tests (Aubert et al., 2000). Therefore as mentioned before, specific antigens of tachyzoite form can open the way for more rapid and accurate diagnosis of toxoplasmosis.

In this study, the recombinant P22 of *T. gondii* tachyzoite was prepared and the result showed that rP22 can be used as an effective antigen for toxoplasmosis (un published data). Reports of Parmley et al (1992) revealed that all of the sera of patients with acute toxoplasmosis have been developed reaction with rP22, while, sera of chronic patients showed weaker reaction (Parmly et al., 1992). Previous research regarding application of rP22 in ELISA test revealed that rP22 can introduce as an important antigen for diagnosis purposes (Lau and Fong, 2008).

Conclusion

In this study, recombinant P22 was cloned and the screened tests revealed that rP22 can be use as a specific antigen in serological diagnosis.

ACKNOWLEDGEMENTS

This study was supported financially by Shahid Beheshti University of Medical Sciences and Rafsanjan University of Medical Sciences and was implemented in Cellular and Molecular Biology Research Center. The authors declare that they have no conflicts of interest.

REFERENCES

Aubert D, Maine GT, Villena I, Hunt JC, Howard L, Sheu M, Brojanac S, Chovan LE, Nowlan SF, Pinon JM (2000). Recombinant antigens to detect *Toxoplasma gondii* specific Immunoglobalin G and Immunoglobulin M in human Sera by Enzyme Immunoassay. J. Clin. Microbiol. 38(3): 1144 -1150.

Boffey SA (1984). Agarose gel electrophoresis of DNA. In methods in molecular biology vol.2: Nucleic acids. United states: Walker JM, Humana Press.; pp. 43- 50.

Bothwell AL, Yancopulos GD, Alt FW (1990). Methods for cloning and analysis of eukaryotic genes. Spi edition. Boston: Jones and Bartlett pub. pp. 247-260.

Burg JL, Perelman D, Kasper LH, Ware PL, Boothroyd JC (1988). Molecular analysis for gene encoding the major surface antigens of *Toxoplasma gondii*. J. Immunol. 141: 3584-3591.

Cohen SN (1970). Toxoplasmosis in patient receiving immunosuppressive therapy. JAMA; 211(4): 657-660

Feliciello I, Chinali G (1993). A modified alkaline lysis method for the preparation of highly purified plasmid DNA from *Escherichia coli*. Anal. Biochem. 212: 394-401.

Flori P, Chene G, Varlet MN, Sung RT (2009). *Toxoplasma gondii* serology in pregnant woman: characteristics and pitfalls. Ann Biol. Clin. (Paris) 67(2): 125-133.

Frankel JK, Dubey JP, Miller NL (1970). *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocyst. Science, 167: 893-896.

Gaastra W, Jorgensen PL (1984). The extraction and isolation of DNA from gels, in Molecular Biology. Nucleic Acids. John M, edotor. Walker. Chapter, 10: 67-76.

Guerina NG (1994). Congenital infection with *Toxoplasma gondii*, Pediatric. Pediatr. Ann. 23 (3): 138-142,147-151.

Hanahan D (1983). Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 98: 503-517.

Kazemi K, Seyed N, Bandehpour M, Sharifnia Z, Pakzad P (2008) Cloning, Expression and Purification of Truncated Chlamydia Trachomatis Outer Membrane Protein 2 (Omp2) and its Application in an ELISA Assay. Iran. J. Immunol. 5(3): 148-155.

Lau YL, Fong MY (2008). *Toxoplasma gondii*: Serological characterization and immunogenicity of recombinant surface antigen 2 (SAG2) expressed in the yeast *Pichia pastoris*. Exp. Parasitol. 19(3): 373-378.

Lekutis C, Ferguson DJ, Grigg ME, Camps M, Boothroyd JC (2001). Surface antigen of *Toxoplasma gondii* : variation of a theme .Int. J.

Parasitol. 31: 1285-1292.
Luft BJ, Remington JS (1992). Toxoplasmic encephalitis in AIDS. Clin. Infect. Dis. 15(2): 211-222.
Manger ID, Hehl AB, Boothroyd JC (1998). The surface of Toxoplasma tachyzoites is dominated by a family of glycosylphosphatidylinositol-anchored antigens related to SAG1. Infect. Immunol. 66: 2237-2244.
Miller NL, Frenkel JK, Dubey JP (1972). Oral infections with *Toxoplasma gondii* cyst and oocyst in felines, other mammals and in birds. J. parasitol. 58(5): 928-937.
Parmley SF, Sgarlato GD, Mark J, Prince JB, Remington JS (1992). Expression, characterization and serologic reactivity of recombinant surface antigen P22 of *Toxoplasma gondii*. J. Clin. Microbiol. 30: 1127-1133.
Prince JB, Auer KL, Huskinson J, Parmely SF, Araujo FG, Remington JS (1990). Cloning, expression and cDNA sequence of surface antigen P22 from Toxoplasma gondii. Mol. Biochem. Parasitol. 43: 97-106.
Shewry PR, Fido RJ (1998). Protein blotting, pricipales and application. In: Molecular Biomethods Handbook, Rapley R, Walker JM, editors. Chapter, 34: 435-344.
Sibley LD (1995). Invasion of vertebrate cells by *Toxoplasma gondii*. Trend. Cell. Biol. 5: 129-132.
Smith BJ (1984). SDS polyacrylamide gel electrophoresis of protein. In methods in molecular biology vol.1: Proteins. United states : Walker JM, Humana Press.; pp. 41-55.
Spiro MJ, Bhoyroo VD, Spiro RG (1997). Molecular Cloning and Expression of Rat Liver Endo- alfa mannosidase, an *N*-Linked Oligosaccharide Processing Enzyme. J. Biol. Chem. 272(46): 29356-29363.
Weiss LM, Dubey JP (2009). Toxoplasmosis: A history of clinical observations. Int. J. Parasitol. 39(8): 895-901.
Zia-Ali N, Keshavarz-Valian H, Rezaian M, Khorramizadeh MR, Kazemi B, Fazaeli A, Darde M (2005). Molecular Characterization of *Toxoplasma gondii* from BirdHosts. Iran. J. Pub. Health, 34(3): 27-30.