

Original Article

Gene Cloning of 30 kDa *Toxoplasma gondii* Tachyzoites Surface Antigen (SAG1)

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Abstract

Background: *Toxoplasma gondii* is an obligate intracellular parasite and its sexual and asexual cycles, respectively take place in the intestinal epithelial cell of definitive host and tissue of intermediate hosts. Congenital toxoplasmosis is more important when the mother acquired the infection during pregnancy period for the first time. Serological tests are the only methods for diagnosis of toxoplasmosis. Among serological tests, ELISA has specific value and availability of parasite specific antigen increases the specificity of test. This study has designed and performed in the aim of availability to specific antigen of *Toxoplasma*.

Methods: A pair of forward and reverse primers was designed based on published sequence of *T. gondii* SAG1 gene. PCR reaction was performed and PCR product was cloned in the pQE-30 expression vector.

Results: The gene of 30 kDa protein of *Toxoplasma* tachyzoites was cloned in expression vector successfully. Recombinant plasmid was confirmed and is ready to express recombinant protein for further studies.

Conclusion: In this research we cloned P30 gene of *T. gondii* tachyzoites surface protein successfully and is ready to express the recombinant protein.

Keywords: *Toxoplasma tachyzoite*, SAG1, Cloning, pQE-30 expression vector, pTZ57R cloning vector

Introduction

Toxoplasma gondii is an obligate intracellular parasite and its life cycle includes definitive and intermediate hosts. The sexual and asexual cycle of parasite, respectively takes place in the intestinal epithelial cell of the cat (definitive host) and any warm blooded, like mammals and birds (intermediate host) (1). Researches have showed that the main human infection can result by ingestion of material contaminated with infected cat feces, from eating raw or partially cooked beef and transplacental transmission from mother to children (2). Congenital toxoplasmosis is more important in the pregnant women who acquired the infection for the first one (3).

Human infection takes place in two forms: acute infection and chronic infection. After beginning of the infection with initial immune response, tachyzoite (multiply fast) escape to different tissue via blood and lymph, then invert to bradyzoite (multiply slowly) inside tissue cyst (4).

Diagnosis of toxoplasmosis occurs in human by serological techniques because human is intermediate host for the *Toxoplasma*. The groups who are at the risk must be known by the use of serological tests in the beginning of pregnancy period. Therefore it is very important that pregnant women acquire the infection before or after the pregnancy period. Among serological tests, ELISA has a specific value and availability to specific antigen of parasite increase the specific-

ity of test. Thirty kDa of *Toxoplasma* tachyzoites surface protein is 3% to 5% of the total parasite proteins (5) and proposed for the diagnosis of acute acquired toxoplasmosis. Cesbron *et al.* (6) and Santaro *et al.* (7) have developed a double-sandwich ELISA based on this protein for detection of IgM antibodies.

The aim of this study was cloning the gene of 30 kDa surface protein of *T. gondii* tachyzoites (SAG1) as a recombinant protein using for diagnosis of toxoplasmosis as coating in micro titer ELISA plates.

Materials and Methods

Parasite

Toxoplasma tachyzoites was isolated from peritoneum of infected mice and rinsed by PBS buffer many times to ready for DNA extraction. Because *T. gondii* found to contain no introns (8), we start gene cloning process by genomic DNA.

PCR reaction

We designed a pair of primer based on SAG1 gene sequence (Accession number X14080 M23658) with Kpn I (GGT AC↓C) and Sall (G↓TC GAC) restriction sites at 5' end of forward and reverse primer respectively (ToxP30 F 5'-GGT ACC ATG TTT CCG AAG GCA GTG -3 and ToxP30 R 5'- GTC GAC CGC GAC ACA AGC TGC GAT-3). PCR reaction was performed using 1µg DNA, 40 pico mol each of forward and reverse specific primers, 1.5 mM MgCl₂, 0.2 mM dNTP, 1X PCR buffer, 1.5 unit of Taq DNA polymerase (CinnaGen, Iran) and dH₂O up to 50 µl. PCR reaction was carried out with 30 cycles of denaturation at 94 °C for 40 seconds, annealing at 65 °C for 60 s and extension at 72 °C for 60 s. Before beginning the PCR cycles, reaction was incubated at 94 °C for 5 min and the end of cycles, reaction was incubated at 72 °C for 5 min (9).

Electrophoresis

PCR product was submitted to electrophoresis using 1% agarose gel. Gel was stained by ethidium bromide and DNA band visualized under UV transilluminator (10).

Gene Cloning

PCR product of P30 gene was electrophoresed on 1% low melting point agarose gel (11), DNA band was sliced under long wave UV and recovered by DNA purification kit (Fermentas Cat. No k0513). Recovered DNA was cloned into pTZ57R cloning vector (Fermentas Lithuania) via T/A cloning method using T4 DNA ligase enzyme (12). The ligation reaction was transformed in *E. coli* XLI-blue strain competent cells (13) and dispensed on LB agar plates containing 100 µg/ml ampicillin. Bacterial colonies were screened by agar plate containing X-gal (Fermentas, Lithuania Cat No R0402) and IPTG (Fermentas, Lithuania Cat No R0392) to discriminate between recombinant (white) and no recombinant (blue) plasmid containing ones (14).

White bacterial colony was mass cultured in LB medium and recombinant plasmid was extracted (15), digested by KpnI (Fermentas, Lithuania Cat No ER 0521) and Sall (Fermentas, Lithuania Cat No ER 0641) restriction enzymes and released expected DNA band recovered by DNA purification kit (Fermentas Cat. No k0513). The gene was subcloned in KpnI and Sall digested pQE-30 expression vector (Qiagen USA). Reaction was transformed in *E. coli* and colonies contained recombinant plasmids was mass cultured on LB medium. Recombinant plasmid was extracted and confirmed by restriction analysis.

The PCR product was purified and submitted to Faza biotech company, Iran and sequenced by dideoxy chain termination method.

Results

Toxoplasma tachyzoites genomic DNA was extracted from infected mouse peritoneum and PCR reaction was done. Fig. 1 shows 957 bp PCR product as P30 gene.

PCR product was electrophoresed on low melting point agarose gel and DNA band (SAG1 gene) was sliced and recovered by DNA purification kit and cloned in pTZ57R cloning vector. Reaction was transformed in XLI- Blue *E. coli* strain and recombinant plasmids were screened via

alfa complementation test and white colonies as contained recombinant plasmids were selected (Fig. 2).

Bacterial colony contained recombinant plasmid was mass cultured and submitted to plasmid extraction. Extracted plasmid was digested with KpnI and SalI restriction enzymes and electrophoresed on low melting point agarose gel. Released expected DNA band (P30 gene) (Fig. 3) was puri-

fied by DNA purification kit, sub cloned in pQE-30 expression vector and named BKpQT30 (Fig. 4).

PCR product was sequenced and submitted to GeneBank under accession number: EF140712. Its amino acid sequence was compared with protein blast software in GeneBank data base and shows in Fig. 6:

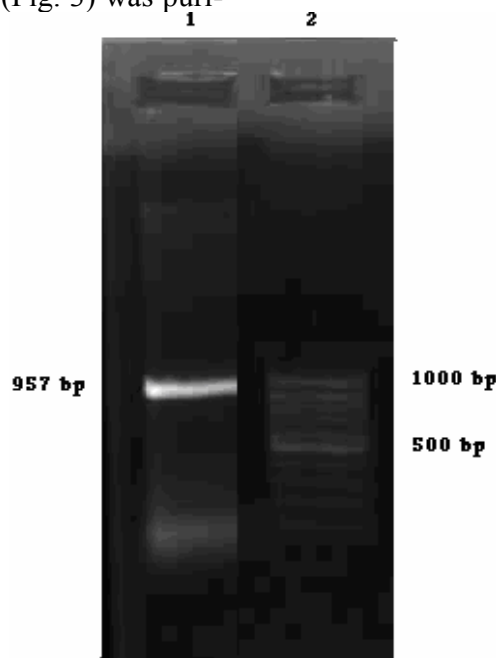


Fig. 1: DNA was extracted of *T. gondii* tachyzoites and PCR reaction was done using P30 gene primer. A 957 bp DNA band related to PCR product along with 100 bp DNA ladder marker is shown on 1% agarose gel electrophoresis
Lane 1: 957 bp PCR product of p30 gene, Lane 2: 100 bp DNA ladder marker

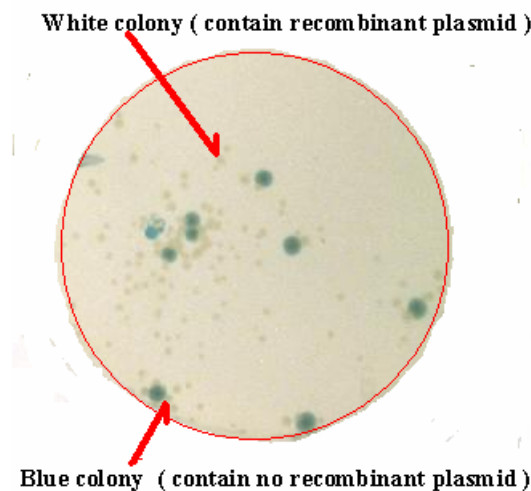


Fig. 2: Alfa complementation test for screening of bacterial colony contain recombinant plasmid.

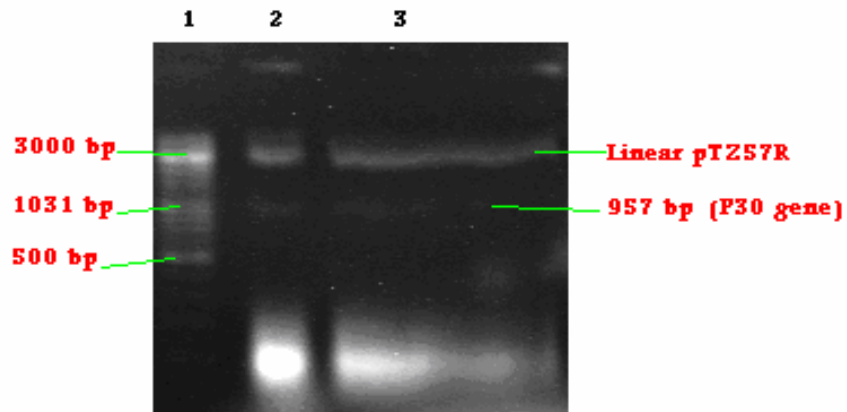


Fig. 3: Electrophoresis on low melting point agarose gel
Lane 1: 100 bp DNA ladder marker, Lanes 2 and 3: Digested recombinant plasmids with KpnI and SalI enzymes.

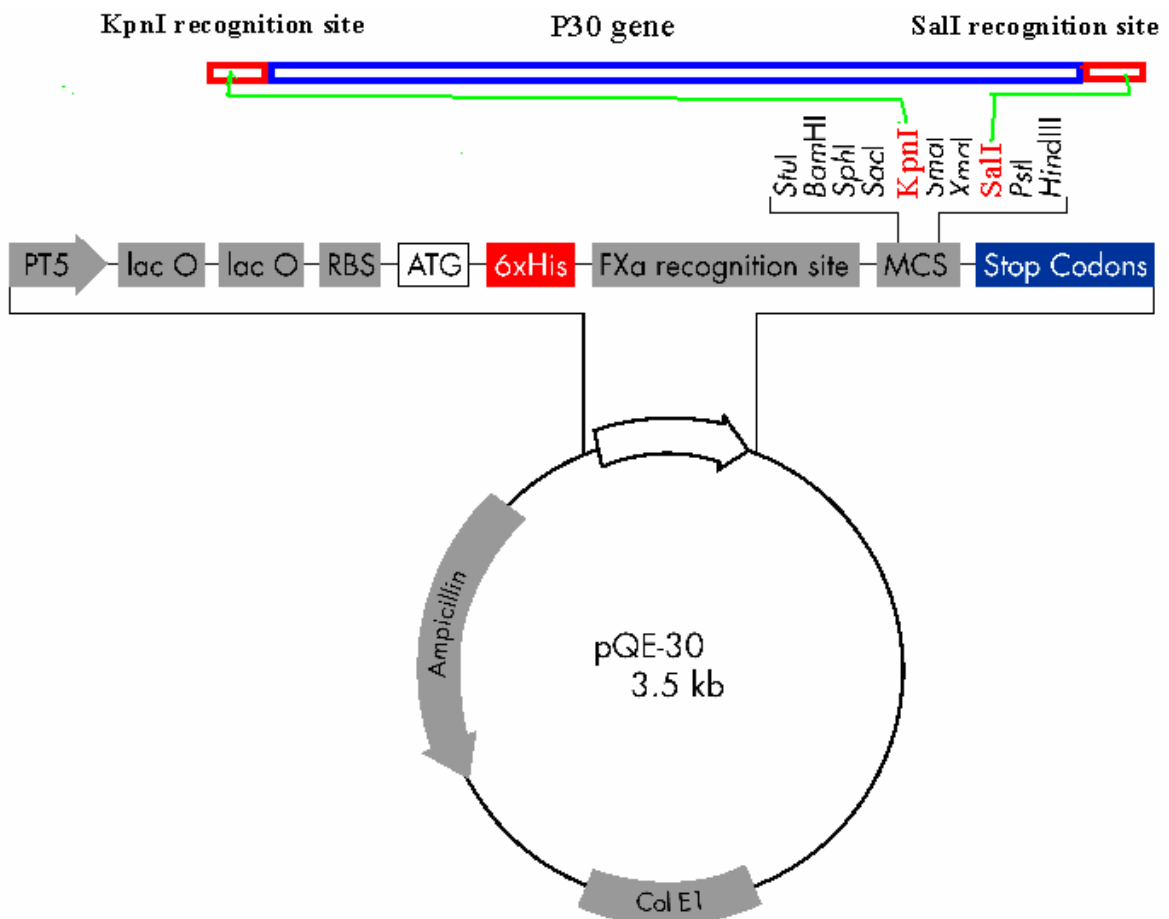


Fig. 4: Schematic of gene cloning in pQE-30

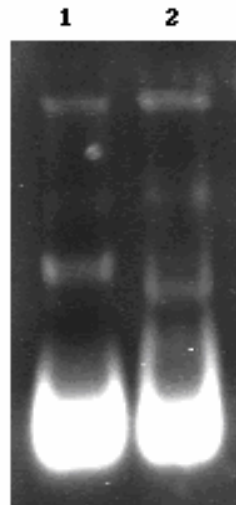


Fig. 5: 0.8% agarose gel electrophoresis
Lane 1: Digested BKpQT30 with Ppuml restriction enzyme, Lane 2: undigested pQE-30

Query	2	GVFAAPTLM SFLRCGAMALEPPLVANQVVTCPDKKSTAAVILTP TENHFTLKCPKTALTE	61
Sbjct	30	GVFAAPTLM SFLRCG MA +PPLVANQVVTCP KKSTAAVILTP TENHFTLKCPKTALTE	89
Query	62	PPTLAYS PNRQICPAGTTSSCTSKAVTLSSLIPEAKDSWWTGDSASLDTAGIKLTVPIEK	121
Sbjct	90	PPTLAYS PNRQICPAGTTSSCTSKAVTLSSLIPEA+DSWWTGDSASLDTAGIKLTVPIEK	149
Query	122	FPVTTQT FVVGCIKGDDAQSCMVT TVQARASSVVMNVARCSYGANSTLGPVKLSAEGPT	181
Sbjct	150	FPVTTQT FVVGCIKGDDAQSCMVT TVQARASSVVMNVARCSYGA+STLGPVK+SAE PT	209
Query	182	TMTLVCGKDGKVPQDNNQYCSGTTLTGCNEKSFKDILPKLSENPWQGNASSDNGATLTI	241
Sbjct	210	TMTLVCGKDGKVPQDNNQYCSGTTLTGCNEKSFKDILPKL+ENPWQGNASSD GATLTI	269
Query	242	NKEAFP AESKSVIIGCTGG SPEKHHCTVQLEVCRGCRVSKIVCGNTSHVFHFRHVTGLIG	301
Sbjct	270	KKEAFP AESKSVIIGCTGG SPEKHHCTV+LE +K G SHV F V GLIG	329
Query	302	SIA 304	
Sbjct	330	SIA 332	

Fig. 6: Amino acid sequence of SAG1 accession number EF140712 compared by amino acid sequence of SAG1 accession number X14080 M23658

Discussion

T. gondii is an obligatory intracellular parasite which has complicated life cycle and almost attacks to all nucleated cells (16). *T. gondii* leads to dangerous manifestation in fetus. The most dan-

gerous effect of congenital toxoplasmosis some times is abortion and premature delivery (17, 18). The congenital infection according to the intensity and variety of the organs contamination has different symptoms. Difference in the intensity of the disease depends on the stage of the preg-

nancy period which the infection occurs (17, 19-21). This parasite is detected in human beings by serological tests, and specific antigen is very essential in diagnosis system. In this study for availability of parasite stage specific antigen, the P30 gene of *Toxoplasma* tachyzoites surface antigen (SAG1) was cloned for diagnosis of *T. gondii* by ELISA system.

Dubremetz *et al.* demonstrated that P30 antigen express on the surface of intra- and extra cellular tachyzoites (22). Rodriguez *et al.* suggested that P30 is the most immunogenic constituent of tachyzoites, and that a single region of this molecule contains most of the immunogenic activity (23). Some epidemiological studies were carried out by this antigen (24-27) and some studies were used this antigen for vaccination (28-31). Others used this antigen for diagnosis of congenital toxoplasmosis (32).

In conclusion the 30 kDa protein gene of *Toxoplasma* tachyzoites surface antigen (SAG1) was cloned in expression vector and is ready to express the protein for diagnosis of toxoplasmosis using ELISA system.

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