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## Gene Cloning of P43 Surface Protein of *Toxoplasma gondii* Tachyzoite and Bradyzoite (SAG3)

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**Abstract:** This study has designed and performed in the aim of cloning a specific toxoplasma antigen for further studies. We have amplified gene of P43 of toxoplasma tachyzoite and bradyzoite surface antigen. PCR product was cloned in pGEMEX-1 expression vector (named pGEM43) and is ready to make the recombinant protein for using as antigen. It can be used for either diagnosis or prevention of parasite in men.

**Key words:** Toxoplasma, tachyzoite, bradyzoite, surface antigen, cloning

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## 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 of the cat (as definitive host) and any warm blooded, like mammals and birds (as intermediate hosts) (Frankel *et al.*, 1970). Previous studies 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 (Miller *et al.*, 1972). Congenital toxoplasmosis is more important in the pregnant women who acquired the infection for the first one (Guerina, 1994). 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 (Hutchison *et al.*, 1970).

Recently, main attention has been attracting to surface molecules of parasite. The surface of tachyzoite and bradyzoite have covered with antigens which is linked to GPI (glycosyl phosphatidyl inositol) (Nagel and Boothroyd, 1989; Tomavo *et al.*, 1989) that are known as SAG (surface antigens) (Lekutis *et al.*, 2000; Boothroyd *et al.*, 1998). Some of these specific molecules are specified stage of parasite life cycle: 30KD (SAG1), 22 kDa (SAG2) and 35 kDa (SRS3) proteins are only in the surface of tachyzoite and are not in the surface of bradyzoite, meanwhile some other proteins such as 43KD (SAG3) and 23 kDa are in the surface of both tachyzoite and bradyzoite (Manger *et al.*, 1998; Lekutis *et al.*, 2001; Burg *et al.*, 1988; Prince *et al.*, 1990). More studies are done about the cloning and expression of genes of toxoplasma surface proteins (Burg *et al.*, 1988; Prince *et al.*, 1990; Cesbron-Delauw *et al.*, 1994). The aim of this study was cloning the gene of P43 surface antigen (SAG3) of toxoplasma tachyzoite and bradyzoite as a recombinant protein.

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## MATERIALS AND METHODS

### Parasite

Toxoplasma tachyzoites were isolated from peritoneum puncture of infected mice and were rinsed by PBS buffer many times. Toxoplasma DNA was extracted as previously described (Zia-Ali, 2005).

### PCR Reaction

We designed a set of primer for amplification of P43 gene with SacI and BamHI restriction sites at 5' end of forward and reverse primers, respectively. PCR reaction contained 0.5 µg DNA, 40 pico mol each of forward and reverse primers, 1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 1X PCR buffer, 1.5 unit of Taq DNA polymerase (CinnaGen, Iran) and dH<sub>2</sub>O up to 50 µL. PCR amplification was carried out with 30 cycles of denaturation at 94°C for 40 sec, annealing at 65°C for 60 sec and extension at 72°C for 60 sec. PCR reaction was incubated at 94 and 72°C for 5 min before and after the PCR cycling respectively (Pherson *et al.*, 2000).

### Electrophoresis

PCR product was submitted to electrophoresis using 1% agarose gel and stained by ethidium bromide. The DNA band was visualized under ultraviolet light (UV transilluminator) (Boffey, 1984).

### Gene Cloning

PCR product was electrophoresed on 1% Low Melting Point (LMP) agarose gel (Gaastra and Jorgensen, 1984) and DNA band was sliced under long wave UV and recovered by DNA purification kit (Fermentas, Cat. No. k0513). Recovered DNA was cloned in pBluescript cloning vector via T/A cloning method. Briefly, EcoRV blunt digested pBluescript was 3' tailed using dTTP by terminal deoxy nucleotidyl transferase (Eun, 1996; Gaastra and Klemm, 1984) and 3' A tailed PCR product was ligated to it (Gaastra and Hansen, 1984). The ligation reaction was transformed in *E. coli* XLI-blue strain competent cells (Hanaham, 1983) and dispensed on LB agar plate containing 50 µg mL<sup>-1</sup> ampicillin. Colonies were screened by X-gal and IPTG and white colonies containing recombinant plasmid were selected (Bothwell *et al.*, 1990).

Recombinant plasmid was digested by SacI and BamHI and released expected DNA band was recovered by DNA purification kit (Fermentas Cat. No. k0513) and subcloned in SacI and BamHI digested pGEMEX-1 expression vector. Reaction was transformed and colonies contained recombinant plasmids were mass cultured on LB medium. Recombinant plasmids were extracted and confirmed by restriction analysis.

## RESULTS

Toxoplasma tachyzoites were isolated by peritoneum puncture of infected mice and rinsed by PBS buffer. DNA was extracted and PCR reaction was carried out. Figure 1 shows 1158 bp as PCR product of toxoplasma P43 gene.

PCR product was ligated in pBluescript via T/A cloning method and recombinant plasmid digested with SacI and BamHI restriction enzyme, Fig. 2 shows digested recombinant plasmid.

Digestion reaction was electrophoresed on LMP agarose gel, released DNA band was purified by DNA purification kit and subcloned in SacI and BamHI digested pGEMEX-1 expression vector and named pGEM43. PstI enzyme has a restriction site at position 748 on P43 sequence but pGEMEX-1 don't cut by this enzyme. For confirmation of recombinant pGEM43, we digested recombinant plasmid by PstI and lineared plasmid is shown in Fig. 3.

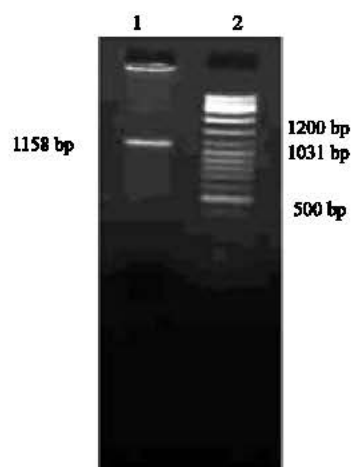


Fig. 1: 1% agarose gel electrophoresis, Lane 1: 1158 bp as PCR product of P43, Lane 2: 100 bp DNA ladder market

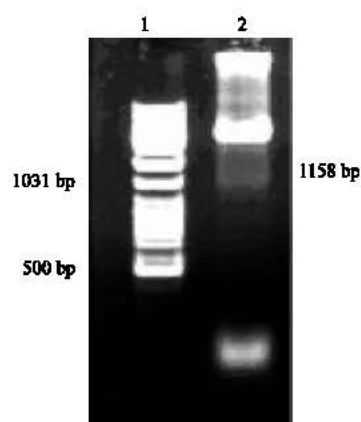


Fig. 2: 1% agarose gel electrophoresis, Lane 1: 100 bp DNA ladder marker, Lane 2: Digested recombinant plasmid by SacI and B am HI

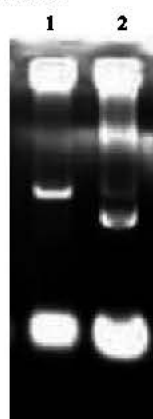


Fig. 3: Confirmation of recombinant plasmid, Lane 1: Recombinant plasmid (digested by PstI), Lane 2: No recombinant plasmid (not digested by PstI)

## DISCUSSION

*Toxoplasma gondii* is an obligatory intracellular parasite which has complicated life cycle. Sexual and asexual cycle respectively takes place in intestinal epithelial cells of cat and tissues of mammals and birds (Frankel *et al.*, 1970). This parasite almost attacks to all host nucleated cells (Sibley, 1995). *Toxoplasma gondii* leads to dangerous manifestation in fetus. The most dangerous effect of congenital toxoplasmosis some times is abortion and premature delivery (Freeman *et al.*, 2005; Dunn, 1999).

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 pregnancy period which the infection occurs (Wallon *et al.*, 2002; Zhao, 1992). This parasite will be detected in human beings by serological tests only and specific antigen is very essential in diagnosis system. P43 (SAG3) is one member of the redundant system of *T. gondii* receptors that act as ligands mediating host cell recognition and involved in the parasite attachment to target cells (Jacque *et al.*, 2001; Dzierszinski *et al.*, 2000). In this study for availability of parasite stage specific antigen, the gene of p43, the tachyzoite and bradyzoite surface antigen was cloned and became ready to make recombinant proteins. It can be used as antigen for detection or prevention of parasite in men.

## CONCLUSION

In this study, the gene of P43 toxoplasma tachyzoite and bradyzoite surface antigen was cloned in expression vector and confirmed. It can be used for either diagnosis or prevention of parasite in men.

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