



***Toxoplasma gondii* rhoptry protein 38 gene: sequence variation among isolates from different hosts and geographical locations**

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ABSTRACT. *Toxoplasma gondii* is an obligate intracellular parasite that is able to infect almost all mammalian species, and may lead to toxoplasmosis of the host. In the present study, we examined sequence variation in rhoptry protein 38 (ROP38) genes among *T. gondii* isolates collected from different hosts and geographical regions. The complete ROP38 gene from 13 *T. gondii* isolates was amplified and sequenced. The results of sequence alignments showed that the lengths of the entire ROP38 gene ranged from 2646 to 2650 bp, with a sequence variation of 0.2-1.1%, among the 13 *T. gondii* isolates. This result

indicated low sequence variation in the ROP38 gene. Phylogenetic analysis of ROP38 sequences using Bayesian inference showed that the clustering of the 13 *T. gondii* isolates was not consistent with their respective genotypes. This result indicates that the ROP38 gene is not a suitable genetic marker for population genetic studies of different *T. gondii* genotypes from different hosts and geographical locations, but may represent a potential vaccine candidate against toxoplasmosis, and hence worthy of further research.

Key words: *Toxoplasma gondii*; Rhoptry protein 38 (ROP38); Toxoplasmosis; Sequence variation

INTRODUCTION

Toxoplasmosis is considered as one of the most important zoonoses worldwide, presenting a major health risk to almost all warm-blooded animals (Dubey, 2008, 2010; Innes, 2010; Tian et al., 2012). Nearly one third of the world's population has been infected with this parasite. In China, about 7.9% of the human population was reported to have been exposed to *Toxoplasma gondii* (between 2001 and 2004), with sustained growing numbers in recent years (Tenter et al., 2000; Hill and Dubey, 2002; Montoya and Liesenfeld, 2004; Zhou et al., 2011). Normally, infections in animals and humans are asymptomatic; however, the parasite may cause severe disease in pregnant women or immunocompromised humans (Fuentes et al., 2001; Djurkovic-Djakovic, 2002; Petersen, 2007; Dubey, 2010; Robert-Gangneux and Dardé, 2012). *T. gondii* infection also has a major economic impact on livestock industry (Dubey and Jones, 2008; Dubey, 2009). However, no effective vaccine is available for protecting humans and domestic animals against toxoplasmosis (Verma and Khanna, 2012).

Rhoptry is a unique secretory organelle that is located in all apicomplexan protozoan parasites, and plays an important role in parasite invasion (Dubremetz, 2007; Reese and Boyle, 2012; Kemp et al., 2013). Previous studies have shown that rhoptry proteins (ROPs) represent potential vaccine candidates against toxoplasmosis, especially the ROP2 protein family (Bhopale, 2003; Dlugonska, 2008; Kur et al., 2009). Sequence variation in different ROP genes is usually low, e.g., 1.7% in ROP7 (Zhou et al., 2012) and 2.0% in ROP13 (Wang et al., 2012). Therefore, a novel rhoptry protein with low sequence variation may be used for the development of new vaccines against *T. gondii* infection.

ROP38 is predicted to be an active kinase that is differentially expressed among *T. gondii* isolates (Peixoto et al., 2010; Melo et al., 2011). Moreover, ROP38 has been observed inside rhoptries, and is associated with the parasitophorous vacuole membrane; hence, it may have an inhibitory effect on host cell transcription by downregulating the mitogen-activated protein kinases signaling track (Peixoto et al., 2010; Melo et al., 2011; Denkers et al., 2012). However, information about the sequence variation in the ROP38 gene for different *T. gondii* isolates is not available. Therefore, this study examined the sequence variation in ROP38 genes of *T. gondii* isolates from different hosts and geographical regions, to assess whether the ROP38 gene may be used as a new marker for population genetic studies of *T. gondii*.

MATERIAL AND METHODS

T. gondii isolates

A total of 13 *T. gondii* isolates originating from different hosts and geographic locations were used for the analysis (Table 1). Genomic DNA (gDNA) of these *T. gondii* isolates was previously prepared and genotyped in our previous studies (Zhou et al., 2009, 2010; Su et al., 2010; Huang et al., 2012).

Table 1. Details of *Toxoplasma gondii* isolates used in the present study.

Strain	Host	Geographical origin	Genotype*
GT1	Goat	United States	Reference, Type I, ToxoDB #10
PTG	Sheep	United States	Reference, Type II, ToxoDB #1
CTG	Cat	United States	Reference, Type III, ToxoDB #2
TgCatBr5	Cat	Brazil	Reference, ToxoDB #19
MAS	Human	France	Reference, ToxoDB #17
TgCatBr64	Cat	Brazil	Reference, ToxoDB #111
TgCgCa1	Cougar	Canada	Reference, ToxoDB #66
TgWtdSc40	White-tailed deer	USA	Type 12, ToxoDB #5
RH	Human	France	Reference, Type I, ToxoDB #10
Prugniaud (PRU)	Human	France	Type II, ToxoDB #1
QHO	Sheep	Qinghai, China	Type II, ToxoDB #1
SH	Human	Shanghai, China	Type I, ToxoDB #10
TgC7	Cat	Guangzhou, China	ToxoDB #9

*Based on genotyping results of Zhou et al. (2009, 2010), Su et al. (2010), and Huang et al. (2012).

PCR amplification

gDNA of individual isolates was used as a template for the amplification of all ROP38 gene sequences. Primers ROP38F (5'-ATGAAAATACTCTGTTGTACCTGTTGGT-3') and ROP38R (5'-CTGTGTATGAAAATACAGGGAATGGCGTA-3') were designed based on the ROP38 gene sequence of *T. gondii* ME49 strain that is available in the ToxoDB database (TGME49_242110). The amplification reaction was carried out in 25 µL containing 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 3 mM MgCl₂, 250 µM of each dNTP, 0.2 µM of each primer, 100-200 ng template DNA, and 0.25 U *Ex Taq* polymerase (TaKaRa). The PCR was carried out in a thermocycler (BioRad, USA) with an initial denaturation step at 94°C for 10 min, followed by 35 cycles at 94°C for 40 s (denaturation), 55°C for 30 s (annealing), 72°C for 2 min and 35 s (extension), and a final extension step at 72°C for 10 min. The PCR amplifications were confirmed by electrophoresis on 1% (w/v) agarose gel, and stained with GoldenView™.

Sequencing of the ROP38 amplicons

The ROP38 PCR products were purified using the PCR-Preps DNA Purification System (Promega, USA) following manufacturer protocols. After ligated the products into the pMD18-T vector (TaKaRa), the recombinant vectors were transformed into JM109 competent cells (Promega). Following identification by PCR amplification and enzymatic digestion, the positive colonies were sequenced by Shanghai Songon Biological Engineering Biotechnology Company, China.

Sequence analysis and phylogenetic reconstruction

The obtained ROP38 gene sequences from different *T. gondii* strains were aligned using the Multiple Sequence Alignment Program, Clustal X 1.83 (Thompson et al., 1997), and sequence variation was determined among the examined *T. gondii* strains. Phylogenetic reconstructions based on the complete ROP38 gene sequences from different *T. gondii* strains were performed by Bayesian inference (BI). BI analyses were conducted with 4 independent Markov chains run for 10,000,000 metropolis-coupled generations, sampling a tree every 10,000 generations in MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). The first 250 trees were omitted as burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities.

RESULTS AND DISCUSSION

The complete ROP38 gene sequences were 2646 bp for *T. gondii* strain CTG, 2647 bp for WtdSc40, 2648 bp for TgC7 and QHO, 2650 bp for GT1, and 2649 bp for the other 9 *T. gondii* isolates. The A+T content ranged from 47.07 to 47.32%. Sequence variations in the ROP38 gene among the 13 examined *T. gondii* isolates were 0.2 to 1.1%, which is lower compared to the ROP7 and ROP13 genes (Wang et al., 2012; Zhou et al., 2012). Furthermore, there were 37 variable positions at the coding region of the gene, indicating very low genetic variation in the ROP38 gene. There were 58 transitions (C↔T, and A↔G) and 10 transversions (A↔T, G↔T, A↔C and C↔G) in the entire genomic sequences of the ROP38 gene ($R = \text{transversion/transition} = 17.24$); 32 transitions (A↔G and C↔T) and 4 transversions (A↔T and C↔G) in the coding region of the ROP38 gene ($R = \text{transversion/transition} = 12.5$). These results were similar to those found in previous studies for PLP1 (Yan et al., 2011) and ROP7 (Zhou et al., 2012).

Phylogenetic analysis using the BI of the 13 examined *T. gondii* isolates based on the ROP38 gene sequences showed that *T. gondii* isolates of the same genotypes were clustered in different clades (Figure 1). This result indicates that the ROP38 gene is not an ideal genetic marker for population genetic studies of *T. gondii* isolates. This result was not consistent with some previous studies using other genetic markers, such as GRA5 (Chen et al., 2012).

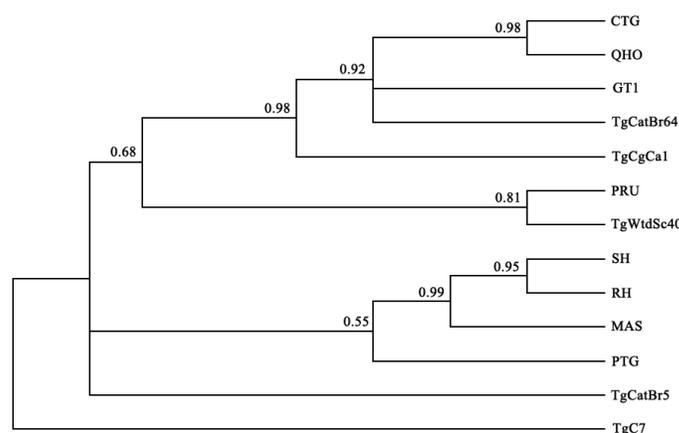


Figure 1. Phylogenetic analysis (Bayesian inference) of 13 *Toxoplasma gondii* isolates based on the entire sequences of the ROP38 gene.

The present study examined sequence variation in ROP38 gene sequences, and demonstrated the presence of low variability in the ROP38 gene among the examined *T. gondii* isolates from different hosts and geographical regions. In conclusion, the ROP38 gene is not a suitable marker for population genetic studies of *T. gondii* isolates, but may represent a potential vaccine candidate against *T. gondii* infection.

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