INTRODUCTION

Toxoplasma gondii, the direct pathogenic factor of toxoplasmosis, is an obligate intracellular protozoan parasite of the phylum Apicomplexa which can infect all warm-blooded vertebrates, including humans, livestock, and marine mammals [1]. Although most infections are clinically asymptomatic, the parasite can cause severe disease in immunocompromised populations and congenitally infected individuals [1,2]. In addition, infections in domestic animals may result in economic losses as well as bring enormous psychological troubles, since it can cause abortion, stillbirth, and neonatal loss [3].

Unlike many other Apicomplexa parasites which exhibit stronger host specificity, T. gondii faces vastly numerous hosts and can adapt to various environmental conditions during its complex life cycles, which can be contributed to many different T. gondii strains and genotypes [4]. It was popularly believed that T. gondii had a clonal population structure with 3 predominant lineages, namely types I, II, and III [5-7]. Besides these isoforms, it also exists in atypical and recombinant strains [8,9]. To better understand the population genetics and molecular epidemiology of this parasite, and to develop more strategies for vaccination, diagnosis, and treatment of toxoplasmosis, it is necessary to study the genetic diversities in T. gondii [10,11].

Superoxide dismutase (SOD), an important enzyme that widely exists in many organisms, including animals, plants, and microorganisms, can promote the conversion of superoxide (O₂⁻) into hydrogen peroxide and oxygen [12,13]. In view of SOD that can eliminate extra superoxide (O₂⁻) anion in the cells and protect cells from oxidative damages, it has potential applications in medicine, food industry, and agriculture [12,14,15]. In T. gondii, limited studies have shown that SOD is a typical FeSOD and its activity might be essential for the intracellular growth of both bradyzoite and tachyzoite forms [16]. To our knowledge, no report has described the sequence variation of SOD gene in different T. gondii strains. We hereby examined sequence variation of SOD gene among 10 T. gondii
isolates from different hosts and geographical regions (different countries), and assess SOD could be used as a new marker for genetic study or a potential vaccine candidate against *T. gondii*.

**MATERIALS AND METHODS**

*T. gondii* strains

A total of 10 genotyped *T. gondii* strains were utilized as shown in Table 1, and the genomic DNA was prepared as described previously [17].

PCR amplification

The SOD gene was amplified by PCR from genomic DNA of *T. gondii* with 1 pair of primers: 5’-ATGGATCTACATGCCC-CCGCT-3’ (forward prime) and 5’-TCAATTTAGGATCTCTCAAG-3’ (reverse primer). The design of primers was based on SOD gene of *T. gondii* RH isolate available in GenBank database (AF029915). The amplification reaction was performed in a volume of 20 μl containing 2 μl template DNA, 10 μl 2×1 Step buffer (0.5 U *Taq* polymerase), 1 μl of each primer, and 6 μl RNase-free dH2O. The target DNA was amplified under the following conditions: 94˚C for 30 sec, 63.6˚C for 1 min, and 72˚C for 1 min. The PCR amplification products were confirmed by electrophoresis in a 1.5% agarose gels and staining with ethidium bromide followed by visualization under UV.

The analysis of PCR-RFLP

The SOD PCR amplification products from representative *T. gondii* strains were digested with restriction enzymes *Xba*I and *EcoR*I, respectively, and incubated at 37˚C for 3 hr. The restriction fragments were separated by electrophoresis in 1.5% agarose gels.

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Table 1. *Toxoplasma gondii* strains subjected to SOD gene sequence analysis

| Strain       | Host   | Geographical origin | Genotype*               |
|--------------|--------|---------------------|-------------------------|
| RH           | Human  | France              | Reference, Type I, ToxoDB #10 |
| GT1          | Goat   | United States       | Reference, Type I, ToxoDB #10 |
| PTG          | Sheep  | United States       | Reference, Type II, ToxoDB #1     |
| Prugniaud (PRU) | Human  | France              | Reference, Type II, ToxoDB #1     |
| CTG          | Cat    | United States       | Reference, Type III, ToxoDB #2    |
| TgCgCa1      | Cougar | Canada              | Reference, ToxoDB #66         |
| MAS          | Human  | Brazil              | Reference, ToxoDB #19         |
| TgCatBr5     | Cat    | Brazil              | Reference, ToxoDB #111        |
| TgCatBr64    | Cat    | Brazil              | Reference, ToxoDB #66         |
| TgToucan (TgRsCr1) | Toucan  | Costa Rica          | Reference, ToxoDB #52         |

*aBased on genotyping results of Su et al. [17].
rose gel and staining with ethidium bromide, followed by visualiza-
tion under UV.

Sequence analysis and reconstruction of phylogenetic relationships

The SOD PCR products were purified with the DNA purifi-
cation kit (TransGen Biotech, Beijing, China) and ligated with the pEAY-T1 vector (TransGen Biotech) according to the manufacturer’s instructions, and then transformed into Esche-
richia coli DH5α competent cells. The transformed cells carry-
ing the insert were successively selected by blue-white screen-
ing, PCR, and restriction enzyme digestion. The positive colo-
nies were sequenced by Beijing Genomics Institute Company (Beijing, China). The obtained SOD gene sequences from dif-

Table 2. Nucleotide polymorphisms of the SOD gene coding region within *Toxoplasma gondii* strains

|       | RH  | CTG  | GT1  | MAS  | PRU  | PTG  | TgCatBr5 | TgCatBr64 | TgCgCa1 | TgToucan |
|-------|-----|------|------|------|------|------|-----------|-----------|----------|----------|
| 1403  | A   | *    | *    | *    | *    | *    | C         | *         | *        | *        |
| 1437  | A   | *    | *    | *    | *    | *    | T         | *         | *        | *        |
| 1480  | G   | *    | *    | *    | *    | *    | C         | *         | *        | *        |
| 1501  | G   | *    | *    | *    | *    | *    | *         | T         | *        | *        |
| 1503  | G   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1504  | G   | *    | *    | *    | *    | *    | T         | *         | *        | *        |
| 1518  | A   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1519  | C   | *    | *    | *    | *    | A    | *         | *         | *        | *        |
| 1520  | T   | *    | *    | *    | *    | C    | *         | *         | *        | *        |
| 1521  | G   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1532  | C   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1535  | C   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1545  | A   | *    | *    | *    | *    | *    | *         | G         | *        | *        |
| 1547  | G   | *    | *    | *    | *    | *    | C         | *         | *        | *        |
| 1549  | A   | *    | *    | *    | *    | *    | *         | G         | *        | *        |
| 1555  | C   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1556  | A   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1557  | T   | *    | *    | *    | *    | C    | *         | *         | *        | *        |
| 1558  | C   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1566  | A   | *    | *    | *    | *    | G    | *         | *         | *        | *        |
| 1567  | A   | *    | *    | *    | *    | *    | *         | T         | *        | *        |
| 1568  | C   | *    | *    | *    | *    | *    | *         | T         | *        | *        |
| 1574  | G   | *    | *    | *    | *    | A    | *         | *         | *        | *        |
| 1575  | G   | *    | *    | *    | *    | A    | *         | *         | *        | *        |
| 1584  | T   | *    | *    | *    | *    | C    | *         | *         | *        | *        |
| 1585  | C   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1586  | G   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1587  | A   | *    | *    | *    | *    | G    | *         | *         | *        | *        |
| 1590  | G   | *    | *    | *    | *    | A    | A         | *         | *        | *        |
| 1599  | G   | *    | *    | *    | *    | *    | *         | A         | *        | *        |
| 1601  | C   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1614  | G   | *    | *    | *    | *    | A    | *         | *         | *        | *        |
| 1616  | T   | *    | *    | *    | *    | G    | *         | *         | *        | *        |
| 1618  | G   | *    | *    | *    | *    | T    | *         | *         | *        | *        |
| 1620  | G   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1628  | T   | *    | *    | *    | *    | A    | *         | *         | *        | *        |
| 1635  | G   | *    | *    | *    | *    | A    | *         | *         | *        | *        |
| 1637  | C   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1668  | A   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1669  | C   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1679  | G   | *    | *    | *    | *    | *    | *         | *         | *        | *        |
| 1685  | C   | *    | *    | *    | *    | A    | *         | *         | *        | *        |
| 1793  | C   | *    | *    | *    | *    | *    | *         | *         | *        | *        |

Numbers in the left column indicate positions of variable nucleotides. Asterisks (*) indicate identical nucleotides related to the sequence of RH (first column). Dashes (-) indicate deletions.
Table 3. Amino acid changes of the SOD gene coding region among ten *Toxoplasma gondii* strains

| Position | RH | CTG | GT1 | MAS | PRU | PTG | TgCatBr5 | TgCatBr64 | TgCgCa1 | TgToucan |
|----------|----|-----|-----|-----|-----|-----|----------|-----------|---------|----------|
| 66       | N  | *   | *   | *   | *   | Y   | *        | *         | *       | *        |
| 80       | S  | *   | *   | *   | *   | T   | *        | *         | *       | *        |
| 87       | G  | *   | *   | *   | *   | *   | *        | *         | *       | *        |
| 88       | G  | *   | *   | *   | *   | C   | *        | V         | *       | *        |
| 93       | T  | *   | *   | *   | *   | *   | *        | *         | *       | *        |
| 94       | G  | *   | *   | *   | *   | W   | *        | *         | *       | *        |
| 102      | K  | *   | *   | *   | *   | N   | *        | E         | *       | *        |
| 103      | E  | *   | *   | *   | *   | *   | *        | G         | *       | *        |
| 105      | T  | *   | *   | *   | *   | I   | *        | *         | *       | *        |
| 106      | S  | *   | *   | *   | *   | F   | *        | *         | *       | *        |
| 109      | N  | *   | *   | *   | *   | *   | *        | *         | I       | *        |
| 112      | D  | *   | *   | *   | *   | N   | *        | *         | *       | *        |
| 115      | S  | *   | *   | *   | *   | *   | *        | P         | *       | *        |
| 116      | K  | *   | *   | *   | *   | *   | *        | E         | *       | *        |
| 117      | V  | *   | *   | *   | *   | I   | *        | *         | *       | *        |
| 120      | G  | *   | *   | *   | *   | *   | *        | *         | S       | *        |
| 125      | G  | *   | *   | *   | *   | S   | *        | *         | *       | *        |
| 126      | W  | *   | *   | *   | *   | F   | *        | *         | *       | *        |
| 127      | A  | *   | *   | *   | *   | *   | *        | *         | T       | *        |
| 129      | L  | *   | *   | *   | *   | H   | *        | *         | *       | *        |
| 132      | D  | *   | *   | *   | *   | *   | *        | *         | H       | *        |
| 143      | T  | *   | *   | *   | *   | N   | *        | *         | *       | *        |
| 148      | T  | *   | *   | *   | *   | N   | *        | *         | *       | *        |
| 151      | T  | *   | *   | *   | *   | *   | *        | *         | *       | *        |

Numbers in the left column indicate positions of variable amino acids. Asterisks (*) indicate identical amino acid related to the sequence of RH (first column). Dashes (-) indicate deletions.
polies of all trees based on nucleotide sequences inferred by 2 different methods were similar, with only small differences of bootstrap values.

DISCUSSION

SOD widely exists in many organisms and plays a crucial role in eliminating the extra superoxide anion \( \text{O}_2^- \) in the cells to avoid oxidative damages [12-15]. In \textit{T. gondii}, SOD is an iron-containing type, which correlates with the protection of the parasite [16]. However, SOD enzymes from C and RH strain tachyzoites do not appear to be the basis for differences in virulence to mice [20]. In the present study, we cloned and sequenced the partial genome sequence of SOD gene among 10 \textit{T. gondii} isolates from different hosts and geographical regions and examined genetic diversity of SOD locus by the techniques of PCR-RFLP, sequence analysis, and phylogenetic reconstruction. The results revealed nucleotide polymorphisms at 43 positions and amino acid polymorphisms at 24 positions, suggesting low sequence variability among all the tested isolates.

PCR-RFLP has been widely used in analysis of specific genetic loci for \textit{T. gondii} genotyping. Multilocus PCR-RFLP marker is a high resolution for identification of parasites, although it requires a huge investment of time to test and optimize each marker [11]. By contrast, single PCR-RFLP marker is simply and more convenient. Then, studies on single marker loci (e.g. GRA5, GRA6, and ROP17) have shown signs of positive selection, and could be sufficient for genotyping of \textit{T. gondii} isolates. [21-23].

In our study, however, 3 clonal lineages (types I, II, and III) cannot be differentiated in 10 examined \textit{T. gondii} strains using a single PCR-RFLP marker SOD. One possible reason is that the sequence variation of SOD could not be located in restriction enzyme sites, to some degree, leading to loss of many polymorphisms in this situation [24].

The direct sequencing of genomic regions can detect small deletions and insertions (e.g., indels) and single nucleotide polymorphisms (SNPs) in the genomic regions, and hence it is capable of testing more genetic diversity compared with PCR-RFLP. Based on the partial sequences of the SOD locus, phylogenetic analysis revealed 2 major clusters with only a little difference of bootstrap values, and it cannot differentiate \textit{T. gondii} strains to their genotypes, implying a low genetic diversity. Our results were similar to some studies such as MIC13, elf4A [25,26] and different from other genetic markers including GRA5, GRA6, and ROP17 [21-23]. The low sequence variation (0-1.0%) in the partial SOD gene suggests that SOD gene could not be an ideal genetic marker for differentiation of the \textit{T. gondii} strains or intraspecific phylogenetic analyses.

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**CONFLICT OF INTEREST**

We have no conflict of interest related to this work.

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