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Expression of Toxoplasma Gondii Recombinant Protein and Its Application in ELISA Detection

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Abstract: *Toxoplasma gondii* is a prevalent parasite with global distribution capable of infection various warm-blooded animals, potentially causing clinical manifestations such as dirrhea and miscarriage. Investigation its protein expression through exerimental approached can enhance scientific understanding of this pathogen. This study mainly focuses on the epidemiological samples of *T. gondii* using the enzyme-linked immunosorbent assay (ELISA) to detect protein expression. Experimental procedures included PCR amplification. protein induction, expression, and purification, with recombinant protein validation performed through protein elextrophoresis. Results demonstrated that among 60 tested samples, a high proportion of false positives was observed, which did not correlate with the expected positivity rate (4 confirmed cases). This discrepancy may stem from sample contamination during non-standardized experimental procedures. These findings provide a reference for future *T. gondii* research and contribute to vaccine development efforts against this parasite.

1. Introduction

T. *gondii* is a uboquitous parasite affection various warm-blooded animals, such as cats, pigs, and humans, with cats serving as its definitive hosts. The parasite spreads through consumption of undercooked meat or contaminated water. In most cases, infected animals develop latent infections without apparent symptoms. however, immunocompromised hosts--such as pregnant women or HIV-positive individuals--may experience severe comlications including encephalitis, miscarriage, or stillbirth. [1] Therefore, research on *T. gondii* is crucial for disease prevention and control.

Current, diagnostic methods include pathogen isolation, serolobical tests, and mplecular assays. Among these, the ELISA has become a preferred method dus to its operational simplicity, high sensitivity, and specificity, making it a widely used technique for *T. gondii* detection. There are previous studies, have extensively investigated this approach; for instance, Miao et al. conducted ELISA-based detection of *T. gondii* antigens using a large number of diverse samples. [4]

This study amis to analyze the expression of recombinant *T. gondii* proteins and their detection via ELISA, providing a foundation for developing rapid diagnostic tools, therapeutic strategies, and preventive measures against toxoplasmosis. Additionally, the findings may contribute to future vaccine development against *T. gondii* infections.

2. Materials and Methods

2.1 Materials

The materials used in this experiment are shown in Table 1.

Table 1 Materials

Items	source
50 mL centrifuge tubes	GeneBrick Biotechnology Co., Ltd
Filter tips	Biosharp Life Science
Microplate reader	Bio Tek
Protein Electrophoresis Apparatus	BIORAD
Gel staining machine	GenScript
Ultrasonic Cell Disruptor	Ningbo Xin Zhi Biotechnology Co., Ltd.
Centrifuge	Thermo Scientific
Chromogenic substrate	Li Ji Biology

2.2 Method

2.2.1 Reviving Recombinant Bacteria

2.2.1.1 Preparation of liquid LB medium

To prepare LB liquid medium, first mix LB Broth 2.5 mg of meat extract powder with 100 mL of double-distilled water (ddH2O) and dissolving it completely. We wrapped the bottle, which contained the LB liquid with aluminum foil to prevent contamination then labeled it with name and date. We put it in the autoclave to sterilize 121 degrees and 15-20 min, took out and cooled down, then the preparation is completed.

2.2.1.2 Recovery of recombinant bacteria

We added 200 μL of kanamycin to the sterilized LB liquid medium and mixed it. Using a sterile inoculation loop, we took a little bit of recombinant bacteria and inoculated it into the LB liquid medium with antibiotics. We put it in a shaker and cultured it for 12 hours and see the results. The process of inoculating recombinant bacteria and adding antibiotics were done in a laminar flow cabinet to keep everything sterile.

2.2.2 Verification of Recombinant Plasmid

2.2.2.1 Practical operation of PCR amplification of genes

PCR is a technique used to amplify and amplify specific DNA fragments and molecular biology [3]. In this experiment, the total reaction preparation system was 25 mL, including template DNA, primers (forward and reverse), dNTPs, Taq DNA polymerase, and reaction buffer. We added 5.5 μL of ddH2O, 5 μL of Toxoplasma gondii template, 1 μL of each primer, 12.5 μL of reaction buffer, mixed them in a PCR tube. We put the PCR tube into the DNA amplifier and setted the program: 95 ℃ for 5 minutes to pre-denature, followed by 35 cycles of 94 ℃ for 15 seconds to denature, 55 ℃ for annealing, and 68 ℃ for 40 seconds to extend. We then extended at 72 ℃ for 5-10 minutes. The amplified DNA sample was obtained and subjected to electrophoresis.

2.2.2.2 Preparation of AGAR gel

We mixed 1 g of agarose powder with 100 mL buffer solution and heated it in the microwave until the agarose was completely dissolved. We added nucleic acid stain and mixed it completely. We poured it into a gel mold and inserted a comb. We waited for 30-50 minutes until the agarose gel solidified completely.

2.2.2.3 Electrophoresis

We checked the electrophoresis apparatus whether it was correctly connect to the electrophoresis tank and poured buffer solution into the tank. We placed the prepared agarose gel into the tank. Prepared the DNA samples and a marker (a standard for comparison). We loaded the marker into the first lane and the DNA samples into the remaining lanes in order. We turned on the electrophoresis and observeed the movement of the dye and DNA samples for about 30-40 minutes.

2.2.3 Induction and Expression of Recombinant Protein

2.2.3.1 Preparation and Washing of Recombinant Bacteria

We took the prepared recombinant bacteria and put them into a centrifuge tube. We centrifuged the tube at 8000 rpm for 10 minutes to precipitate the bacterial cells at the bottom of the tube. We removed the supernatant. We add 10 mL of IPTG to the tube, shaked it to mix, and washed the cells. We centrifuged again at 8000 rpm for 10 minutes. We discarded the supernatant, and get the precipitate which was the recombinant bacterial cells.

2.2.3.2 Formulation of imidazole in different concentrations

We prepare different concentrations of imidazole as shown in Table 2. To make the imidazole solution, two different buffers were needed: Buffer 1 (Elution Buffer, 500 mL) and Buffer 2 (Tris-Cl, 400 mL).

Table 2 Imidazole Concentration

Imidazole Concentration	Buffer 1 volume	Buffer 2 volume
10mM	2mL	48mL
20mM	2mL	23mL
40mM	4mL	21mL
60mM	6mL	19mL
80mM	8mL	17mL
100mM	10mL	15mL
120mM	12mL	13mL
200mM	20mL	5mL

2.2.4 Purification of Recombinant Protein

2.2.4.1 Purification and Analysis of Recombinant Proteins

We added 10 mL of buffer solution to the centrifuge tube with the precipitated bacterial cells and shook it to mix the cells with the buffer. We put the tube in an ice bath to keep the temperature low during ultrasonication. We placed the tube in the ultrasonic machine and sonicated it for 10 minutes, pulsing 5 seconds on and 5 seconds off to break the cells. We centrifuged the tube at 10000 rpm for 15 minutes to separate the cell debris. We collected the supernatant and filtered it through a 0.45 μm

filter.

We poured the buffer solution into the column and collected the filtrate in a centrifuge tube. We added 6 mL of 20 mM imidazole to the column and filtered it for later use. We poured the supernatant into the column and collected the filtrate that passed through the column. We repeated this step to ensure proper binding. Then we added 1 mL of the prepared imidazole solutions of different concentrations to the column one by one and collected the eluates in separate centrifuge tubes. We repeated this step for all imidazole concentrations.

We prepared 10 centrifuge tubes, including the supernatant filtrate, washed liquids with different imidazole concentrations, and the initial supernatant filtrate, 10 mM wash liquid, and the precipitate of the sonicated cells. We added 5 μL of lysozyme and 25 μL of the wash liquid, filtrate, or 10 mM wash liquid to each tube. We took a little bit of the cell precipitate, put it into a centrifuge tube, and shook it to mix. We added 10 μL of lysozyme and 40 μL of water to the tube with the precipitate to dissolve it completely.

2.2.4.2 Protein electrophoresis

We boiled the water and heated all the samples from the centrifuge tubes, then cooled them in ice. We prepared the protein electrophoresis gel, placed it in the electrophoresis tank, and added electrophoresis buffer. We loaded the marker into the first lane and the samples into the remaining lanes following the increasing concentration. We turned on the electrophoresis and set the voltage to 80 V. After approximately 1 hour, we took out the gel and stained it to see the protein bands. We put the gel into the gel imaging system to capture the image.

2.2.4.3 SDS-PAGE Electrophoresis of Purified Protein

We heated the purified protein samples for 5 minutes to denature the proteins and then cooled them down. To prepare the protein electrophoresis gel, we put the marker into the first lane and added the purified protein samples into the remaining lanes in increasing order. We placed the gel into the electrophoresis apparatus, added the appropriate amount of electrophoresis buffer, turned on the electrophoresis, and set the voltage to 80 V. After about 1 hour, we took out the gel, stained it for about 10 minutes, and analyzed the results.

2.2.4.4 Coating of ELISA Plate with Antigen

We diluted the Toxoplasma gondii antigen samples with coating buffer to the working concentration and coated them onto the microplate wells, adding 100 μL to each of the 19 wells (since we only had 19 samples). We placed the coated antigen samples in the refrigerator at 4 ℃ overnight.

2.2.4.5 Blocking, Primary Antibody Binding, Washing, and Secondary Antibody Binding

We took out the coated antigen samples, discarded the liquid, and washed them three times with PBST, adding about 200 μL each time and washing for about 5 minutes. We made sure to remove all the liquid after the last wash. We added 100 μL of blocking buffer to each well, then added 2 μL of serum samples (primary antibody) in order, and incubated them at 37 ℃ for 30 minutes. We discarded the liquid from the plate, washed it five times with PBST again, and repeated the previous steps. We added the blocking buffer again and added HRP-labeled secondary antibody, incubating at 37 ℃ for 1 hour. We discarded the liquid from the plate and washed it five times again. We added 100 μL of chromogenic substrate to each well and covered it to avoid light for 15 minutes. Finally, we added 50 μL of stop solution to each well.

2.2.4.6 Substrate Development

We discarded the liquid from the plate and washed it five times again. We added 100 μL of chromogenic substrate to each well and covered it to avoid light for 15 minutes. We added 50 μL of stop solution to each well.

2.2.4.7 Data Analysis and Processing

We used an OD450 to read the results and analyzed them.

2.2.5 Preparation and Principle of Reagent

We prepared reagents in the laboratory, which required advance understanding of the reagents or other substances. The preparation of the solution needed an understanding of their properties, including physical and chemical properties. We added the corresponding volume of solvent according to the required concentration, stirred fully until dissolved, labeled it with their names and date, and stored it in the relevant environment.

3. Experimental Results and Analysis

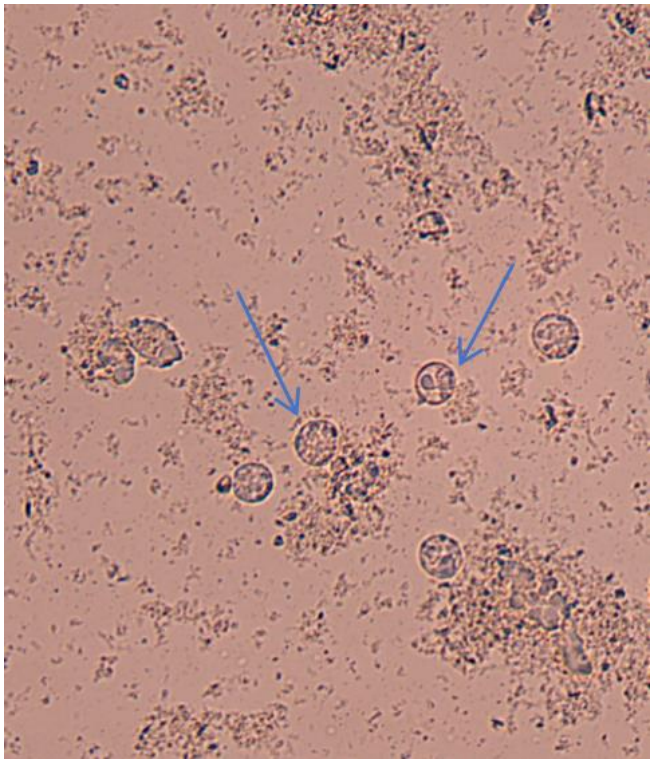

Figure 1: *T. gondii* Oocysts Under Microscope.

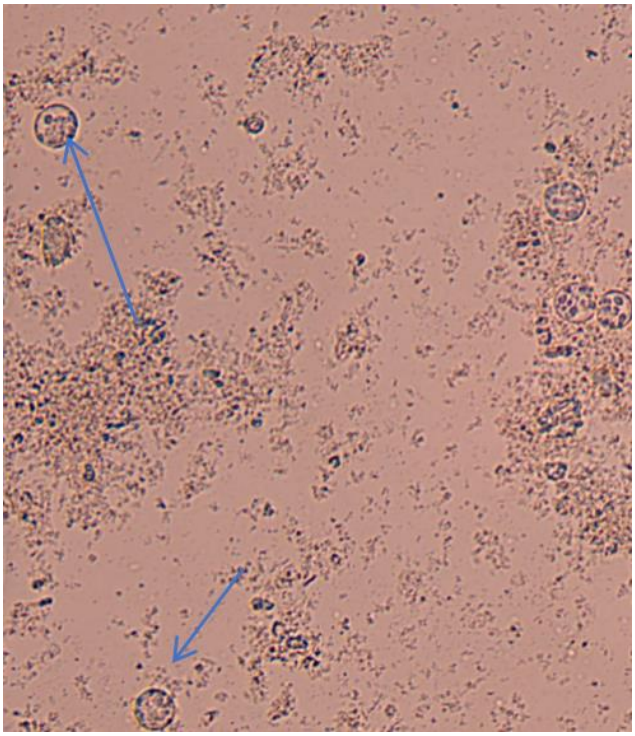

Figure 2: *T. gondii* Oocysts Under Microscope

The final hosts of Toxoplasma gondii, which is cats, often excrete feces containing *T. gondii* oocysts. These figures show the oocysts under a microscope. The oocysts of Toxoplasma gondii are oval to spherical, with a diameter of about 8-12 micrometers, and their oocyst wall is very dense. [2] In Figure 1 and Figure 2, the round cells are the oocysts of *T. gondii*. Since there are many impurities in the feces, some irregular cells can be seen.

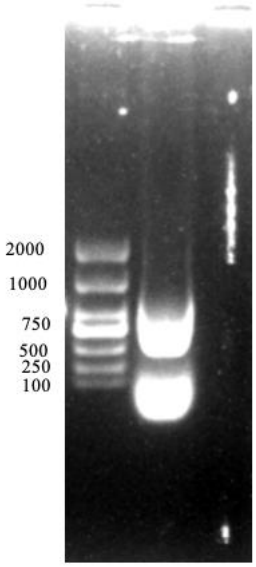

Figure 3: Electrophoresis Image of Recombinant Bacteria Target DNA

Electrophoresis can analyze DNA fragments in order to determine the size and concentration of molecular weight. Figure 3 shows the imaging under electrophoresis after PCR reaction of

recombinant bacteria. Due to the small sample size, only lane 1 was imaged. It shows two strips, the upper band is about 500, the lower band is about 100, indicating that there is an abnormal result, possibly caused by the primer is larger than the standard amount or improper operation. Normally, only one strip demonstrates the presence of specific amplification.

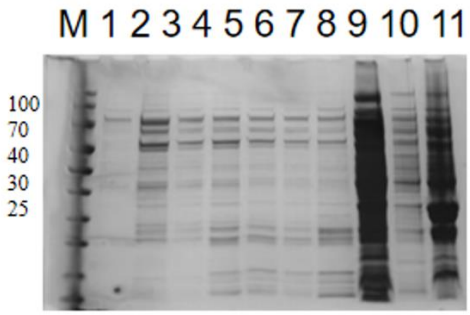

Figure 4: Protein Electrophoresis of Recombinant Bacteria

Table 3 Different imidazole correspond with different lane

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9	Lane 10	Lane 11
marker	20 mM Imidazole	40 mM Imidazole	60 mM Imidazole	80 mM Imidazole	100 mM Imidazole	120 mM Imidazole	200 mM Imidazole	filtrate	10 mM Imidazole	percipitate

According to Figure 4 and Table 3, except for lanes 9 and 11, the other lanes all show relatively uniform band images. The bands between 70-100 are relatively obvious, but they are different from the normal state. This might because the protein binding was not accurate. The samples of the filtrate and precipitate show darker colors because of higher protein concentration or some non-specifically bound proteins.

Table 4: OD450 Detection Results of Toxoplasma gondii Antigen

	1	2	3	4	5	6	7	8
A	0	1.243	1.077	1.21	0	0	0	0
B	0	0.983	1.109	1.14	0	0	0	0
C	0	0.822	1.29	1.087	0	0	0	0
D	0	1.381	1.223	0.741	0	0	0	0
E	0	1.302	1.345	1.117	0	0	0	0
F	0	0.984	1.091	1.214	0	0	0	0
G	0	0.722	1.031	0.042	0	0	0	0
H	0	1.358	0.663	0.045	0	0	0	0

Table 4 shows the OD values of the *T. gondii* antigen samples at 450 nm. The results indicate elevated OD values, even in wells without samples, suggesting a high incidence of false positives. This means that many samples are false positives, which doesn't match the real situation. The reason might be improper operation during the experiment, leading to sample contamination and inaccurate data, causing a large number of false-positive samples.

4. Conclusion and Future Work

This study conducted a serological examination of *T. gondii*, encompassing the revival and expression of recombinant bacteria, verification of recombinant plasmids, induction and expression of recombinant proteins, also the purification of recombinant proteins. Through this experiment, not only the basic techniques of molecular biology and protein engineering are mastered but also a deeper understanding of the serological detection of *T. gondii* has been gained. In the stage of expressing and

reviving recombinant bacteria, we used of genetic cloning technology to insert the specific antigen gene of Toxoplasma gondii into the expression vector and transformed it into Ecoli. After revival culture, the successfully obtained bacterial strains containing recombinant plasmids. The key to this step is to ensure the correct insertion of the gene fragment and optimize the transformation efficiency. This confirmed the successful construction of the recombinant plasmid through colony PCR. In the stage of inducing and expressing recombinant proteins, the IPTG to induce the expression of recombinant proteins is used. Through SDS-PAGE electrophoresis analysis, the results showed the target protein bands, indicating the expression of recombinant proteins even though the data is not that successful. In this process, the optimization of induction time and IPTG concentration has a significant impact on protein expression. It determined the best induction conditions through consulting literature and the experience of professors to ensure the efficient expression of recombinant proteins. In the stage of purifying recombinant proteins, we use affinity chromatography to purify the recombinant proteins. Through the ELISA method, it removed impurities and obtained the recombinant proteins. The purified proteins have immunoreactivity and can be used for subsequent serological detection experiments.

Through this experiment, the molecular biology experiments are rigor and complex. Every steps need a precise control, and even the slightest error can affect the final result. At the same time, teamwork is also important, especially in experimental design and data analysis. The guidance of professors and the cooperation of team members played a key role in the smooth progress of the experiment. This experiment did not achieve perfect results, and there are still many areas for improvement. In the process of expressing and purifying recombinant proteins, other expression systems (such as yeast or mammalian cells) can be used to improve the activity or modification degree of the proteins. In addition, exploring the immunogenicity of other antigen proteins of Toxoplasma gondii can help to provide a theoretical basis for the development of multivalent vaccines.

In conclusion, this experiment has laid an experimental foundation and sparked the strong interest in biology. In the future, the development of pathogenic mechanisms of Toxoplasma gondii and its detection methods and contribute to the prevention and control of related diseases can be improved.

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