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ABSTRACT

Identification of Toxoplasma Gondii Epitopes Using Phage Display Libraries for Serodiagnostic Purposes

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Background: Toxoplasmosis is a parasitic disease caused by Toxoplasma gondii that can infect humans and animals. Currently, the diagnosis of toxoplasmosis can be performed through various methods, including serological and molecular methods. However, while molecular methods are highly sensitive and specific, they require highly specialized laboratory equipment and high BSL (biosafety levels), which may not be readily available in many settings. Moreover, molecular methods require the use of genetic material, which can pose a risk of infection for laboratory personnel. On the other hand, serological methods are more accessible and affordable, and they can detect the presence of antibodies against T. gondii, which indicates a past or current infection. Additionally, serological methods can identify specific T. gondii antigens or epitopes, which can be used to develop diagnostic tools without the need for live parasites. Thus, there is a need to explore the potential of serological methods for the diagnosis of toxoplasmosis and their potential applications in the development of safer and easier diagnostic tools. The identification of specific epitopes using phage display libraries can improve the specificity and sensitivity of diagnostic assays for T. gondii infection in cats and humans.

Objectives: the research aims to identify specific epitopes that can be used in the diagnosis of Toxoplasma gondii infection using M13 phage display libraries, and then to analyze the sequences of the isolated phages using next generation DNA sequencing and bioinformatics tools.

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Methods: The method of using phage display libraries to identify specific epitopes for the diagnosis of Toxoplasma gondii in cat serum involves several major steps. First, a phage display library is prepared by inserting DNA sequences encoding random peptides into bacteriophages, which are then displayed on the surface of the phage particles. The phage library is then screened with cat serum containing cat's antibodies against T. gondii; to identify phages that display peptides that bind in high specificity to the target antibodies. The selected phages are then amplified and purified, and the DNA sequence of the displayed peptide is determined using next-generation sequencing. Finally, the identified epitopes are analyzed using bioinformatics tools to identify their sequences and potential applications in diagnostic assays. This approach has the potential to improve the sensitivity and specificity of current diagnostic methods for T. gondii infection.

1. Construction of pIII-Displayed Peptide Libraries using M13KE Phage:

The first step is the preparation of phages and screening. In this step, a phage display library is constructed by genetically fusing the gene encoding the peptide (an epitope) to the gene encoding the coat protein of a bacteriophage. The New England Bio lab M13 Phage Display Library 12 amino acids kit will be used for the display and screening of small peptides. The procedure involves the construction of a library of phages that display a 12 amino acid peptide sequence. This will be achieved by cloning the peptide-encoding DNA sequences into the M13 bacteriophage genome. The library can then be applied to a solid surface, such as a nitrocellulose membrane, to create a phage display library.

2. Selection of Highly Specific Phages by Bio-Panning Using T. gondii-Positive Cat Serum:

The phage display library in this step subjected to multiple rounds of the screening process, a process that involves incubating the library with the target antibodies (IgG and IgM in the pooled cats' serum that was taken from pre-exposed cats for Toxoplasma

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gondii) and selecting for phages that bind specifically to the antibodies. This process is repeated several times to enrich for phages that bind strongly to the antibodies.

3. Isolation of the reactant clones and amplification through ER2738 culture:

In this step, the phages that bind specifically to the antibodies will be isolated (eluted). These clones with the highest binding affinity to the antibodies will be selected and amplified in large quantities for further analysis. The amplification process occurs by the insertion of the eluted phages into the E. coli ER2738 strain culture, in which the phages will be amplified.

4. DNA Extraction from the Amplified Clones for NGS Sequencing and Bioinformatics Analysis:

The final step is NGS and bioinformatics analysis of the isolated and amplified epitopes. In this step, the amino acid sequences of the selected clones are analyzed using next generation sequencing (NGS) and bioinformatics tools to identify the specific epitopes that are possible to use as diagnostic tools for Toxoplasma gondii infection. These epitopes can then be synthesized and used as diagnostic markers in laboratories.

DNA will be extracted from the phages that bind in high specificity to the target antibodies in the serum to explore the sequence of the peptides displayed on the surface of these phages using Next-Generation Sequencing (NGS). Following rounds of bio panning to selectively enrich phages displaying peptides with desired binding properties, the amplified phage population is subjected to DNA isolation. This process involves breaking down the phage particles and extracting their genetic material. Subsequently, the isolated DNA serves as the foundation for library preparation tailored for NGS. The DNA undergoes meticulous processing, including fragmentation, end repair, adapter ligation, and PCR amplification, culminating in the creation of a high-quality DNA library ready for sequencing. Leveraging advanced sequencing platforms, such as Illumina, NGS is executed to generate vast amounts of short DNA sequences representing the diverse repertoire of displayed peptides.

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Results: The expected results is to find displayed epitopes bind in high specificity with the IgG in the serum, and To get the sequence of the isolated epitope\s using NGS and analyze the results using bioinformatics tools.

Conclusion: This project employs the M13 phage random phage display library to systematically identify new epitopes associated with Toxoplasma gondii, which can be used in the development of diagnostic tools. By inserting exogenous DNA fragments into the M13 phage genome, a diverse peptide will be expressed on the phage surface. Then, the generated peptides will be subsequently exposed to positive Toxoplasma gondii cat serum containing IgG and IgM antibodies, allowing for the selective capture of immunoreactive peptides potentially representing specific T. gondii epitopes. This can help in understanding specific interactions between the phage-displayed epitopes and antibodies present in the serum in addition to identifying robust candidates for diagnostic applications. Accordingly, this strategic use of the phage display technology, besides immune serum, not only enhances our understanding of Toxoplasma gondii epitopes but also lays the foundation for the development of an effective diagnostic tool for the timely detection of this parasitic infection.

Key words: Phages, Phage display libraries, Epitopes, IgG and IgM antibodies, Toxoplasma gondii, Toxoplasmosis infection

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