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Notice for the PhD Viva Voce Examination

Ms Kaviya P K (Registration Number: 1940088), PhD scholar at the School of Sciences, CHRIST (Deemed to be University), Bangalore will defend her PhD thesis at the public viva-voce examination on Tuesday, 20 February 2024 at 10.30 am in Room No. 044, Ground Floor, R & D Block, CHRIST (Deemed to be University), Bengaluru - 560029.

Title of the Thesis	:	**Cloning and Characterization of an Exported Protein Present in the RD7 Region of Clinical Isolates of *Mycobacterium Tuberculosis***
Discipline	:	**Biotechnology**
External Examiner (Outside Karnataka)	:	**Dr Vijaya Anand** Professor Department of Human Genetics and Molecular Biology Bharathiyar University Coimbatore Tamil Nadu – 641046
External Examiner (Within Karnataka)	:	**Dr Manjula K R** Associate Professor Department of Biotechnology Rukmini Knowledge Park Yelahanka Kattigenahalli, Bengaluru Karnataka
Supervisor	:	**Dr Suma S** Associate Professor Department of Life Sciences School of Sciences CHRIST (Deemed to be University) Bengaluru Karnataka-560029

The members of the Research Advisory Committee of the Scholar, the faculty members of the Department and the School, interested experts and research scholars of all the branches of research are cordially invited to attend this open viva-voce examination.

Registrar

Place: Bengaluru

Date: 15 February 2024

ABSTRACT

The bacterium *Mycobacterium tuberculosis* is responsible for causing the disease tuberculosis in mammals, which is regarded as one of the oldest diseases haunting the human race. The only available tuberculosis vaccine Bacillus Calmette-Guerine (BCG), is effective against childhood tuberculosis but is regarded as having low efficacy in conferring protection in the case of tuberculosis in adults. A comparison of the *M. tuberculosis* H37Rv strain and clinical isolates from Kerala had earlier revealed that the clinical strains have a distinctive 4.5 kb genomic sequence that is lacking from the H37Rv strain in the RD7 region. The RD7 is a distinctive genomic region that is absent in *M. tuberculosis H37Rv* and *Mycobacterium bovis* BCG strain. The 4.5 kb genomic sequence is projected to include 6 potential ORFs by NCBI ORF prediction tool, one of which Novel Hypothetical Protein (NHP2) is anticipated to encode an exported protein with a length of 268 amino acids. Studies demonstrate that *Mycobacterium tuberculosis* secretory proteins such as the Ag85 complex, the ESAT-6 family protein, and the PE-PPE family proteins were effective vaccine candidates because they trigger T cells. Here, we present an in-depth analysis of the exported protein, which is 268 amino acids long. The putative exported protein with a gene 807 bp long was PCR amplified and cloned in the expression vector pET-32a for expression. The protein was over expressed using Isopropyl ß-D-1-thiogalactopyranoside (IPTG) and was isolated and purified using column chromatography. Bioinformatics studies were conducted to study the characteristics of the expressed protein.

A novel putative mycobacterial protein discovered by subtractive hybridization was studied for its potential as a vaccine candidate using cutting-edge computer technologies. Novel Hypothetical Protein 2 (NHP2), which is found in the RD7 area of *Mycobacterium tuberculosis* clinical strains, was examined for its physical, chemical, immunological, and structural characteristics using a variety of computational methods. The NHP2 protein was shown to be functionally active and to have a potential antibiotic binding domain by Pfam and Gene Ontology analyses. Different computational methods used to evaluate the protein's toxicity, allergenicity, and antigenicity revealed that the protein was antigenic in nature. The T and B cell determinants of the protein were investigated using Immune Epitope Database (IEDB) technologies. Bioinformatics tools were used to create the protein's secondary and tertiary structures. To comprehend the protein's potential to be a candidate for a tuberculosis vaccine, it was further tested for its capacity to bind and activate the T-cell and B-cell epitopes. To determine the effectiveness of the protein as a vaccine candidate, molecular docking and molecular dynamic investigations of the protein with the human TLR3 receptor were conducted. The study contributed to our understanding of the NHP2 protein and its potential as a multiepitope or subunit vaccination candidate.

Keywords: *Mycobacterium tuberculosis*, Polymerase Chain Reaction, Cloning, Column Chromatography, SDS PAGE, NCBI BLAST, Molecular Docking, MD Simulation, Vaccine candidate.

Publications:

1. **Kaviya Parambath Kootery,** Suma Sarojini., *In silico* analysis of potential promoters in the N4.5 genomic locus of *Mycobacterium tuberculosis.* **Submitted to *Biointerface Research in Applied Chemistry***
2. **Kaviya Parambath Kootery,** Suma Sarojini., Cloning and expression of a novel 28 kDa protein encoded by the NHP2 locus in the RD7 region of clinical strains of *Mycobacterium tuberculosis* from India. **Submitted to *Journal of microbiology, biotechnology and food sciences.* (Impact factor: 0.9)**
3. **Kaviya Parambath Kootery,** Suma Sarojini., *In silico* analysis of NHP2 membrane protein, a novel vaccine candidate present in the RD7 region of *Mycobacterium tuberculosis.* **Accepted by *Biologia.* (Impact factor: 1.653)**
4. **Kaviya Parambath Kootery,** Suma Sarojini., Structural and functional characterization of a hypothetical protein in the RD7 region in clinical isolates of *Mycobacterium tuberculosis*-an i*n silico* approach to candidate vaccines", ***Journal of Genetic Engeneering and Biotechnology***, 20,55. 2022
5. DOI: 10.1186/s43141-022-00340-5 **(Impact factor: 3.818)**