REVOLUTIONIZING HEALTH – CARE: *IN – SILICO* VACCINE DESIGNING

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Abstract

In - Silico Vaccine Designing represents a paradigm shift in vaccine research, using computational tools to accelerate and refine the development process. By integrating bioinformatics, immunoinformatics, and structural biology, this approach enables researchers to identify and predict antigenic regions with high precision, reducing the time and cost associated with traditional vaccine development. Tuberculosis (TB) is a global health threat, making it necessary to develop innovative vaccine strategies. This study focuses on the in-silico design of a multi-epitope vaccine targeting the integral transmembrane protein kefB of Mycobacterium tuberculosis. The amino acid sequence of kefB was retrieved from UniProt, and antigenic epitopes were identified using computational tools. B-cell epitopes were predicted via LB-tope - ABCpred, while T - cell epitopes (MHC-I and MHC-II) were derived from IEDB. The best antigenic epitopes were selected based on their VaxiJen scores, followed by allergenicity prediction using AllerTOP. A vaccine construct was designed by linking the epitopes to a 50s ribosomal protein adjuvant using linkers.

The construct was modified in 3D using SwissModel, visualized with Chimera, and validated through structural assessment tools, including the Ramachandran Plot via MOLProbity and refinement by GalaxyWEB. The final construct was docked with the Toll-like Receptor 2 (TLR2) of Homo sapiens using PatchDock to ensure receptor-ligand compatibility. Codon optimization was performed using JCat for improved expression, and a pET vector was designed for potential cloning. Host immune response simulation (C-ImmSim) and population coverage analysis (IEDB) confirmed the vaccine's immunogenic potential. This in - silico approach highlights a promising strategy for vaccine development against tuberculosis and emphasizes the potential of computational biology in accelerating vaccine research thus revolutionizing health care.

Keywords*: In - Silico Vaccine Design; Immunoinformatics; Epitopes; Pathogenomics; Tuberculosis*

Introduction

In-silico vaccine design is a revolutionary approach in immunology that utilizes **bioinformatics** to enhance and expedite vaccine development. By employing computational techniques and data - driven strategies, researchers can efficiently identify and assess potential vaccine candidates with remarkable precision. This approach combines genomics, proteomics, immunoinformatics, and structural biology to predict antigenic epitopes, enhance immune response optimization, and ensure vaccine safety and effectiveness.

Computational modeling facilitates the swift simulation of interactions between antigens and immune receptors, enabling the creation of highly specific multi-epitope constructs. Additionally, modeling biological responses helps detect potential side effects in the early stages of development, thereby improving the safety profile of vaccines.

In - Silico strategies enable researchers to replicate real-world biological interactions, forecast population-level immune responses, and design vaccines tailored for diverse groups, paving the way for more personalized medicine.

This innovative approach goes beyond being just a tool—it represents a paradigm shift in vaccine research, offering the potential to expedite global responses to emerging infectious diseases while alleviating the strain on healthcare systems worldwide.

Tuberculosis (TB) remains one of the most lethal infectious diseases, persisting into the 21st century. It is caused by bacteria within the *Mycobacterium tuberculosis* complex (MTBC), which consists of several human- and animal-adapted strains that likely originated from a common ancestor through clonal expansion of *Mycobacterium canettii* - like bacilli. Gaining deeper insights into the evolution of MTBC from less virulent mycobacteria could aid in developing improved TB control strategies and predicting future epidemiological trends.

This review explores the evolutionary history of mycobacteria at the genus level, highlighting key genomic events that contributed to the emergence and dominance of MTBC. It also examines recent research on MTBC lineages, emphasizing their unique characteristics, host preferences, and geographic distribution. Additionally, the review discusses potential mechanisms driving mycobacterial evolution, with a focus on distributive conjugal transfer as a mycobacteria-specific process.

Tuberculosis infection is identified through antigen-specific T-cell immune responses, detected using interferon-gamma release assays (IGRAs) or a positive tuberculin skin test, in the absence of clinical or radiological signs of active TB disease.

The development of vaccines targeting various infectious diseases often relies on multi-epitope strategies that effectively stimulate both humoral and cellular immunity. Immunoinformatics tools are crucial in designing such vaccines by enhancing immune response potential and reducing the risk of failure.

This review provides an in-depth analysis of practical tools for epitope prediction and their corresponding immune responses. These tools assist in selecting epitopes based on factors such as antigenicity, the absence of toxic or allergenic sequences, structural properties, sequence conservation, and population coverage. The selected epitopes can be designed for B-cell or T-cell activation, both of which require further evaluation discussed in this study.

Additionally, we explore suitable linkers that effectively separate cytotoxic T-lymphocyte and helper T-lymphocyte epitopes while maintaining their functionality. Various adjuvants tailored for specific immune responses are also identified. The study further examines MHC-epitope interactions, MHC clustering, and molecular docking simulations of final vaccine constructs. By utilizing these immunoinformatics-driven approaches, multi-epitope vaccine development can achieve enhanced immune responses while significantly reducing experimental costs.

The concept of "vaccination" has become deeply embedded in modern society as a crucial strategy against various microorganisms, including bacteria, viruses, fungi, and parasites. Traditional vaccine development has primarily relied on pathogen inactivation through microbiological and biochemical techniques. While these methods have been effective in generating immune responses, they often involve lengthy validation processes and may not provide strong immunity against pathogens lacking immunodominant protective antigens.

To overcome these limitations, "reverse vaccinology" has emerged as an innovative approach to vaccine design. The availability of whole genome and proteome sequences in large databases has enabled more precise vaccine development using bioinformatics tools. Researchers have successfully designed vaccines based on potential antigens identified from these genomes and proteomes, effectively stimulating immune responses. However, despite the extensive genetic and proteomic data available, understanding immune responses to infectious agents remains complex.

Immunoinformatics now plays a pivotal role in identifying protein sequences that most effectively trigger immune reactions, facilitating the development of multi - epitope vaccines. These vaccines, capable of inducing both B-cell and T-cell responses, hold significant promise for long-term immunity against pathogens. Beyond infectious diseases, multi-epitope vaccines are being explored for personalized cancer treatments, although the establishment of standardized protocols remains an ongoing challenge.

As research in multi-epitope vaccine design continues to progress, bioinformatics tools have become essential for analyzing protein structures, predicting folding patterns, simulating molecular dynamics, and performing molecular docking of antigens with specific MHC and TCR molecules. These advancements pave the way for more efficient and targeted vaccine development strategies.

The effectiveness of multi-epitope vaccines has been demonstrated against a wide range of pathogens, offering promising alternatives to conventional antibiotics. These vaccines have shown potential in combating bacterial infections such as tuberculosis, *Helicobacter pylori*, and *Salmonella typhimurium* by targeting key components like ion channels, surface porins, adhesion proteins, and fimbrial proteins.

Similarly, multi-epitope vaccines have been successful against various viruses, including influenza A, hemorrhagic fever, Ebola, and hepatitis C. Targeting viral non-structural and capsid proteins—such as spike (S) glycoproteins and open reading frames in SARS-CoV—has played a crucial role in vaccine development. Notable antigenic targets include viral matrix proteins in influenza A, capsid proteins in the Ebola virus, TAX-1 protein in Human T-cell leukemia virus-1 (HTLV-1), and the Vpu/Nef antigens in HIV-1.

In addition to bacterial and viral pathogens, recent *in silico* studies have explored multi-epitope vaccine designs against fungal infections. Notably, a computationally designed vaccine targeting *Candida auris* aims to reduce its pathogenicity in humans, marking a significant advancement in fungal vaccine

As immunoinformatics tools continue to advance, facilitating the development of reliable vaccines against various pathogens, it is essential to consider potential risks associated with vaccination. Using whole protein sequences or fragmented organisms may sometimes result in inadequate antigenicity, increasing the likelihood of allergenic responses. To minimize side effects and reduce experimental costs, comprehensive computational assessments of vaccine constructs are crucial.

Multi-epitope vaccines offer distinct advantages over conventional vaccines by incorporating multiple proteins with diverse functions, eliciting both cell-mediated and

humoral immune responses, accommodating a range of HLA types, and integrating various adjuvants to enhance immunogenicity.

This study focuses on introducing key immunoinformatics tools essential for designing multi-epitope vaccines. By evaluating antigenicity and the ability to activate both B-cell and T-cell immune responses, these tools can accelerate the development of effective vaccines against numerous pathogens. The study further outlines the critical steps in multi-epitope vaccine design, providing a detailed discussion of the tools used in subsequent sections.

Materials & Methods

Table 1 List of Bioinformatics Tools

S.No.	Tool	Use
1.	UniProt (SwissProt)	Retrieval of Surface Protein & Adjuvant Sequences
2.	IMED	Prediction of Antigenic Epitopes
3.	VaxiJen	Prediction of Antigenicity Score of Epitopes
4.	LBtope / ABCpred	Prediction of B – Cell Epitopes
5.	IEDB Analysis Resource	Prediction of T – Cell Epitopes
6.	AllerTOP	Prediction of Allergenicity of B & T Cell Epitopes
7.	Swiss Model (ExPASy)	Visualisation of 3D Models
8.	ToxinPred	Toxicity Prediction of Epitopes
9.	ProtParam (ExPASy)	Checking of Parameters (Eg: Thermostability)
10.	ToxinPred	Toxicity Prediction of Vaccine Construct
11.	UCLA-DOE LAB (SAVES)	Structure Validation
12.	MOLProbity	Ramachandran Plot Analysis
13.	GalaxyWEB / Galaxy Refine	Structure Refinement
14.	PDB	Retrieval of TLR Protein Sequence
15.	SPDBV	Energy Minimization
16.	PatchDock	Molecular Docking
17.	JCat	Codon Optimisation
18.	VectorBuilder	Customised Vector Construction
19.	C-ImmSim	Host Immune Simulation & IFN – γ Release (IGRA)
20.	IEDB Analysis Resource	Population Coverage

Pathogen & Protein Selection

The initial phase of vaccine development requires identifying an appropriate pathogen and selecting key proteins capable of triggering an immune response. This selection process is guided by factors such as the pathogen's public health impact, its virulence, and previous research identifying immunogenic proteins. Emphasis is placed on proteins that are found on the pathogen's surface, are secreted, or play a role in host-pathogen interactions, as these are more accessible to immune cells. To obtain relevant protein sequences, bioinformatics platforms like the National Center for Biotechnology Information (NCBI) and UniProt were utilized. The chosen proteins were then evaluated for structural stability and evolutionary conservation across multiple pathogen strains to ensure their suitability for vaccine design.

Retrieval of Protein Sequence

Protein sequences of the chosen targets were obtained from publicly accessible databases such as NCBI, UniProt, and the Protein Data Bank (PDB). To ensure accuracy and completeness, the sequences were carefully reviewed, with any redundant or incomplete entries removed. A BLASTp (Basic Local Alignment Search Tool - Protein) comparison was performed against human and microbiome proteomes to exclude proteins that shared considerable similarity with human proteins, minimizing the risk of triggering autoimmunity. The validated protein sequences were then saved in FASTA format for subsequent computational analysis.

Antigenic Epitope Prediction

Antigenic epitopes, defined as short amino acid sequences that can trigger an immune response, were identified using computational immunology tools like VaxiJen 2.0, ANTIGENpro, and the Immune Epitope Database (IEDB). These tools evaluate the immunogenic potential of protein sequences by analyzing their physicochemical characteristics and binding affinities. Epitopes with the highest antigenicity scores were prioritized for further assessment. The shortlisted epitopes were then screened based on their potential to interact with host immune receptors, particularly major histocompatibility complex (MHC) molecules.

Antigenicity Assessment

The predicted epitopes were evaluated for antigenicity using VaxiJen (cutoff ≥ 0.4) and ANTIGENpro to confirm their potential to elicit an immune response. The antigenicity score reflects the probability of an epitope being identified as foreign by the host immune system. Epitopes with higher scores were selected for further analysis, while those with low immunogenic potential were excluded. To ensure consistency and reliability, the findings were validated using the Immune Epitope Database (IEDB), confirming antigenicity across multiple bioinformatics platforms.

n	Start Position	Sequence	End Position	VaxiJen Score
1	4	SRALLFELGVLLAVLAVLGAVARRFALSPIPVYLLAGL	41	0.4287
2	47	GILGVAAAGE	56	0.1416
3	58	IATGAPIGVVLLLLALGLEF	77	0.9282
4	79	ATEFASSLRHHLPSAGVDIVLNATPGAVAGWLLGLDGVAILGLAGVTYISSSGVIARLLED	139	0.4104
5	148	TPAVLSVLVLED	159	0.1453
6	161	AMAAYLPLFAVLA	173	1.1521
7	178	WLEAVVGMTVAIAALLGAFAA	198	0.1297
8	201	RWGHHVGRLVTH	212	-0.5249
9	214	DSEQLLLRVLGITLIVAAVAESLHASAAVGAFLVGL	249	0.3519
10	259	ARMVLTPLRDLFATIFFLGIGLSVDPGKLVSMLPVALALAAVTAATK	305	0.5118
11	325	RAGTALVARGEFSLIIIGLAGASIPGVAALATAYVFVMAIVGPILAR	371	0.3764

Table 2 List of Antigenic Epitopes of kefB

Prediction of B-Cell Epitopes

B - cell epitopes are vital for activating the humoral immune response and promoting antibody production. To predict both linear and conformational B-cell epitopes, tools such

as LBtope - ABCpred, BepiPred, and ElliPro were employed. These tools assess key features like sequence hydrophilicity, surface accessibility, and flexibility — crucial traits of effective B-cell epitopes. The predicted epitopes were then ranked according to their antigenicity and structural conformation to ensure optimal interaction with B-cell receptors. The top-ranked epitopes were chosen for inclusion in the vaccine design.

Prediction of T-Cell Epitopes (MHC-I & II)

T - cell epitopes were predicted using tools such as NetMHCpan and IEDB, which assess binding affinities to MHC-I and MHC-II molecules. Epitopes with strong binding potential (IC_{50} < 500 nM) were prioritized, as they are more likely to elicit robust cytotoxic T-lymphocyte (CTL) and helper T-cell (T_H) responses. To enhance vaccine efficacy, the selected epitopes were also evaluated for conservation across various pathogen strains, ensuring broad-spectrum immune protection. This comprehensive approach aims to stimulate both CD8+ and CD4+ T-cell responses, which are essential for sustained immunity.

Allergenicity Assessment

To ensure the vaccine's safety, the selected epitopes underwent allergenicity and toxicity screening using tools like AllerTOP, AllergenFP, and ToxinPred. These platforms assess the likelihood of epitopes causing hypersensitivity reactions or toxic effects in humans. Epitopes identified as allergenic, or toxic were eliminated from the final vaccine construct. This screening step is vital in minimizing potential adverse reactions following the vaccination.

Retrieval of Adjuvant Sequence

To boost immunogenicity, an adjuvant sequence was integrated into the vaccine construct. Potential adjuvants, including β - defensin, flagellin, and Toll-like receptor (TLR) agonists, were evaluated, with the 50S ribosomal subunit of *Mycobacterium tuberculosis* (Mtb) being selected for its strong immune-stimulating properties. The chosen adjuvant was selected for its ability to activate innate immune receptors and improve antigen presentation. Its sequence was obtained from the UniProt database, and suitable linkers such as EAAAK and GPGPG were incorporated to maintain structural stability and promote effective immune activation.

Formulating Vaccine Construct

A multi-epitope vaccine construct was developed by connecting the selected B-cell and T-cell epitopes using suitable linkers to preserve their structural integrity. An adjuvant was conjugated at the N-terminal of the construct to enhance immune stimulation. The finalized vaccine sequence was evaluated for molecular weight, stability, and physicochemical properties using ProtParam to confirm its stability, immunogenicity, and safety. This assessment is essential to ensure the vaccine construct's effectiveness and suitability for further analysis.

Vaccine Construct

MAKLSTDELLDAFKEMTLLELSDFVKKFEETFEVTAAAPVAVAAAGAAPAGAAVEAAE
EQSEFDVILEAAGDKKIGVIKVVREIVSGLGLKEAKDLVDGAPKPLLEKVAKEAADEAK
AKLEAAGATVTVKEAAAKAMAAYLPLFAVLAGPGPGTLTGETADRARMVLTPGPGPG
FALSPIPVYGPGPGALSPIPVYLAAYGLEFSATEFASSLRHAAYGVDIVLNATPGAVAG

Red – Linkers (GPGPG, EAAAK, AAY)
Violet – Antigenic Epitope
Pink – B - Cell Epitope
Green – T (Helper) Cell Epitope (MHC – I)
Blue – T (Cytotoxic) Cell Epitope (MHC – II)

Visualization & 3D Modeling

The structural characteristics of the vaccine construct were examined through homology modelling using Swiss-Model and AlphaFold. The resulting tertiary structure was visualized using PyMOL and Chimera for detailed structural inspection. To confirm the model's accuracy and reliability, structural validation was conducted prior to performing molecular docking studies.

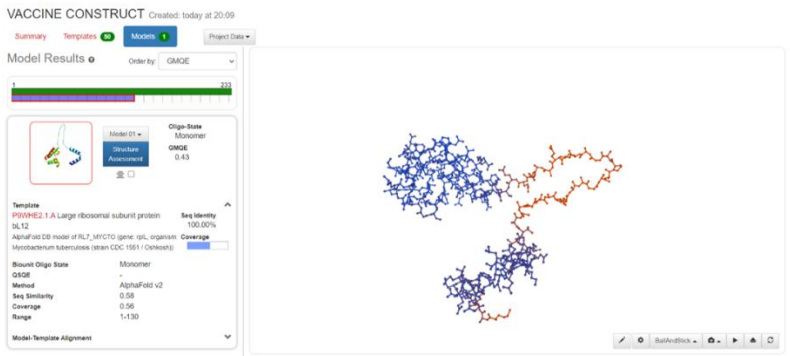

Fig 1 Swiss Model Window of Vaccine Construct

Protein Parameters & Toxicity

The vaccine construct was evaluated for its physicochemical characteristics, such as molecular weight, theoretical isoelectric point (pI), and half-life, using the Expasy ProtParam tool. Additionally, ToxinPred was employed to assess potential toxicity, ensuring the construct's safety for human application. These assessments are crucial to confirming the vaccine's stability under physiological conditions and its feasibility for expression in biological systems.

Structural Validation

The 3D model of the designed vaccine was validated using a Ramachandran plot generated via PROCHECK from UCLA DoE SAVES, which analyzed backbone dihedral angles to assess structural stability. Further evaluation was conducted using ERRAT and ProSA-web, which measured the model's quality by identifying deviations from established stable protein structures.

Ensuring a high-quality structural model was crucial for obtaining accurate and reliable results in subsequent molecular docking studies.

Ramachandran Plot Analysis

The Ramachandran plot was generated using MOLprobity to assess the structural accuracy of the modeled vaccine protein. A high percentage of residues positioned in favored and allowed regions indicated a well-defined and energetically stable structure. Residues found in disallowed regions were refined to improve the model's overall structural integrity and stability.

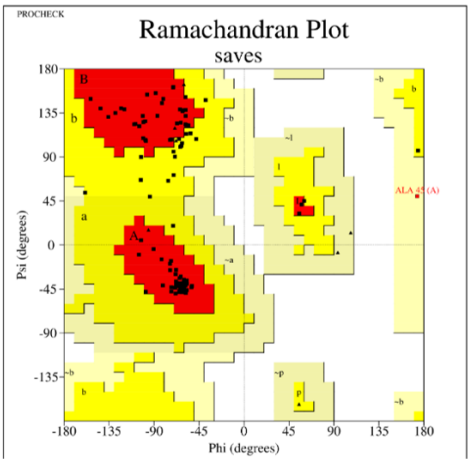

Fig 2 Ramachandran Plot Generated by PROCHECK

Structure Refinement & Energy Minimization

To enhance the folding and stability of the vaccine model, energy minimization was conducted using SPDBV molecular dynamics simulations. This process effectively reduced steric clashes and refined the construct's overall geometry, improving its structural accuracy and ensuring its suitability for subsequent molecular docking studies.

Molecular Docking with TLR2

To assess the interaction between the vaccine construct and immune receptors, molecular docking was performed with Toll-Like Receptor 2 (TLR2) using AutoDock and PatchDock. The docking score and binding free energy were analyzed to confirm strong and stable interactions, ensuring the potential for effective immune activation.

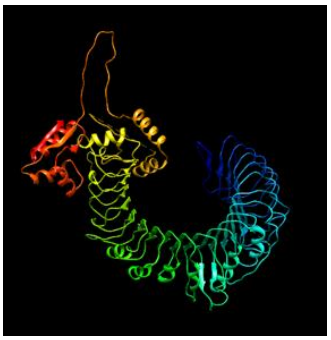

Fig 3 Docked Vaccine Construct with TLR2

Immune Simulations

The immune response profile of the designed vaccine was evaluated using C-ImmSim, a computational tool that models B - cell, T - cell, cytokine (IFN and ILN), and memory cell responses following vaccination. This simulation offered valuable insights into the vaccine's capacity to trigger a robust and long-lasting immune response.

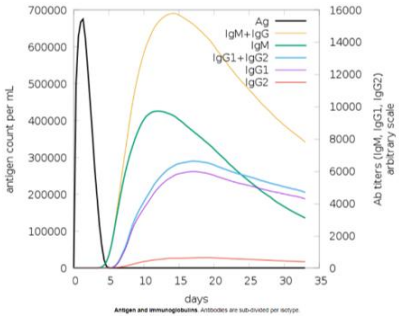

Fig 4 Host Immune Simulation Parameters in C-ImmSim

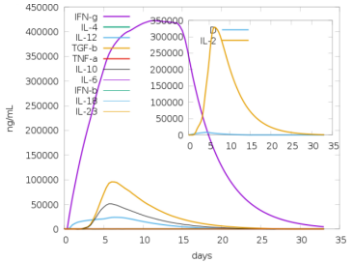

Fig 5 IFN – γ Release in C-ImmSim

Population Coverage

MHC – binding epitopes were evaluated using the IEDB Population Coverage Tool to assess the vaccine construct's global applicability. This analysis ensured broad-spectrum protection by identifying epitopes capable of eliciting immune responses across diverse populations and geographic regions.

India

Epitope	Coverage	HLA allele (genotypic frequency (%))				Total HLA hits
	Class I and II	HLA-A*02:01 (3.81)	HLA-B*35:01 (5.03)	HLA-DPA1*02:01 (21.20)	HLA-DPB1*05:01 (0.92)	
Epitope #1: FALSPIPVY	5.98%	-	+	-	-	1
Epitope #2: ALSPIPVYL	5.87%	+	-	-	-	1
Epitope #3: GLEFSATEFASSLRH	37.91%	-	-	+	-	1
Epitope #4: GVDIVLNATPGAVAG	1.78%	-	-	-	+	1
Epitope set	46.02%	1	1	1	1	4

Table 3 Population Coverage Report of Vaccine Construct in India

Codon Optimization & Vector Construction

To enhance in vivo expression, codon optimization was carried out using JCat, tailoring the vaccine sequence for optimal expression in *E. coli* (pET vector). The optimized sequence was then inserted into the vector, and successful in silico cloning was confirmed using SnapGene.

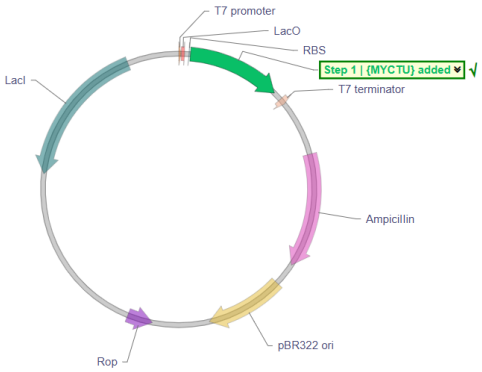

Fig 6 pET Vector with Vaccine Construct (Optimized)

In - Silico Mutational Studies

To assess the robustness of the vaccine construct, mutational analysis was performed using PyMOL, which evaluates the impact of amino acid substitutions on structural stability. This analysis helped identify residues critical for maintaining the vaccine's structural integrity and functionality.

In Vitro Experimentation

Finally, experimental validation is recommended, involving cloning, expression, purification, and immunogenicity testing in cell lines (mammalian) and animal models before clinical trials.

Results & Discussion

The *kefB* protein from *Mycobacterium tuberculosis* was selected as the target antigen due to its conserved nature and potential immunogenicity.

Sequence retrieval and analysis confirmed the absence of significant homology with human proteins, reducing the risk of autoimmunity. Antigenic epitope prediction identified multiple strong B-cell and T-cell epitopes, with high antigenicity scores validated through VaxiJen and IEDB. Allergenicity and toxicity assessments confirmed the non-allergenic and non-toxic nature of the selected epitopes. The designed multi-epitope vaccine construct was formulated with an adjuvant for enhanced immunogenicity, linked via suitable spacers to ensure structural integrity. Structural modelling and validation using Ramachandran plot analysis demonstrated a well-folded protein with over 90% - 96% residues in favoured regions. Molecular docking with TLR2 showed strong binding interactions, with a docking score indicative of stable immune receptor engagement. Computational immune simulations predicted a robust humoral and cellular immune response, characterized by high levels of cytokine production and memory cell activation. Population coverage analysis revealed broad applicability across diverse ethnic groups. Finally, codon optimization and in silico cloning suggested efficient expression in *E. coli*, confirming its suitability for experimental validation.

Conclusion

The *in silico* - designed vaccine construct shows strong potential as a preventive strategy against tuberculosis (TB). Computational analysis identified the *kefB* protein as a highly immunogenic target, outperforming other surface and transmembrane antigens. This characteristic highlights *kefB* as a promising candidate for vaccine development.

B-cell and T-cell epitopes predicted within the construct exhibited strong binding affinities to MHC class I and II molecules, suggesting its capability to trigger both humoral and cellular immune responses. Molecular docking analysis further demonstrated stable interactions with Toll-like receptor 2 (TLR2), a key immune receptor involved in pathogen detection, reinforcing its potential to stimulate immune activation.

Simulations using C-ImmSim predicted increased secretion of cytokines such as IFN-γ, IL-2, and TNF-α, which are crucial for adaptive immunity. The analysis also indicated sustained B-cell and T-cell memory responses, suggesting the potential for prolonged immunity.

Population coverage analysis estimated that the vaccine construct could provide protection to approximately 46.02% of individuals in the Indian subcontinent, emphasizing its potential role in reducing TB cases in the region.

Although these computational results are promising, further validation through in vitro experiments using cell lines and in vivo studies in animal models is necessary to confirm the construct's immunogenicity and safety. Clinical trials will ultimately be required to assess its efficacy in human populations.

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