Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2024**; 29(5): 176
https://doi.org/10.31083/j.fbl2905176

Original Research

Designing a Multiepitope Vaccine against the Foodborne Pathogenic Bacteria *Listeria monocytogenes* Using Subtractive Immunoinformatics Approaches

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Academic Editors: Teresa Semedo-Lemsaddek and Marcello Iriti
Submitted: 24 October 2023 Revised: 31 December 2023 Accepted: 26 February 2024 Published: 9 May 2024

Abstract

Background: *Listeria monocytogenes*, a Gram-positive bacterium, is a prominent foodborne pathogen that causes listeriosis and poses substantial health hazards worldwide. The continuing risk of listeriosis outbreaks underlies the importance of designing an effective prevention strategy and developing a robust immune response by reverse vaccinology approaches. This study aimed to provide a critical approach for developing a potent multiepitope vaccine against this foodborne disease. **Methods**: A chimeric peptide construct containing 5 B-cell epitopes, 16 major histocompatibility complex I (MHC-I) epitopes, and 18 MHC-II epitopes were used to create a subunit vaccination against *L. monocytogenes*. The vaccine safety was evaluated by several online methods, and molecular docking was performed using ClusPro to determine the binding affinity. Immune simulation was performed using the C-ImmSimm server to demonstrate the immune response. **Results**: The results validated the antigenicity, non-allergenicity, and nontoxicity of the chimeric peptide construct, confirming its suitability as a subunit vaccine. Molecular docking showed a good score of 1276.5 and molecular dynamics simulations confirmed the construct's efficacy, demonstrating its promise as a good candidate for listeriosis prophylaxis. The population coverage was as high as 91.04% with a good immune response, indicating good antigen presentation with dendritic cells and production of memory cells. **Conclusions**: The findings of this study highlight the potential of the designed chimeric peptide construct as an effective subunit vaccine against *Listeria*, paving the way for future advances in preventive methods and vaccine design.

Keywords: *Listeria*; virulent proteins; food safety; vaccine; immunoinformatics

1. Introduction

In 1924 Murray, Webb, and Swann for the first time identified *Listeria monocytogenes* as a gram-positive bacillus responsible for epidemic cases of mononucleosis, a disease that affects laboratory rabbits and guinea pigs; they named it *Bacterium monocytogenes* [1,2]. In Denmark after a couple of years, another scientist named Nyfeldt isolated a similar bacteria that causes mononucleosis [3]. *L. monocytogenes* is considered one of the most dangerous foodborne pathogens causing listeriosis in humans. This bacterium poses a serious threat to food safety because of its incredible capacity to proliferate, endure, and flourish in a variety of environmental conditions such as high salinity, low temperature (refrigerator), and a wide range of pH values [4–6].

Given the potentially dangerous outcomes of listeriosis, an in-depth study is needed to identify the virulent proteins responsible for the pathogenicity of *Listeria* and design prevention strategies for this foodborne illness [7,8]. With its ability to cross the blood–brain barrier, *L. monocytogenes* poses a particular threat to the central nervous system [9]. The ability of *Listeria* to grow at refrigerated temperatures complicates food safety procedures even more. It has a mortality rate of about 20–30%. This bacteria has

been linked to a variety of food products, including dairy, ready-to-eat meats, and vegetables, due to its tolerance to many settings. Every year, an estimated 1600 people die from listeriosis in the United States alone, with specific demographic groups, such as pregnant women and persons with compromised immune systems, being at a higher risk [10,11].

Considering the serious consequences that listeriosis may lead to, we must comprehend the virulent proteins that are crucial to the pathogenicity of *Listeria*. Because this bacterium can adapt to a variety of conditions, it becomes a complex task to ensure food safety [12,13]. The study sets out on a complex expedition utilizing subtractive immunoinformatic techniques to reveal the molecular complexities of *Listeria*'s pathogenicity factors. The ultimate objective is to design a chimeric multiepitope vaccine strategically, a novel approach that has the potential to mitigate the harmful effects of *L. monocytogenes* on human health. The goal of this study is to improve our understanding of foodborne pathogens and proactively come up with novel ways to protect public health [14–16]. Advances in computational biology and bioinformatics have enabled the rapid and cost-effective design of vaccines against *L. monocytogenes* using *in silico* approaches. For instance, bioinformatics-based predictive algorithms can be employed to identify potential antigens with the capacity to elicit an effective immune response against this pathogen. Additionally, data-driven approaches such as systems vaccinology can be utilized to analyze large datasets of immunological parameters to identify potential vaccine targets [17–21]. Such approaches have the potential to significantly accelerate the development of effective vaccines against *L. monocytogenes*, which can have a major impact on public health [7].

In this study, computational approaches, more precisely reverse vaccinology approaches, were used to develop a chimeric vaccine construct against *L. monocytogenes* infections. The multiepitope strategy has been used for development and design purposes, and the efficiency of the designed construct has been validated by computational means [22,23]. The results confirm that the designed vaccine construct has the potential to be used as a considerable prevention strategy against this foodborne infection and a robust, long-lasting immune response was simulated [24]. This study presents a promising approach to combat *L. monocytogenes* foodborne infections. The designed vaccine construct was found to be safe and to possess a positive immunological response. Future objectives include progressing to preclinical trials, modifying the vaccine formulation based on new data, and investigating possible uses for other foodborne infections, all of which will contribute to ongoing efforts to improve global food safety. The field of *in silico* or computational vaccine development, and in particular reverse vaccinology, is a potential way to overcome the drawbacks of conventional approaches.

2. Material and Methods

2.1 Selection of Proteins from UniProt

The first step in designing an *in silico* multiepitope vaccine is the selection of protein. For this purpose, the proteins must be related to the disease of interest and must be antigenic enough to be recognized by the immune system [25,26]. Proteins chosen as vaccine targets must be expressed in the target organism, exhibit immunogenic properties, and possess an optimal length for antibody binding, while also ensuring the comprehensive coverage of antigenic sites [27]. These criteria help prioritize proteins with the highest potential to stimulate effective immune responses, contributing to the development of successful vaccines. To this end, the UniProt Database was queried to retrieve the complete proteome of *L. monocytogenes*, identified by the UniProt ID UP000000817.

The antigenicity or immunogenicity of the identified proteins was predicted using the online tool VaxiJen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). The tool is based on the Antigenic Index (AI), which considers the hydrophobicity and flexibility of the amino acid residues present in the protein sequence. The AI score of a protein sequence ranges between 0 and 1, with a score of 1 indicating a higher antigenicity of the protein [28]. Moreover, the physiochemical properties of selected proteins were analyzed using the ExPASy-Protparam tool (https://web.expasy.org/protparam/) [29].

2.2 Selection of B-Cell Epitopes

Once the antigen of choice is identified, the next step is to identify regions of the protein that can be used as potential vaccine epitopes. The target proteins are shortlisted and B-cell epitopes are predicted from the The Immune Epitope Database (IEDB) database (http://tools.iedb.org/main/bcell/). This can be done by using algorithms such as a combinatorial peptide library or artificial neural network to identify the optimal peptides that can be recognized by the B-cell receptors [30].

2.3 Selection of T-Cell Epitopes

In addition to B-cell epitopes, T-cell epitopes must also be identified and selected to design a successful multiepitope vaccine. The T-cell epitopes are typically identified using an algorithm that considers the context of the protein sequence, as well as the major histocompatibility complex (MHC) class I or class II alleles that are expressed by the target population [8,31]. It is important to select T-cell epitopes that are conserved across different strains of the pathogen, as well as epitopes that are recognized by different MHC alleles [28]. The *in silico* multiepitope vaccine design is to select the T-cell epitopes from the IEDB (http://tools.iedb.org/main/tcell/). This can be done by using algorithms such as the support vector machine or hidden Markov Model to identify the optimal peptides that can be recognized by the T-cell receptors [32].

2.4 Immunogenicity Analysis

The analysis of immunogenicity is an important step in vaccine design, and is performed using an immunoinformatics tool such as Vaxijen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). This tool can be used to identify the most immunogenic epitopes and to evaluate the potential of the vaccine to create an effective and safe vaccine [28].

2.5 Vaccine Construct and Assembly

A multiepitope vaccine construct can be generated by combining potential epitopes, together with an adjuvant such as 50S ribosomal protein L7/L12 (UniProt ID: Q8Y3L4) attached to the N-terminal of the construct [33]. Linkers of type EAAAK, GPGPG, and AAY can be used, and a 6xHis tag can be added to the C-terminus to enable the expression and binding of the histidine protein. To reduce the size of the vaccine construct, overlapping epitopes from B-cell, cytotoxic T lymphocyte (CTL), and helper T lymphocyte epitopes can be merged. The sequence of these epitopes can then be used to form the final vaccine construct and its corresponding region in the protein [27].

2.6 Physiochemical Evaluation

When the multiepitope constructions have been successfully synthesized, it is critical to conduct an analysis of the constructs' physiochemical characteristics, antigenicity, immunogenicity, allergenicity, and toxicity. To this purpose, the antigenicity of the constructions may be evaluated using Vaxigen v2.0 (http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html), and the allergenicity of the constructs can be evaluated using AllerTOP v2.0 (https://www.ddg-pharmfac.net/AllerTOP/). In addition, the toxicity of the constructions may be evaluated using ToxinPred (https://webs.iiitd.edu.in/raghava/toxinpred/protein.php/), which can be found at this URL. In addition, the physiochemical characteristics of the constructions may be analyzed with the ExPASy-Protparam tool (https://web.expasy.org/protparam/) and the constructs' levels of purity can be measured with the use of solubility tests.

2.7 Population Coverage of Vaccine Construct

The IEDB provides a tool to evaluate the population coverage of a vaccine construct based on the genetic diversity of the target population. This tool uses data on more than 7 million known alleles of the MHC molecules and provides estimates of the population coverage of a given vaccine construct [34]. The tool accepts either a list of epitopes or a protein sequence as an input and compares them with the IEDB's MHC allele information.

2.8 Secondary and Tertiary Structure Prediction and Validation

A structural study of the construct is required to verify the design of the multi-epitope vaccine [35]. PSI-blast based secondary structure PREDiction 4.0 (PSIPRED 4.0) (http://bioinf.cs.ucl.ac.uk/psipred) for secondary structure prediction and trRosetta (https://yanglab.qd.sdu.edu.cn/trRosetta/) for tertiary structure prediction may be used to do this. These tools build a three-dimensional (3D) model of the construct based on the protein sequence and expected immunogenicity [36]. The model may then be used to assess the construct's stability and verify the design [28]. After the 3D model of the project has been created, it must be refined to guarantee that it is correct and sturdy [37]. To this end, Galaxy Refine (http://galaxy.seoklab.org/), a protein structure refining tool, may be used. To enhance the 3D model of the build, this program employs a mix of energy reduction and residue-level optimization. This phase is critical to ensuring that the construct is stable and capable of eliciting a significant immunological response [30]. The last stage in structure prediction is to verify the vaccine's structure. The vaccine's tertiary structure was confirmed further using ProSA-web (https://prosa.services.came.sbg.ac.at/prosa.php) and SAVES 6.0 tools (https://saves.mbi.ucla.edu/). ProSA-web developed a quality model from low-resolution input, as well as z-scores, and showed residue energies. SAVES was used to evaluate non-bonded interactions and generate a Ramachandran map, which depicts the permitted and forbidden areas for amino acid moieties based on phi and psi angles. The structure's quality was assessed by examining the z-score and Ramachandran score using online tools [32].

2.9 Molecular Docking and Molecular Dynamics Simulations

Molecular docking is a computer tool for analyzing the interaction of a ligand with its target molecule. To further assess the vaccine's effectiveness, molecular docking was done using the E-cadherin receptor ligand binding domain as the target using the ClusPro 2.0 tool (https://cluspro.org/). Molecular dynamics (MD) simulations (https://imods.iqfr.csic.es/) were also used to analyze the vaccine's dynamic behaviour [38]. These simulations aid in determining the vaccine's potential effectiveness and intensity of the antibody response it induces.

2.10 Codon Optimization and Expression Analysis

The sequence was reverse-translated using EMBOSS 6.0.1 backtranseq software (https://www.ebi.ac.uk/Tools/emboss) to aid in the production of the chimeric protein in an expression vector. The generated sequence was subsequently codon-optimized using the Java Codon Adaption tool (JCat), an online web-based server (http://www.jcat.de/). The report included information about the optimized codons' GC content % and codon AI (CAI) score, which may be used to evaluate their likelihood for optimal expression. The recommended range for GC content is 30% to 70% since levels outside this range might have a deleterious influence on transcriptional and translational performance. The PbR322 vector was selected for expres-

sion, and PfoI and AflIII restriction sites were added to the construct's ends using the SnapGene program (https://www.snapgene.com/), allowing it to be cloned into the vector. To amplify the construct, *in silico* polymerase chain reaction (PCR) was performed using SnapGene.

2.11 Immune Simulations

Comprehensive immunological simulations using the C-ImmSim server aided in the full assessment of the vaccine's potential immunogenicity. These simulations were carefully designed to precit the vaccine's expected immunological response, which included immune system activities such as antigen presentation, processing, and T-cell activation. The simulations demonstrated the dynamic movements of major cell types such as dendritic cells, macrophages, epithelial cells, B lymphocytes, CD4 Helper T-Cells (HTLs), CD8 CTLs, natural killer cells, and cytokines.

3. Results

3.1 Analysis of Protein Sequences

The complete genome sequence of the betacoronavirus was obtained from the UniProt Database with the UniProt ID UP000000817. Additionally, the highly antigenic extracellular membrane protein was retrieved from the same source using the UniProt ID Q8Y3L4. Selection of the protein was based on different evaluating parameters that make it suitable for vaccine design. Analysis of the physiochemical properties of the antigenic protein was conducted with the ExPASy-Protpram tool. The instability index (II) of the protein was calculated as 26.51, its aliphatic index as 82.73, and the grand average of hydropathicity (GRAVY) as –0.394.

3.2 Identification of B-Cell Epitopes

To predict B-cell epitopes, the IEDB Linear Epitope Prediction program version 2.0 was used. The antigenicity of the protein was analyzed using the Kolaskar and Tongaonkar techniques for predicting antigenicity. This approach is based on the physico-chemical characteristics of the amino acids that make up the protein. It was discovered that the protein had an antigenic propensity that ranged from a low of 0.4535 up to a high of 1.138. The average was determined to be 0.73. It was decided that a value of 0.5 would serve as the antigenic determination threshold for the protein, and any value larger than 0.5 would indicate the presence of probable antigenic determinants. It was discovered that nine epitopes met the threshold value and were anticipated to have the ability to produce a B-cell response. Of these nine epitopes, only two were antigenic; therefore, they were eliminated from consideration as shown in Table 1.

3.3 Recognition of T-Cell Epitopes

The stabilized matrix method was used to predict a distinct set of MHC human leukocyte antigen alleles in humans. Of the 766 selected epitopes, 50 were chosen based on their maximum binding affinity to MHC-I alleles. Then the selected epitopes were assessed for their antigenicity, toxicity, and allergenicity, and those found to have a score less than 0.4 or deemed toxic or allergenic, were excluded. The remaining 16 epitopes were further analyzed for their conservancy, antigenic nature, allele binding, and surface accessibility. Additionally, 630 epitopes were predicted to have IC_{50} values less than 200, of which 30 were chosen based on their interaction with more than 4 MHC class II alleles. The final selected epitopes were used to construct a vaccine chimera, with both MHC-I and MHC-II bindings being analyzed to identify potential epitopes to increase vaccine efficacy as shown in Table 2.

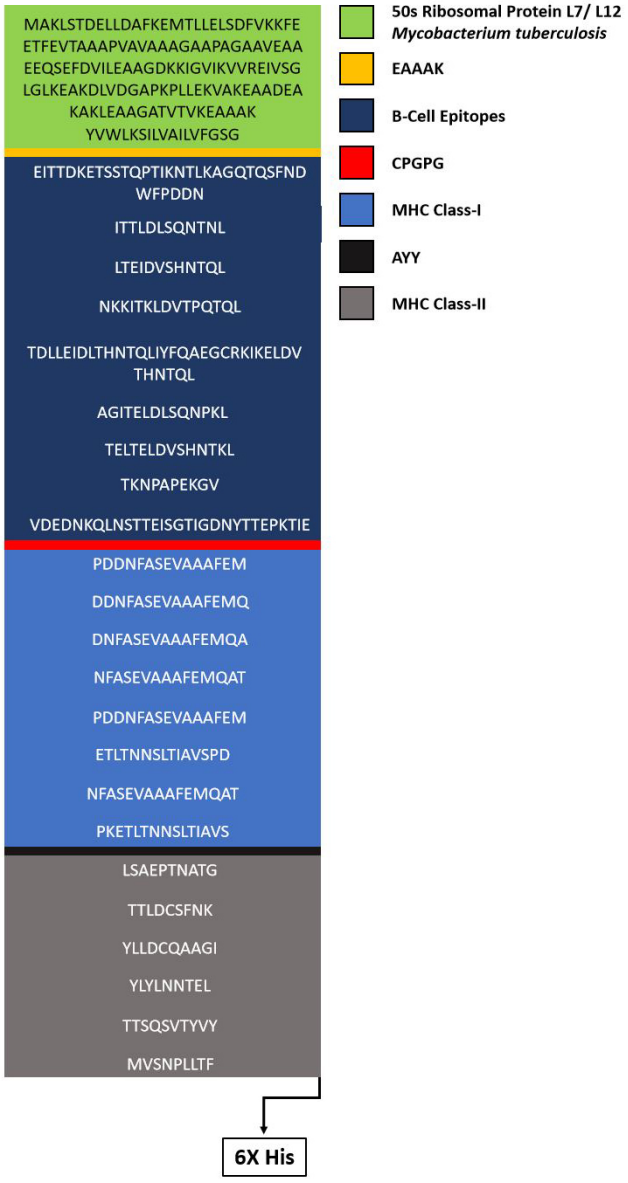

Fig. 1. The final vaccine construct by joining the Adjuvant, Linkers, B-Cell, MHC-I and MHC-II Epitopes. MHC, major histocompatibility complex.

Table 1. Prediction of linear B-cell epitopes by Immune Epitope Database (IEDB) antibody epitope prediction resource analysis.

Epitopes	Length	Antigenicity
EITTDKETSSTQPTIKNTLKAGQTQSFNDWFPDDN	35	0.3440 (Probable NON-ANTIGEN)
ITTLDLSQNTNL	12	0.8194 (Probable ANTIGEN)
LTEIDVSHNTQL	12	0.8984 (Probable ANTIGEN)
NKKITKLDVTPQTQL	15	1.0751 (Probable ANTIGEN)
TDLLEIDLTHNTQLIYFQAEGCRKIKELDVTHNTQL	45	0.7778 (Probable ANTIGEN)
AGITELDLSQNPKL	14	1.1387 (Probable ANTIGEN)
TELTELDVSHNTKL	14	1.0134 (Probable ANTIGEN)
TKNPAPEKGV	10	0.0424 (Probable NON-ANTIGEN)
VDEDNKQLNSTTEISGTIGDNYTTEPKTIE	30	0.4535 (Probable ANTIGEN)

Table 2. The finalized and selected MHC-I and MHC-II epitopes on the basis of antigenicity scores.

MHC-I		
Epitopes	Length	Antigenicity
PDDNFASEVAAAFEM	15	0.4208 (Probable ANTIGEN)
DDNFASEVAAAFEMQ	15	0.7668 (Probable ANTIGEN)
DNFASEVAAAFEMQA	15	0.5962 (Probable ANTIGEN)
NFASEVAAAFEMQAT	15	0.9263 (Probable ANTIGEN)
PDDNFASEVAAAFEM	15	0.4208 (Probable ANTIGEN)
ETLTNNSLTIAVSPD	15	0.4828 (Probable ANTIGEN)
NFASEVAAAFEMQAT	15	0.9263 (Probable ANTIGEN)
PKETLTNNSLTIAVS	15	0.3668 (Probable NON-ANTIGEN)
MHC-II		
Epitopes	Length	Antigenicity
LSAEPTNATG	10	0.7925 (Probable ANTIGEN)
TTLDCSFNK	9	0.3722 (Probable NON-ANTIGEN)
YLLDCQAAGI	10	0.7415 (Probable ANTIGEN)
YLYLNNTEL	9	0.9089 (Probable ANTIGEN)
TTSQSVTYVY	10	0.6815 (Probable ANTIGEN)
MVSNPLLTF	9	–0.1206 (Probable NON-ANTIGEN)

3.4 Construction of Multiepitope Subunit Vaccines

The final vaccine construct consisted of a 50S ribosomal protein L7/L12 (Uniprot ID: P9WHE3) as an adjuvant, attached at the N-terminus of a peptide sequence via an EAAAK linker to induce a specific immune response. After the adjuvant, five B-cell epitopes were attached at the previous EAAAK linker. Then 16 MHC-I epitopes and 18 MHC-II epitopes were connected by CPGPG and AAY linkers, respectively, and a 6xHis tag was fused to the C-terminus for protein purification and identification. The chimeric peptide sequence had a total of 504 amino acids and a calculated molecular weight of 54,266.40 Da. Fig. 1 shows the construction of the vaccine by joining the adjuvant, epitopes, and linkers [39].

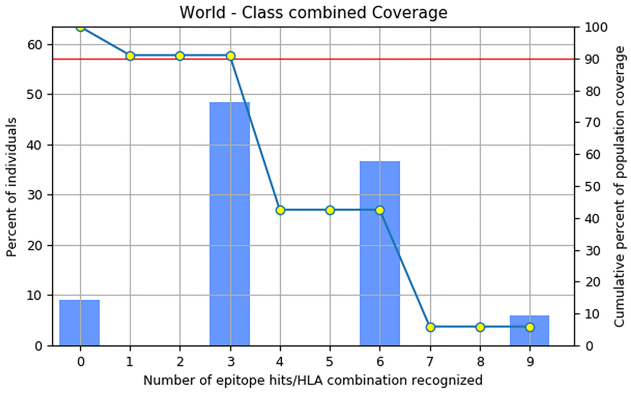

Fig. 2. The graph of the worldwide population coverage analysis obtained by the IEDB Population Coverage Analysis Tool.

3.5 Evaluation of the Antigenicity and Allergenicity of Vaccine Proteins

The antigenicity of the whole vaccine sequence was determined using the Vaxijen 2.0 server and found to be 0.6507, indicating that the sequence is antigenic. The antigenicity of the same sequence without the adjuvant was also tested using Vaxijen 2.0 and the score was 0.6761, confirming antigenicity regardless of the presence or absence of the adjuvant. Additionally, AllerTOP v2.0 was utilized to ensure the vaccine sequence is non-allergenic. The toxicity of the vaccine construct was predicted to be nontoxic using the ToxinPred.

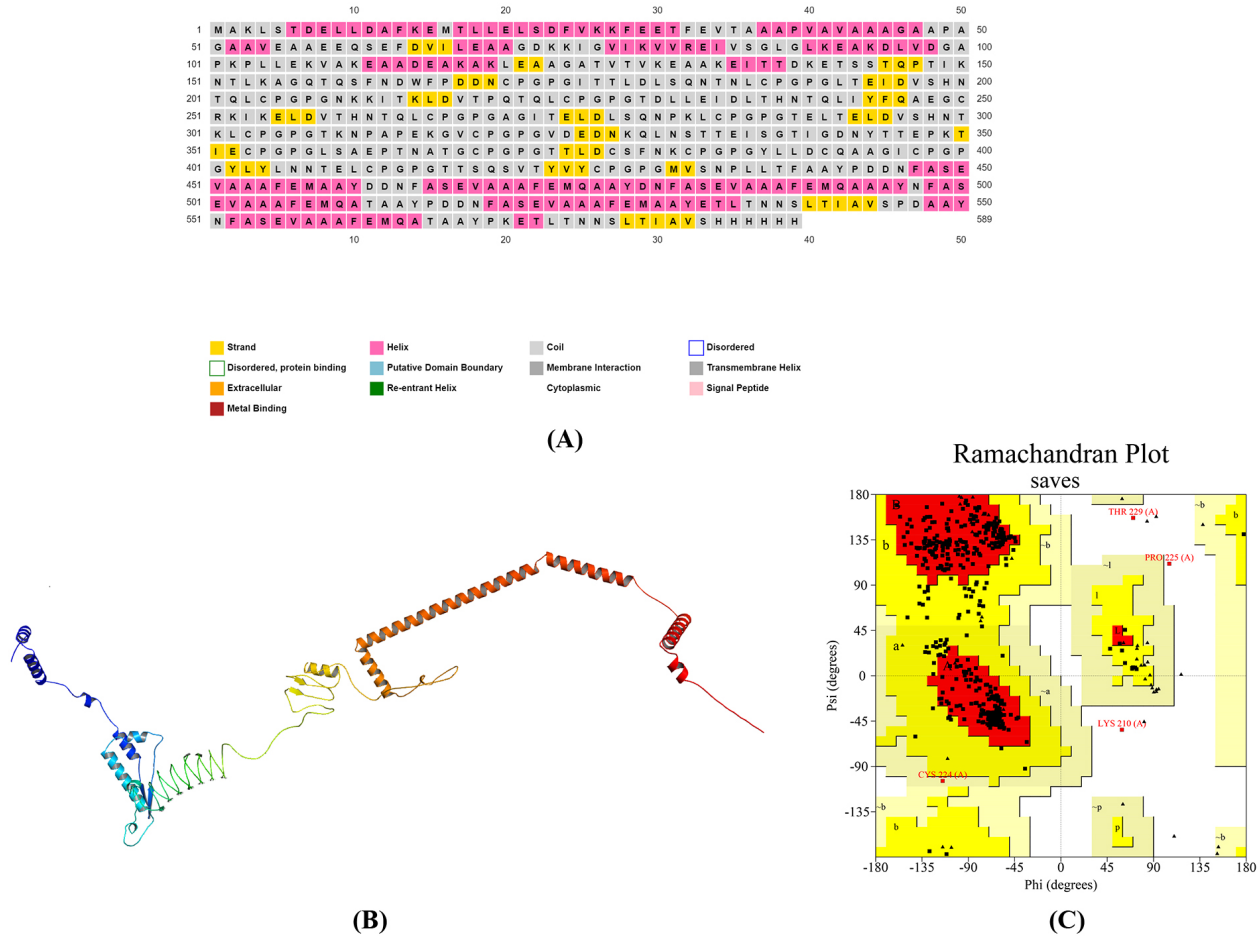

Fig. 3. Protein structure prediction and quality assessment. (A) The secondary structure of the vaccine predicted by PsiPred. (B) Tertiary structure of the vaccine construct. (C) Ramachandran plot constructed by PROCHECK, indicating the good quality of the predicted protein model.

3.6 Analysis of Solubility and Physiochemical Properties of Multiepitope Subunits

The molecular weight of the specific vaccine construct was found to be 72,455.26 Da using the ExPASy-ProtParam, and the theoretical isoelectric point value (PI) was 8.44. The half-life of the protein construct was determined to be 30 h (mammalian reticulocytes, *in vitro*), >20 h (yeast, *in vivo*), and >10 (*Escherichia coli*, *in vivo*). The instability index (II) for the protein was predicted by Protpram to be 24.87, suggesting that it is a stable model. The estimation of the aliphatic index was 82.80, indicating its thermostability. The GRAVY was –0.240, demonstrating the protein's hydrophobic nature.

3.7 Population Coverage of the Vaccine Construct

The population coverage of the combination alleles of MHC-I and MHC-II was projected globally, and the 91.04% coverage was examined by the IEDB tool as shown in Fig. 2. This indicates that the chosen epitopes are promising candidates for the prevention of this bacterium.

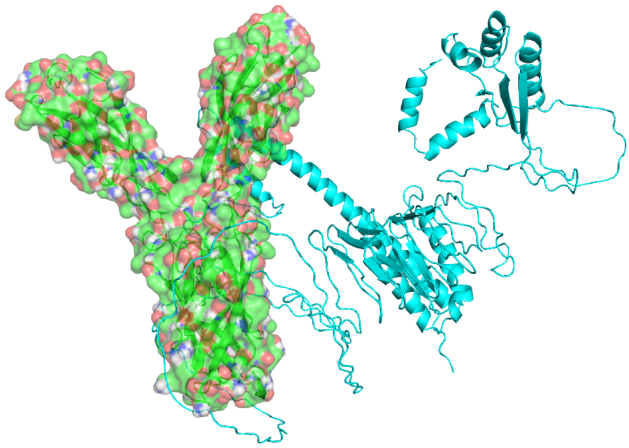

Fig. 4. Docked complex of vaccine construct with the receptor E-Cadherin indicating a good and stable interaction.

3.8 Extrapolation of Secondary and Tertiary Structures

The secondary structure of the chimeric vaccine construct was estimated with the help of the PSIPRED service. The results of this estimation showed that the amino acid

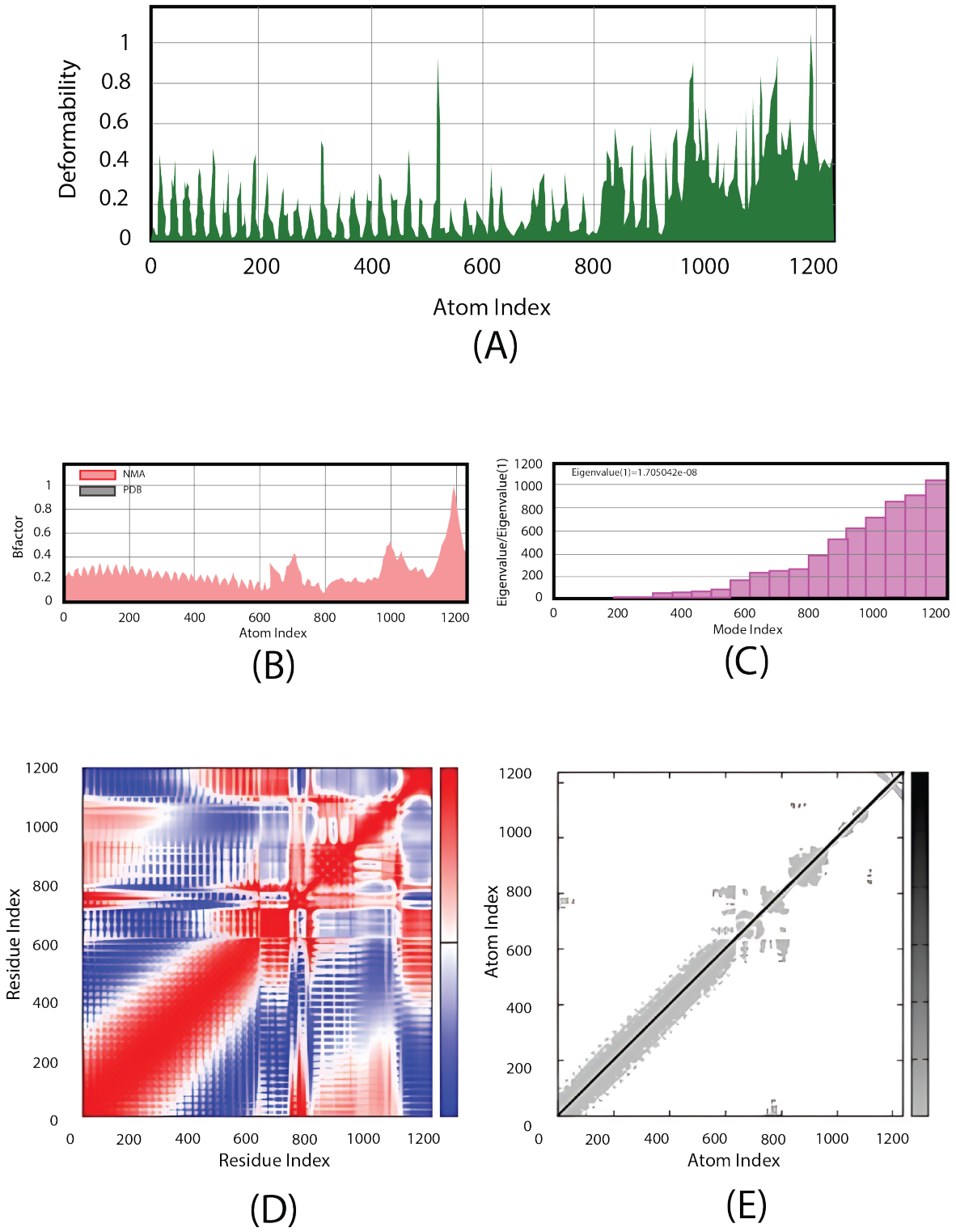

Fig. 5. The results of the Molecular Dynamic Simulation study of vaccine construct and E-Cadherin docked complex. (A) Deformability, (B) Normal Mode Analysis (NMA) Mobility, (C) Eigenvalue, (D) co-variance map (correlated (red), uncorrelated (white), or anti-correlated (blue) motions), (E) elastic network (darker grey regions indicate stiffer regions).

residues were likely to form helices, beta strands, and coils, as shown in Fig. 3A. The anticipated secondary structure is shown in Fig. 3B. The tertiary structure of the construct was predicted utilizing the Rosetta server, which resulted in a 3D model. The GalaxyRefine service was used to further develop the tertiary structure of the predicted model. A total of five models were developed, and the one that proved to be the most successful was selected based on criteria including Global Distance Test-High Accuracy (0.8884), root-mean-square deviation (0.630), and MolProbity (1.493).

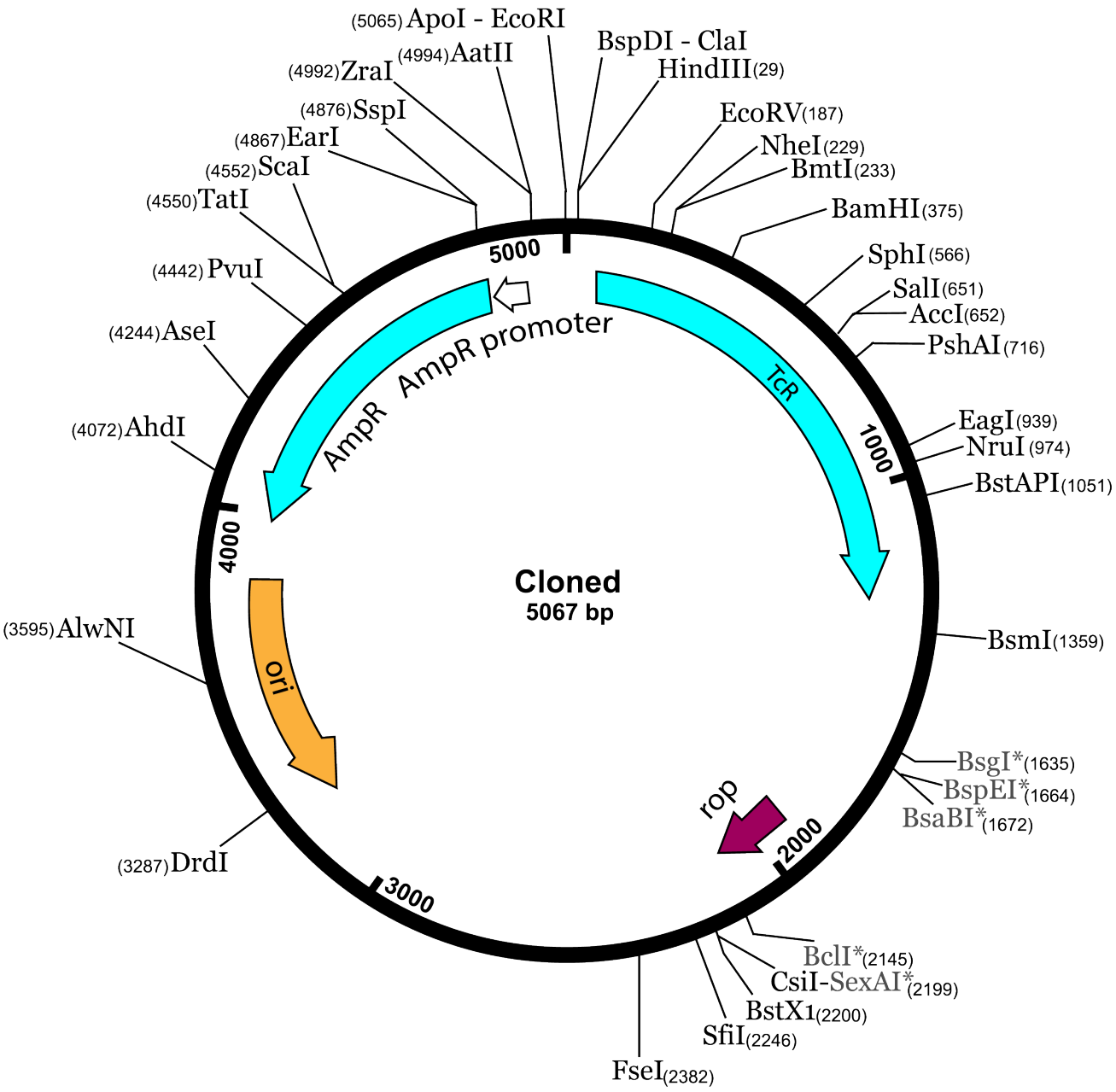

Fig. 6. Cloned vaccine construct in PbR322 Vector using SnapGene.

Scores of 4.3 and 0.2 for collision and poor rotamer, respectively, were achieved by the model that was selected. The Ramachandran plot predicted a score of 95.9% for the participant. The SAVES server was used to confirm the tertiary structure refinement, which resulted in an enhanced Ramachandran plot. Before refining, 82.3% of the amino acids were in ramafavoured regions, 14.7% in the additionally allowed regions, and 3% in the disallowed region. After refining, 95.9% of the amino acids were in the ramafavoured regions, 3.3% in the additionally allowed region, and 0.8% in the disallowed region. The Fig. 3C represents the Ramachandran plot of the designed vaccine construct. The ProSA-web, on the other hand, predicted a negative Z-score of about 5.64.

3.9 Molecular Docking with the Ligand-Binding Domain of Toll-Like Receptor E-Cadherin

With the online ClusPro tool, we were able to determine the strength of the interaction that exists between the improved structure of the vaccine construct and E-cadherin. The 10 findings were prioritized, and the best-docked model that could be constructed from those results had a docking score, interaction energy, and cluster size. It was determined that the complex had an atomic interaction score of 1276.5, which was negative. Fig. 4 depicts a 3D schematic illustrating hydrogen bonds and interaction bumps, as well as the interaction angles and docking position. MD simulations were performed to gain a deeper understanding of the interaction.

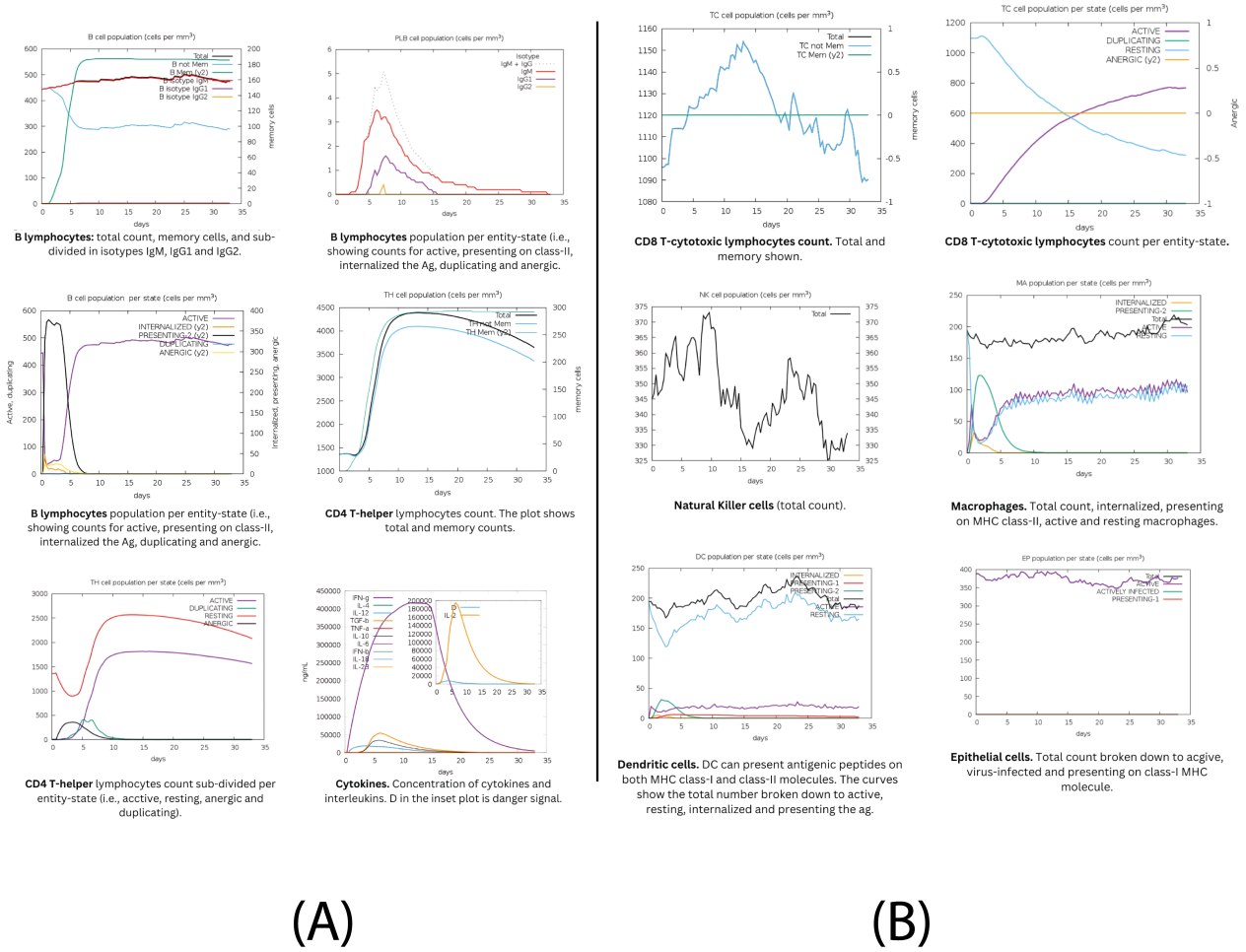

Fig. 7. Immune Simulation and Immunogenic Profiling of the designed vaccine construct. (A) Cell counts are displayed. Act = active, Intern = internalized ag, Pres II = presenting on MHC II, Dup = in the mitotic cycle, anergic = anergic, resting = not active. (B) The symbols shown in the illustration above. (C) The virus, immunoglobulins, and immunocomplexes. (D) Cytokine and interleukin concentration.

3.10 MD Simulations

It is necessary to understand particle motion to have a better grasp of the docking outcomes. The MD online application was used to simulate the motion of particles derived from amino acid residues implicated in protein–protein interactions in docking studies. MD Online implements an energy reduction method in the input stage and then presents the final model findings. The docked model is processed, and the energy is minimized. The system is given a copy of the complex, and the force field of the complex is modified at various time intervals. The resulting model has low energy for all particles and enhanced interactions for all residues (Fig. 5).

3.11 Codon Optimization of Expression Analysis

To improve protein expression, the JCat was used to optimize codons. The optimized codon sequence was 2019 nucleotides long, with a codon adaption index (CAI) of 0.94 and a GC content of 55.6%. Since the ideal GC content range is between 30% and 70%, these values indicate that vector expression will be steady. Then the optimized sequence was cloned into a PbR322 (+) vector to generate a recombinant plasmid, which was amplified *in silico* using SnapGene software (Fig. 6).

3.12 Immune Simulations

The immunological simulation findings produced on the C-ImmSim server provide convincing quantitative insights into the vaccine's potential immunogenicity. The simulations addressed a wide range of immune system activities, including antigen presentation, processing, and T-cell activation, intending to predict the vaccine's immunological response. Dendritic cells, macrophages, epithelial cells, B lymphocytes, CD4 T-helper lymphocytes, CD8 T-cytotoxic lymphocytes, natural killer cells, and cytokines were identified as having distinct dynamics. Antigen presentation by dendritic cells, for example, increased significantly, reaching a numerical value of 230 mm^3, suggesting increased immunological activation. The simulations also demonstrated a coordinated response, with a quantifiable association between CD4 T-helper cell activation and cytokine production, indicating a robust and organized immune response. Fig. 7 depicts the detailed numerical results, which not only improve our understanding of the vaccine's immunogenic potential but also give a quantitative basis for evaluating its efficacy and immunostimulatory capabilities, directing future vaccine development and optimization techniques.

4. Discussion

The development of an *in silico* vaccine against Listeria is an important area of research for the prevention of foodborne infection. An *in silico* vaccine is a computer-generated vaccine that is designed to target a specific pathogen, such as *Listeria*. *In silico* vaccines are created by combining the genomic sequence of the pathogen with the genetic sequence of its host. This combination of information is used to generate a vaccine that is designed to induce an immune response against the pathogen. *In silico* vaccine design is the computational optimization and design of vaccines [40]. This computational approach has been used to predict potential vaccine candidates, design optimal vaccine formulations, and assess vaccine efficacy. In recent years, there has been increasing interest in applying this approach to the design of vaccines against *Listeria* [41], a Gram-positive bacterium that is a major cause of foodborne disease [42].

Many studies have focused on the identification of potential vaccine targets for *Listeria* [43]. For example, a study by Cordero *et al*. [44] used bioinformatics methods to identify potential *Listeria* vaccine targets by analyzing the sequence of its outer membrane protein. The authors identified many potential targets, including several cell wall-associated proteins and many flagellar proteins. Another study by Peña-González *et al*. [45] used a computational approach to predict the antigenic potential of *Listeria* proteins. The authors identified several potential vaccine targets, including several surface proteins and lipoproteins. In addition to identifying potential vaccine targets, *in silico* vaccine design has also been used to optimize vaccine formulations. For example, a study by Dutta *et al*. [46] used computational methods to identify optimal combinations of *Listeria* antigens for a potential vaccine formulation. The authors identified a combination of antigens that provided the highest level of protection against the bacteria.

In 2015, Geyer *et al*. [43] published a research that focused on discovering vaccine targets for *Listeria*. This sort of study is critical because it aids in the creation of vaccines by identifying particular proteins or antigens that can elicit an immune response against the virus [47]. Naveed *et al*. [39] published another important study in the discipline of vaccine design and development. They designed a vaccine construct utilizing the M protein of SARS-Cov-2 using bioinformatics techniques. They discovered numerous possible vaccine targets, including cell wall-associated proteins and flagellar proteins. Computer analysis was used to narrow down possible candidates for future experimental examination in this technique [48].

In silico vaccine design has also been used to assess the efficacy of potential vaccine formulations. For example, a study by Liang *et al*. [49] used computational modeling to evaluate the efficacy of a potential *Listeria* vaccine. The authors found that the vaccine was able to induce a strong immune response and to reduce the levels of bacteria in infected animals. Overall, the current state of the field of *in silico* vaccine design for *Listeria* is rapidly advancing. By using computational methods to identify potential vaccine targets, optimize vaccine formulations, and assess the efficacy of vaccine candidates, researchers are making significant progress toward the development of effective vac-

cines against this pathogen. In addition, hypothetical proteins also could be a considerable source for vaccine development according to a recent study [50].

The use of *in silico* approaches for vaccine design could lead to the development of novel vaccines that are tailored to the specific needs of a particular host. This could potentially reduce the cost and time required for vaccine production and allow for the development of more effective vaccines. In a recent study, the use of an *in silico* approach to identify potential antigenic epitopes on the surface of *Listeria monocytogenes* was shown to be more effective than traditional methods of vaccine design [51]. This study demonstrated that the *in silico* approach was able to identify a greater number of antigenic epitopes than traditional methods, which resulted in a more effective vaccine, indicating that the *in silico* approach may be a valuable tool for the design of more effective vaccines against *Listeria*. Molecular docking methods could be potential strategies to study environmental contamination effects as well [52].

5. Conclusions

The development of an *in silico* vaccine against Listeria is an important area of research that has the potential to provide a safe and effective means of preventing infection. The use of *in silico* approaches for vaccine design could lead to the development of novel vaccines that are tailored to the specific needs of a particular host. The developed chimeric peptide construct has the potential to be used as a subunit vaccine to prevent *Listeria* if synthesized *in vitro*. It was found to be safe and secure to use with maximum immunogenic score as per the computational predictions. Therefore, this could be a breakthrough for future scientists who want to work on the *in vitro* development of vaccines against *Listeria* infections.

Availability of Data and Materials

All the data generated in this research work has been included in the manuscript.

Author Contributions

Conceptualization: TA, MN, MAS, KJ, ZY; Methodology: JMR, AH, AAK, KJ, ZY; Software: AAK; Validation: AZ and THA; Resources: TA; Data Curation: MAS; Formal Analysis: TA; Investigation: MN and TA; writing—original draft preparation: MAS; writing— review and editing: TA; visualization: ZY; Supervision: JMR; funding acquisition: ZY. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

The authors greatly acknowledge and express their gratitude to the Researchers Supporting Project number (RSPD2024R568), King Saud University, Riyadh, Saudi Arabia.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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