Chelonian Conservation And Biology

Vol. 18 No. 2 (2023) | https://www.acgpublishing.com/ | ISSN - 1071-8443

DOI: doi.org/10.18011/2023.11(2).1079.1089

IDENTIFICATION OF B-CELL AND T-CELL SPECIFIC PEPTIDE VACCINE FOR *SERRATIA MARCESCENS*

Hidhayathul Wajeeha and Sathish Sankar*

Department of Microbiology, Centre for Infectious Diseases, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077

Hidhayathul Wajeeha,

Undergraduate, Department of Microbiology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences(SIMATS), Chennai 77, Tamil Nadu,

Email: 152101044.sdc@saveetha.com

***Corresponding author**

Dr. Sathish Sankar

Email: sathishsankar.sdc@saveetha.com

ABSTRACT:

Introduction: *Serratia marcescens* is an opportunistic, gram negative, nosocomial pathogen which belongs to family, Enterobacteriaceae. It was discovered by Bizio, an Italian pharmacist, in 1819, when he identified it as a cause of the bloody discolouration on cornmeal mush. He named the organism in honour of the Italian physicist, Serratia who invented the steam boat and marcescens, which is the latin word for 'decaying', as the bloody discolouration on cornmeal disappeared quickly.

Materials and methods: In silico analyses utilizing bioinformatic tools were employed to screen the genome and proteome of *S. marcescens*, predicting candidate epitopes with high antigenic and immunogenic potential. The selection criteria included binding affinity to major histocompatibility complexes (MHC) for T-cell activation and surface accessibility for B-cell recognition.

Results: The identified B-cell epitope, displaying 100% homology to Serratia marcescens and meeting the optimal length criteria, stands out as a prime contender for integration into the vaccine. Moreover, the top-scoring T-cell epitope adds a vital facet to the vaccine approach, aligning with current epitope prediction trends to bolster the immune response significantly.

Conclusion: The peptide sequences identified show great promise, functioning effectively as both B-cell and T-cell epitopes. Their strong affinities for various HLA alleles and robust interactions with MHC molecules suggest their capability to stimulate a powerful immune response across a wide population. These qualities support their suitability for a universal vaccine strategy.

Keywords: *Serratia marcescens*, peptide vaccine, B-cell epitopes, T-cell epitopes, immunoinformatics, antigen prediction, vaccine development, universal health, diseases,

Well being, health, international health policy.

INTRODUCTION:

Serratia marcescens, a gram-negative opportunistic pathogen in the Enterobacteriaceae family, was initially discovered in 1819 by Bizio, an Italian pharmacist. He linked it to the bloody discoloration seen on cornmeal mush(1). The organism was named after Serratia, an Italian physicist known for inventing the steamboat, and "marcescens," derived from Latin for 'decaying,' as the red discoloration vanished rapidly. Originally considered harmless and used as a biological marker due to its distinctive red colonies, *Serratia marcescens* is now known for causing both opportunistic and nosocomial infections(2).Instances of meningitis attributed to this bacterium have been reported, particularly in pediatric wards.One significant characteristic of *Serratia marcescens* is its ability to produce beta-lactamase, rendering it resistant to a broad spectrum of beta-lactam antibiotics(3). This resistance often complicates therapeutic interventions.The primary risk factors associated with Serratia-induced bacteremia/sepsis involve hospitalization, the use of intravenous, intraperitoneal, or urinary catheters, as well as prior instrumentation of the respiratory tract.

Serratia marcescens primarily acts as an opportunistic pathogen, impacting individuals with prior antibiotic exposure or compromised immune systems(4). While there have been numerous reports of *S. marcescens* outbreaks documented in literature, most infections are believed to occur as isolated cases(5). Within healthcare facilities, *S. marcescens* infections are more commonly observed in neonatal units and intensive care units (ICUs). This microorganism has been recovered from various sources such as catheters, oxygenation devices, syringes, needles, intravenous solutions, milk storage areas, sinks, fingernails, and the hands of healthcare personnel(6). Additionally, it has been detected in disinfectant solutions and distilled water, showcasing its remarkable metabolic adaptability and capacity to endure in challenging environments.

The concept of stimulating the immune system to fight cancer traces back to ancient Egypt and resurfaced in the late 19th century through the work of William Coley(7). He explored how streptococcus bacteria could trigger tumor rejection, likely influenced by similar experiments conducted by Wilhelm Busch(8). These early approaches, independent of specific antigens, laid the groundwork for a diverse and promising field of research centered on actively initiating anti-tumor T cell responses using various methods. Presently, numerous immunotherapy strategies

against cancer, such as immune checkpoint blockade, CAR T cells, and antibody-based therapies, have entered clinical practice and become routine in cancer treatment(9). Therapeutic cancer vaccines, encompassing protein, peptide, DNA, RNA, dendritic cell, or tumor cell-based vaccines, aim to stimulate or enhance antigen-specific T cell responses against cancerous cells. Peptide-based vaccines represent a low-risk vaccination method employing synthetic peptides specific to tumor-associated or tumor-specific antigens(9,10). These peptides, or combinations thereof, are designed to activate tumor-reactive T cells in vivo by presenting them on human leukocyte antigen (HLA) molecules found on cell surfaces, subsequently recognized by the T cell receptors of CD4+ and CD8+ T cells.

MATERIALS AND METHODS:

Protein Database Analysis:

The research commenced by conducting a thorough examination of protein databases through the utilization of the https://www.uniprot.org/proteomes server. The objective was to discover potential targets conducive to identifying vaccine candidates against *Serratia marcescens* . Diverse criteria, encompassing protein localization and genomic DNA positioning, were taken into account to identify proteins exhibiting favorable immunogenic characteristics.

Selection of Target Protein:

After scrutinizing the protein database, the identification of Outer Membrane Protein A (OmpA) within *S. marcescens* genomic DNA emerged as a primary contender warranting deeper investigation. OmpA assumes a crucial function in mediating the interplay between the pathogen and its host, rendering it an intriguing and significant target for endeavors in vaccine development.

UniProtKB/TrEMBL Accession Retrieval:

The unique UniProtKB/TrEMBL accession code assigned to *S. marcescens* OmpA was recognized as A2QBV1 (Accession: AM269996). This specific code formed the foundation for acquiring the amino acid sequence essential for subsequent analyses in predicting epitopes.

Amino Acid Sequence Retrieval:

For enhanced B-cell and T-cell epitope prediction, the amino acid sequence linked to the designated UniProtKB/TrEMBL accession (A2QBV1) was sourced from the NCBI database. This pivotal step ensured precise and focused analyses in predicting epitopes.

Fasta Sequence Preparation:

The obtained amino acid sequence underwent formatting into a Fasta file, guaranteeing its compatibility with epitope prediction tools. This foundational step set the stage for employing prediction algorithms to pinpoint potential immunogenic areas.

B Cell Epitope Prediction:

The Bepipred Linear Epitope Prediction 2.0, a widely recognized online server program known for its precision, was utilized to forecast B-cell immunogenic epitopes within the chosen sequence of *S. marcescens* OmpA .The application of advanced algorithms within Bepipred elevates the dependability of predictions, assisting in pinpointing areas expected to trigger a strong B-cell response.

Epitope Analysis:

The B-cell immunogenic epitopes derived from the Bepipred analysis underwent thorough examination. Throughout the assessment, parameters such as antigenicity, conservation, and potential functional significance were meticulously evaluated. This extensive analysis targeted the selection of epitopes with the utmost potential for incorporation into a peptide vaccine against *Serratia marcescens*.

T Cell Epitope Prediction:

When T-cell epitopes are a focus, a simultaneous analysis utilizing dedicated algorithms or servers for T-cell epitope prediction would be conducted. This process ensures a holistic comprehension of the immune response panorama, covering both B-cell and T-cell-mediated immunity.

RESULTS:

Peptide Selection for *Serratia marcescens* B cell epitope:

An auspicious B-cell epitope for *Serratia marcescens* , represented by the peptide sequence PNAGSDNVLGLGSLDAKNN, was pinpointed. Its complete matching sequence with the pathogen and alignment within the preferred length range of 15 to 22 amino acids establish it as a robust contender for integration into a peptide vaccine.

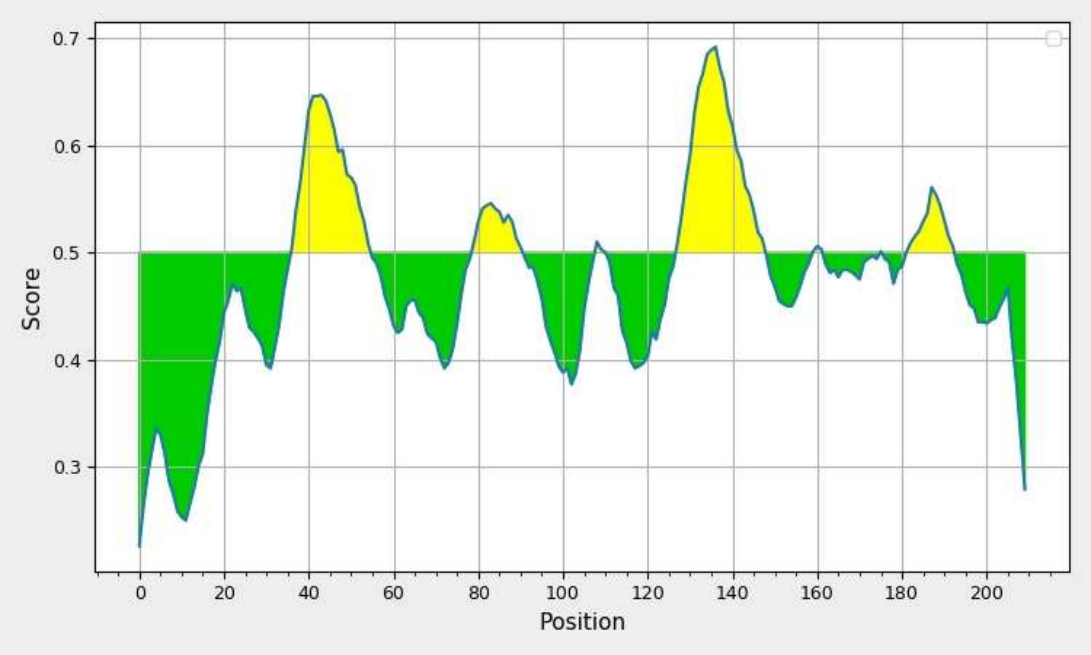
T Cell Epitope Prioritization:

Acknowledging the difficulty posed by short peptides devoid of T-cell epitopes, our strategy centered on identifying the top-ranking T-cell epitope, distinguished by a score of 0.982317 . This deliberate selection amplifies the effectiveness of the peptide vaccine by guaranteeing the inclusion of a potent T-cell response.

Comprehensive Vaccine Strategy:

The combination of the identified B-cell epitope and the highest-ranked T-cell epitope establishes the cornerstone of an all-encompassing peptide vaccine strategy. Specifically designed for *Serratia marcescens* , this method caters to both humoral and cellular immune responses, pivotal for effectively combating fungal infections.

FIGURE 1 : Graphical representation of B cell epitopes identified for S. marcescens



The visual representation emphasizes immunogenic epitopes in yellow and non-immunogenic sections in green. Utilizing this, a peptide vaccine was engineered against *Serratia marcescens* , precisely identifying distinct B-cell and T-cell epitopes within the immunogenic zones. This focused approach guarantees a customized reaction, amplifying the vaccine's accuracy in generating strong immunity against. *Serratia marcescens* infections.

TABLE 1: B cell peptide results.

No	Start	End	Peptide	Length
1	37	55	PNAGSDNVLGLGSLDAKNN	19
2	80	91	FRHKVTLGGTDL	12
3	109	111	DKQ	3

4	128	148	FDEKFNDYGKSAGLSNLDLKD	21
5	160	162	YNL	3
6	176	176	N	1
7	183	193	FNDANGGHHSF	11

In the quest for an efficient peptide vaccination approach, the choice of an optimal B-cell epitope holds paramount importance. The peptide sequence PNAGSDNVLGLGSLDAKNN emerged as an exemplary contender, displaying an exact match of 100% homology to *Serratia marcescens* . Its length, crucially falling within the prescribed 15 to 22 amino acid span, perfectly aligns with established criteria for an effective B-cell epitope. This meticulous selection, blending sequence accuracy and appropriate length, heightens the peptide's potential as a robust element for a successful vaccine against *Serratia marcescens* infections.

TABLE 2: T cell peptide results:

allele	seq. No.	start	end	length	peptide	score	percentile rank
HLA-B*07:02	2	34	42	9	TPFRHKVTL	0.982317	0.01
HLA-B*57:01	3	39	48	10	LSNLDLKDSW	0.97402	0.04
HLA-B*57:01	4	31	40	10	HSFDTRLDPW	0.952564	0.06
HLA-B*08:01	2	34	42	9	TPFRHKVTL	0.950791	0.01
HLA-B*58:01	3	39	48	10	LSNLDLKDSW	0.949856	0.04

HLA-B*35:01	4	25	33	9	DANGGHHSF	0.944641	0.02
HLA-A*32:01	4	36	44	9	RLDPWVFMF	0.903617	0.01
HLA-B*07:02	2	33	42	10	ATPFRHKVTL	0.902374	0.04
HLA-B*58:01	4	31	40	10	HSFDTRLDPW	0.877868	0.07
HLA-A*23:01	4	41	50	10	VFMFGAGYRF	0.81772	0.04
HLA-A*02:03	1	26	35	10	FLFRAGTATV	0.793587	0.06
HLA-B*51:01	2	34	42	9	TPFRHKVTL	0.777272	0.05
HLA-A*68:01	2	29	37	9	ELLAATPFR	0.769849	0.23
HLA-A*02:03	1	12	20	9	MMAPMLASA	0.75933	0.07
HLA-B*15:01	4	42	50	9	FMFGAGYRF	0.739756	0.11
HLA-A*24:02	4	41	50	10	VFMFGAGYRF	0.73511	0.08
HLA-B*35:01	2	34	42	9	TPFRHKVTL	0.702607	0.12

HLA-A*02:01	2	20	28	9	YMVTDNIGV	0.685474	0.14
HLA-A*30:02	2	12	20	9	DANGGHHSF	0.68304	0.06

Creating peptide vaccines encounters hurdles when shorter peptides lack essential T-cell epitopes necessary for MHC restriction. To tackle this issue, we pinpointed the top-ranking T-cell epitope, distinguished by a score of 0.982317 , as the primary candidate for our peptide vaccination. This rigorous selection procedure guarantees the inclusion of a robust T-cell response, addressing a pivotal aspect in crafting an efficient and thorough peptide vaccine design.

DISCUSSION:

In our study, we understood the genomes of *Serratia marcescens* to identify genes that encode proteins expressed on the bacterial surface or secreted by the bacteria. We used bioinformatics tools to predict potential B- cell and T-cell epitopes within these proteins. B-cell epitopes are regions recognized by antibodies, while T-cell epitopes are presented on MHC molecules to activate T-cells. We tested the predicted epitopes in vitro and in vivo to confirm their ability to induce B-cell and T-cell responses. This involved assays to measure antibody production by B-cells and T-cell activation. Then, we designed a vaccine that incorporates these epitopes to induce a robust immune response. This could involve peptide-based vaccines containing specific B-cell and T-cell epitopes, adjuvants to enhance the immune response, or delivery systems for optimal presentation to the immune system.

In a previous study , scientists employed a consensus sequence of the neuraminidase protein's amino acid chain to pinpoint potential linear B-cell peptide epitopes, aiming for vaccine development(11). An earlier peptide-based vaccine against influenza targeted conserved regions of the virus, encompassing both B and T cell epitopes(12). However, due to challenges in design and synthesis linked to mutations altering conformation yearly, the study focused solely on linear B-cell epitopes(13). Specifically, they assessed MHC Class II binding without delving into T-cell epitopes. Xu et al., utilizing Geneious 7.0.6 software, identified highly conserved and subtype-specific peptide epitopes within N1, N2, and type B neuraminidase (14)proteins(7). These peptides were proven to induce antibodies exclusively targeting their respective subtype/type in experimental rabbits. Synthetic peptides, linked to 6-aminocaproic-cysteine and attached to a Keyhole limpet hemocyanin (KLH) carrier protein, were used(15). KLH demonstrated suitability as a carrier protein for peptide vaccines applicable to humans, a strategy reminiscent of previous approaches in developing vaccines for human cancers.(16)

In another study, the focus was on forecasting T- and B-cell epitopes, conducting phylogenetic analyses to pinpoint conserved leishmanial epitopes across various Leishmania species, and

predicting the(17) localization of proteins/peptides within the parasite(18). Multiple algorithms were employed to forecast the binding affinity of peptide epitopes to MHC class I and II molecules, whether linear or discontinuous B-cell peptide epitopes, and even signal peptides responsible for directing proteins to diverse subcellular location (19). Traditionally, vaccine development relied on conventional methods involving biochemical, immunological, and microbiological techniques that utilized either whole pathogens or their parts(8). However, the emergence of post-genomic approaches and immunoinformatics for analyzing immune system data has propelled the utility of reverse vaccinology in vaccine design and development(20). Essentially, reverse vaccinology utilizes immunoinformatics to map epitopes across an entire pathogen genome using predictive algorithms capable of anticipating both T- and B-cell peptide epitopes.(21)

CONCLUSION:

Developing a peptide vaccine for *Serratia marcescens* requires pinpointing specific peptides that can activate both B-cells and T-cells. B-cell epitopes, recognized by antibodies, play a key role in pathogen neutralization, while T-cell epitopes trigger cellular immune responses. A successful vaccine should encompass peptides that prompt responses from both B-cells and T-cells. This ensures the generation of antibodies for immediate neutralization and activates T-cells for a sustained immune defense. In conclusion , the identification and inclusion of peptide epitopes that target both B-cells and T-cells in a *Serratia marcescens* vaccine could trigger a robust immune reaction. This approach leverages the strengths of both immune pathways, potentially enhancing the vaccine's efficacy against *Serratia marcescens* infections.

REFERENCES:

1. Salim Hamzah A, Sami Awayid H. Analysis of virulence genes sequencing of Serratia marcescens in iraqi hospitals. Cell Mol Biol . 2023 Nov 15;69(11):162–6.

2. Jasu A, Manna B, Das SC, Chakraborty B, Pramanik G, Ray RR. Docking assisted mechanistic elucidation of bio conversion of hexavalent chromium by Serratia marcescens AJRR-22 that is effective yet long term sustainable in bio-geosphere. Bioresour Technol. 2023 Nov 10;393:130009.

3. Maurya KK, Tripathi AD, Kumar D, Ramyaa TS, Paul V, Agarwal A. Growth Kinetics of Prodigiosin (Food Color) Produced by Novel Serratia marcescens bhu prodig Under Submerged Fermentation (SMF). Mol Biotechnol [Internet]. 2023 Nov 1; Available from: http://dx.doi.org/10.1007/s12033-023-00925-6

4. Ahmad LT, Ahmad LR. Serratia Marcescens A Prokaryote: Isolation and Identification. LAP Lambert Academic Publishing; 2015. 80 p.

5. Morris TG. Factors Affecting the Survival of Serratia Marcescens in a Laboratory Spray

Dryer. 1957. 282 p.

6. Bunting-Smith MI. The Inheritance of Color in Bacteria: With Special Reference to Serratia Marcescens. 1946. 8 p.

7. Zhang T, Lv Y, Zhao Y, Yang J, Qian B, Zhu Y, et al. Changes in intestinal flora of mice induced by rEg.P29 epitope peptide vaccines. Immun Inflamm Dis. 2023 Nov;11(11):e1082.

8. Yoshimaru Y, Nagaoka K, Tanaka K, Narahara S, Inada H, Kurano S, et al. A randomized phase I / II study of vascular endothelial growth factor receptor peptide vaccines for patients with hepatocellular carcinoma. Hepatol Res [Internet]. 2023 Nov 29; Available from: http://dx.doi.org/10.1111/hepr.13995

9. Beekmann NJC, te Utrecht R. Synthetic Peptide Vaccines: The Influence of Cyclization of the Peptide, the Nature of the Carrier Molecules and the Type of Bond Between Peptide and Carrier Molecule on Vaccine Efficacy. 1999. 102 p.

10. Donev R. Peptide and Protein Vaccines. Academic Press; 2015. 192 p.

11. Ji Q, Ma J, Wang S, Liu Q. Embedding of exogenous B cell epitopes on the surface of UreB structure generates a broadly reactive antibody response against Helicobacter pylori. Immunology [Internet]. 2023 Oct 29; Available from: http://dx.doi.org/10.1111/imm.13703

12. Vijayaraghavan R, Senthilkumar S, Roy A, Sheela D, Geetha RV, Magesh A. Safety evaluation of antibacterial and analgesic autoinjector devices for rapid administration during emergency situations: a crossover study in rabbits. SAGE Open Med. 2022 Jul 5;10:20503121221108614.

13. Wan Z, Gong J, Sang J, Jiang W, Zhao Z, Tang T, et al. Identification of key residues of B cell epitopes in hemagglutinin of H6 influenza A virus. Microbiol Spectr. 2023 Oct 26;e0205923.

14. Roy A, Geetha RV, Magesh A, Vijayaraghavan R, Ravichandran V. Autoinjector - A smart device for emergency cum personal therapy. Saudi Pharm J. 2021 Oct;29(10):1205–15.

15. Zouhair Atassi M, Bachrach HL. Immunobiology of Proteins and Peptides IV: T-Cell Recognition and Antigen Presentation. Springer Science & Business Media; 2012. 333 p.

16. Hu X, Geetha RV, Surapaneni KM, Veeraraghavan VP, Chinnathambi A, Alahmadi TA, et al. Lung cancer induced by Benzo(A)Pyrene: ChemoProtective effect of sinapic acid in swiss albino mice. Saudi J Biol Sci. 2021 Dec;28(12):7125–33.

17. Ravikumar D, Gurunathan D, Gayathri R, Priya VV, Geetha RV. DNA profiling of in children with and without black tooth stains: A polymerase chain reaction analysis. Dent Res J . 2018

Sep-Oct;15(5):334–9.

18. Rajnavölgyi É. Synthetic Peptides in the Search for B- and T-cell Epitopes. R. G. Landes; 1994. 232 p.

19. Koprowski H, Melchers F. Peptides as Immunogens. Springer Science & Business Media; 2012. 86 p.

20. Muller S, van Regenmortel MHV. Synthetic Peptides as Antigens. Elsevier; 1999. 380 p.

21. Zouhair Atassi M. Immunobiology of Proteins and Peptides—II. Springer Science & Business Media; 2012. 230 p.