

Research Article



Insilco Approach for Unraveling Flagellin as a Promising Antigen for Next-Gen DNA Vaccines

Ammar Mehfooz¹, Zahid Hussain², Humaira Zafar^{3*}

¹Industrial Biotechnology, Institute of Industrial Biotechnology, Government College University, Lahore.

²Associate Professor of Biotechnology, Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan.

³Professor of Pathology, Consultant Microbiologist, Al Nafees Medical College & Hospital, Islamabad.

*Corresponding author: Humaira Zafar, Professor of Pathology, Consultant Microbiologist, Al Nafees Medical College & Hospital, Islamabad.

Abstract

Objective: To identify and characterize the best possible vaccine candidate among entire proteome of *Salmonella typhimurium* on the basis of its suitability as an immunogen for DNA vaccine development, emphasizing its non-allergenic properties and its potential to elicit elevated antibody titers.

Methodology: It was an exploratory, Insilco experimental study. It was performed at the Pathology laboratory, Department of Biotechnology, Institute of Industrial Biotechnology, GC University Lahore, from January 2020 to October 2020.

This study employed the powerful approach of reverse vaccinology to investigate the proteome of *Salmonella Typhimurium*. Our primary focus was directed towards flagellin, a protein previously considered hypothetical, which bears a striking resemblance to a conserved extracellular phage-encoded counterpart (accession number AEF07831.1).

Results: Total sample size of study was 207(N). Our investigation unveils the remarkable ability of flagellin to elicit both innate and adaptive immune responses. This dual role in orchestrating immune reactions, as predicted by Vaxijen, C-Immsim, Allertop and Properd significantly enhances the potential value of flagellin as a potentially strong DNA vaccine candidate.

Conclusion: With the anticipation of flagellin as a potent immunogen, molecular cloning within a non-pathogenic host, we envision a new paradigm in defense against *Salmonella Typhimurium*. Nonetheless, the imperative for empirical validation remains, guiding us forward on our journey fueled by the allure of uncharted scientific discovery.

Keywords: *Salmonella typhimurium*; flagellin; hypothetical proteins; immune simulations; reverse vaccinology; insilco characterization

Introduction

The relentless march of progress in sanitation practices, coupled with groundbreaking advances in vaccination techniques and the discovery of novel antibiotics, has wielded a transformative influence on the well-being and living standards of humanity. Within the realm of vaccine development, a pivotal concern looms large: the cost-benefit ratio of safeguarding lives against an array of pernicious diseases [1]. In the contemporary landscape, the arsenal of cutting-edge technologies for identifying optimal vaccine candidates against targeted pathogens includes recombinant DNA technology, chemical conjugation, reverse vaccinology, structural biology, and systems biology [2,3]. The amalgamation of reverse vaccinology, recombinant DNA technology, molecular cloning, and genome editing opens vistas for combatting obstinate maladies and fostering novel

avenues for vaccine design and production [4,5]. Vaccines generated through reverse vaccinology and recombinant DNA technology bear the distinction of being non-allergenic and non-reactive, heralding a new era of immune protection with minimal adverse effects [6, 7].

The triumvirate of bacteremic maladies spearheaded by *Salmonella typhimurium*, *Streptococcus pneumoniae*, and *Neisseria meningitides* stand as chief targets for multifaceted defenses [8]. Among all of them an array of *Salmonella* species, notably *Salmonella typhi*, and the notorious *Salmonella typhimurium* are reported as most hazardous [9, 10]. The gravity of typhoid fever is unequivocally portrayed by the staggering statistics that emerged from a comprehensive survey conducted in 2018. A harrowing toll of approximately 269,000 lives was claimed by this affliction, with an alarming annual incidence of about 26.9 million reported

cases, as outlined in the authoritative WHO report [11]. The urgency for effective intervention becomes abundantly clear. In this innovative landscape, microbial proteins emerge as prime candidates for antigen sourcing. Their merits, spanning high yields, rapid microbial growth on cost-effective media, consistent production, ease of modifiability, economic viability, and structural stability, place them at the forefront of vaccine development strategies [12]. Such proteins offer a promising trajectory towards robust, scalable, and effective vaccine solution [13]. So, the convergence of reverse vaccinology, DNA synthesis, and microbial protein selection not only accelerates our response to existing threats but also holds the key to tackling future infectious adversaries with unparalleled precision and efficacy. The landscape of immunization is evolving, and these advancements propel us towards a safer and healthier world. This study aims to identify most antigenic and least allergic vaccine candidate among whole proteome of *Salmonella* species using reverse vaccinology and other Insilco techniques for development of DNA vaccine against *Salmonella typhi* and *Salmonella typhimurium*, which pose significant threats to public health. Microbial proteins, due to their advantages like high yields, rapid growth, and structural stability, emerge as ideal antigens for vaccine development. So, by combining reverse vaccinology, DNA synthesis, and microbial protein selection, this research accelerates responses to existing threats and equips us to combat future infectious adversaries effectively. This evolving landscape of immunization promises a safer and healthier world through these advancements.

Methodology

The complete research work was performed at the Pathology lab, Department of Biotechnology, Institute of Industrial Biotechnology, GC University Lahore, under the approval number granted by IRB i.e., 031O-MPHIL-BT-18, from January, 2020 to October 2020. This was an Exploratory Insilco experimental study in which the cutting-edge approach of reverse vaccinology was applied for sample analysis. Total 207 (N) hypothetical proteins were selected from 1964 protein of *Salmonella typhimurium* found on NCBI server by mean of Vaxign tool. A pioneering journey was started within the genome of *Salmonella typhimurium*, strategically by selecting hypothetical proteins to sculpt a novel

vaccine candidate primed for the creation of a robust DNA-based vaccine. The foundation of Inclusion criteria was set on the basis of a meticulously crafted bioinformatics algorithm, intricately woven with critical parameters [14]. These parameters encompass an optimal fusion of antigenic prowess and adhesion potential, the precise subcellular or membrane localization of the proteins, a judicious restriction of trans-membrane helices (set at a threshold of one or less), a resolute divergence from the human proteome through non-homology and non-similarity, and an emphasis on inciting formidable immunogenic responses. All those vaccine candidates which didn't match the vaccine candidacy criteria of Vaxign, Allertop as well as Vaxijen were excluded accordingly. Among the instrumental tools, Vaxign emerges as a beacon of innovation, standing tall as an online system that spearheads vaccine design endeavors. A simple digital portal (www.violinet.org/vaxign) unveils a realm where the algorithmic principles of reverse vaccinology are harnessed to unearth the gems of potential vaccine candidates [15]. From the initial bounty of 201 proteins, an arduous winnowing process unfolds, governed by the principles of scrutiny and precision. Ultimately, a curated assembly of 50 proteins emerges victorious from the crucible of our exacting standards, each exuding the hallmarks of an ideal vaccine candidate. Beyond their individual merits, these selected proteins collectively span a broad spectrum of *S. typhimurium* strains, infusing versatility into their potential application. A meticulous extraction effort yielded a trove of nearly 50 robust candidates from the annals of *Salmonella typhimurium*, meticulously cherry-picked for their remarkable resilience and potency when juxtaposed with their 155 counterparts. After the identification of promising hypothetical proteins through predictive methods, the spotlight now turns to their refinement via a meticulous shortlisting process based on subcellular localization. Then we employed two potent tools: PsortB [16] and CELLO [17] each an embodiment of precision in the realm of subcellular localization assessment. Then next task was evaluation of antigenicity which stands as a cornerstone in the meticulous curation of a robust vaccine candidate. Central to this endeavor is the indispensable role of a tool aptly named Vaxijen, accessible at the following web address: www.ddg-pharmfac.net/vaxijen. The remarkable potency of Vaxijen's predictions finds its quantifiable footing, with reported accuracy range spanning approximately 70% to 89% [18]. The

evaluation of allergenicity among the chosen candidates underwent meticulous scrutiny through the utilization of the cutting-edge tool known as Allertop. While Vaxign primarily targets human similarity searches, the supremacy of Allertop reigns when it comes to deciphering allergenicity indices within the realm of the proposed hypothetical proteins [19]. Imbued with an impressive sensitivity rate of 94%, allertop emerges as a beacon of accuracy in allergenicity determination. C-immsim breathes life into the process of assessing hypothetical proteins for their potential as robust antigens, capable of eliciting a robust immune response [20]. The orchestration of C-immsim's operation was elegantly simple yet profoundly powerful. It examined a shortlisted protein named flagellin on the basis of its antigenic potential to generate antibody titer with least allergic response, after that prediction of its B & T cell epitopes were performed the formidable predictive capabilities of Propred-1 [21] and Propred-2 tools. This intricate model enabled the identification of shared epitopes capable of binding to both MHC I and MHC II molecules. A subsequent meticulous scrutiny and tallying of interacting MHC alleles culminated in comprehensive epitope assessment.

Results

The reverse vaccinology algorithm, harnessed through the bioinformatics powerhouse known as Vaxign, was deployed to discern potential vaccine candidates against the formidable *Salmonella typhimurium*. 207 (N) out of the expansive pool of 1964 proteins exhibited the promise of inducing immunity and serving as prime candidates for inclusion in vaccine design. Notably, the sequences of these hypothetical proteins were diligently retrieved and meticulously archived in the FASTA format files, via NCBI ensuring their availability for comprehensive analysis. Out of those 205 proteins only 40 revealed their residence in the coveted outer membrane or outer layers of the bacterial realm, hold the keys to an

efficacious vaccine. Their strategic positioning facilitates encounters with vigilant immune cells. With the field narrowed down to these 40 proteins, the next chapter unfolded: antigenicity assessment. The criteria were stringent, as proteins with an antigenicity score breaching the 50% threshold emerged as the cream of the crop, primed for vaccine design. However, not all could bear the weight of this lofty standard.

After meticulous examination, 11 proteins from this elite group were deemed non-antigenic, their antigenicity scores falling below the 50% threshold, and thus were judiciously excluded from further consideration. Then we harnessed the predictive prowess of Allertop to predict allergenicity of shortlisted proteins. The outcome was both revealing and cautionary, as the digital oracle pinpointed 10 proteins awash in the hues of allergenicity and triumphant majority of 30 proteins emerged untarnished, bearing the mantle of non-allergens, fortifying our endeavor's foundation. From this pool of 30 proteins, 6 were singled out based on their remarkable antigenicity scores, surpassing a threshold of 0.83 and to delve deeper into their immunogenic potential, we harnessed the power of C-immsim. The chosen roster of protein candidates for immune simulations encompassed an array of intriguing entities: the outer-membrane fimbrial usher protein, cryptic curlin major subunit, cell envelope integrity inner membrane protein TolA, major curlin subunit precursor, flagellin, and outer membrane protein S. By inputting the FASTA sequences of these distinguished proteins, we initiated a cascade of immune simulations, materialized in the form of intricate graphs. These visual representations illuminated that among all selected proteins, A protein named flagellin having accession number AEF07831.1.1 showed highest number of antibody titer and can generates strong immunity by considering it as a potentially strong immunogen with antigenicity score of 1.214 predicted by Vaxijen. This protein has no allergic potential and possessed maximum number of B & T Cell epitopes.

Table 1: Characteristics of flagellin

S. No	Characteristics	Values Attained
1	Protein I	AEF07831.1
2	Antigenicity Score	1.214
3	Allergenicity	Non-Allergen
4	Molecular weight	53269.67

The comparative analysis of antibody titer of predicted primary immune simulations after first, second, third and fourth immunization of all those selected candidates are shown in graphs below and flagellin was the only antigen having maximum

number of antibody titers. It was found out that the antigenicity and antibody titer is correlated and higher the antibody titer higher will be the antigenicity score.

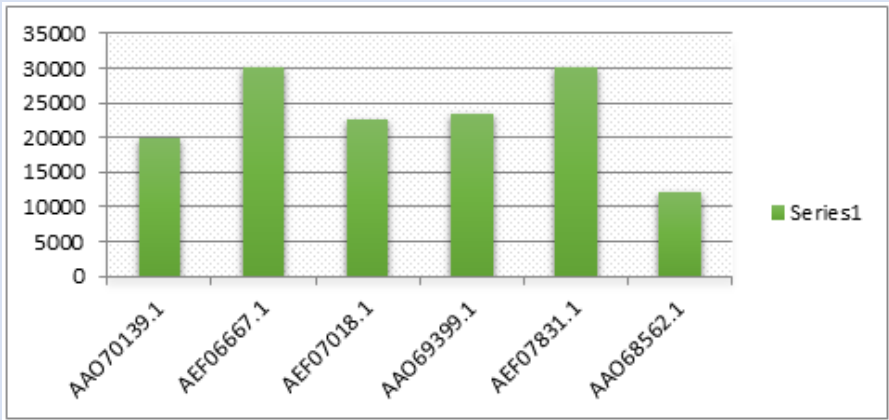


Figure 1: Showing the results of first immunization of the selected proteins where protein flagellin with accession number AEF07831.1 has maximum antibody titer.

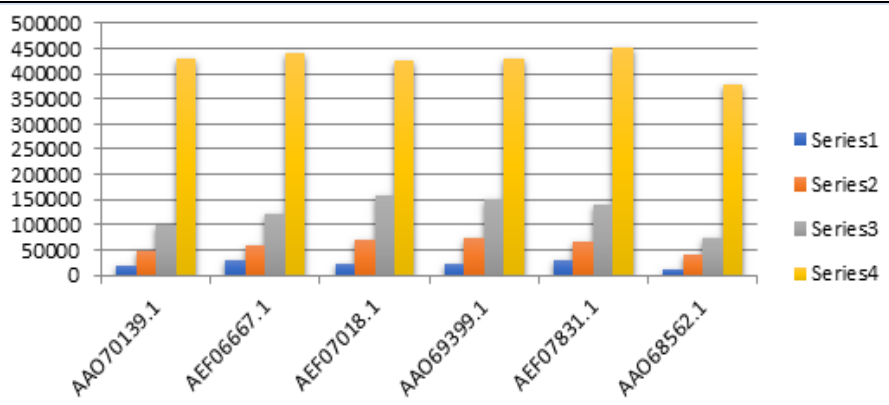


Figure 2: Showing difference among first, second, third and fourth immunization where protein flagellin with accession number AEF07831.1 still has maximum antibody titer proving flagellin as a best candidate for DNA vaccine development against *Salmonella typhimurium*

Discussion

Salmonella typhimurium remains a significant public health concern, warranting the development of effective preventive measures, including vaccines. In this study, we employed a reverse vaccinology approach using the Vaxign algorithm to identify potential vaccine candidates from a vast pool of 1964 proteins. Our findings have provided valuable insights into the selection of promising candidates, their antigenicity, allergenicity, and immunogenic potential. Moreover, we have compared our results with existing literature to contextualize the significance of our findings [20-22]. Our initial screening identified 207 proteins as potential vaccine candidates, but we focused on the 40 proteins residing in the outer membrane or outer layers of *Salmonella*

typhimurium, as these proteins are strategically positioned to interact with the host immune system. Subsequently, we subjected these 40 proteins to stringent antigenicity assessments, with only those surpassing the 50% threshold being considered for further evaluation. This rigorous criterion allowed us to select the top 30 antigenic proteins, which formed the basis for subsequent analyses. To ensure the safety of our vaccine candidates, we utilized Allertop to predict allergenicity. This step revealed that 10 out of the 30 proteins exhibited potential allergenic properties. However, the majority of 30 proteins were deemed non-allergenic, providing a solid foundation for our research [22]. From this pool of 30 proteins, we further narrowed down our selection to six proteins based on their exceptional antigenicity scores, exceeding a threshold

of 0.83. To explore their immunogenic potential, we turned to C-immsim, a tool that allowed us to simulate the immune response to these proteins. The results of our simulations, presented graphically, showed that flagellin, with the accession number AEF07831.1.1, stood out prominently among the selected proteins. Flagellin not only exhibited the highest number of antibody titers but also possessed a remarkable antigenicity score of 1.214, as predicted by Vaxijen. Moreover, it was identified as a non-allergen, further enhancing its appeal as a vaccine candidate. Additionally, flagellin was found to contain the maximum number of B and T cell epitopes, which are crucial for inducing a robust immune response [22, 23]. Comparatively, our findings align with previous research indicating that reverse vaccinology is a positive approach for designing DNA vaccine. Tao et al, [22] in his study also performed the same technique and applied an in-silico, in-vitro and in-vivo process in order to screen various cell-wall-associated proteins because available vaccines that were used at that time against *Salmonella typhimurium* were not able to act against all serotypes of *Salmonella typhimurium* that's why, they have designed a special unbiased as well as genome-wide technique, BacScan, and utilizes bacterial-specific serum to for identification of potentially immunogenic as well as stable proteins. Their technique resembles with our methodology of finding a specific immunogenic protein for DNA vaccine development [22-23].

Khan et al. [23] applied C-Immsim and other computational approaches for designing a stable, most antigenic and safe vaccine against the pathogen species of *Salmonella* and find that simulations of antibody titer generated by this tool has high precision rate and found quite helpful in finding a vaccine candidate. They used multiple immunoinformatic approaches and other computational approaches for designing a stable yet save vaccine against species of *Salmonella* [23]. A correlation between antigenicity and antibody titers of flagellin has also been reported by a study by Kumar et al., who identified the multifaceted potential of flagellin [24]. The prediction of both B-cell and T-cell epitopes augments its promise as a formidable candidate, capable of inciting responses from both the innate and adaptive arms of the immune system. Their vision was to extend the transition of this DNA vaccine through rigorous clinical trials in human subjects, envisaging its potential to emerge as a potent defense against *Salmonella typhimurium*, marked by its

capacity to induce a potent antibody response [24]. Another study by Loomba et al., shows that flagellin holds immense potential in generating a robust DNA vaccine that not only sidesteps harm to the host but also promises efficacy. They found the flagellin and Outer membrane protein C (omp C) as important vaccine candidate as they were resulted in reducing colony forming units and, in an animal sepsis model it reduces mortality [24].

Conclusion

With the anticipation of flagellin as a potent immunogen, molecular cloning within a non-pathogenic host, we envision a new paradigm in defense against *Salmonella typhimurium*. The journey from computational predictions to tangible DNA vaccine candidates signifies a leap towards safer and more effective immunization strategies.

References

1. Patterson TJ, Gaudelli W. Pedagogy of Global Events: Insights from Concerts, Film Festivals and Social Network Happenings. Taylor & Francis.
2. Maslow JN. (2019). Zika vaccine development—current progress and challenges for the future. *Tropical medicine and infectious disease*, 4(3):104.
3. Yin BB, Sun WK, Zhang XY, Liew KM. (2022). Deciphering structural biological materials: Viewing from the mechanics perspective and their prospects. *Composit Part B Eng*, 245:1102-1113.
4. Rappuoli R, Gregorio DE, Giudice DG, Phogat S, Pecetta S, Pizza M, et al. (2021). Vaccinology in the post- COVID-19 era. *Proc Natl Acad Sci USA*, 118(3):1-7
5. Choi M, Hegerle N, Nkeze J, Sen S, Jamindar S, Nasrin S, et al. (2020). The diversity of lipopolysaccharide (O) and capsular polysaccharide (K) antigens of invasive *Klebsiella pneumoniae* in a multi-country collection. *Front. Microbiol*, 11:1249.
6. Bisgin A, Sanlioglu AD, Eksi YE, Griffith TS, Sanlioglu S. (2021). Current update on severe acute respiratory syndrome coronavirus 2 vaccine development with a special emphasis on gene therapy viral vector design and construction for vaccination. *Hum Gene Ther*, 32(12):541-562.
7. Khalid K, Poh CL. (2023). The Promising Potential of Reverse Vaccinology-Based Next-Generation Vaccine Development over

- Conventional Vaccines against Antibiotic-Resistant Bacteria. *Vaccines*, 11(7):1264.
8. Nayak A. (2023). Recombinant Vaccines: The Revolution Ahead. Springer Nature Singapore.
 9. Gan BH, Gaynord J, Rowe SM, Deingruber T, Spring DR. (2021). The multifaceted nature of antimicrobial peptides: Current synthetic chemistry approaches and future directions. *Chem Society Rev*, 50(13):7820-7880.
 10. Sharma P, Mondal K, Kumar S, Tamang S, Najari IN, Das S, et al. (2022). RNA thermometers in bacteria: Role in thermoregulation. *Biochim Biophys Acta Gene Regul Mech*, 1865(7):194871.
 11. Hancuh M, Walldorf J, Minta AA, Benissan TC, Christian KA, Nedelec Y, et al. (2023). Typhoid fever surveillance, incidence estimates, and progress toward typhoid conjugate vaccine introduction—worldwide, 2018–2022. *MMWR*, 72(7):171.
 12. Gómez LA, Oñate AA. (2018). Plasmid-based DNA vaccines. *Plasmid*.
 13. Rodrigo AP, Costa PM. (2019). The hidden biotechnological potential of marine invertebrates: *The Polychaeta case study*. *Environ Res*, 173:270-280.
 14. Arega AM, Pattanaik KP, Nayak S, Mahapatra RK. (2021). Computational discovery and ex-vivo validation study of novel antigenic vaccine candidates against tuberculosis. *Acta Tropica*, 217:105870.
 15. Guerra R, Pedregal J, Maréchal JD. (2018). PyChimera: use UCSF Chimera modules in any Python 2.7 project. *Bioinformatics*, 34(10):1784-1785.
 16. Singh G, Tyagi R, Singh A, Kapil S, Parida PK, Scarcelli M, et al. (2023). Protein Language Model for Prediction of Subcellular Localization of Protein Sequences from Gram-negative bacteria (ProtLM. SCL). *BioRxiv*.
 17. Jones TS, Oliveira SM, Myers CJ, Voigt CA, Densmore D. (2022). Genetic circuit design automation with Cello 2.0. *Nat Protoc*, 17(4):1097-1113.
 18. Aslam S, Ashfaq UA, Zia T, Aslam N, Alrumaihi F, Shahid F, et al. (2022). Proteome based mapping and reverse vaccinology techniques to contrive multi-epitope based subunit vaccine (MEBSV) against *Streptococcus pyogenes*. *Infect Genet Evol*, 100:105259.
 19. Subramaniam MD, Venkatesan D, Iyer M, Subbarayan S, Govindasami V, Roy A, et al. (2020). Biosurfactants and anti-inflammatory activity: A potential new approach towards COVID-19. *Curr Opin Environ Sci Health*, 17:72-81.
 20. Saha R, Ghosh P, Burra VP. Designing a next generation multi-epitope based peptide vaccine candidate against SARS-CoV-2 using computational approaches. *Biotech*, 11(2):47.
 21. Singhvi N, Singh Y, Shukla P. (2020). Computational approaches in epitope design using DNA binding proteins as vaccine candidate in *Mycobacterium tuberculosis*. *Infect Genet Evol*, 83:104357.
 22. Tao P, Dong J, Zhang Q, Yang J, Zhao Y, Miao Z, Pei S, Qin H, Wen G, Zhang A. (2023). BacScan: An Unbiased and Genome-Wide Approach to Identify Bacterial Highly Immunogenic Proteins. *bioRxiv*.
 23. Vijay-Kumar M, Bovilla VR, Yeoh BS, Golonka RM, Saha P, Joe B, Gewirtz AT. (2023). Bacterial flagellin is a dominant, stable innate immune activator in the gastrointestinal contents of mice and rats. *Gut Microbes*, 15(1):2185031.
 24. Khan K, Burki S, Alsaiani AA, Alhuthali HM, Alharthi NS, Jalal K. (2023). A therapeutic epitopes-based vaccine engineering against *Salmonella enterica* XDR strains for typhoid fever: a Pan-vaccinomics approach. *Journal of Biomolecular Structure and Dynamics*, 9:1-5.

Cite this article: Mehfooz A, Hussain Z, Zafar H. (2023). Insilco Approach for Unraveling Flagellin as a Promising Antigen for Next-Gen DNA Vaccines, *Clinical Research and Reports*, BioRes Scientia Publishers. 1(1):1-6. DOI: 10.59657/2995-6064.brs.23.006

Copyright: © 2023 Humaira Zafar, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Article History: Received: September 07, 2023 | Accepted: September 22, 2023 | Published: September 29, 2023