Evaluation of Egg Drop Syndrome Virus Fiber Protein as a Vaccine Candidate: In Silico Analysis, Expression, Purification and Its Stability

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Keywords: Egg drops syndrome virus, Fiber protein, *In silico*, Purification, Vaccines

Abstract

The egg drop syndrome virus (EDSV), an avian adenovirus, triggers a sharp decline in both egg production and quality in infected chickens, resulting in significant economic impacts for the poultry sector. Based on previous studies, EDSV fiber protein could be a candidate for subunit vaccine against EDSV. Present research focuses on the expression, purification, and thermal stability evaluation of recombinant fiber protein as a vaccine against EDSV. In an *in silico* approach, we investigated the fiber protein structure and expressed it in *Escherichia coli (E. coli)* as a vaccine candidate. We also evaluated the thermal stability of the expressed protein. The protein was expressed predominantly as soluble trimeric proteins in *E. coli* and expression level after nickel-affinity purification was 15 mg/L. Structural analysis using immunological and bioinformatics tools Confirmed retention of the native trimeric conformation in the recombinant

protein. Based on the thermal stability evaluation on this recombinant protein, the protein showed good thermal stability, which can be a good candidate for a vaccine against EDSV.

Abbreviations

BSA: Bovine serum albumin
CAI: Codon adaptation index
CE: Conformational epitopes
EDSV: Egg drop syndrome virus
GRAVY: Grand average of hydropathicity
MFE: Minimum free energy
PBS: Phosphate-buffered saline
TMB: Tetramethylbenzidine

Introduction

Egg drop syndrome virus (EDSV), a member of the Atadenovirus genus [1], represents a significant threat to poultry health worldwide. First identified in the Netherlands in 1976 [2], this non-enveloped, double-stranded DNA virus causes substantial economic losses through its characteristic clinical manifestation: the production of thin-shelled, soft, or shell-less eggs in laying hens. The virus demonstrates broad host specificity, affecting not only chickens but also turkeys, geese, and quails, making it a particularly challenging pathogen to control [3]. The viral capsid comprises 11 structural proteins, among which the fiber protein plays a pivotal role in host cell interaction and viral entry [4]. This trimeric protein consists of three distinct domains tail, shaft, and knob with the knob domain being particularly crucial for initial host cell binding [5]. The immunodominant nature of this protein, combined with its essential function in viral pathogenesis, makes it an ideal candidate for recombinant vaccine development [6]. Current vaccination strategies rely on inactivated vaccines produced in duck eggs, a method that presents several limitations. The dependence on non-specific pathogen-free duck eggs carries inherent risks of pathogen contamination and spread, compounded by the known susceptibility of ducks to

various avian pathogens [7, 8]. These challenges highlight the urgent need for alternative vaccine production methods that can ensure both safety and efficacy. Subunit vaccines comprising viral surface protein components can circumvent these limitations [9, 10]. Recent advances in recombinant DNA technology have opened new avenues for vaccine development, as evidenced by the successful commercialization of numerous recombinant viral vector vaccines against major avian diseases. As reported in a review by Hein *et al*, more than 15 commercially available recombinant viral vector vaccines have been developed against key avian diseases, including Newcastle disease, infectious laryngotracheitis, infectious bursal disease, avian influenza, and Mycoplasma gallisepticum [11]. The potential of subunit vaccines, particularly those targeting key viral surface proteins like the fiber protein, offers a promising solution to the limitations of traditional vaccine production methods [12]. This study addresses these challenges through three primary objectives: 1) Comprehensive *in silico* analysis of the fiber protein structure to identify key immunogenic elements, 2) Development of an efficient expression and purification system for the recombinant fiber protein, and 3) Evaluation of the thermal stability properties of the purified protein. By combining computational biology with experimental validation, we aim to establish a foundation for developing a safer, more effective alternative to current EDSV vaccines. The significance of this work extends beyond EDSV control, as the methodologies developed may be applicable to other avian adenoviruses. Furthermore, the shift to recombinant production methods could significantly improve vaccine safety profiles while reducing production costs and complexity. Our approach aligns with global trends in vaccinology that emphasize rational, structure-based vaccine design and production.

Results

Physicochemical parameter evaluation

Molecular weight, the number of amino acids, and numbers of positively and negatively charged residues of the fiber protein were 28659.33 Da, 268, 15 and 16, respectively. The extinction coefficient of was 34630 M^{-1} cm^{-1} at 280 nm measured in water. The aliphatic index and Grand Average of Hydropathicity (GRAVY) of the chimeric protein were 77.16 and 0.007, respectively. The bio computed half-life was 5.5 h (mammalian reticulocytes, in vitro), 3 min (yeast, in vivo), and 2 min (*E. coli*, *in vivo*). Moreover, the instability index, and pI were 23.39, and 6.14, respectively (Table 1). Additionally, protein solubility (0.840895) was estimated through the SOLpro.

Secondary structures Evaluation

Analysis using the GOR IV server revealed that the secondary structure in our protein contains 7.46% α-helix, 35.07% β-sheet, and 57.46% coiled-coil. According to GOR IV results, it can be seen that random coils are dominant in the fusion protein sequence, followed by extended strands and alpha helices (Fig. 1). Also, using the PSIPRED server, were shown the secondary structure and graphical representation of our protein.

3D structures prediction of the fiber protein and validation

Phyre2, I-TASSER and Galaxy servers were used for 3D structure modeling. The evaluation of the results of different servers showed that the I-TASSER server is suitable for modeling the 3D structure of our protein. The scoring system of I-TASSER server is based on C-score, C-score range is usually between -5 and 2, models with higher C-score (-1.23) were selected for further evaluations. (Fig. 2). Fiber protein refinement was done using GalaxyRefine server to optimize quality. Regarding the role of trimer structure in antigenicity and binding to host cell, trimer

structure of the mentioned construct was modeled by the Galaxy Web server. The evaluation of Ramachandran diagram showed that, 81.2, 15.7, 1.3and 1.8% of residues are located in favored, allowed, generously allowed and disallowed regions, respectively. In order to determine the potential errors in the 3D structures, the analysis of the models has been done by ProSA-webserver (Wiederstein & Sippl, 2007). ProSA z-score of input model was -4.38 (Fig. 3).

Antigenicity evaluation

ANTIGENpro and Vaxijen servers predicted antigenicity probabilities of 93% and 100%, respectively. The results obtained from the above servers showed that with high probability, engineered fiber protein is antigenic.

Prediction of B-cell epitopes

The linear epitope prediction results of the target protein by Ellipro and SVMTriP server are shown in the Tables 2 and 3. B-cell epitopes were computationally ranked by prediction scores derived from ABCpred's neural network model (Table 4). Prediction scores directly correlate with epitope probability (threshold= 0.5), with all shown peptides exceeding this cutoff value. The results of discontinuous epitope prediction by Ellipro server are shown in (Table 5) Predicted conformational epitopes including: conformational epitopes 1 (CE1) and conformational epitopes 2 (CE2), conformational epitopes 3 (CE3), conformational epitopes 4 (CE4), which having score greater than 0.5. Three discontinuous epitopes were identified with substantial surface accessibility: CE1 (68 residues, 68.5% exposed), CE2 (35 residues, 65.9% exposed), and CE3 (55 residues, 63.7% exposed). The significant solvent exposure of these regions (all scores >0.63) supports their inclusion in vaccine design.

MHC-I binding prediction analysis

MHC-I processing tool identified 259 potential nonamer peptides from the fiber protein that may bind MHC-I and trigger immune responses. Three peptide fiber protein sequence *i.e.*, QESIRFGLV and GELTLAYDS and TENGLALKV were determined, which are likely to be to bind with MHC-I alleles, with percentage rank ≤ 2 (Table 6).

MHC-II binding prediction analysis

For MHC class II binding prediction, the fiber protein sequence was fragmented into 254 overlapping 15-mer peptides using NetMHCIIpan 4.0. LTRIISMGNNLFDSG were considered to be strong MHC-II binders. The optimal peptide binding core of this peptide was IISMGNNLF (Table 7).

Prediction of mRNA structure

Based on the results of the RNA fold server, the minimum free energy of the RNA structure is (-255.60 kcal/mol), which can be concluded that the gene structural sequence can form a stable RNA secondary structure. (Fig. 4).

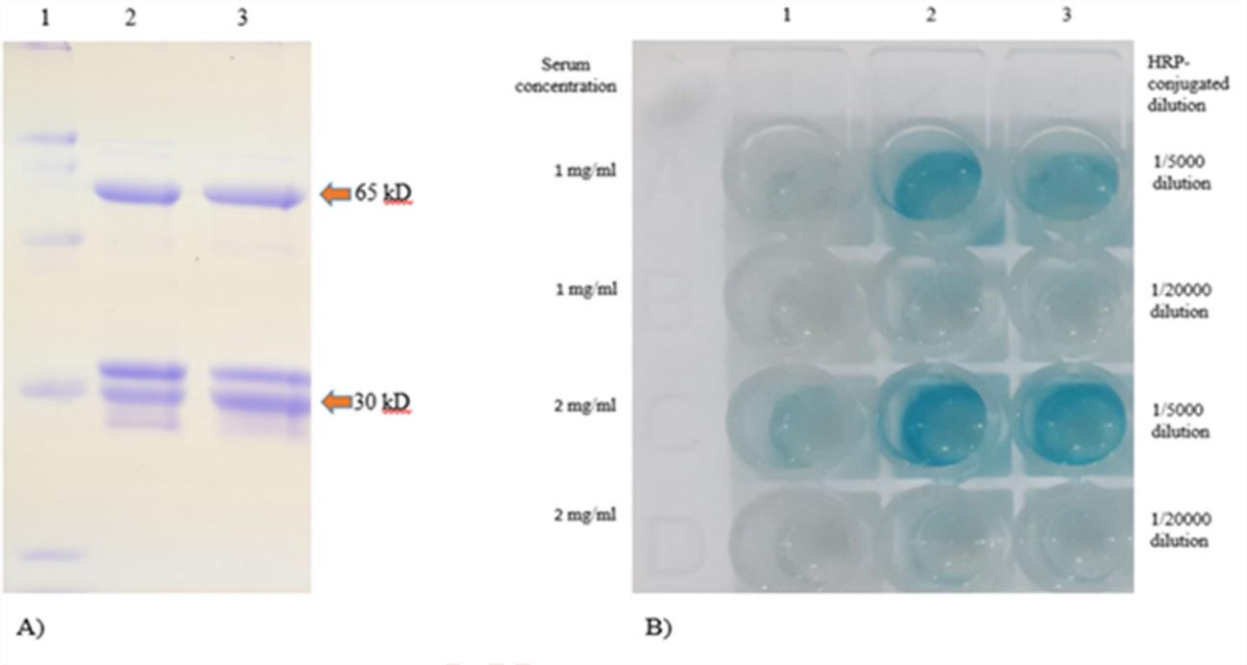
Gene expression and protein purification

Codon adaptation of fiber protein was performed by Java codon adaptation tool (JCAT) in order to codon optimization in eukaryotic expression system. The optimized codon sequence length for our construct with 268 aa was 804 nucleotides. CAI value for optimized nucleotide sequence was 0.99, and CG-content of sequence was 53%. It shows the possibility of expression of protein in eukaryotic system (Fig. 5). The pET-fiber protein construct was successfully transformed into E. coli SHuffle cells, a strain optimized for disulfide bond formation in cytoplasmic proteins. Following induction, we observed robust expression of the target protein, with SDS-PAGE

analysis revealing a prominent band at approximately 30 kDa, consistent with the predicted molecular weight of the monomeric fiber protein subunit (Fig. 6).

Thermal stability evaluation

Analysis of the recombinant fiber protein by SDS-PAGE revealed distinct oligomeric states under different treatment conditions (Figs. 6A, 7). Under native (non-boiled) conditions, the protein migrated as two predominant bands corresponding to molecular weights of 30 kDa (monomeric form) and 65 kDa (trimeric form), with the trimeric form representing the primary conformation. This trimeric structure demonstrated remarkable stability, maintaining its oligomeric state even in the presence of SDS at ambient temperature. However, thermal denaturation by boiling prior to electrophoresis resulted in complete dissociation of trimers, yielding exclusively monomeric 30 kDa bands. Notably, pre-incubation of samples at different temperatures (4°C *vs* 37°C) prior to analysis did not affect these migration patterns, suggesting temperature-independent stability of the protein's oligomeric states under non-denaturing conditions. Parallel ELISA results revealed that the purified recombinant fiber protein maintained strong antigenic properties under both tested storage conditions (4°C and 37°C), as shown in the Figure 7B.



Discussion

The development of effective vaccines against avian pathogens remains challenging due to the time-consuming and costly nature of traditional vaccine production methods [13]. Recent advances in computational immunology and structural biology have enabled more rational vaccine design approaches, as demonstrated in this study through the comprehensive bioinformatics and experimental characterization of the EDSV fiber protein as a promising vaccine candidate. Our work aligns with emerging trends in epitope-based vaccine design, building upon successful examples such as immunoinformatics approach for MARV and cross-protective vaccine for Mpox and EBOV. These studies collectively highlight the transformative potential of computational tools in identifying stable, immunogenic vaccine candidates while significantly reducing development timelines [14-16]. Key structural findings from our study provide crucial insights for vaccine development. We confirmed the stability of the trimeric conformation through both computational prediction using the Galaxy server [17] and experimental validation via SDS-PAGE, with the

protein maintaining structural integrity at physiological temperatures (37°C for 14 days). This stability was further supported by favorable physicochemical parameters, including an aliphatic index of 77.16 and instability index of 23.39. Our construct strategically incorporated both the knob domain and partial shaft region (268aa) based on previous studies demonstrating their critical role in maintaining proper trimer formation and antigenicity [7, 18]. The preservation of conformational epitopes with >63% surface exposure (CE1-CE3) suggests strong potential for eliciting protective immune responses. From a production standpoint, the recombinant protein demonstrated several advantageous characteristics. We achieved high-yield expression in *E. coli* Shuffle [19], with clear identification of both monomeric (30 kDa) and trimeric (65 kDa) forms. The mRNA stability (MFE= -255.60 kcal/mol) and retained antigenicity post-temperature challenge (as validated by ELISA) further support the practical utility of this vaccine candidate. These results are particularly encouraging given the well-documented challenges of maintaining adenovirus fiber protein stability during production and storage. However, several important questions remain to be addressed in future studies. The compatibility of our antigen with various adjuvants requires systematic evaluation, as adjuvant selection can significantly impact both immunogenicity and stability [20]. Comprehensive *in vivo* studies in chicken models will be essential to confirm the protective efficacy suggested by us *in silico* and *in vitro* results. Additional investigations into long-term storage stability and large-scale production optimization will be crucial for translational applications [10]. Structural refinement through techniques like cryo-EM could provide atomic-level insights into the trimer conformation and potential stabilization strategies [21]. This study makes significant contributions to multiple aspects of vaccine development. It demonstrates the power of integrating computational and experimental approaches for rational vaccine design, particularly for veterinary applications. Our focus on thermal stability

addresses a critical limitation in vaccine distribution, especially relevant for resource-limited settings. The framework established here for EDSV could be readily adapted for other avian pathogens, potentially transforming poultry vaccine development pipelines [22]. While the current results are promising, we acknowledge certain limitations. The absence of *in vivo* immunogenicity data represents a key gap that will be addressed in subsequent studies. The effects of adjuvants on both stability and immunogenicity remain to be characterized, and scale-up production parameters need optimization. Nevertheless, our multidisciplinary approach exemplifies how modern computational tools can accelerate and improve vaccine development while maintaining rigorous scientific standards [14]. By combining robust computational predictions with systematic experimental validation, we have established a strong foundation for developing an effective EDSV vaccine while minimizing animal use in preliminary testing stages. Future work will focus on translating these promising results into practical solutions for avian health, with potential implications for vaccine development against related pathogens.

Conclusion

In this study, fiber protein was investigated by *in silico* method. Based on the structural and immunological results, this recombinant protein can be used as a preventive vaccine, although more experimental studies are needed, and if confirmed using experimental studies, it can be used as a vaccine candidate against EDSV in the future.

Materials and Methods

Sequence selection and design of construct

This study utilized the amino acid sequence of the partial fiber protein A0A482J640, obtained from the UniProt Knowledgebase (https://www.uniprot.org/) in FASTA format, which comprises the knob domain and a segment of the shaft region.

Prediction of physicochemical properties

Various physicochemical parameters of the designed fiber protein were predicted using ProtParam, encompassing molecular weight, theoretical isoelectric point (pI), amino acid composition and count, atomic composition, chemical formula, extinction coefficients, estimated half-life, aliphatic index, instability index, and grand average of hydropathicity (GRAVY). Additionally, protein solubility was evaluated through the SOLpro (http://scratch.proteomics.ics.uci.edu/) and Protein-Sol (https://protein-sol.manchester.ac.uk/) servers.

Secondary structure prediction of fiber protein

Bioinformatic analysis of secondary structure was performed using the amino acid sequence as input for GOR IV and PSIPRED. These programs calculated probabilities for α-helix, β-sheet, and random coil at every amino acid position, with the highest probability structure chosen as the predicted conformation [23, 24].

3D structures prediction

Protein 3D structure prediction was performed using Phyre2, I-TASSER, and GalaxyWeb servers [25-27].The I-TASSER server, which provides a confidence score (C-score) for model evaluation, was chosen for final modeling based on comparative results. The Galaxy server generated the trimer structure. Model quality was verified through Ramachandran plot analysis and ProSA-web evaluation [28, 29].

Antigenicity prediction

Antigenicity Evaluation of the fiber protein was performed using VaxiJen version 3.0, which is available at (https://www.ddg-pharmfac.net/vaxijen3/home/) and can predict antigenicity based solely on Physical and chemical properties of antigens [30]. ANTIGENpro server was also used to evaluate antigenicity.

B-cell epitopes prediction

Computational prediction identified both continuous (linear) and discontinuous (structural) B-cell epitopes in the fiber protein sequences. (knob + shaft) using the EliPro server. EliPro server scoring is based on a score of 0 to 1 and a cutoff of 0.5, where a score higher than 0.5 is considered an epitope and a score less than 0.5 is considered a non-epitope [31]. SVMTriP server [32]. were also used. ABCpred Prediction Server predict B cell epitopes in a protein sequence [33], using artificial neural network.

MHC class I and II binding prediction

The NetMHCcons 1.1 server was employed to predict MHC class I binding affinities. The threshold for strong binding peptides was determined as an IC50 value of less than 2 nM, and cutoff of 50 nM has been set for weak binders, that is, peptides with IC50< 2 nM were considered as strong binders and those with IC50 < 50 nM as weak binders [34]. Fiber protein sequences (shaft + knob) were submitted to the NetMHCcons 1.1 server. The NetMHCIIpan 4.0 algorithm was employed to identify potential MHC-II binding epitopes. The fiber protein was cleaved into peptides with a length of 15 amino acids in the server. Among these peptides, only those peptides that were strong binders (%Rank < 2) for MHC alleles were selected [35]. In this study, based on previous studies the allele (HLA*B 40:06) was used for MHC class I and the allele (DRB1:1482) was used for MHC class II [36]. Using MHCcluster software, Thomsen et al showed that human MHC-I/II alleles are the best substitute for its homologues in chicken [37].

mRNA structure prediction

The RNA fold server was used to analyze the minimum free energy (MFE), which is a loop-based energy model presented by Zucker et al., for the secondary structures formed by RNA molecules [38].

Optimization of the synthetic gene

Codon optimization and reverse translation were conducted using the Java Codon Adaptation Tool (JCat) online software, with parameters set to maximize the codon adaptation index (CAI) for cloning and expression in *E. coli* K12. [39]. NEB Cutter Server (New England Biolabs) was used to find the presence of common restriction enzyme sites in the nucleotide sequence of the designed construct (http://www.labtools.us/nebcutter-v2-0/). Parameters such, codon adaptation index (CAI), and GC content were also analyzed and codon adaptation index (CAI), and GC content are also evaluated.

Protein expression and purification protocol

Following gene synthesis by ShineGene Co., the fiber protein gene was subcloned into the pET-28a vector (Novagen, 2023) to generate recombinant pET-fiber plasmids. These constructs were subsequently transformed into *E. coli* SHuffle strain competent cells [19] via heat shock method [40]. Transformed colonies were selected through overnight growth at 37°C in LB medium supplemented with 50 μg/mL kanamycin. For protein expression, starter cultures were diluted 1:100 into fresh LB-kanamycin medium and grown at 37°C with shaking until reaching mid-log phase (OD_{600}= 0.4-0.6), typically requiring 3-5 hours. Protein expression was then induced by adding 0.5 mM, IPTG [41], followed by overnight incubation at 17°C with 150 rpm shaking to enhance proper protein folding. After induction, following centrifugation at 5,000 ×g for 10 min, cell pellets were collected and resuspended in lysis buffer for subsequent protein extraction. Initial

expression analysis was performed by resolving whole cell lysates on 12.5%, SDS-PAGE gels. The recombinant protein was subsequently purified under native conditions using Ni-NTA Sepharose Fast Flow resin (ARG Biotech, Iran) to isolate the His-tagged fusion protein."

Thermal stability of the recombinant protein

For thermal stability analysis of the recombinant protein, purified samples were subjected to two storage conditions: 1-incubation at 37°C for 14 days to simulate physiological temperature stress, and 2- refrigeration at 4°C as a stability control. Protein integrity under both conditions was assessed through 12.5%, SDS-PAGE analysis. Antigenic properties were examined using a standardized ELISA protocol with the following steps: 96-well microplates were coated overnight at 4°C with 1 µg/mL of recombinant fiber protein in carbonate-bicarbonate coating buffer (100 mM $NaHCO_3$, pH 9.6). After four washes with PBS, plates were blocked for 1 hour with 1%, bovine serum albumin (BSA) in PBS-T (PBS containing 0.05%, Tween 20). Serial dilutions (1 mg/mL and 2 mg/mL) of chicken immune serum (containing EDSV-specific antibodies from inactivated vaccine immunization) were added and incubated for 1 hour at room temperature. Post-washing with PBS (3×5 min), plates were incubated with horseradish peroxidase-conjugated goat anti-chicken IgG secondary antibody for 60 minutes at 25°C with gentle shaking (Razi Biotech Co. Iran) at two working dilutions (1:5000 and 1:20000 in PBS-T). Tetramethylbenzidine (TMB) substrate (0.01% 3,3',5,5'-Tetramethylbenzidine, 99% Water, 0.1% Hydrogen peroxide, pH 3.3-4.0), which is a common substrate for horseradish peroxidase (HRP) and suitable for enzyme-linked immunosorbent assay (ELISA), was added for color development. Then, this reaction was terminated with 2M, H_2SO_4, and absorbance was measured at 450 nm using a microplate spectrophotometer.

References

1. Benkő M, Aoki K, Arnberg N, Davison AJ, Echavarría M, Hess M, et al. ICTV Virus Taxonomy Profile: Adenoviridae 2022. J Gen Virol. 2022;103(3).
2. Cha SY, Kang M, Moon OK, Park CK, Jang HK. Respiratory disease due to current egg drop syndrome virus in Pekin ducks. Vet Microbiol. 2013;165(3-4):305-11.
3. Swayne DE BM, Logue CM, McDougald LR, Nair V, Suarez DL. Diseases of Poultry. 14ed: Wiley-Blackwell; 2020.
4. San Martín C. Latest insights on adenovirus structure and assembly. Viruses. 2012;4(5):847-77.
5. Song Y, Wei Q, Liu Y, Feng H, Chen Y, Wang Y, et al. Unravelling the receptor binding property of egg drop syndrome virus (EDSV) from the crystal structure of EDSV fiber head. Int J Biol Macromol. 2019;139:587-95.
6. Graham BS, Gilman MSA, McLellan JS. Structure-Based Vaccine Antigen Design. Annu Rev Med. 2019;70:91-104.
7. Fingerut E, Gutter B, Gallili G, Michael A, Pitcovski J. A subunit vaccine against the adenovirus egg-drop syndrome using part of its fiber protein. Vaccine. 2003;21(21-22):2761-6.
8. Alexander DJ. An overview of the epidemiology of avian influenza. Vaccine. 2007;25(30):5637-44.
9. Li D, Huang Z, Zhong J. Hepatitis C virus vaccine development: old challenges and new opportunities. National science review. 2015;2(3):285-95.
10. Abdelaziz K, Helmy YA, Yitbarek A, Hodgins DC, Sharafeldin TA, Selim MSH. Advances in Poultry Vaccines: Leveraging Biotechnology for Improving Vaccine Development, Stability, and Delivery. Vaccines (Basel). 2024;12(2).
11. Hein R, Koopman R, Garcia M, Armour N, Dunn JR, Barbosa T, et al. Review of Poultry Recombinant Vector Vaccines. Avian Dis. 2021;65(3):438-52.
12. Plotkin S, Robinson JM, Cunningham G, Iqbal R, Larsen S. The complexity and cost of vaccine manufacturing - An overview. Vaccine. 2017;35(33):4064-71.
13. Farhadi T, Nezafat N, Ghasemi Y, Karimi Z, Hemmati S, Erfani N. Designing of complex multi-epitope peptide vaccine based on omps of Klebsiella pneumoniae: an in silico approach. International Journal of Peptide Research and Therapeutics. 2015;21:325-41.
14. Al-Zayadi FQJ, Shakir AS, Kareem AS, Ghasemian A, Behmard E. Design of a novel multi-epitope vaccine against Marburg virus using immunoinformatics studies. BMC Biotechnology. 2024;24(1):45.
15. Hensley LE, Mulangu S, Asiedu C, Johnson J, Honko AN, Stanley D, et al. Demonstration of cross-protective vaccine immunity against an emerging pathogenic Ebolavirus Species. PLoS Pathog. 2010;6(5):e1000904.

16. Saadh MJ, Ghadimkhani T, Soltani N, Abbassioun A, Daniel Cosme Pecho R, Taha A, et al. Progress and prospects on vaccine development against monkeypox infection. Microb Pathog. 2023;180:106156.

17. Ko J, Park H, Heo L, Seok C. GalaxyWEB server for protein structure prediction and refinement. Nucleic Acids Res. 2012;40 (Web Server issue):W294-7.

18. Song Y, Wei Q, Liu Y, Bai Y, Deng R, Xing G, et al. Development of novel subunit vaccine based on truncated fiber protein of egg drop syndrome virus and its immunogenicity in chickens. Virus Res. 2019;272:197728.

19. Lobstein J, Emrich CA, Jeans C, Faulkner M, Riggs P, Berkmen M. SHuffle, a novel Escherichia coli protein expression strain capable of correctly folding disulfide bonded proteins in its cytoplasm. Microb Cell Fact. 2012;11:56.

20. Moyle PM, Toth I. Modern subunit vaccines: development, components, and research opportunities. ChemMedChem. 2013;8(3):360-76.

21. Nogales E, Scheres SH. Cryo-EM: A Unique Tool for the Visualization of Macromolecular Complexity. Mol Cell. 2015;58(4):677-89.

22. Schachner A, Matos M, Grafl B, Hess M. Fowl adenovirus-induced diseases and strategies for their control - a review on the current global situation. Avian Pathol. 2018;47(2):111-26.

23. Sen TZ, Jernigan RL, Garnier J, Kloczkowski A. GOR V server for protein secondary structure prediction. Bioinformatics. 2005;21(11):2787-8.

24. Buchan DWA, Jones DT. The PSIPRED Protein Analysis Workbench: 20 years on. Nucleic Acids Res. 2019;47(W1):W402-7.

25. Baek M, Park T, Heo L, Park C, Seok C. GalaxyHomomer: a web server for protein homo-oligomer structure prediction from a monomer sequence or structure. Nucleic Acids Res. 2017;45(W1):W320-4.

26. Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. Nat Protoc. 2015;10(6):845-58.

27. Yang J, Yan R, Roy A, Xu D, Poisson J, Zhang Y. The I-TASSER Suite: protein structure and function prediction. Nat Methods. 2015;12(1):7-8.

28. Williams CJ, Headd JJ, Moriarty NW, Prisant MG, Videau LL, Deis LN, et al. MolProbity: More and better reference data for improved all-atom structure validation. Protein Science. 2018;27(1):293-315.

29. Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res. 2007;35(Web Server issue):W407-10.

30. Doytchinova IA, Flower DR. VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. BMC bioinformatics. 2007;8(1):4.

31. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, et al. The Immune Epitope Database (IEDB): 2018 update. Nucleic Acids Res. 2019;47(D1):D339-43.

32. Yao B, Zhang L, Liang S, Zhang C. SVMTriP: a method to predict antigenic epitopes using support vector machine to integrate tri-peptide similarity and propensity. PLoS One. 2012;7(9):e45152.

33. Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res. 2017;45(W1):W24-9.

34. Karosiene E, Lundegaard C, Lund O, Nielsen M. NetMHCcons: a consensus method for the major histocompatibility complex class I predictions. Immunogenetics. 2012;64(3):177-86.

35. Andreatta M, Karosiene E, Rasmussen M, Stryhn A, Buus S, Nielsen M. Accurate pan-specific prediction of peptide-MHC class II binding affinity with improved binding core identification. Immunogenetics. 2015;67(11-12):641-50.

36. Valdivia-Olarte H, Requena D, Ramirez M, Saravia LE, Izquierdo R, Falconi-Agapito F, et al. Design of a predicted MHC restricted short peptide immunodiagnostic and vaccine candidate for Fowl adenovirus C in chicken infection. Bioinformation. 2015;11(10):460-5.

37. Thomsen M, Lundegaard C, Buus S, Lund O, Nielsen M. MHCcluster, a method for functional clustering of MHC molecules. Immunogenetics. 2013;65(9):655-65.

38. Lorenz R, Bernhart SH, Höner Zu Siederdissen C, Tafer H, Flamm C, Stadler PF, et al. ViennaRNA Package 2.0. Algorithms Mol Biol. 2011;6:26.

39. Grote A, Hiller K, Scheer M, Munch R, Nortemann B, Hempel DC, et al. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. Nucleic Acids Res. 2005;33(Web Server issue):W526-31.

40. Green MRaS, J. . Molecular cloning : a laboratory manual 4th ed: Cold Spring Harbor Laboratory Pres; 2012.

41. Studier FW. Stable expression clones and auto-induction for protein production in E. coli. Methods Mol Biol. 2014;1091:17-32.

Figures and Tables

Figure 1. Fiber protein secondary structure predicted by GOR4 server. (A) The composition of the secondary structure in percent (B) fiber protein secondary structure map (h: alpha-helix; e: extended strand; c: coil). (C) diagram of fiber protein secondary structure (the blue color represents the alpha-helices; the red color represents beta strands, and the purple color was the coils)

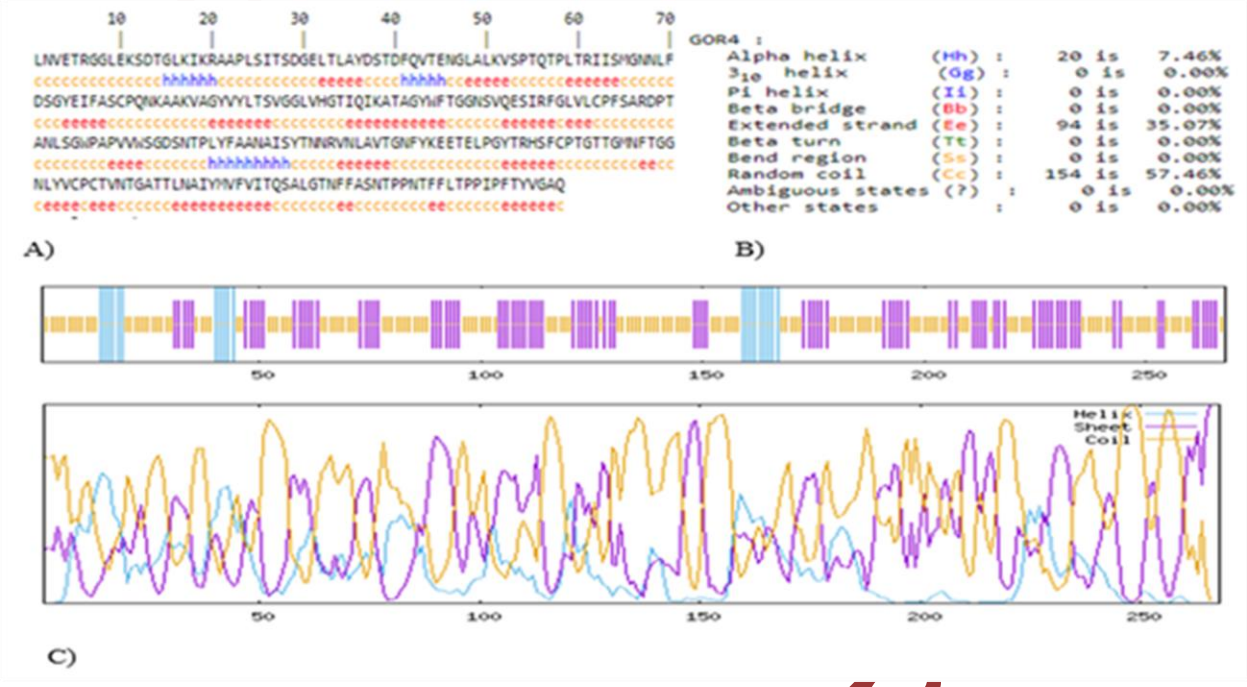


Figure 2. Tertiary structure prediction. A probabilistic structural model for fiber protein by online servers: A) Monomer prediction by I-TASSER server; B); prediction of trimer structure by Galaxyhomomer

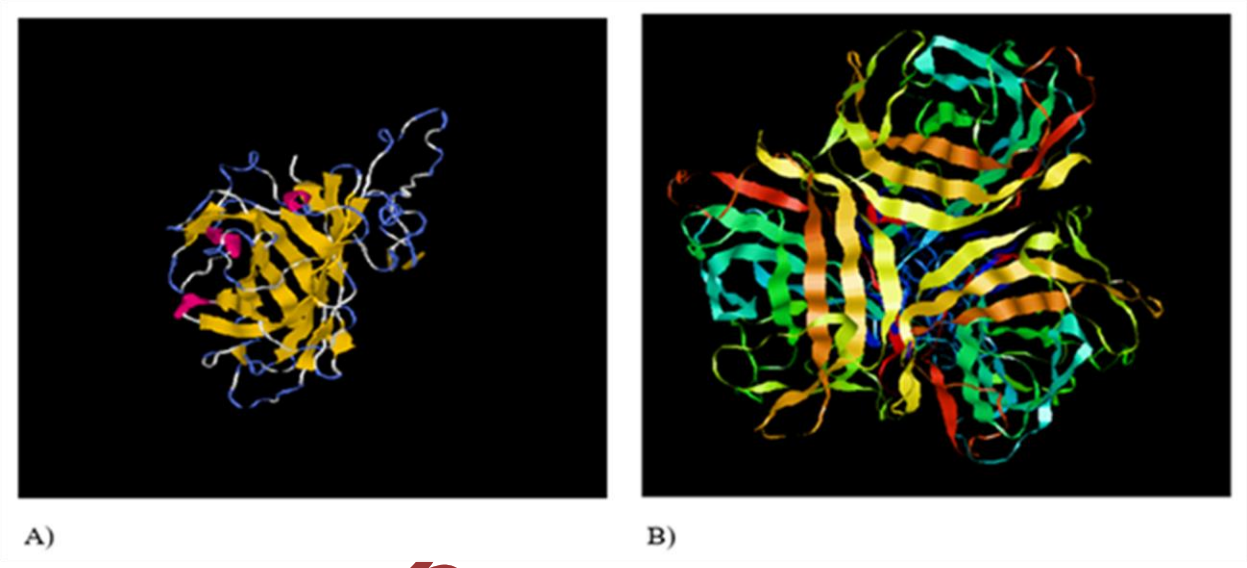


Figure 3. Model stability was evaluated based on Ramachandran plot for fiber protein: A) Ramachandran plots for trimer structure fiber protein by PROCHEC; B) Fiber protein structure analysis by ProSA- web server

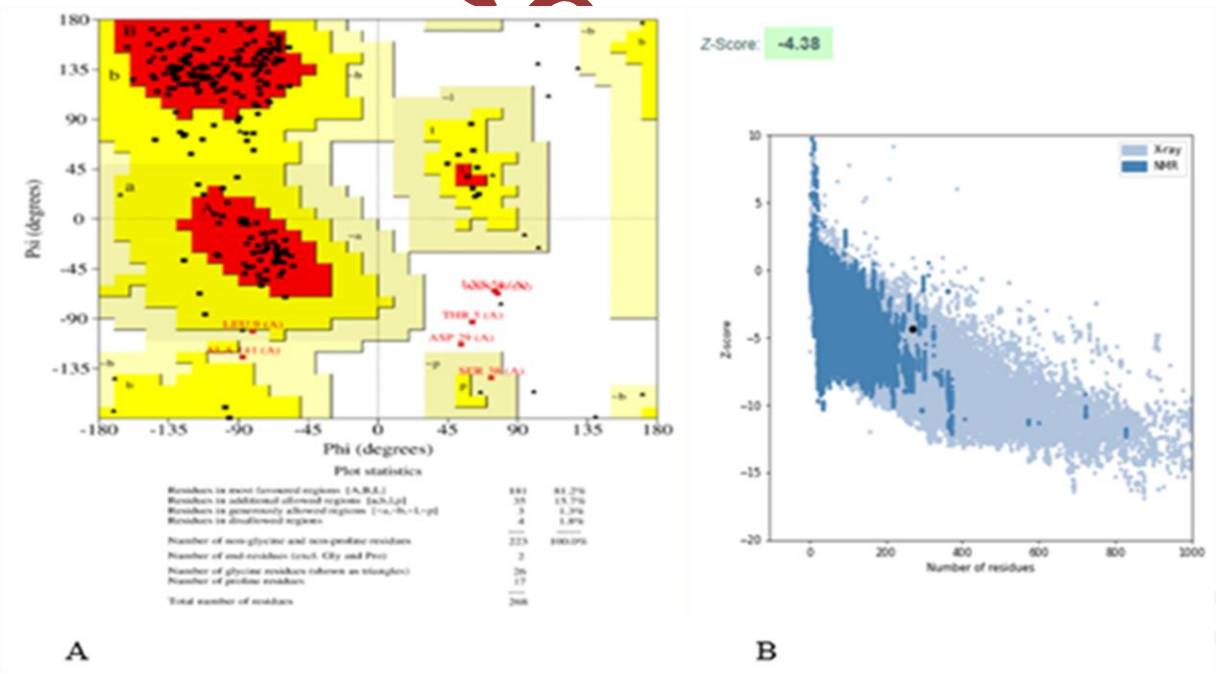


Figure 4. Predicted mRNA secondary structure by RNA fold server: A) Evaluation of the minimum free energy. The optimal secondary structure with the minimum free energy is indicated in the dot-bracket notation, which is colored by the positional entropy. The shown interactive drawing represents the RNA structure with the minimum free energy, colored based on the probabilities of base pairing. [((())) stem; …. loop; ,,,,,, internal loop]; B) The mountain plot of the MFE structure, RNA structures thermodynamic ensemble, and the centroid structure. Plateaus correspond to loops in the mountain plot (with hairpin loops representing peaks), while slopes represent helices. (The red, green and blue lines referred to the minimum free energy (MFE), the probability of the pair (PF) and, the centroid algorithm, respectively. Also, the positional entropy was indicated below; C) The mRNA secondary structure.

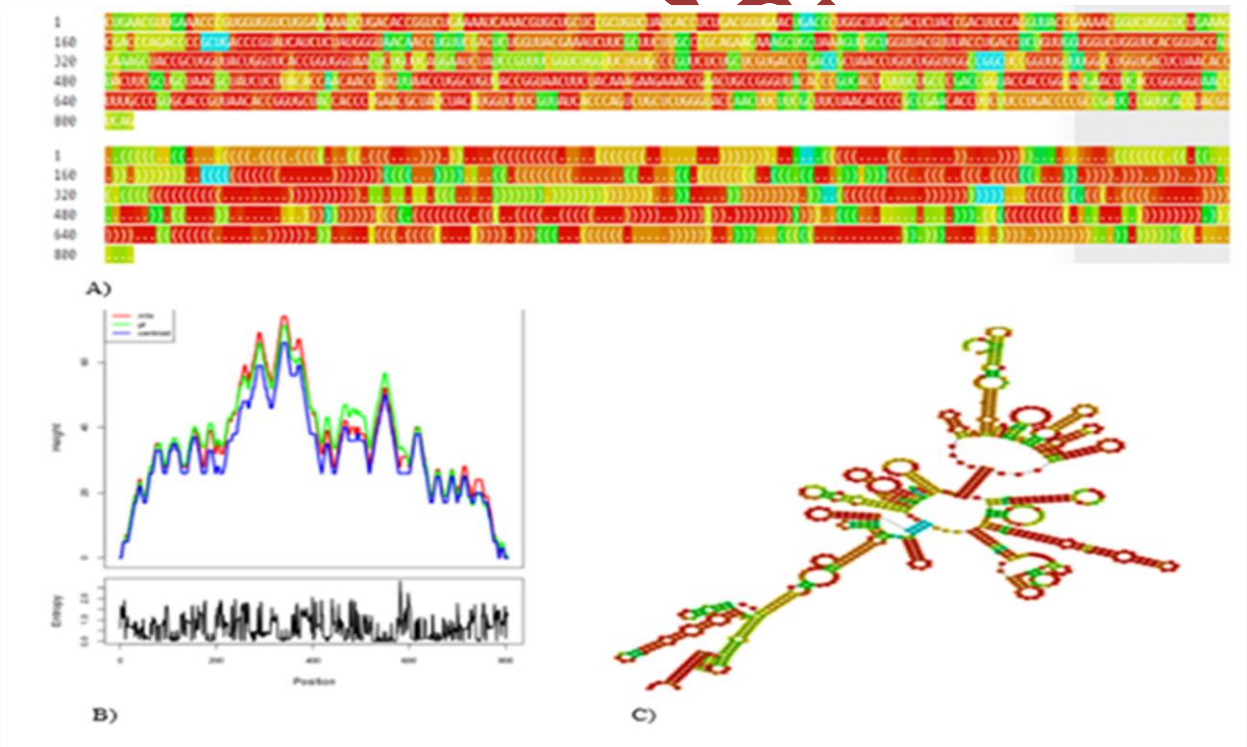
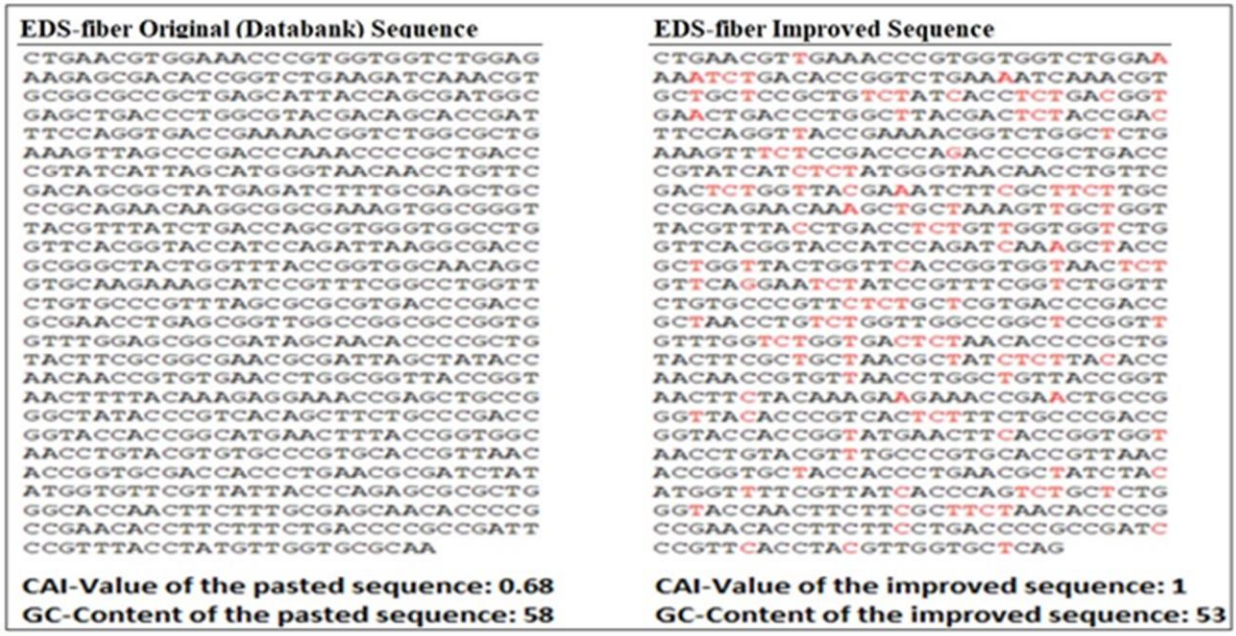


Figure 5. Optimization of the synthetic gene for expression of the recombinant protein: A) Optimization by Jcat; B) Analysis by GenRCA Rare Codon Analysis Tool.



A)

Index	Description	Range	Value
GC3	GC Content at the Third Position of Synonymous Codons	0~1	0.76
GC	GC Content		0.59
GC1	GC Content at the First Position of Synonymous Codons		0.51
CAI	Codon Adaptation Index	0~1	0.91
CFD	Codon Frequency Distribution	0~1	0
FOP	Frequency of Optimal Codons	0~1	0.74
CBI	Codon Bias Index	-1~1	0.61

B)

Figure 6. Protein pattern of the LB crude extract and purified fiber protein in the SDS-PAGE: A) SDS-PAGE analysis of level of expression of fiber protein in LB broth, Lane1= Protein molecular weight marker (77-14 kDa), lane2= Soluble part of supernatant containing fiber protein, Lanes3 and 4= Pellet of *E. coli* shuffle including pET-fiber not and induced with IPTG respectively, grown in LB broth; B) Expression and purification of fiber protein (PET28-fiber) as a candidate vaccine in *E. coli* cells by

SDS-PAGE, Lane 7= Protein molecular weight marker; lane 9= Non induced, lane8= Induced *E. coli* cells bearing fiber protein-PET28 plasmid, lane 1 to 6= Elution 1 to 6.

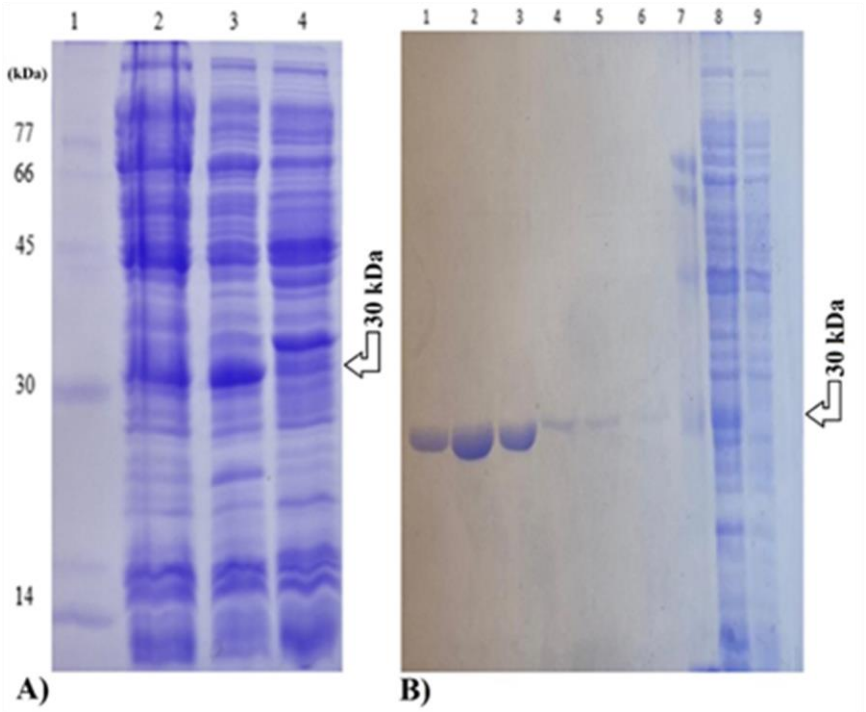


Figure 7. Thermal stability evaluation by SDS-PAGE and ELISA. A) SDS-PAGE of purified fiber protein: lane 1, Protein molecular weight marker (77-14 kDa), lane2; Protein fiber stored in 4° C, lane3; Protein fiber stored in 37° C. B) ELISA assay in order to evaluate antigenicity. lane1; negative control, lane2; Protein fiber stored in 4°C that coated as antigen, lane3; Protein fiber stored in 37° C that coted as antigen**.**

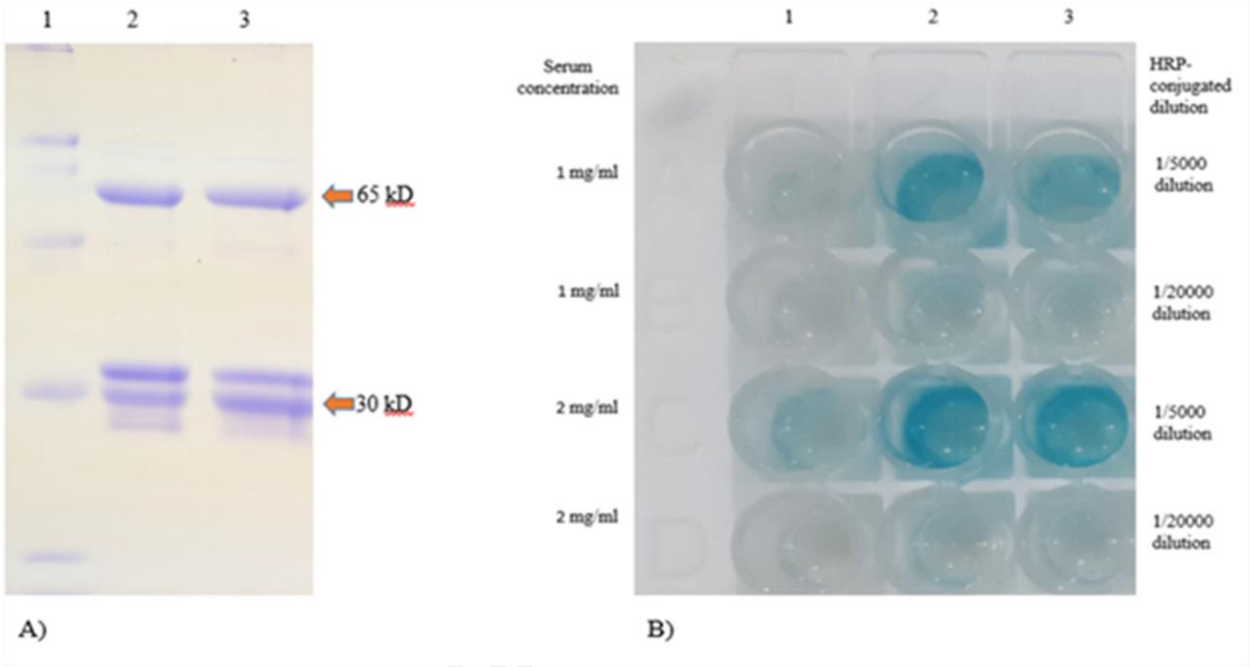


Table 1. Parameters analyzed by the Expasy ProtParam server

Sequence length	Mw*	T pI	-R	+R	EC	II	AI	GRAVY
268	28659.33	6.14	16	15	34630	23.39	77.16	0.007

*Mw, Molecular weight; T pI, Theoretical Isoelectric point; -R, Number of negative charged residues; +R, Number of positive charged residues; EC, Extinction coefficient at 280 nm; II, Instability index; AI, Aliphatic index; GRAVY, Grand Average Hydropathy

Table 2. Linear B-cell epitope of fiber protein predicted using the SVMTriP server

Rank	Location	Epitope	Score
1	91 – 110	YVYLTSVGGLVHGTIQIKAT	1
2	173 – 192	RVNLAVTGNFYKEETELPGY	0.807
3	131 – 150	LCPFSARDPTANLSGWPAPV	0.605

Table 3. Linear B-cell epitope of fiber protein predicted using the ElliPro server

No.	Start	End	Peptide	Residues No.	Score
1	35	59	AYDSTDFQVTENGLALKVSPTQTPL	25	0.79
2	236	254	TQSALGTNFFASNTPPNTF	19	0.79
3	218	226	TVNTGATTL	9	0.784
4	200	208	TGTTGMNFT	9	0.737
5	135	141	SARDPTA	7	0.663
6	110	124	TAGYWFTGGNSVQES	15	0.661
7	165	192	NAISYTNNRVNLAVTGNFYKEETELPGY	28	0.646
8	68	75	NLFDSGYE	8	0.597
9	9	24	LEKSDTGLKIKRAAPL	16	0.591
10	153	158	SGDSNT	6	0.567
11	26	29	ITSD	4	0.548

Table 4. Predicted Epitope by ABCpred Prediction Server

Rank	Sequence	Start position	Score
1	GTTGMNFTGGNLYVCP	201	0.93
2	GNSVQESIRFGLVLCP	118	0.92
3	SGDSNTPLYFAANAIS	153	0.9

3	LSGWPAPVVWSGDSNT	143	0.9
4	ANAISYTNNRVNLAVT	164	0.89
5	GNLYVCPCTVNTGATT	210	0.88
5	KATAGYWFTGGNSVQE	108	0.88
6	GELTLAYDSTDFQVTE	30	0.87
7	VSPTQTPLTRIISMGN	52	0.86
7	NFFASNTPPNTFFLTP	243	0.86
7	TQSALGTNFFASNTPP	236	0.86
8	SGYEIFASCPQNKAAK	72	0.85
8	VETRGGLEKSDTGLKI	3	0.85
8	HGTIQIKATAGYWFTG	102	0.85
9	RIISMGNNLFDSGYEI	61	0.83
10	PFSARDPTANLSGWPA	133	0.8

Table 5. Conformational B-cell epitope of fiber protein vaccine predicted using the ElliPro server

No.	Peptide regions and residues number	Residues No.	Score
1	A:N67, A:N68, A:L69,A:F70, A:D71, A:S72, A:G73, A:Y74, A:E75, A:Q82, A:N83, A:K84, A:T110, A:A111, A:G112, A:Y113, A:W114, A:F115, A:T116, A:G117, A:G118, A:N119, A:S120, A:V121, A:Q122, A:E123, A:S124, A:I125, A:A177, A:V178, A:T179, A:G180, A:N181, A:F182, A:Y183, A:K184, A:E185, A:T187, A:E188, A:P199, A:T200, A:G201, A:T202, A:T203, A:G204, A:M205, A:N206, A:F207, A:T208, A:T236, A:Q237, A:S238, A:A239, A:L240, A:G241, A:T242, A:N243, A:F244, A:F245, A:A246, A:S247, A:N248, A:T249, A:P250, A:P251, A:N252, A:T253, A:F254	68	0.685
2	A:F134, A:S135, A:A136, A:R137, A:D138, A:P139, A:T140, A:A141, A:N165, A:A166, A:I167, A:S168, A:Y169, A:T170, A:N171, A:N172, A:R173, A:N175, A:L189, A:P190, A:G191, A:Y192, A:T218, A:V219, A:N220, A:T221, A:G222, A:A223, A:T224, A:T225, A:L226, A:N227, A:G266, A:A267, A:Q268	35	0.659
3	A:N2, A:T5, A:R6, A:G7, A:G8, A:L9,A:E10, A:K11, A:S12, A:D13, A:T14, A:G15, A:L16, A:K17, A:I18, A:K19, A:R20, A:A21, A:A22, A:P23, A:L24, A:S25, A:I26, A:T27, A:S28, A:D29, A:G30, A:E31, A:L32, A:T33, A:A35, A:Y36, A:S38, A:T39, A:D40, A:F41, A:Q42, A:V43, A:T44, A:E45, A:N46, A:G47, A:L48, A:A49, A:L50, A:K51, A:V52, A:S53, A:P54, A:T55, A:Q56, A:T57, A:P58, A:L59, A:T60	55	0.637
4	A:V151, A:S153, A:G154, A:D155, A:S156, A:N157, A:T158	7	0.548

Table 6. MHC-I binding epitopes predicted by the NetMHCcons 1.1 server

Allele	Start	Length	Peptide	1-log50k (aff)	Affinity (nM)	% Rank
HLA-B40:06	29	9	GELTLAYDS	0.247	3441.22	2.00
HLA-B40:06	43	9	TENGLALKV	0.330	1407.46	1.50
HLA-B40:06	121	9	QESIRFGLV	0.287	2244.37	1.50

Table 7. MHC-II binding epitopes predicted by the NetMHC 4.0 server.

Allele	Start	Peptide	Core Sequence	Score EL	PR*
DRB1_1454	13	DTGLKIKRAAPLSIT	LKIKRAAPL	0.367271	3.55
DRB1_1454	14	TGLKIKRAAPLSITS	IKRAAPLSI	0.340573	4.11
DRB1_1454	15	GLKIKRAAPLSITSD	IKRAAPLSI	0.419096	2.66
DRB1_1454	58	PLTRIISMGNNLFDS	IISMGNNLF	0.393941	3.04
DRB1_1454	59	LTRIISMGNNLFDSG	IISMGNNLF	0.464146	2.10
DRB1_1454	60	TRIISMGNNLFDSGY	IISMGNNLF	0.358547	3.73
DRB1_1454	101	VHGTIQIKATAGYWF	IQIKATAGY	0.336206	4.22
DRB1_1454	102	HGTIQIKATAGYWFT	IQIKATAGY	0.370812	3.48
DRB1_1454	116	TGGNSVQESIRFGLV	VQESIRFGL	0.370514	3.48
DRB1_1454	117	GGNSVQESIRFGLVL	VQESIRFGL	0.367819	3.54

* Percentile Rank

ارزیابی پروتئین فیبری ویروس سندرم کاهش تخم مرغ به عنوان کاندیدای واکسن: آنالیز بیوانفورماتیکی، بیان، خالص سازی و پایداری آن

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چکیده

ویروس سندرم افت تولید تخم مرغ(EDSV) ، یک آدنوویروس پرندگان، که باعث کاهش شدید تولید و کیفیت تخم مرغ در مرغ‌های آلوده می‌شود و در نتیجه تأثیرات اقتصادی قابل توجهی بر بخش طیور دارد. بر اساس مطالعات قبلی، پروتئین فیبر EDSV می‌تواند کاندیدایی برای واکسن زیر واحد علیه EDSV باشد. تحقیق حاضر بر بیان، خالص‌سازی و ارزیابی پایداری حرارتی پروتئین فیبر نوترکیب به عنوان واکسن علیه EDSV تمرکز دارد. در یک رویکرد *in silico* ، ما ساختار پروتئین فیبر را بررسی کردیم و آن را در Escherichia coli (*E. coli*) به عنوان کاندیدای واکسن بیان کردیم. ما همچنین پایداری حرارتی پروتئین بیان شده را ارزیابی کردیم. پروتئین عمدتاً به صورت پروتئین‌های تریمری محلول در *E. coli* بیان شد و سطح بیان پس از خالص‌سازی با میل ترکیبی نیکل 15 میلی‌گرم در لیتر بود. تجزیه و تحلیل ساختاری با استفاده از ابزارهای ایمونولوژیکی و بیوانفورماتیک نشان داد که پروتئین نوترکیب ساختار تریمری طبیعی را حفظ می‌کند. بر اساس ارزیابی پایداری حرارتی بر روی این پروتئین نوترکیب، این پروتئین پایداری حرارتی خوبی را نشان داد که می‌تواند کاندیدای خوبی برای واکسن علیه EDSV باشد.

واژگان کلیدی: پروتئین فیبر، خالص سازی، درون رایانه ای، سندرم افت تولید تخم مرغ، واکسن