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Bioinformatics tools based Comparison of Avian Pathogenic *E. coli* isolates using Phylogenetic analysis, 3D structure prediction, ADMET analysis, Molecular Docking and Molecular Dynamics Simulation

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Abstract: - This study examines the comparative analysis of Avian Pathogenic Escherichia coli (APEC) isolates through various bioinformatics tools, including phylogenetic analysis, 3D structure prediction, ADMET evaluation, molecular docking and molecular dynamics simulation. APEC strains significantly impact poultry health, leading to colibacillosis and subsequent economic losses in the poultry industry. The study underscores the importance of in silico methodologies in vaccine development, aiding in identifying antigens and optimizing vaccine design. We isolated *E. coli* strains from table and hatchable eggs using Tryptic Soy Agar, MacConkey Agar and Eosin Methylene Blue Agar media. The genomic analysis revealed approximately 4,288 protein-coding genes, highlighting the organism's complex structure and diverse antigenic types contributing to its virulence. The classification of *E. coli* based on serogroups and phylogroups enables a deeper understanding of its pathogenicity. By employing advanced bioinformatics techniques, we aim to elucidate the virulence factors associated with APEC strains and leverage this knowledge to enhance disease prevention strategies in poultry. This comprehensive analysis could pave the way for more effective vaccine formulations and improved management practices to mitigate the impact of APEC-related diseases in avian populations.

Keywords: Avian Pathogenic *E. coli* (APEC), Phylogenetic analyses, Ecotin, 3D Protein Structure, Molecular Docking.

Introduction

Escherichia coli is a rod-shaped, Gram-negative bacterium classified with in the Enterobacteriaceae family (Jhang *et al*., 2017). Its genome consists of circular DNA molecule, approximately 4.6 million base pairs in length (Mellata, 2013). Colibacillosis represents an extraintestinal disease predominantly caused by avian pathogenic *Escherichia coli* (APEC), which results in considerable morbidity and mortality in poultry, particularly among chickens and turkeys, leading to significant economic repercussion with in the poultry industry (Malik *et al*., 2021). At present, there is no practical method for prevention of colibacillosis. Efforts aimed at prevention and control emphasize minimizing risk factors with in flocks, which include administrating vaccines against immunosuppressive diseases, enhancing environmental conditions and upholding hygiene standards in hatcheries (Davies, 2005). In silico methodologies, encompassing computational biology, bioinformatics and molecular dynamic simulations, have proven vital in expediting the development of vaccines against *E. coli* infections. These approaches are essential throughout various stages of vaccine development, from identifying antigens to designing and optimizing vaccines (Soltan *et al*., 2022).

E. coli typically exists harmlessly with in the intestines of chickens, however, an imbalance in the gut microbiota can lead to its overgrowth and subsequent infections in sites outside the intestine

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(Pasquali *et al*., 2015). Avian pathogenic ***Escherichia coli*** (APEC) strains are implicated in colibacillosis, which is associated with a range of extraintestinal diseases, such as omphalitis, yolk sac infections, respiratory tract infections, pericarditis and aerosacculitis. In general, *E. coli* infections initiate in the respiratory tract, where the bacteria breach mucosal barriers, enter the bloodstream and induce conditions such as septicemia, airsaculitis and pericarditis. Pre-exiting viral or mycoplasma infection frequently exacerbate the severity of these infections (Sola-Gines *et al*., 2015).

The *E. coli* genome comprises approximately 4,288 protein-coding genes, organized into 2,584 operons. In addition, it contains seven ribosomal RNA (rRNA) operons and 86 transfer RNA (tRNA) genes (Mellata, 2013). *E. coli* is classified into various antigenic types which include 180 O antigens, 60 H antigens and 80 K antigens. The K antigen is associated with virulence while, H and K antigens are heat-sensitive. O antigens, part of the lipopolysaccharides (LPS) on the bacterial surface, are crucial in identifying APEC strains. These antigens interact with the immune system and bacteriophages, helping to protect the bacteria from host defenses. The diversity of O antigens allows *E. coli* adapt to different environments and evade immune responses (Wilczynski *et al*., 2022).

E. coli was traditionally differentiated into serogroups based on identifying significant O antigens using specific antisera.Common serogroups associated with APEC include O1, O2 and O78. In contemporary practices, phylo-grouping is amployed, utilizing a triplex PCR method that classifies *E. coli* into seven distinct phylogroups (A, B1, B2, C, D, E and F) based on the presence or absence of specific genes (Kravik *et al*., 2023). The virulence of APEC strains is influenced by various factors including adhesions (such as papC, papG alleles, as well as fimH), invasins (such as afaD, ibeA), protectins (including iss and traT), toxins (such as hlyF, sat) and system for iron acquisition (like iroN, iutA) these factor facilitate the bacteria's ability to persist and evade the immune response at extraintestinal sites.[10] Furthermore, *Escherichia coli* possess a serine protease inhibitor known as Ecotin, located in its periplasm and can inhibit proteolytic enzymes suchas chymotrypsin, trypsin and elastase (McGrath et al., 1991).

Materials and Method

Source of Bacteria

Tryptic Soy Agar (TSA), MacConkey Agar (MA) and Eosin Methylene Blue Agar (EMB) media were utilized to isolate and identify *E. coli* from the surfaces of table and hatchable eggs (Mehmood *et al*., 2024). A partial eco gene of *E. coli*, measuring 585bp was successfully amplified using the primers F: 5'GACCTCGGTTTAGTTCACAGA3' and R: 3'CACACGCTGACGCTGACCA5'.

Phylogenetic Analysis

The PCR amplicon was sent to APICAL SCIENTIFIC SDN. BHD, Malaysia for sequencing. Each sequence was analyzed using the Basic Local Alignment Search Tool (BLAST) to compare it with similar sequences submitted to the National Center for Biotechnology Information (NCBI). A phylogenetic comparison was conducted by constructing a maximum likelihood phylogenetic tree using MEGA 11 Software and Clustal W alignment algorithm. iTOL v6 was utilized for further visualization, annotation, and management of the phylogenetic tree.

Protein Selection and Structural Refinement

UniProt database was used to obtain the protein sequence of reference isolates in FASTA format. ExPASy Prot.Param was utilized to examine the physiochemical properties of protein. To predict the structure of the protein, three different methods were performed. Homology modeling predicts the 3D structure of a query protein through sequence alignment with template proteins. Modeller 9.25 software was used for homology modeling. Furthermore, we moved towards threading and ab-initio methods. The ab-initio method predicts protein structures based only on sequence information without using templates and for ab-initio, we used the online server trRosetta. Then we used the threading method and we used the online server I-TASSER. For protein 3D structure visualization, PyMOL (3.0.3) was used.

Selection and Retrieval of Ligands

We conducted a thorough literature review to identify compounds previously recognized as effective against the disease. We successfully retrieved the 2D chemical structure of the Neutrophil Elastase Inhibitor from PubChem and confidently transformed it into a 3D chemical structure using PyMOL (3.0.3) software. This conversion allows us to thoroughly analyze the inhibitor's three-dimensional conformation, paving the way for comprehensive docking and interaction studies.

Molecular Docking

We obtained protein -ligand complexes through molecular docking to explore the interactions between inhibitors and their receptors and identify binding sites. We utilized PyRx (0.8) software for the docking simulations to predict the optimal binding pockets of the inhibitors with the target proteins. Subsequently, PyMOL (3.0.3) was used to analyze the resulting protein-ligand complexes, allowing us to visualize and understand the detailed interactions and binding sites.

Toxicity Analysis

After analyzing the docking results, we performed toxicity analysis. We used several tools to see if Ecotin could be a good target for vaccines. We checked its potential as a vaccine with VaxiJen and tested for allergenicity using AllerTOP v.2.0.

Lead Identification

The most effective inhibitors were identified by evaluating docking scores, protein-ligand interactions and toxicity assessments. The assessments considered various factors, including molecular weight, the number of hydrogen bond donors and acceptors, logP, polar surface area, the number of rotatable bonds, the presence of rings, blood-brain barrier penetration and overall toxicity. The compounds with the lowest binding affinity, favorable drug-like properties and optimal interactions were selected for further studies.

Molecular Dynamics Simulation

Molecular dynamics simulations were conducted for 20 nanoseconds using Desmond from Schrödinger LLC. The initial protein–ligand complexes were derived from docking studies, which predict ligands binding under static conditions. Simulations aimed to evaluate ligand binding in a physiological environment.

The complexes were prepared with the Protein Preparation Wizard in Maestro, then optimization and minimization. The System Builder tool was used to set up the systems with in an orthorhombic box and utilized the TIP3P solvent model. The OPLS_2005 force field was applied; counter ions were added to neutralize the system. To mimic physiological conditions, 0.15 M NaCl was added to the system. The simulations were performed under an NPT ensemble, maintaining constant moles, pressure, and temperature at 300 K and 1 atm. The models were relaxed before the simulation and trajectories were recorded every 50 ps. The stability of the simulations was assessed by calculating the root mean square deviation (RMSD) of the protein and ligand over time (Rasheed *et al*., 2021).

Results

The indigenous isolates OP1- OP10 of *E. coli* 585bp amplicon size were submitted in NCBI database and their accession numbers are OR493517, OR423060, OR452423, OR712913, PP327376, PP327377, PQ518654, PQ525289, PQ525288 and PQ525287. In the context of phylogenetic analysis, it was observed that all the indigenous isolates of Ecotin strains are situated in the same region. The strain MS6198-OP7 exhibits the most extended clade value (0.06244438), indicating that it is the most genetically distinct strain. In contrast, DX6 presents a significantly shorter clade value (0.00248246), while, Ecotin PF15-OP9 has a clade value of 0.00430187. Notably, several strains including CP55 Sichuan-OP10, DAX4, DH1OB, Res13-Lact-PER13-34, 1919D3-OP8, CP66-6 Sichuan and MS6192 share identical clade values (0.00000483) as shown in Figure 1.

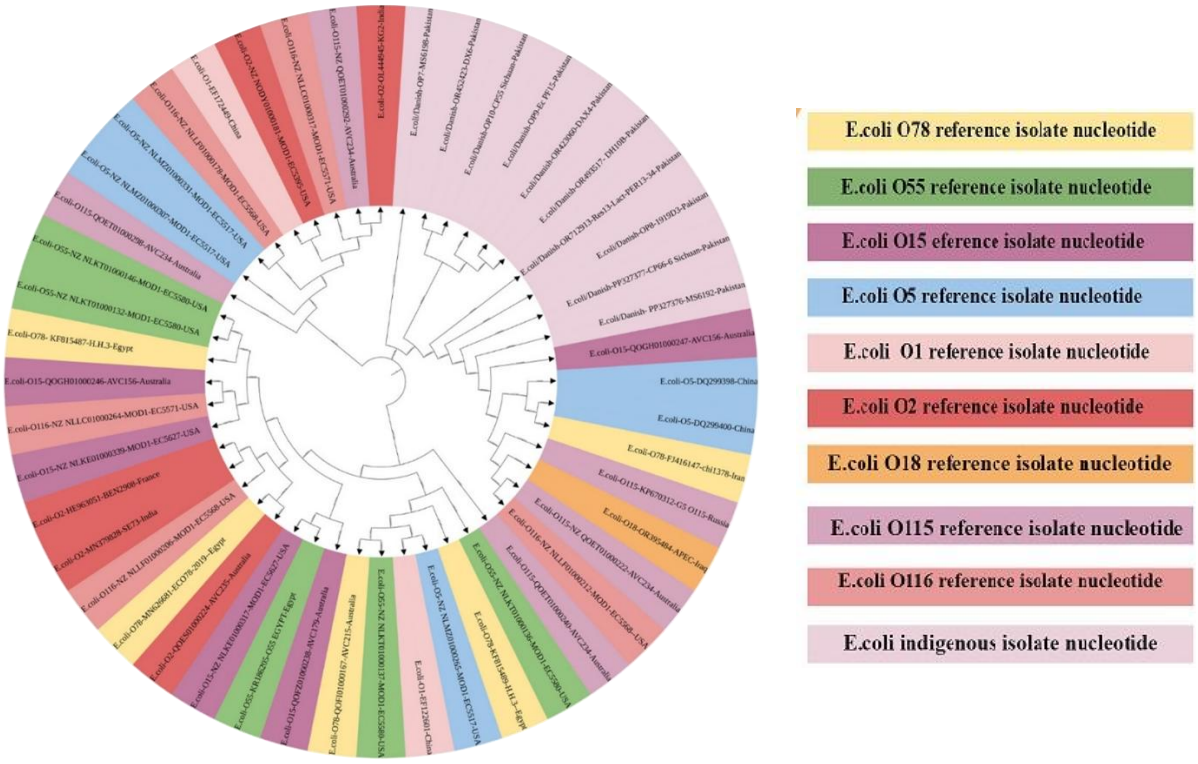

Figure 1: Phylogenetic tree of various Escherichia coli reference and indigenous isolates of nucleotide sequence constructed by maximum likelihood method and aligned by Clustal W. The tree is color-coded by serotype including O78, O55, O15, O1, O2, O18, O115, O116, O18 and indigenous isolates.

In the phylogenetic analysis of amino acid sequence isolates, all the Ecotin strains lie in the same region. MS6198-OP7 has the most extended clade value (0.25146677). CP66-6 Sichuan has a clade value of 0.00725753. Other strains including DX6, CP55 Sichuan-OP10, Ec PF15-OP9, DAX4, DH1OB, Res13-Lact-PER13-34, 1919D3-OP8 and MS6192, have same clade values (0.00001461) as shown in Figure 2.

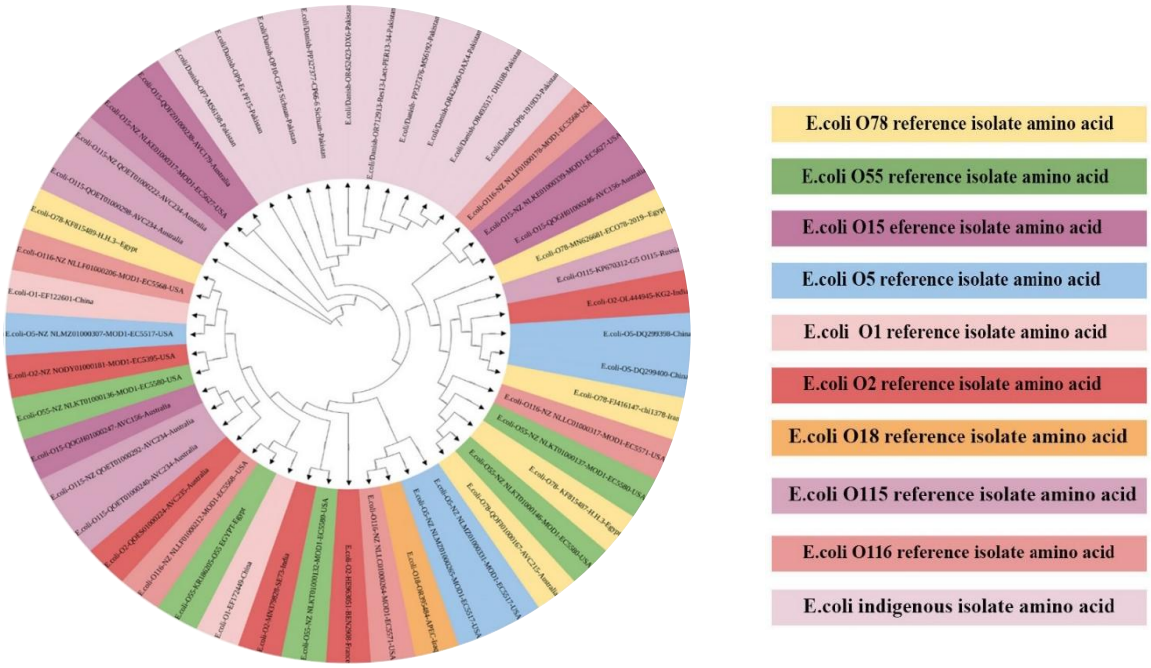

Figure 2: Phylogenetic tree of various Escherichia coli reference and indigenous isolates of amino acid sequence constructed by maximum likelihood method and aligned by Clustal W. The tree is color-coded by serotype including O78, O55, O15, O1, O2, O18, O115, O116, O18 and indigenous isolates.

In our investigation, the 3D dimensional structure of Ecotin (DH1OB) predicted through homology modeling and ab-initio methodologies, yielded comparable results. However, the structure prediction generated by I-TASSER exhibited discrepancies due to incorporating and additional glutamine (Glu) residue as illustrated in figure 3. The model of Ecotin (DH1OB) derived from the Homology Modelling and trRosetta comprised 140 amino acids resulting in a molecular weight of 15,220.65 Da. In contrast, I-Tasser prediction comprised 141 amino acids with molecular weight of 15,349.76 Da. In the case of Ecotin (DX6) the 3 dimensional structures produced by homology modeling, ab-initio and threading methods were varied, however, their physiochemical properties remained consistent, with no difference detected in the amino acid sequence. Likewise, the structural predictions for Ecotin (DX6) by Homology Modelling, trRosetta and I-Tasser included 134 amino acids yielded a molecular weight of 14,548.83 Da as explained in figure 4.

The predicted structure of Ecotin (DH1OB) show some differences based on the modelling methods used. The homology modeling and trRosetta prediction indicate that Ecotin (DH1OB) have five beta sheets and seven alpha hielices while, the I-TASSER prediction shows five beta sheets and five alpha helices. For Ecotin (DX6), the structure predicted by homology modeling contains three beta sheets and five alpha helices. In contrast, the trRosetta prediction includes two beta sheets and five alpha helices while, the I-TASSER prediction has two beta sheets and four alpha helices.

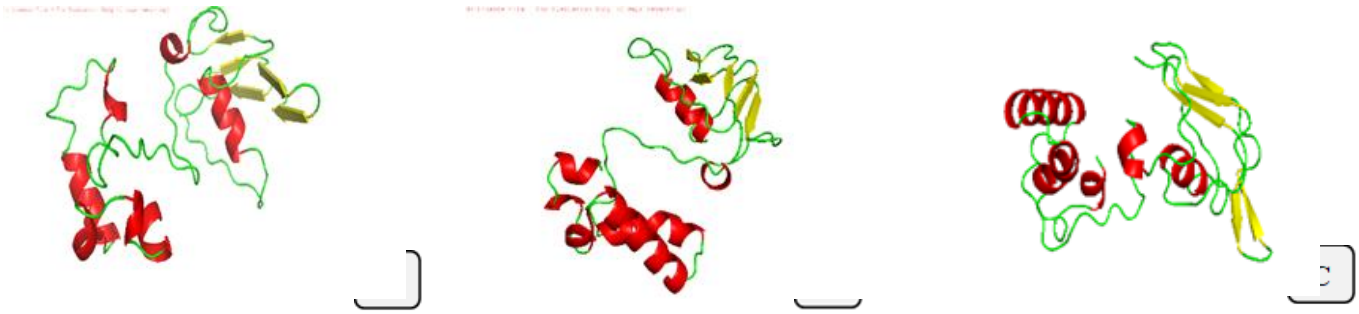

Figure 3: 3D Ecotin (DH1OB) protein structure of *E. coli* Accession # OR493517 through **A:** Homology modelling, **B:** Ab initio (Rosetta) and **C:** Threading (I-taser)

Figure 4: 3D Ecotin (DX6) protein structure of *E. coli* Accession #: OR452423 through **A:** Homology modelling, **B:** Ab initio (Rosetta) and **C:** Threading (I-taser)

The protein properties were analyzed using ExPASy ProtParam, focusing on critical parameters such as the extinction coefficient, half-life, instability index, aliphatic index and GRAVY (Grand Average of Hydropathicity). The extinction coefficient measures how much light protein absorbs at a wavelength of 280nm. Typically, protein extinction coefficients range from 4.0 to 24.0. For Ecotin (DH1OB), the extinction coefficient are 0.557 and 0.552, while for Ecotin (DX6), the coefficient is 0.583. Based on half-life evaluation this indicates that Ecotin (DH1OB) is more stable than Ecotin (DX6). The instability index indicates protein stability and has an ideal value of 40 or lower. All models analyzed reflect stable structures. The instability index values for Ecotin (DH1OB) are 23.10 and 23.16 whereas, for Ecotin (DX6) it is 21.96, suggesting that both variants are stable. The aliphatic index measures the relative volume of aliphatic side chains in a protein indicating its thermostability. This index typically ranges from 61.09 to 83.59. The aliphatic index values for Ecotin (DH1OB) are 97.43 and 96.74 while, for Ecotin (DX6) it is 98.88 indicating good thermostability. Finally, the GRAVY score assesses a protein's hydrophilicity or hydrophobicity. Negative values suggest the protein is hydrophilic whereas, positive values indicate hydrophobicity. In this analysis, both Ecotin (DH1OB) and Ecotin (DX6) are hydrophilic, as evidenced by their negative GRAVY scores, as shown in Table 2.

Table 1: Physiochemical Properties of Ecotin (DH1OB) and Ecotin (DX6)

Properties	**Ecotin (DH1OB) Homology Modelling, Ab-Initio**	**Ecotin (DH1OB) Threading**	**Ecotin (DX6)**
Extinction Co-efficient	Trp. residue found Abs=0.1% **0.557** Ext. coefficient = 8480	Trp. residue found Abs=0.1% **0.552** Ext. coefficient = 8480	Trp. residue found Abs=0.1% **0.583** Ext. coefficient = 8480
Half-Life	30 hrs. (Mammalian reticulocytes, in vitro). 20 hrs. (Yeast, in vivo). 10 hrs. (*E. coli*, in vivo).	30 hrs. (Mammalian reticulocytes, in vitro). 20 hrs. (Yeast, in vivo). 10 hrs. (*E. coli*, in vivo).	5.5 hrs. (Mammalian reticulocytes, in vitro). 3 min (yeast, in vivo). 2 min (*E. coli*, in vivo).
Instability Index	23.10 (Stable)	23.61 (Stable)	21.96 (Stable)
Aliphatic Index	97.43	96.74	98.88
GRAVY	-0.041 Hydrophilic	-0.065 Hydrophilic	-0.036 Hydrophilic

We conducted molecular docking studies to investigate how different variants of Ecotin (DH1OB and DX6) interact with a Neutrophil Elastase inhibitor. We utilized PyRx to predict the optimal binding sites and affinities while, PyMOL was employed to visualize these interactions in detail. Our analysis identified key binding sites and interactions, offering insights into the mechanism of the inhibitor and its potential therapeutic applications. The visual representations illustrate the predicted binding modes and the critical residues involved, enhancing our understanding of the Ecotin-inhibitor complex and its implications for drug development, as displayed in Figure 5.

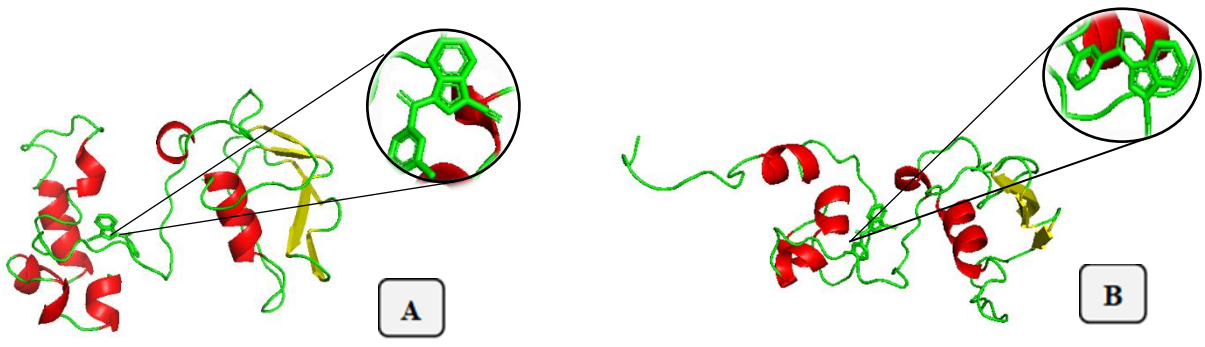

Figure 5: Ecotin Protein structure and Ligand molecule complex Neutrophil Elastase Inhibitor of Indigenous isolates **A:** Ecotin (DH1OB) **B:** Ecotin (DX6)

Neutrophil Elastase Inhibitor (ligand ID: 71818549) was based on binding affinity. The Binding affinity of Neutrophil Elastase Inhibitor is -7.3.

The VaxiJen results for Ecotin (DH1OB) showed that the bacterial proteins were classified as antigens meaning they could trigger an immune response. This suggests they might be good vaccine candidates since the immune system is likely to recognize them. On the other hand AllerTOP results classify these bacterial proteins as non-allergens meaning they are unlikely to cause allergic reactions. This makes them safer for vaccines and treatments, reducing the risk of allergic side effects. However, VaxiJen also classified Ecotin (DH1OB) as antigen (0.4314), meaning these proteins might not provoke a strong immune response. This suggests that more research may be needed to find better antigens for vaccine development, as shown in Table 3.

Table *3*: Results of VaxiJen(Antigenicity) and AllerTOP(Allergenicity)

Sr. No	**VaxiJen (std.value:0.4)**	**AllerTOP**
Ecotin(DH1OB)	Antigen (0.4314)	Non-Allergen
Ecotin(DX6)	Non-antigen (0.3685)	Non-Allergen

Molecular Dynamics Simulation of Ecotin (DH1OB)

The Desmond simulation trajectories were analyzed and the root mean square deviation (RMSD), root mean square fluctuation (RMSF) and protein–ligand contacts were calculated from the molecular dynamics (MD) trajectory analysis. Figure 6 showed the evolution of RMSD values over time for the backbone atoms of the ligand-bound protein. The RMSD plot of the complex indicates that it shows good interaction within the first 4 ns during the 20-nanosecond simulation. Figure 7 revealed the residue-wise RMSF values of the protein bound to the ligand. Higher peaks correspond to loop regions or the N and C-terminal zones as seen in the MD trajectories (Figure 7). Low RMSF values at the binding site indicate stable ligand binding to the protein.

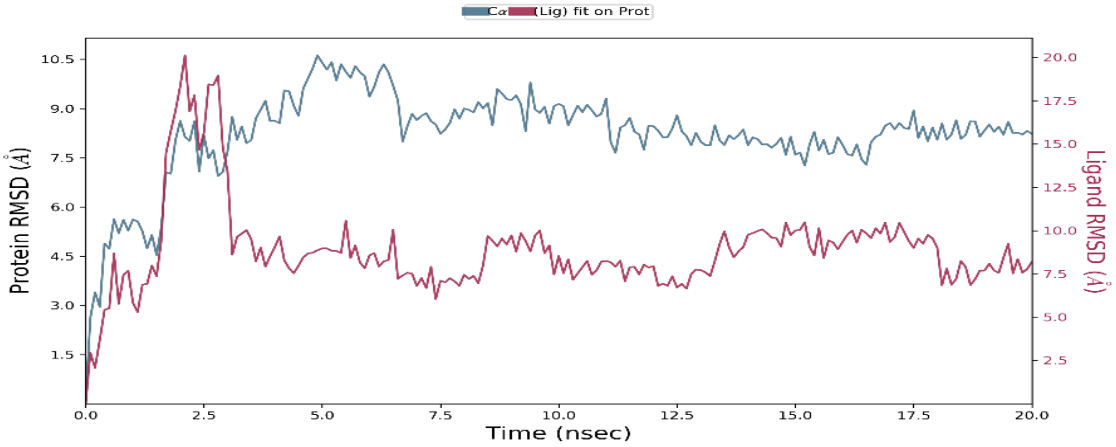

Figure 6: Root mean square deviation (RMSD) of the backbone atoms of protein and the ligand with time. The left Y-axis shows the variation of protein RMSD through time. The right Y-axis shows the variation of ligand RMSD through time.

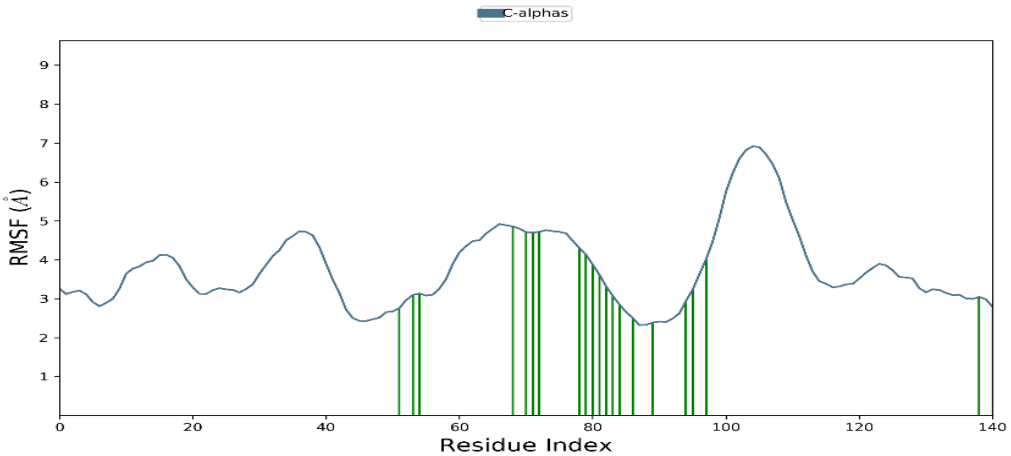

Figure 7: Residue wise Root Mean Square Fluctuation (RMSF) of protein

Protein ligand histogram contact displayed most of the hydrophobic interaction than hydrogen interactions between the protein and the ligand as shown in Figure 8. TYR_87 are most important in terms of hydrophobic interactions and hydrogen interactions.

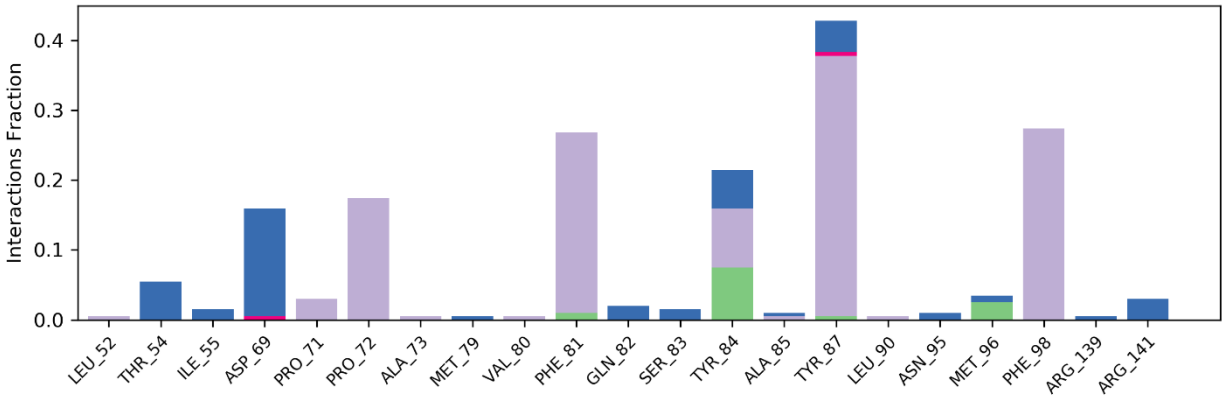

Figure 8: Protein-ligand contact histogram..

The percentage SSE graph in figure 9 illustrated that in Ecotin (DH1OB) Beta-Strands and Alpha Helix are present. This can affect the stability and functionality of the protein.

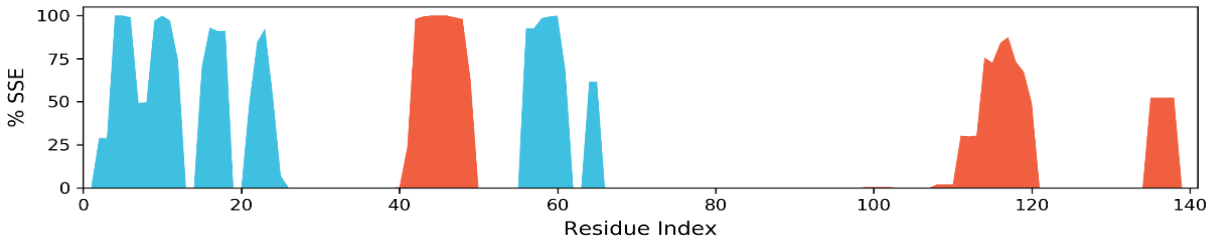

Figure 9: Protein Secondary Structure element distribution by residue index throughout the protein structure. Red columns indicate alpha helices, and blue columns indicate beta-strands.

Molecular Dynamics Simulation of Ecotin (DX6)

The Desmond simulation trajectories were analyzed the root mean square deviation (RMSD), root mean square fluctuation (RMSF) and protein–ligand contacts were calculated from the molecular dynamics (MD) trajectory analysis. Figure 10 indicated the evolution of RMSD values over time for the backbone atoms of the ligand-bound protein. The RMSD plot of the complex suggested that it showed good interaction within the first 11 ns and then again between 17.5 ns and 19 ns during the 20-nanosecond simulation. Figure 11 displayed the residue-wise RMSF values of the protein bound to the ligand. Higher peaks correspond to loop regions or the N and C-terminal zones, as seen in the MD trajectories (Figure 11). Low RMSF values at the binding site indicate stable ligand binding to the protein.

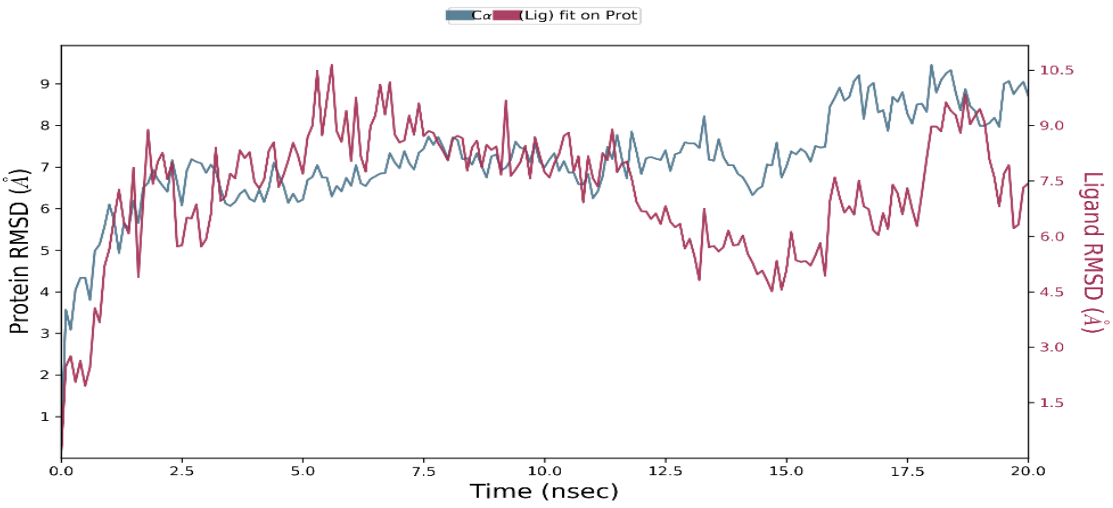

Figure 10: Root mean square deviation (RMSD) of the backbone atoms of protein and the ligand with time. The left Y-axis shows the variation of protein RMSD through time. The right Y-axis shows the variation of ligand RMSD through time.

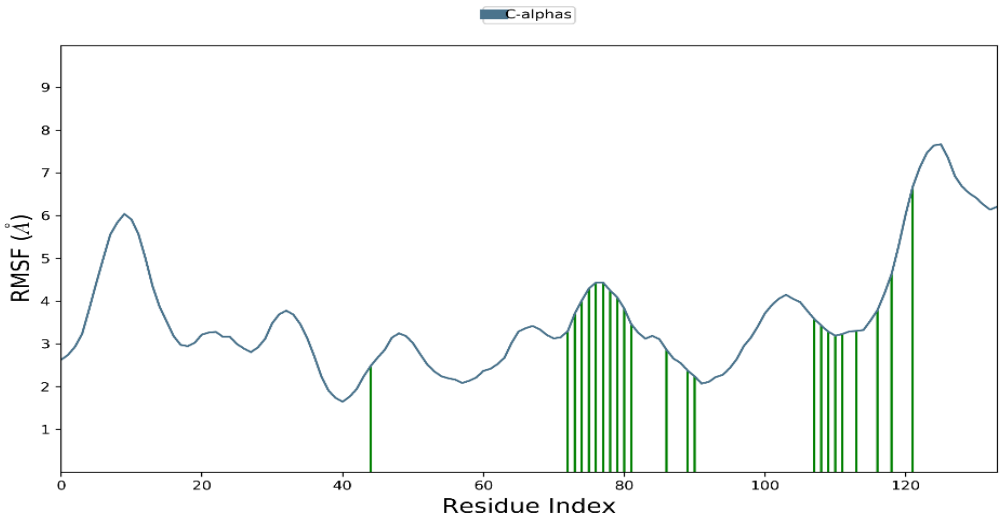

Figure 11: Residue wise Root Mean Square Fluctuation (RMSF) of protein.

Protein ligand histogram contact indicated both hydrophobic interaction and hydrogen interactions between the protein and the ligand as shown in Fig 12. TYR_82 are most important in terms of hydrophobic interactions and hydrogen interactions.

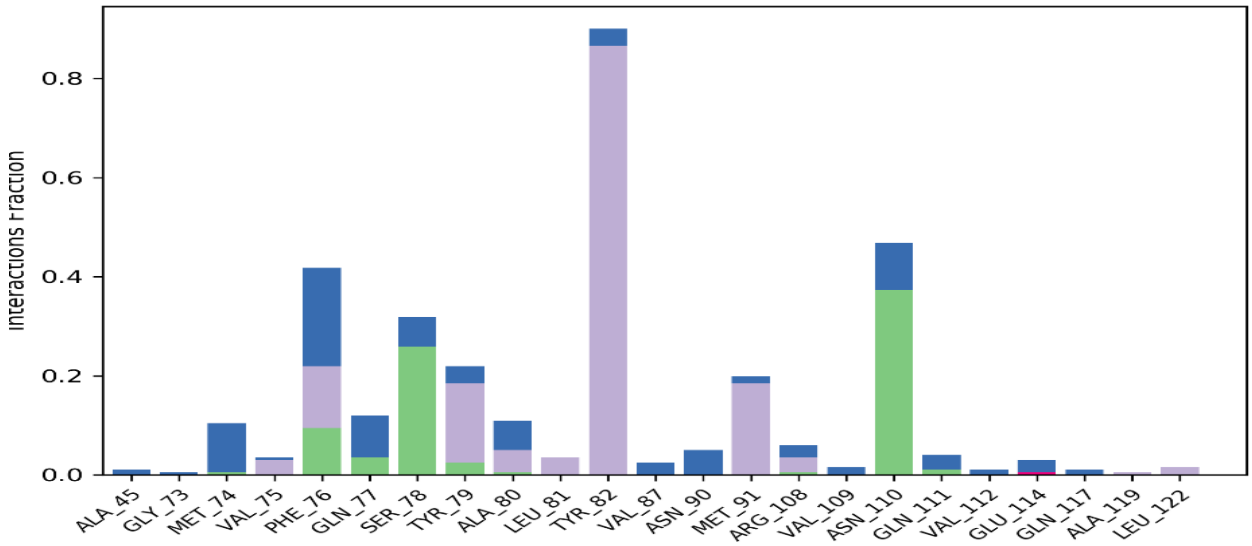

Figure 12: Protein-ligand contact histogram.

The percentage SSE graph in figure 13 illustrated that in Ecotin(DX6) Beta-Strands and Alpha Helix are present. This can affect the stability and functionality of the protein.

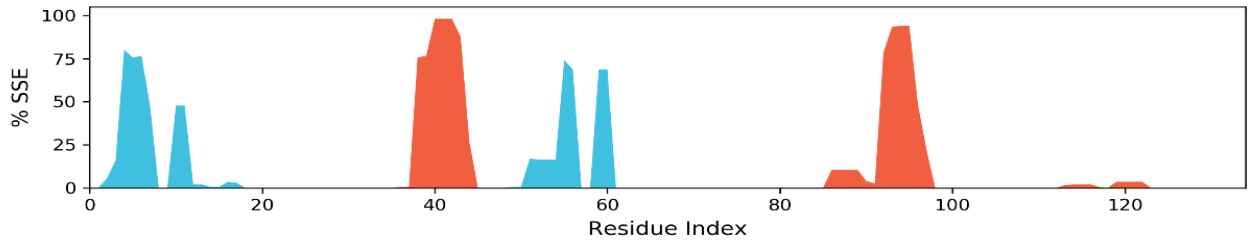

Figure 13: Protein Secondary Structure element distribution by residue index throughout the protein structure. Red columns indicate alpha helices, and blue columns indicate beta-strands.

Discussion

Avian pathogenic *E. coli* (APEC) infections pose a significant challenge to animal health in poultry and lead to substantial economic losses for farmer worldwide (Smailek *et al*., 2020). Effective control strategies for avian colibacillosis include implementing robust management practices, infection control measures and vaccination programs (Jeong *et al*., 2021)

Phylogenetic analysis of APEC strains indicates that while all strains are located within the same region, they display different clade values. In the phylogenetic tree based on nucleotide sequences, strains such as MS6198-OP7, DX6, and Ec PF15-OP9 show distinct clade values. In contrast, other strains, including CP55 Sichuan-OP10, DAX4, DH1OB, Res13-Lact-PER13-34, 1919D3-OP8, CP66-6 Sichuan, and a specific plate number, share similar clade values.In the phylogenetic analysis of amino acid sequences, MS6198-OP7 and CP66-6 Sichuan exhibit different clade values. At the same time, several strains such as DX6, CP55 Sichuan-OP10, Ec PF15-OP9, DAX4, DH1OB, Res13-Lact-PER13-34, 1919D3-OP8, and another specified plate number are grouped with the same clade values. This research includes predicting 3D structures for APEC strains and employing bioinformatics analyses aimed at identifying and evaluating potential new inhibitors or regulators. Various in silico methods were utilised, including homology modeling, ab-initio modeling, threading, binding site analysis, and molecular docking analysis (Kanwal *et al*., 2017). Currently, the 3D structures of APEC strains are not available in the Protein Data Bank (PDB), and the amino acid sequences were sourced from UniProt in FASTA format.

The 3D structures of the APEC strains were predicted using three different methods: homology modeling, ab-initio modeling, and threading, as shown in Table 1 (Azmal *et al*., 2024). Homology modelling is a technique that constructs a 3D model of a target protein by leveraging its sequence similarity to one or more proteins with established structures, known as templates. To assess the accuracy of the predicted structures, we utilised ExPASy ProtParam to analyse the physicochemical properties of the proteins. The analysis indicates that Ecotin (DH1OB) exhibits more excellent stability compared to Ecotin (DX6) when considering half-life. However, according to the instability index, Ecotin (DX6) demonstrates higher stability than Ecotin (DH1OB). Notably, both proteins are characterised as hydrophilic.

Molecular docking is a crucial technique employed in computer-aided drug design (CADD) to evaluate the binding affinity of potential inhibitors. The analysis of the docking results revealed that a lower docking score indicates a higher binding affinity, suggesting that the resulting complex possesses greater stability over time. Based on these findings, the Neutrophil Elastase Inhibitor was chosen for further investigation, demonstrating a binding affinity of -7.3 (Zani & Moreau, 2010).

Subsequently, a toxicity analysis was conducted to evaluate the antigenicity and allergenicity of two variants of Ecotin, namely Ecotin(DH1OB) and Ecotin(DX6), utilising bioinformatics tools such as VaxiJen and AllerTOP (Micheal *et al*., 2022). The outcomes indicated that Ecotin(DH1OB) is classified as an antigen but not an allergen, suggesting its potential as a favourable candidate for vaccine development. Conversely, Ecotin(DX6) was found to be neither an antigen nor an allergen, which implies that it is unlikely to elicit a robust immune response, as detailed in Table 3.

We conducted a Molecular Dynamics (MD) simulation throughout 20 nanoseconds, followed by an analysis of the MD trajectory to determine the Root Mean Square Deviation (RMSD) and Root Mean Square Fluctuation (RMSF) values. In the case of Ecotin (DH1OB), the RMSD plot reveals that the complex maintains strong interactions during the initial four nanoseconds of the simulation. For Ecotin (DX6), the RMSD plot indicates stable interactions for the first 11 nanoseconds and again between 17.5 and 19 nanoseconds. TYR_87 in Ecotin (DH1OB) plays a crucial role in hydrophobic and hydrogen bonding interactions, while TYR_82 in Ecotin (DX6) fulfils a similar function. The percentage of secondary structure elements graph indicates that both variants contain beta sheets and alpha helices, highlighting their structural complexity and stability throughout the simulation (Perdih et al., 2007).

Conclusion

In the results, we found that the Ecotin (DH1OB) variant is stable based on its half-life, while Ecotin (DX6) is stable when considering its instability index. Ecotin (DH1OB) can act as an antigen but, Ecotin (DX6) is non-antigen. Both are non-allergen. While during simulation the Ecotin (DH1OB) complex shows strong interactions within the first 4 nanoseconds. In contrast, the Ecotin (DX6) complex interacts well at two points: up to 11 nanoseconds and again between 17.5 and 19 nanoseconds. Ecotin (DH1OB) mostly engages through hydrophobic interactions while as, Ecotin (DX6) involves both hydrophobic and hydrogen bond interactions.

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