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In-Silico Assessment of Lethal Factor (LF) of *Bacillus anthracis* Exotoxin for its Suitability as a Diagnostic Candidate for Anthrax in Livestock

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A B S T R A C T

Anthrax is a highly fatal disease of animals and humans caused by *Bacillus anthracis*. The bacteria is a Gram-positive capsulated spore forming which harbors two plasmids pX01 and pX02, responsible for the virulence factors encoding for exotoxins and capsule respectively. Although several studies in the past deciphered the various aspects of the disease etiology and pathogenesis, but these have mainly been focused on development of a suitable field applicable rapid diagnostic assays for the detection of antigen and/or antibodies. The exotoxins of bacteria, protective antigen (PA) and lethal factor (LF) have been reckoned as key candidates for the development of diagnostics and vaccines. In this study, the gene encoding for lethal factor has been analyzed with *in-silico* approach using various prediction tools to assess the antigenic nature and its suitability as a candidate for diagnosis and prophylaxis. In comparative sequence analysis, anthrax vaccine strain (sterne) and the field isolates/strains from other countries available in NCBI database were analyzed by multiple sequence alignment. The LF protein was found to be highly conserved across the isolates/strains with 95.4-100% identity at the sequence level. The prediction tools revealed the presence of B cell and T cell epitopes. The LF protein lacks allergenic characteristics as revealed from the absence of IgE epitopes. The protein was predicted to be soluble in recombinant form with absence of super coil structure which may lead to aggregative tendency. In conclusion, the lethal factor (LF) protein of *Bacillus anthracis* was predicated to have all the essential characteristics to be an ideal diagnostic candidate.

Introduction

Anthrax is a zoonotic bacterial disease that has haunted both human and animal populations for centuries, and stands as a testament to the relentless battle between pathogens and scientific inquiry. Its etiological agent, *Bacillus anthracis*, not only wreaks havoc on biological systems but has also served as a fundamental model for understanding infectious diseases as well as mode of action of exotoxins. Anthrax, with its presence recorded in various civilizations, has been intertwined with human history since antiquity. The causative organism, *Bacillus anthracis* is a capsulated spore forming Gram-positive bacteria, with virulence factors encoded by plasmids. These plasmids, pX01 (174 kb) and pX02 (94 kb), carry genes responsible for three exotoxins and capsule production respectively, essential for the bacterium's survival and pathogenicity (Green *et al.,* 1985; Mikesell *et al.,* 1983). The severity of disease underscores the importance of understanding its mechanisms of action and developing effective countermeasures. Central to anthrax's pathogenesis is its arsenal of virulence factors, notably the tripartite toxin composed of protective antigen (PA), lethal factor (LF), and edema factor (EF) encoded by the pX01 plasmid (Kim and Yoon, 2006). During the pathogenesis process, protective antigen of *B. Anthracis* first binds to cellular receptors of cell namely ANTX1 and ANTX 2. Binding facilitates the entry of both lethal factor and edema factor into the cytoplasm of host cell. Zinc metalloprotease activity of LT cleaves mitogen-activated protein kinase kinases (MAPKK) and adenylate cyclase activities of EF converts ATP to cyclic AMP (cAMP) (Park *et al.,* 2002; Popov *et al.,* 2002). Both innate and acquired immune responses inhibited by the effects of toxins on the cell lead to survival and multiplication of bacteria in the host (WHO, 2008). These components work synergistically to disrupt host cellular processes, evade immune surveillance, and promote bacterial proliferation (Kim and Yoon, 2006).

In the quest to combat anthrax, researchers had focused towards understanding host immune responses and developing diagnostic and therapeutic interventions. Detection of antibodies against anthrax toxins, particularly LF and PA, serves as a vital tool for diagnosis and surveillance (WHO, 2008). Development of a suitable field diagnostic kit for the antigenic and serological detection of anthrax in livestock population is much needed to control the disease in animal population of India. The Sterne live spore vaccine which is currently being used is the only vaccine of choice for the control of anthrax in domestic animals despite sporadic reports of adverse reaction in sensitive species such as goats, and incompatibility with antibiotics are some of the limitations of the vaccine (Ndumnego *et al.,* 2018). Hence, the development of a safer and potent native /recombinant antigen based subunit vaccine may ensure strategic implementation of effective control measures. In the current study, focus on molecular characterization of lethal factor (LF) gene and *in silico* analysis for it's a diagnostic potential.

Materials and Methods

Comparative LF sequence analysis

LF toxin gene encoded by *lef* gene to Sterne vaccine strain (34F2), Indian *Bacillus anthracis* strains (n = 4) and strain from different geographical location of world (n = 20) and of different host species available in the NCBI data base were retrieved (Table 1).

Multiple sequence alignment was performed using ClustalWprogramme of MEGA X. The obtained aligned sequences were used to construct a phylogenetic tree using the neighbor-joining (NJ) method with a bootstrap value of 1000 in MEGA X. The identities and divergence at nucleotide level as well as deduced amino acid sequences in these strains were also compared using DNASTAR software.

Prediction of Antigenicity

VaxiJen sever was used for predicating the antigenic nature of the LF protein (Doytchinova *et al.,* 2007). Predication is based on physical and chemical properties of the protein sequence without using sequence alignment. Threshold of 0.4 was used for differentiate between antigenic and non-antigenic proteins (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html).

Prediction of B-Cell Epitopes

B cell epitope was predicted using the EliPro server, a web-based application for the prediction of antibody epitopes (Ponomarenco *et al.,* 2008). Modeled protein PDB structure was used for input. The scoring of the EliPro server ranges from 0 to 1 with a cut-off of 0.5. A score of less than 0.5 is considered as a non-epitope sequence, and a score of greater than 0.5 is considered as an epitope sequencev (http://tools.iedb.org/ellipro/).

Prediction of T-Cell Epitopes

Online server, CTL Pred was used to predict cytotoxic T cell epitopes in the chimeric protein (Bhasin and Raghava, 2004). Furthermore, the Net MHC 1 server was also used to predict the affinity for cytotoxic T cell epitopes with the predicted MHC I molecule.

The CTL pred database predicts a maximum score of 1. A score of greater than 0.5 was considered as a CTL epitope (https://webs.iiitd.edu.in/raghava/ctlpred/).

Prediction of the Allergenic epitopes

AlgPred software was used to predict the allergenicity of the LF protein (Saha *et al.,* 2006). The server facilitates BLAST search against allergen-representative peptides (ARPs) and assign a protein allergen). LF protein sequence was imported and allergens were predicted based on similarity of known epitope (https://webs.iiitd.edu.in/raghava/algpred/submission.html).

Prediction of the Coiled-coils motif

The MultiCoil program was used to predict the coiled-coil regions in amino acid sequences. The programme classify the predictions as dimeric or trimericusing PairCoil algorithm (https://cb.csail.mit.edu/cb/multicoil/cgi-bin/multicoil.cg).

The Multicoil scoring form was used for assessing the presence of coiled-coils motif and presence of dimeric and trimeric coiled-coils (Wolf *et al.,* 1997).

Protein Solubility Prediction

The solubility of the anthrax LF protein was estimated by SoluProt web application which is standalone software for prediction of soluble protein expression in *Escherichia coli* based on Target Track database carefully filtered to keep only targets expressed in *Escherichia coli* (Hon *et al.,* 2021).

The predictor is based on gradient boosting machines and employs 96 sequence-based features, e.g., amino acid content, sequence identity to PDB sequences and several aggregated physico-chemical properties (https://loschmidt.chemi.muni.cz/soluprot/).

Results and Discussion

Comparative sequence analysis

Multiple sequence alignment revealed the high sequence identity between the LF gene of different stains. Sterne vaccine strain had homogeneity of 95.4 to 100% at nucleotide level with the other *B. anthracis* isolates.

Phylogenetic tree showed the clustering of strains into two major cluster (Fig. 1). At deduced amino acid sequence of LF, the protein was found highly conserved across the various strains used in study (identity >95%).

Prediction of allergenicity

No allergen was predicated in the LF protein sequence with the existing allergen model used for the prediction. The lethal factor protein was predicted to be non-allergenic for the host.

Mapping of the IgE did not reveal any experimentally proven IgE epitopes in the protein sequence.

Prediction of Antigenicity

The LF protein was predicated to have antigenic epitopes. Amino acid sequence generated a score 0.436 which was above the threshold score of 0.4.

Prediction of the B and T cell epitopes

Prediction of B in LF protein revealed 10 linear and 11 dis-continuous B cell epitopes. All the B cell epitopes had a score above the cut off score of 0.5. Similarly three T cell epitopes were predicted having a score above the cut off score of 0.51 (Table 2).

Solubility and Coiled-coil prediction

Solubility analysis of the protein revealed that protein expression in *Escherichia coli* would yield soluble protein. Predicative score is 0.8 which is above the cut off criteria of 0.5. The coiled- coil prediction by the multi-coil form indicated absence of any super coil structures and thereby almost negating the scope of formation of oligomers. The multi-coil graph is represented in Fig 2. The absence of super coil structure also decreases the aggregative tendency of the proteins during over expression and purification.

Anthrax is highly fatal zoonotic disease, continues to be a public and animal health problem in several agro-livestock dependent countries including India. In endemic regions, anthrax can be a serious problem in unvaccinated herbivores animals especially large and small ruminants like cattle, buffalo, sheep and goat. People usually develop anthrax following exposure to infected animals and or animal products. The anthrax toxins are composed of three different proteins, designated as protective antigen, the lethal factor and the edema factor. PA factors interact with LF and EF and form active toxins lethal toxin (LT) and edema toxin (ET) respectively (Collier and Young, 2003). The lethal factor suppresses the immune cell activity and prevents microbial clearance from host body (Tournier *et al.,* 2009). Various researchers have evaluated the PA and LF proteins for diagnosis and vaccine use. Detection of anti-LF antibodies could be a better diagnostic marker of infection in the early stage of infection due to higher IgG antibody titers (Brenneman *et al.,* 2011). Host production of antibodies against PA antigens is delay and generally produces after 11 days and 25 days, in inhalational anthrax and cutaneous anthrax respectively.

However, IgG antibodies against LF could be detected as early as day 4 after the infection in the host (Brenneman *et al.,* 2011). Hence, anti-LF IgG titer in serum can be explore as better diagnostic marker for detection of anthrax infection in the early stage. In this study, we performed the comparative sequence and *in-silico* analysis of the lethal factor protein for its suitability as a diagnostic or vaccine candidate by using the *lef* gene sequence of Anthrax Sterne vaccine strain as backbone for the comparative sequence analysis and the various prediction models.

The resolution of the crystal structure of the anthrax LF protein has provided critical insight towards interaction with PA protein and its critical role in pathogenesis. The LF protein (molecular mass 90000) is a highly specific zinc metallo protease that cleaves of the mitogen-activated protein kinase (MAPKK) family at amino termini, leading to the inhibition of one or more signaling pathways. Studies indicated that the LF protein consists of four domains: domain I interacts with the membrane-translocating component of anthrax toxin. Domains II, III, and IV collectively form an extended trench capable of accommodating the 16-residue N-terminal tail of MAPKK-2 prior to cleavage. Domain IV exhibits a distant relation to the zinc metalloprotease family and encompasses the catalytic center (Pannifer *et al.,* 2001). The well-defined domains of LF gave an opportunity to customize various recombinant fusion proteins. Despite, the enormous amount of information and knowledge with regards to the toxin proteins of anthrax, most of the fundamental and diagnostic research is limited to its use in humans. The molecular dynamics of the toxin in the diverse livestock host species needs to be critically evaluated in order to come up with a diagnostic/prophylactic solution to combat the disease in the livestock population of India and elsewhere.

Our study was initiated with a comparative sequence analysis of the LF toxin gene and amino acid sequences available in the NCBI database. Multiple sequence alignment of 4 Indian and 20 isolates from other countries and anthrax sterne vaccine strain revealed the highly conserved nature of the lethal factor toxin across time and space. Strong genetic stability of the immunogenic *lef* gene of the *B. anthracis* strains from different regions of the world covering different susceptible species including humans was evident through this study. The anthrax sterne vaccine strain had a sequence identity of 95.4-100% with the other isolates suggestive of the immunogenic compatibility with isolates used in the study. Limited studies on the comparative sequence analysis of anthrax LF protein highlighting the evolution of the LF protein remained a challenge, however conserved nature of the *B. anthracispag* gene, showed a sequence identity of > 99.9% among the different species isolates (Chandranaik *et al.,* 2017). Similar studies on *P. multocida* for identifying gene homology of specific target sequences and comparative homology modeling have been carried out for selective expression of the candidate protein and subsequent use in diagnostics and vaccine (Shivachandra *et al.,* 2014). The LF protein was predicted to be non-allergenic, thus the recombinant over expressed LF protein may be used for raising of antibodies and experiments for protective efficacy. The allergenicity analysis has mostly used in field of drug discovery in various studies (Dana *et al.,* 2020) could be suitably used for the preliminary analysis of the proteins in recombinant and chimeric form for safety before administration in host.

Prediction of B-cell, epitopes remains challenging; however reliable predication is highly desirable for the vaccines and immunodiagnostics design. Antigenicity of the protein under study was analyzed for the presence of B cell epitopes before moving forward towards it over expression and *in-vivo* studies.

Table.1 Details of strains sequences used for constructing phylogenetic tree.

S. No	Accession ID	Country	Host	Strains	Year
1.	M29081	United States	NA	pLF74	1995
2.	KM019142	India	elephant	IVRI	1975
3.	MN536455	Russia	NA	1055/38	1993
4.	MN536473	Russia	NA	STI-1	1940
5.	MN536472	Russia: Volgograd	Human	**strain 1298**	2020
6.	MN536471	Estonia	Cattle	**strain 157(B-1107)**	1978
7.	MN536470	Russia: Chechen Republic	meat	**strain 914/213**	1988
8.	MN536469	Russia	Cow	Strain 1173	1995
9.	MN536468	Russia: Chechen Republic	NA	48/29	1968
10.	MN536467	Russia: Chechen Republic	NA	47/28	1968
11.	MN536466	Russia	NA	Strain 44	
12.	MN536465	Turkmenistan	Yak	**Strain 11**	1971
13.	MN536464	Russia: Tatarstan	Cow	**Strain 8(2099)**	1971
14.	MN536463	Uzbekistan	Cattle	**Strain 219/6**	1976
15.	MN536462	Russia: Kabardino-Balkar Republic	Soil	strain="118	1998
16.	MN536461	Russia: Karachay-Cherkessia	Russian	1030/213	
17.	MN536460	Russia: Chechen Republic	Human	**Strain 822/7**	1986
18.	MN536459	Russia	NA	592/10	1982
19.	MN536458	Russia: Orenburg region	Human	555/288	1982
20.	CP010853.1	USA		A1144	2016
21.	CP009340.1	USA	Pig	Ohio ACB	2014
22.	CP060195.1	India	Bovine	**Kanchipuram**	2019
23.	CP141534.1	India Odisha	Cattle	OK-29	2023
24.	CP139445.1	India: Odisha, Koraput	Bubalus Bubalis	OK-05	2023

Figure.1 Phylogenetic tree of LF sequences of the Anthrax isolates.

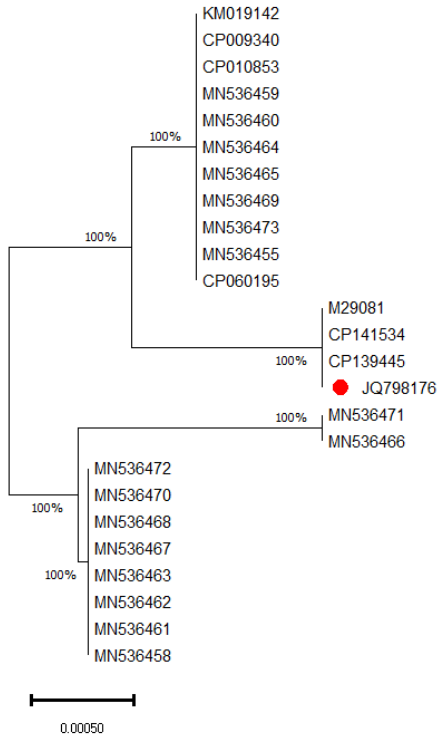
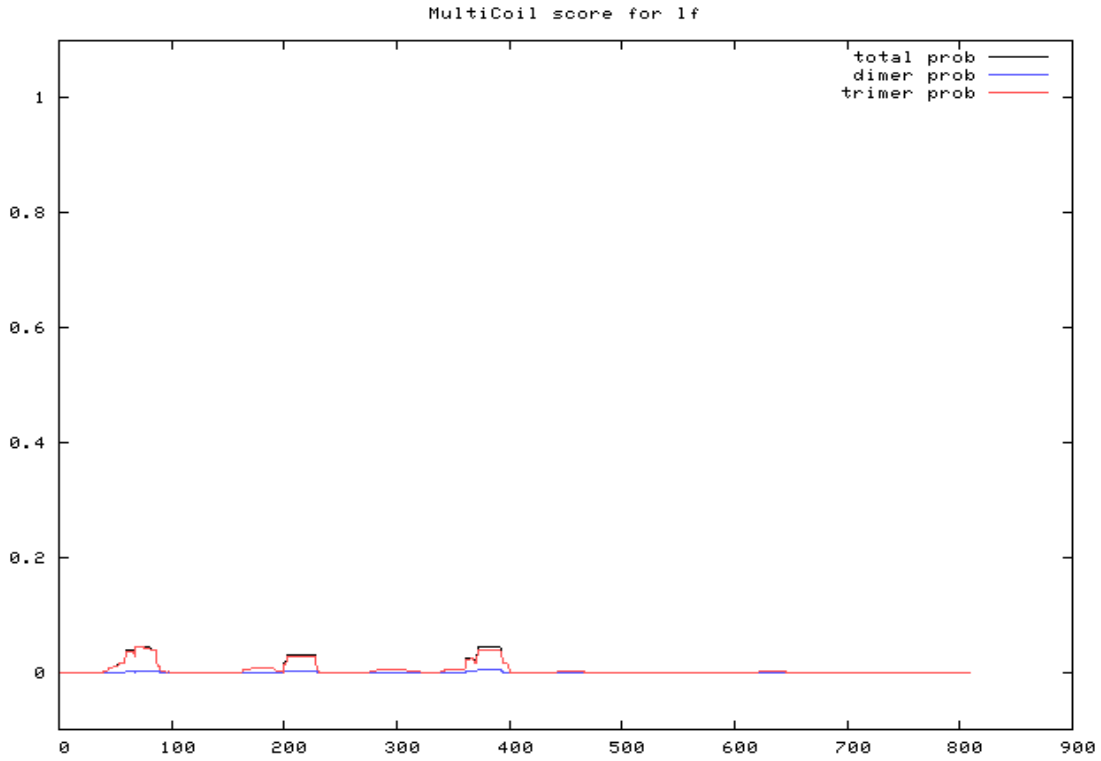


Table.2

Predicted Linear Epitope(s): B cell					
No	**Start**	**End**	**Peptide**	**Number of residues**	**Score**
1	299	326	PIEPKKDDIIHSLSQEEKELLKRIQIDS	28	0.857
2	740	773	AFRLMHSTDHAERLKVQKNAPKTFQFINDQIKFI	34	0.787
3	79	147	GKIYIVDGDITKHISLEALSEDKKKIKDIYGKDALLH EHYVYAKEGYEPVLVIQSSEDYVENTEKALNV	69	0.759
4	692	730	VDDYAGYLLDKNQSDLVTNSKKFIDIFKEEGSNLTS YGR	39	0.743
5	335	379	KEFLKKLQIDILSEKEKEFLKKL	23	0.735
6	37	66	KEIMKHIVKIEVKGEEAVKKEAAEKLLEKV	30	0.72
7	567	582	NQEWNKALGLPKYTKL	16	0.714
8	178	200	KNASDSDGQDLLFTNQLKEHPTD	23	0.699
9	27	34	ERNKTQEE	8	0.676
10	404	432	INLDVRKQYKRDIQNIDALLHQSIGSTLY	29	0.618
Predicted Discontinuous Epitope(s): Bcell					
1	-	-	-	51	0.779
2	-	-	-	87	0.774
3	-	-	-	108	0.733
4	-	-	-	23	0.717
5	-	-	-	7	0.702
6	-	-	-	3	0.657
7	-	-	-	59	0.655
8	-	-	-	5	0.599
9	-	-	-	8	0.578
10	-	-	-	3	0.551
11	-	-	-	3	0.532
Predicted T cell epitope					
1	36	-	GHGDVGMHV	-	1
2	365	-	TEEKEFLKK	-	1
3	380	-	DSLSEEEKE	-	1

Figure.2 Multicoil graph depicting the presence and absence of coiled coil super structure.



Assessment of the antigenicity and epitopes assist in target selection and selective expression of the antigenic regions for productive results. Predication of B and T cell epitopes in the LF protein sequence indicated the antigenic nature of protein. The protein was predicted with 10 linear and 11 discontinuous B cell epitopes and three T cell epitopes.

The data is indicative of good antigen which could serve as very effective diagnostic and prophylactic candidate. The result indicates the suitability of LF protein in diagnostics. Similar analysis of antigenic epitopes of putative protein of bacteria and viruses using various *in-silico* platforms has been depicted in several studies (Mohanty *et al.,* 2019; Shivachandra *et al.,* 2014).

The prediction of the solubility characters of a protein is crucial before over expression in any system and could significantly affect the outcome and the direction of the experimental design. The prediction of the solubility of the LF protein indicated the protein to be soluble in nature and the prediction of the absence of the coiled coils deters form aggregative tendency. The description of the crystal structure in prior studies does not indicate presence of super coils and the same has been validated in the prediction study. It is predicated the soluble nature of LF protein in expression system which helps in further purification and characterization without denaturing the protein.

Earlier studies also found that protein sequence having coiled coil motifs have aggregative tendency in expressed recombinant proteins (Chacko *et al.,* 2015). Hence, based on the analayis of sequences and predication of various regions, lethal factor (LF) protein of *Bacillus anthracis* can be predicated for highly suitable candidate for diagnostic platform.

Further, use of suitable vector and heterologous expression host system could enhance the better purification of recombinant LF protein without the issues of hydrophobic interactions and formation of aggregate, which is a key factor in bulk production and down-stream processing of antigens.

The study represents a preliminary approach of evaluation of the anthrax lethal factor protein for its suitability either on a diagnostic and / or vaccine platform. The evaluation of any candidate protein on immunogenic, phylogenic and solubility parameters

serves as the backbone for successful downstream application. Analysis of the candidate protein will help in selection of epitopic target regions and anticipated behavior of recombinant protein after expression.

On the preliminary evaluation of the lethal factor, it was noted that the LF could be a suitable candidate for diagnostic and vaccine research for anthrax in livestock. The studies targeting the depicted epitopes could also be used in recombinant protein expression system and subsequent purification for development of recombinant LF based epi-diagnostics/ subunit vaccines for livestock.

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Author Contributions

Sandeep Kumar Singh: Conceived the original idea and designed the model, rough draft of the paper.; Nihar Nalini Mohanty: Assisted in Designing the model, analysis of data.; Awadhesh Prajapati: Also assisted in Designing the model, collection of relevant literature.; Sathish Bhadravati Shivachandra: Assisted in data analysis, drafting of paper.; Amar Prakash Garg: Assisted in data analysis, finalization of model and final drafting of paper.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Bhasin, M and Raghava, G. P. S. (2004). Prediction of CTL epitopes using QM, SVM and ANN techniques. *Vaccine,* 22: 3195-3204 https://doi.org/10.1016/j.vaccine.2004.02.005

Brenneman, K. E *et al.,* **(**2011). The early humoral immune response to *Bacillus anthracis* toxins in patients infected with cutaneous anthrax. *FEMS Immunology and Medical Microbiology,* 62:164 –172. https://doi.org/10.1111/j.1574-695x.2011.00800.x

Chacko, N *et al.,* (2015). A coiled-coil motif in non-structural protein 3 (NS3) of bluetongue virus forms an oligomer. *Virus Genes*, 51: 244-251. https://doi.org/10.1007/s11262-015-1230-9

Chandranaik, B. M *et al.,* (2017). Pag gene based phylogenic characterization of Bacillus anthracis from sheep. *The Indian Journal of Animal Sciences*, 87(1): 25-28. https://doi.org/10.56093/ijans.v87i1.66823

Collier, R. J. and Young, J. A. T. (2003). Anthrax toxin. Annu. Rev. Cell Dev. Biol. 19: 45–70. http://dx.doi.org/10.1146/annurev.cellbio.19.111301.140655

Dana, H. *et al.,* (2020). In silico analysis, molecular docking, molecular dynamic, cloning, expression and purification of chimeric protein in colorectal cancer treatment. *Drug Design, Development and Therapy*, 309-329. https://doi.org/10.2147/dddt.s231958

Doytchinova, I. A. and Flower D. R. (2007). VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines. *BMC Bioinformatics*, 8: 4. https://doi.org/10.1186/1471-2105-8-4

Green, B. D *et al.,* (1985). Demonstration of a capsule plasmid in *Bacillus anthracis*. *Infection and Immunity,* 49: 291-297. https://doi.org/10.1128/iai.49.2.291-297.1985

Hon, J. *et al.,* (2021). SoluProt: prediction of soluble protein expression in *Escherichia coli*. *Bioinformatics*, 37 (1): 23–28. https://doi.org/10.1093/bioinformatics/btaa1102

Kim, J. and Yoon, M. Y. (2006). Anthrax lethal factor: Critical virulence factor of pathogenesis of anthrax toxins. *Toxin Reviews*, 25(1): 109-124.

Mikesell, P *et al.,* (1983). Evidence for plasmid mediated toxin production in Bacillus anthracis. *Infection and Immunity,* 39: 371-376. https://doi.org/10.1128/iai.39.1.371-376.1983

Mohanty, N. N *et al.,* (2019). An efficient production of hybrid recombinant protein comprising non-structural proteins (NS 1 & NS 3) of bluetongue virus in prokaryotic expression system. *Protein Expression and Purification*, 155: 15-20. https://doi.org/10.1016/j.pep.2018.09.005

Ndumnego, O. C *et al.,* (2018). Immunogenicity of anthrax recombinant peptides and killed spores in goats and protective efficacy of immune sera in A/J mouse model. *Scientific Reports,* 8(1): 16937. https://doi.org/10.1038/s41598-018-35382-8

Pannifer, A. D *et al.,* (2001). Crystal structure of the anthrax lethal factor. *Nature*, 414: 229-233. https://doi.org/10.1038/n35101998

Park, J. M *et al.,* (2002). Macrophage apoptosis by anthrax lethal factor through p38 MAP kinase inhibition. *Science,* 297: 2048- 2051. https://doi.org/10.1126/science.1073163

Ponomarenko, J. V *et al.,* (2008). ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC Bioinformatics,* 9: 514. https://doi.org/10.1186/1471-2105-9-514

Popov, S. G. *et al.,* (2002). Lethal toxin of Bacillus anthracis causes apoptosis of macrophages. *Biochemical and Biophysical Research Communications,* 293: 349- 355. https://doi.org/10.1016/s0006-291x(02)00227-9

Saha, S. and Raghava, G. P. S. (2006). AlgPred: prediction of allergenic proteins and mapping of IgE epitopes. *Nucleic Acids Research*, 34: 202-209. https://doi.org/10.1093/nar/gkl343

Shivachandra, S. B *et al.,* (2014). Immunogenicity of highly conserved recombinant VacJ outer membrane lipoprotein of *Pasteurella multocida. Vaccine,* 32(2): 290-296. https://doi.org/10.1016/j.vaccine.2013.10.075

Tournier, J. N *et al.,* (2009). Anthrax toxins: a weapon to systematically dismantle the host immune defenses. *Molecular Aspects of Medicine*, 30(6): 456-466. https://doi.org/10.1016/j.mam.2009.06.002

Wolf E *et al.,* (1997). MultiCoil: A Program for Predicting Two- and Three-Stranded Coiled Coils. *Protein Science*, 6: 1179-1189. https://doi.org/10.1002/pro.5560060606

World Health Organization (WHO), (2008). Anthrax in humans and animals. *Ed*World Health Organization, Food and Agriculture Organization of the United Nations & World Organization for Animal Health. 208.

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