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INSILICO DESIGN AND DEVELOPMENT OF VACCINE BY REVERSE VACCINOLOGY APPROACH FOR ANTHRAX

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ABSTRACT:
Anthrax is a life-threatening infectious disease that normally affects animals, especially ruminants. Anthrax can be transmitted to humans by contact with infected animals or their products. Bacterial infectious disease remains a major cause of deaths and disabilities in the world. Although conventional vaccinology were successful in conferring protection against several diseases, they failed in providing effective vaccine candidate against major bacterial pathogen. The reverse approach to vaccine development takes advantage of the genome sequence of the pathogen. This approach allows the identification of all the antigens seen by the conventional methods. With the use of computer software possible antigen determinants are predicted.. The main aim of this paper is to Design and Development of vaccine against anthrax by Reverse Vaccinology approach. Antigen determinant was found out through various tools. The protein sequence having less E-value (1) and less identity (24.73%) was chosen for designing the potent vaccine candidate.

Keywords: - zoonotic, Reverse Vaccinology, vaccine candidate, docking, immune response, minimization.

INTRODUCTION

Anthrax is zoonotic disease caused by Bacillus anthracis, primarily affect herbivores including sheep, cattle, horses and other domestic animals [8]. The name "anthrax" comes from *ANTHRAKIS,* the Greek word for coal, because of the large black-crusted sores the disease often causes in humans. In recent years, anthrax has received a great deal of attention as it has become clear that the infection can also be spread by a bioterrorist attack or by biological warfare[9]. In human anthrax may occur in three types depending upon how it enters the body.

1. Cutaneous (skin) – anthrax spores or bacteria enter the skin through a cut or scratch. In one to twelve days a small red raised spot that looks like an insect bite or blister forms and turns into a sore with a thick black scab. Most cases are cured with antibiotics. Without treatment the chances of death are about one in five.

2. Inhalational – anthrax spores are breathed into the lungs. Symptoms may begin two to 45 days but most often within one week. Flu-like symptoms of fever, headache, cough, and muscle aches develop first, followed by sudden rise in fever, shaking chills, trouble breathing and collapse. Vomiting and stomach pain may occur. The chance of death is high unless antibiotics can be started very early in the disease. This type is very rare.

3. Gastro-intestinal – from eating undercooked meat of an animal that had anthrax. Symptoms of nausea, vomiting, diarrhea, stomach pain and fever usually begin two to five days after eating the meat. About half of people with this type may die. Anthrax spores can be produced in vitro and used as a biological weapon [9].

Reverse Vaccinology

The basic idea behind Reverse Vaccinology is that an entire pathogenic genome can be screened using bioinformatics approaches to find

genes [2]. Next, those genes are filtered for desirable attributes that would make good vaccine targets such as outer membrane proteins. Those proteins then undergo normal wet lab testing for immune responses. The principle of the Crick's dogma is also used by RV, in which possession of a gene sequence is searched for the possibility of a probable protein encoded by this sequence to be an antigen capable of stimulating an immune response in a host organism[8].

MATERIALS AND METHOD

Bioinformatics tools were identified for analyzing Anthrax pathogenic proteins for their antigenic properties. The protein sequence of Anthrax bacteria (Bacillus anthracis) in FASTA format from JCVI CMR was extracted (TIGR http://www.tigr.org). For screening the sequence SDSC biological workbench is used. The sequence having less identity was found out by screening the sequence. The sequence which is obtained from screening having less E value and identity is used for finding epitope. The antigenic determinants (epitope) were found out by using EMBOSS antigenic. MAPPP (MHC-I Antigenic Peptide Processing Prediction) is used for binding prediction and proteasome cleavage prediction. This help to predict possible antigenic peptides to be processed and finally presented on the cell surfaces. The antigenic determinant having greater LCV value was chosen to design a molecule by using discovery studio 2.5.

RESULT AND DISCUSSION

Screening:-The protein sequence of Bacillus anthracis is screened and the sequence of least identity is found. The sequence has least identity 24.73% with Accession number ZP_05214185.1 is obtained. The above sequence which is obtained from screening of proteins, having least identity and least E value was chosen and used for finding antigenic determinants using Emboss Antigenic.

Finding Epitope:-By using the above sequence the antigenic determinants were found. Then the LCV values of the determinants were calculated. Based on the LCV value the antigenic determinant having greater value was chosen. (Table1).Results are compared with MAPPP result for the binding of MHC-I molecule (Table.3 & 4). The chosen epitope is used for docking analysis.

Minimization:-The designing of molecule and minimization is done by using discovery studio 2.5.The antigenic determinant TSLVVEVVVESK was designed. The minimization of MHC molecule was done and the energy was found to be -164.664 k cal/mol (Fig.2).

Docking:-Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules. The epitope molecule is docked with MHC I molecule successfully is shown in the figure given below. The epitope molecule docked with MHC-I molecule successfully this shows that MHC I molecule represent the epitope to B cells.Cdocker energy of the interaction was found to be 169.117(Fig.3)

Emboss Result(Table .1)

```
# Sequence: ZP_05214185. from: 1   to:420
# Hit Count: 16
Max score pos at "*"
    (1)    Score 1.288 length 12 at residues 130->141
                          *
        Sequence: TSLVVEVVVESK
                  |          |
                130          141
Max_score_pos: 135
LCV value=50
```

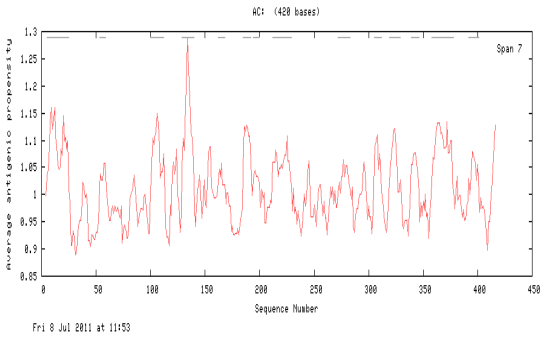
(2) Score 1.161 length 21 at residues 6->26

```
                    *
Sequence: RISSLVLAGIIGLSSTVAVKA
          |                   |
          6                  26
```

Max score pos: 13
LCV value =28.57

(Fig.1) Average antigenic propensity for this protein is 1.0123

Antigenic plot for sequence



(Table.2) There are 14 antigenic determinants in your sequence:

N	Start Position	Sequence	End Position
1	5	KRISSLVLAGIIGLSSTVAVK	25
2	53	EKQAVSK	59
3	100	KNEIVVLEDKVLA	112
4	129	NTSLVVEVVVES	140
5	162	EILRLQE	168
6	185	EASLVVDK	192
7	194	KLAKAQA	200
8	212	NLQTVQAKYNQVASQLNL	229
9	272	EQEALAKAQAEV	283
10	305	KVEPVQPS	312
11	319	REIYVHATAYT	329
12	339	PGQQVYSA	346
13	358	MKLIAVDPSVIPLGSRVYVEG	378
14	392	KGHKIDVLMP	401

(Table .3)MAPPP - Results

Proteasome cleavage prediction result

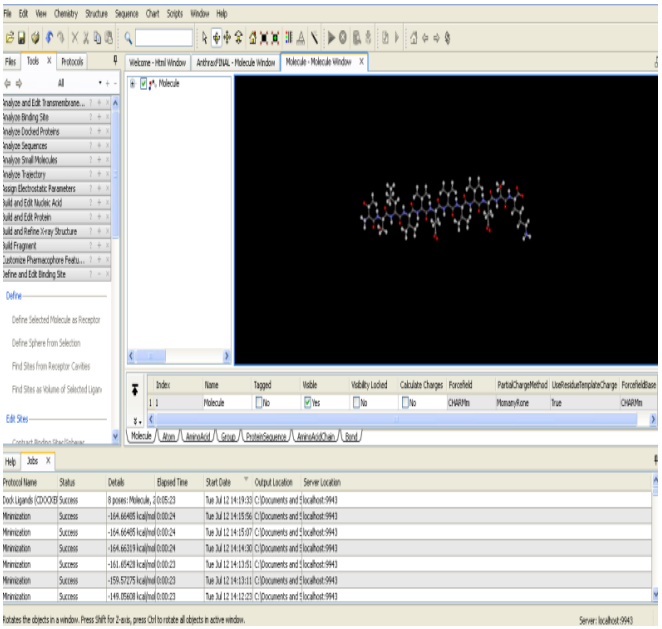
Parameters	
Length of sequence	12
Range for length of peptide fragments	9..11
Min. probability for cleavage after a single residue	0.5
Min. probability for cleavage of a fragment	0.5
Prediction results	
No. of possible fragments	3
Total no. of fragments	9
Percentage (fragments / total fragments)	33.33%
Highest fragment cleavage probability (at pos. 0@1)	1
Highest residue cleavage probability (at pos. 11@1)	0.9487

T S L V V E V V V E S K
1 1 2 3 3 3 3 3 3 2 2 2
T S L V V E V V V (1)
L V V E V V V E S K (1)
V V E V V V E S K (1)

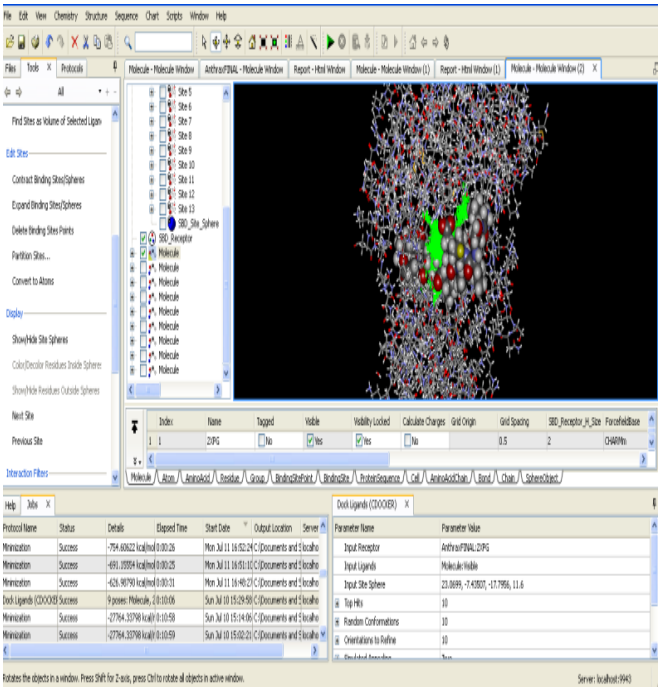
(Table.4) Query results

Query parameters	
Start with	Protein cleavage
Cleavage algorithm	FRAGPREDICT
Min. residue cleavage prob.	0.6
Min. fragment cleavage prob.	0.6
MHC binding matrices	SYFPEITHI
MHC type(s)	ALL
Min. binding score	0.6
Weight (cleavage:binding)	5:5

Query results							
Protein position	**Length**	**Sequence**					
Epitope	**Position**	**MHC type**	**n-mer**	**Overall score**	**Cleavage Probability**	**MHC binding score**	**Group**
0..419	420	MNYFKRISSLVLAGIIGLSS..PDKASSSKW GRKNVKLTILS					
KAESNDEKL	24	H2_Db	9	0.8714	1.0000	0.7429	same length
KAESNDEKL	24	H2_Db	9	0.8714	1.0000	0.7429	c-term. Trimmed
NELHNLNNTI	65	H2_Db	10	0.8939	1.0000	0.7879	c-term. Trimmed
IMRKRMVSV	115	HLA_A_0201	9	0.8333	1.0000	0.6667	same length
SVQNSSNTSL	122	H2_Kd	10	0.8733	0.9967	0.7500	n-term. Trimmed
SVQNSSNTSL	122	H2_Kd	10	0.8750	1.0000	0.7500	same length
RLQEQDLRQI	164	HLA_A_0201	10	0.8529	0.9999	0.7059	c-term. Trimmed



(Fig.2)Minimization of Antigenic Epitope



(Fig.3)Docking result

CONCLUSION

Anthrax is a life-threatening infectious disease that normally affects animals caused by the bacterium *Bacillus anthracis*. Most forms of the disease are lethal. We have retrieved the

complete proteomic sequence of *Bacillus anthracis* and screen it by using SDSC workbench. We got the sequence having less identity 24.73%. Antigen determinants was selected base on the least identity and least E-value and the epitope was predicted i.e. TSLVVEVVVESK. From which we have designed epitope molecule. This molecule binds with MHC 1 molecule successfully, so the selected epitope was docked with the MHC Class1 molecule and the docking energy was found to be 169.117. Therefore from the analysis vaccine is potent and good for further research. From this docking analysis we can conclude that this is the best Vaccine Candidate. The resultant vaccine can be sent to clinical trials and used for further research.

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