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DESIGN & DEVELOPMENT OF POTENT VACCINE TO TUBERCULOSIS BY REVERSE VACCINOLOGY APPROACH

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Abstract- Tuberculosis is a leading killer of young adults worldwide and the global scourge of multi-drug resistant tuberculosis is reaching epidemic proportions. It is endemic in most developing countries and resurgent in developed and developing countries with high rates of human immunodeficiency virus infection. The bacteria causing tuberculosis belong to a diverse group of microorganisms known as mycobacterium. Humans are the only reservoir for the bacterium. The gastrointestinal (GI) tract is also affected by Mycobacterium tuberculosis. The main aim of this paper is to Design and Development of vaccine against tuberculosis by Reverse Vaccinology approach. Mycobacterium tuberculosis is the bacteria which causes tuberculosis, having 8000 odd proteins. One protein was selected from 8000 odd proteins which has less Identity and less E-value. After screening of all the proteins the protein sequence with less identity and less E-value was chosen. From this antigen determinants were found, out of which HRRAPL antigenic determinant was found to be good vaccine candidate through docking analysis.

Keywords- Mycobacterium tuberculosis, Antigenic determinant, gastrointestinal tract, docking.

Introduction

Tuberculosis is the life threatening disease. TB is mainly caused due to mycobacterium tuberculosis. *Mycobacterium TB* also infects oesophagus, stomach and duodenum, jejunum, ileum, large bowel, Appendix, Caecum and ascending colon.

Taxonomically at the scientific name of *Mycobacterium* tuberculosis *complex* are listed 39 different types of bacteria that have been characterized at molecular level (Table 1)

Mainly tuberculosis occurs in:

a. Gastrointestinal tuberculosis
b. Tuberculosis in oesophagus
c. Tuberculosis in stomach and duodenum

Gastrointestinal Tuberculosis: Gastrointestinal tuberculosis is defined as infection of the peritoneum, hollow or solid abdominal organs, and abdominal *lymphatics* with Mycobacterium tuberculosis organisms. [19, 21].

Gastrointestinal TB is divided into 3 categories;

a. The Ulcerative form.
b. The Hypertropic form.
c. Mass like lesions (Ulcerhypertropic).

The Ulcerative form: Among these three forms, the ulcerative form is most likely to cause in human beings .Ulcerative form usually occurs in the human beings with the reduced immune response.

Hypertropic form: This form less likely occurs in human beings**.** *Hypertropic* form usually occurs in those who have enhanced immune system.

Mass like lesions (*Ulcerhypertropic***):** This less likely occurs in human beings as *hypertropic* form. *Ulcerhypertropic* usually results in the formation of ulcer and elevated mass lesions.

Symptoms are reduced immune system, Thickening of bowel wall with scarring, Fibrosis and a rigid mass like appearance that mimics that of malignancies.

[1]. **Tuberculosis in *Oesophagus***:

Oesophageal TB is extremely rare, accounting for only 0.15% of all TB deaths. Infection usually spreads to the *oesophagus* from adjacent disease in the lung, spine, or paraoesophageal glands, 'primary' lesions being rare. [1].

Symptoms are Solitary ulcer, External compression, Pseudodiverticulosis, Intramural dissection. [20]

[2]. Tuberculosis in Stomach and *Duodenum:*

The stomach and duodenum are rarely affected due to a combination of an acidic environment, a sparsity of lymphoid tissue and the rapid passage of swallowed mycobacterium. Gastric TB is reported to be more common in males (2–3 times) and in those aged 20–40 years. The antral-pyloric complex is commonly affected, resulting in gastric outlet obstruction. Symptoms are abdominal pain or dyspepsia, GI bleeding etc. (Ref 9)

REVERSE VACCINOLOGY APPROACH:
This approach starts from the whole genomic sequence of an organism. The prediction from computer helps in determining the antigenic determinants that are likely to be the vaccine candidates. After the identification of vaccine DNA vaccine is prepared. Then recombinant proteins are expressed. Then this vaccine is tested for immunogenicity in animal models. Then the Vaccine is developed. Before the vaccine is goes for marketing it has to undergo Phase 1, Phase 2, Phase 3 and Phase 4 trials, in these trials if it is found to be effective without any side effects then only the Vaccine is commercialized.

RESULTS

SCREENING OF PROTEIN
In screening of proteins the 3 protein sequences with least identity are found.

a. Least identity was 22. 5% with its Accession number is NP_216495.1 and gene ID is found to be 15609116.
b. Least Identity – 24.53, accession number found to be NP_215297.1 and gene ID is 15607923.
c. Least Identity – 23.171, Accession number is 3CXY and Gene ID is 206581993 .

INFERENCE-The Above sequence (1) which is obtained from screening of proteins, having least identity and least E value was chosen and antigenic determinants were found out using Emboss Antigenic. The antigenic determinant with greater LCV value was chosen and compared with mappp results and immunomed results. This antigenic determinant was used to design a molecule and further used in docking analysis.

IMMUNOMED GROUP- This tool is mainly used to find out antigenic determinant (epitope) and the best epitope was identified using docking analysis.

DISCOVERY STUDIO 2.5- This software is mainly used to design a molecule and for its minimization purpose. The molecule is in turn docked thereby helps to find out its interaction energy.

MINIMIZATION OF MHC MOLECULE:
The antigenic determinant HRRAPL was designed. This antigenic determinant was chosen based on the score value. The MHC molecule is designed by minimization process and the minimization energy was found to be 206.3578.

DOCKING: Molecular docking is a method to predict the orientation of one molecule to a second molecule when bound to each other to form a stable complex. Docking can also be used in "lead optimization" process. This helps in increasing the binding potential. In the fig 4 given below shows that the *epitope* molecule is docked successfully with the MHC I molecule successfully

The C *docker* energy of the molecule was found to be 57.886 and C *docker* interaction energy was found to be 82.7527

BLAST
Here sequences of some of the *vertebrates*, non *vertebrates* and *homosapeins* were obtained by using this blast.
Here are some of the organisms and their resultant sequences are obtained by BLAST and some of the Accession number of the Sequence is listed below.

a. Query sequence is given with Accession number NP_216495.1 and Gene ID is 15609116 .
b. Resultant Sequence for Mycobacterium tuberculosis H37RV is found and its accession number is NP_216495.1 and gene ID is 15609116.
c. Similarly Resultant sequence for Mycobacterium Bovis was found and its Acession number is NP_855651.1 and gene ID is 31793158.

Clustal W Dendrogram : This is mainly used to identify the similarities between the organisms**.**
The Clustal W Dendrogram shows that *Mycobacterium tuberculosis* H37RV is closely related with *Mycobacterium Bovis* and *permease* Mycobacterium tuber and are distinctly related with *Borrelia garinii* PBI.

DISCUSSION AND CONCLUSION:
Tuberculosis is the life threatening disease caused by *Mycobacterium Tuberculosis*. It is found that it infects Human beings and some of the animals as well. We retrieved the complete proteomic sequence of *Mycobacterium tuberculosis* and screened it by using SDSC workbench. We got the sequence having the least identity as 24.53 %. *Antigenic* determinant HRRAPL was selected based on the least identity and the *epitope* was predicted .From this *epitope* molecule was designed which binds with MHC I molecule. The *epitope* that was selected was docked with MHC I molecule. And the Docking energy was found to be 55.8876. Therefore from the analysis vaccine is potent and good for further research. The research we have done can be sent to clinical trials and the vaccine can be further developed can be used for curing disease .By docking analysis we found that our vaccine is the best candidate and can be further used for research purposes

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Table 1.Taxon and scientific name

Taxon	Mnemonic	Scientific Name
33894		*Mycobacterium africanum*
1765	MYCBO	*Mycobacterium bovis*
233413		*Mycobacterium bovis AF2122/97*
33892		*Mycobacterium bovis BCG*
413996		*Mycobacterium bovis BCG str. Moreau RDJ*
410289	MYCBP	*Mycobacterium bovis (strain BCG / Pasteur 1173P2)*
78331		*Mycobacterium canettii*

Table 2. *INFERENCE the sequence (1) obtained from screening of proteins was taken and antigenic determinants were found through immunomed group.*

n	Start Position	Sequence	End Position
1	8	GYAIHKLGFCSVVMLGI	24
2	28	IGAGIFLTPGEVIGLAGPFAPMAYVLAGIFAGVVAIVFATAARY	71
3	77	ASYAYTT	83
4	90	IGIYVGVTHAITA	102
5	104	IAWGVLASFFVSTLLRVAF	122
6	129	DAEQLFSVKTLTFLGFIGVLLAI	151
7	167	TVGKAFALSAFIVGGLWIITT	187
8	195	TAWSAYSATPYSLLGVAE	212
9	219	SSMALATIVALYAF	232
10	252	LPRAIPIAIFSVGAIYLLTLTVAML	276
11	285	SDDTVKLAAA	294
12	300	FRTIIVVGALIS	311
13	313	FGINVAASFGA	323
14	325	RLWTALADSGVLPT	338
15	344	NQYDVPMVSFAITASLALAFPLALRFDNLHLTGLAVIARFVQFIIVPIALIALARSQAVEHAAVR	408
16	412	FTDKVLPLVAIVVSVGLAVSYDYRCIFLVRG	442
17	445	NYFSIALIVITFVVVPAMAYLHYYRIIR	472

Table 3. ***MAPPP:*** *From Mappp results the MHC binding probability and MHC cleavage score was found*

Protein position	**Protein position**	**Protein position**					
Epitope	**Position**	**MHC type**	**n-mer**	**Overall score**	**Cleavage Probability**	**MHC binding score**	**Group**
0..480	481	MVGPRTRGYAIHKLGFCSVV..MAYLHYYRIIRRVGDRPSTR					
VLAGIFAGV	51	HLA_A_0201	9	0.8889	1.0000	0.7778	n-term. trimmed
VLAGIFAGV	51	HLA_A_0201	9	0.8889	1.0000	0.7778	n-term. trimmed
VLAGIFAGV	51	HLA_A_0201	9	0.8889	1.0000	0.7778	trimmed twice
VLAGIFAGV	51	HLA_A_0201	9	0.8889	1.0000	0.7778	same length
VLAGIFAGV	51	HLA_A_0201	9	0.8889	1.0000	0.7778	c-term. trimmed
VLAGIFAGV	51	HLA_A_0201	9	0.8889	1.0000	0.7778	c-term. trimmed
GIFAGVVAI	54	HLA_A_0201	9	0.8750	1.0000	0.7500	trimmed twice
GIFAGVVAIV	54	HLA_A_0201	10	0.8824	1.0000	0.7647	n-term. trimmed
GIFAGVVAI	54	HLA_A_0201	9	0.8750	1.0000	0.7500	c-term. trimmed
GIFAGVVAIV	54	HLA_A_0201	10	0.8824	1.0000	0.7647	same length
VLASFFVSTL	107	HLA_A_0201	10	0.9015	0.9500	0.8529	n-term. trimmed
TFLGFIGVLL	139	H2_Kd	10	0.8929	1.0000	0.7857	n-term. trimmed
AYSATPYSLL	198	H2_Kd	10	0.8929	1.0000	0.7857	n-term. Trimmed
PYSLLGVAEI	203	H2_Kd	10	0.8750	1.0000	0.7500	c-term. trimmed
ESIANAAEEM	235	H2_Db	10	0.9242	1.0000	0.8485	same length
ESIANAAEEM	235	H2_Db	10	0.9242	1.0000	0.8485	c-term. trimmed
AIYLLTLTV	264	HLA_A_0201	9	0.8750	1.0000	0.7500	c-term. trimmed
LALRFDNL	364	H2_Kb	8	0.8871	1.0000	0.7742	n-term. trimmed
LALRFDNL	364	H2_Kb	8	0.8871	1.0000	0.7742	n-term. trimmed
AVRRNAFTDK	405	HLA_A3	10	0.8845	0.9999	0.7692	c-term. trimmed

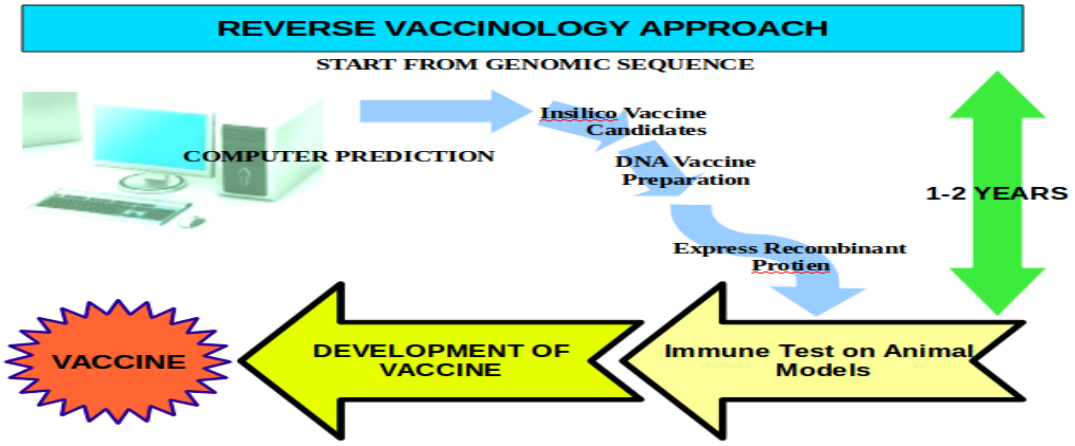

Fig.1 Reverse Vaccinology Approach

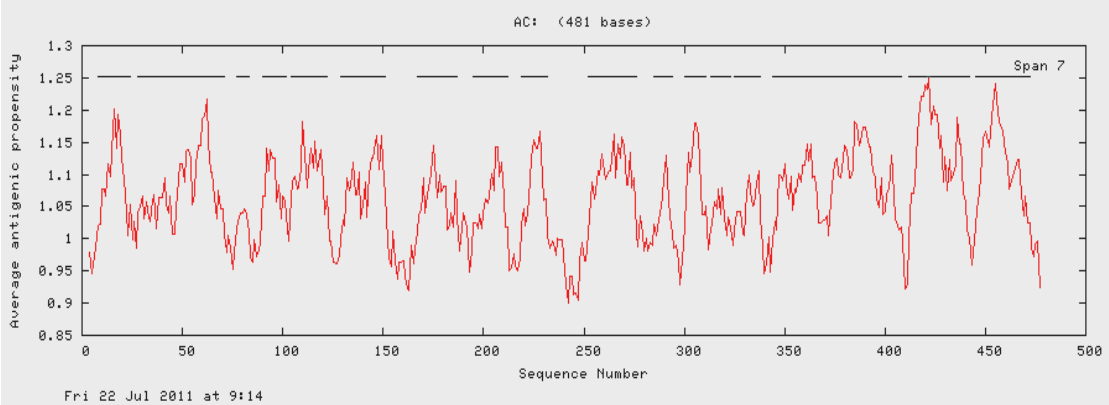

Fig. 2 Average antigenic propensity

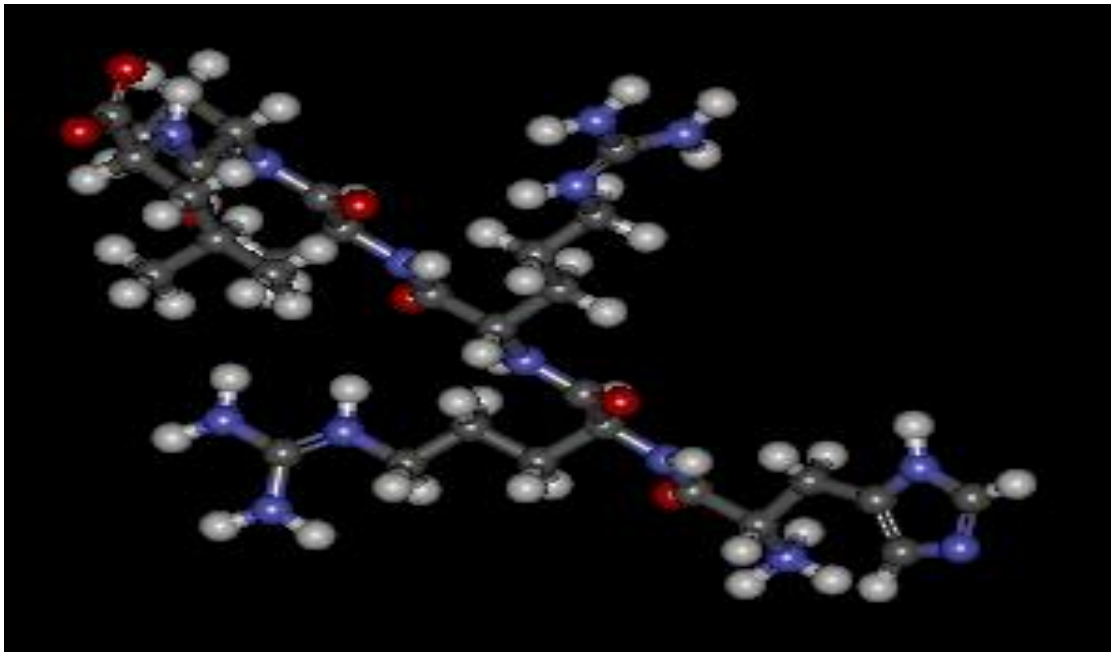

Fig. 3-MHC Molecule

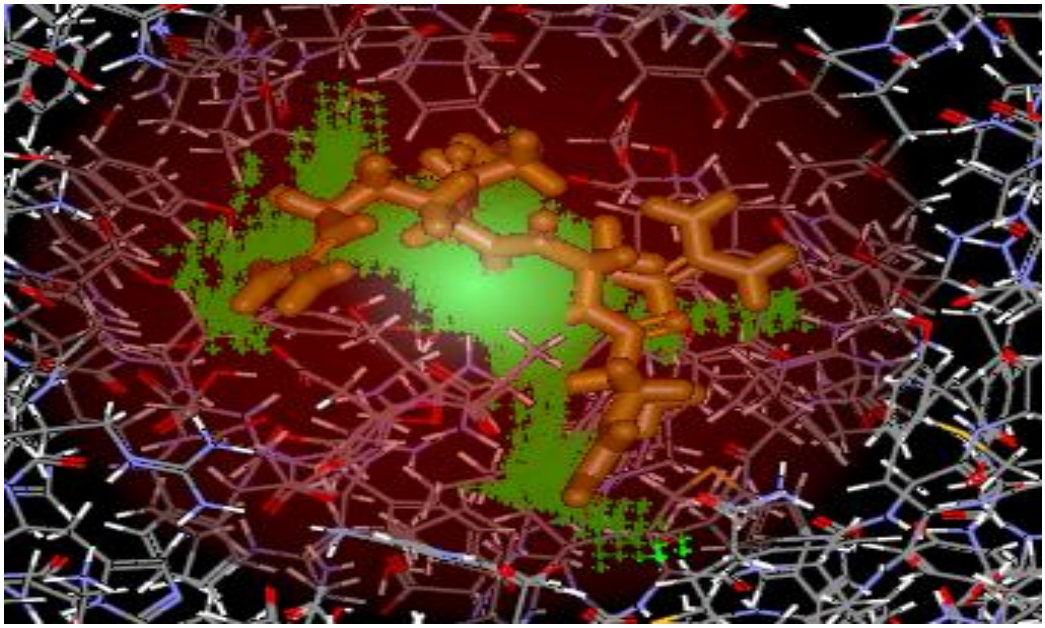

Fig.4-Docked molecule

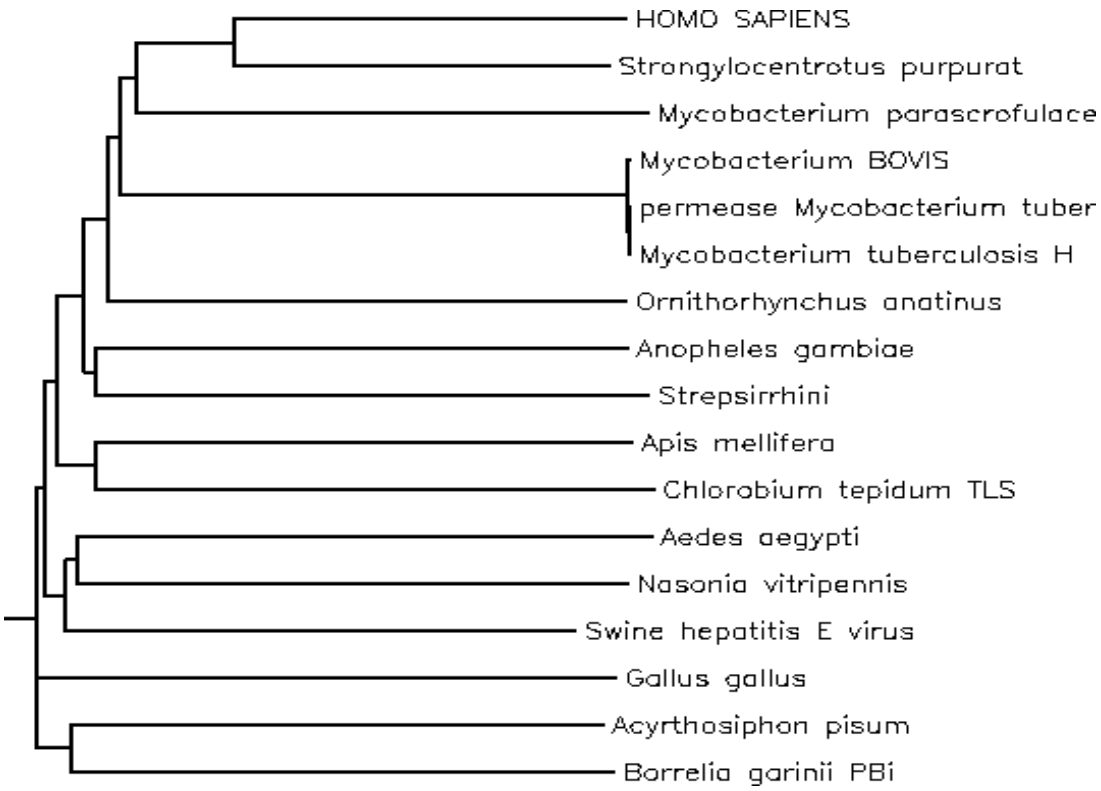

Fig.5- The Clustal W Dendrogram