INSILICO DESIGNING AND DEVELOPMENT OF VACCINE FOR V.CHOLERAE O139 IN CHOLERA DISEASE

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ABSTRACT

V.cholerae was first isolated by Italian anatomist Filipo Panici. V.cholerae is the etiological agent of cholera, a major health concern in most of the developing countries. V cholerae carry strains the encode the cholera toxin. These cholera toxins enters the Epithial cells and after crossing host line of defense it starts colonizing itself in the small intestine. Cholera is usually a non contagious disease.

The main aim of this project is to design and develop a vaccine against cholera. Vibrio Cholerae is a bacterium with 12,865 odd proteins causing cholera. Among these 1 protein sequence was selected having least identity and least E- value. It was then screened by using SDSC workbench tool. Then antigenic determinants were found by using different tools. The sequence with least identity was taken into consideration and then further designed and used for docking studies. From this Docking analysis the epitope molecule LEALVEDL was found to be the best vaccine candidate**.**

1. Introduction

Vibrio cholera also called as kommabacillus is a gram-negative comma shaped bacterium with a polar flagellum that causes cholera in humans. V.cholera follows a fecal-oral infection path. It moves from contaminated food and water and colonizes in the small intestine. V.cholera produces cholera toxin, the enterotoxin which acts on the mucosal epithelium resulting in diarrhea.

There are two major biotypes of v.cholera i.e classical and 'EI Tor' which can be identified by hemagglutination test. They occur both in marine and fresh water surfaces. It causes severe diarrheal disease in humans by food and water. It is one of the most fatal illnesses known as diarrhea.

1.1 Clinical Symptoms:

- Cholera symptoms include watery faces
- With bits of mucus and mild fishy smell
- Vomiting
- Abdominal cramps

- Dehydration
- Fever, it is rare, usually found in children.

The primary symptoms of cholera are profuse painless diarrhea and clear vomiting. These symptoms start within 1 to 5 days after ingestion of bacteria. An untreated person may produce 10-15 lit. of diarrhea a day with fatal results. If severe diarrhea and vomiting are not treated aggressively it may result in life threatening dehydration and electrolytic imbalances.

1.2 Mode of transmission:

Transmission occurs through ingestion of contaminated water and food. Sudden large outbreaks are usually caused by a contaminated water supply. Raw or undercooked seafood may be a source of infection in areas where cholera is prevalent and sanitation is poor. Transmission due to direct person to person contact is rare.

2. Materials and Methods

2.1 Screening of Proteins

SDSC workbench: This bioinformatic tool is very essential in screening of proteins. By this screening the protein with least identity was identified and its antigenic determinant was found.

The following protein ID with least identity was found are ;

- Gene ID was found to be IF2_VIBC3 and its accession number is 172047700 with least identiy 20.62.
- Least Identity – 21.89 , gene ID was found to be – 189046614 and the Accession number is SECA_VIBC3

2.2 IMMUNOMED GROUP

This is mainly used to find out the antigenic determinants of the sequence that has least identity. From this tool the Average antigenic propensity for protein sequence was found to be 1.0097

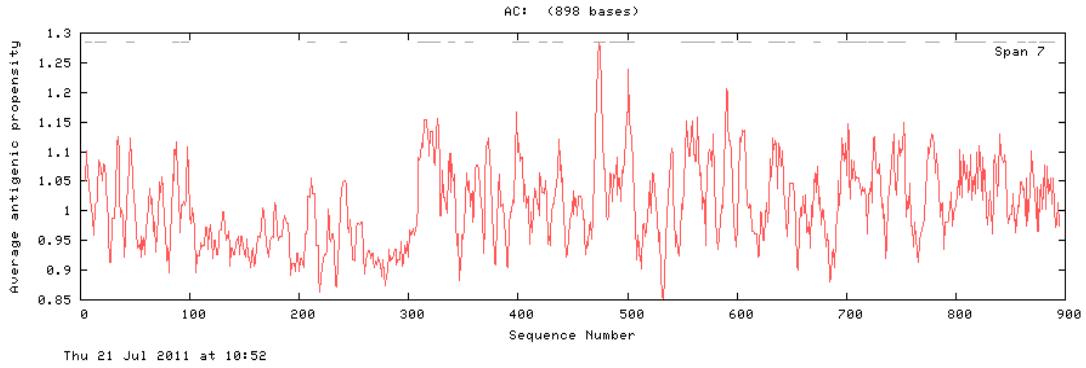

Fig 1. Average Antigenic Propensity

There are 31 antigenic determinants in the sequence:

n	Start Position	Sequence	End Position
1	4	ITVKALS	10
2	13	IGTPVDRLLEQ	23
3	42	KQKLLAHL	49
4	84	KNVQVEV	90
5	92	KKRTYVKR	99
6	208	QLEKVRE	214
7	237	TDYHVTT	243
8	308	DKTAVVAKADVVVGETIVVSE	328
9	336	KATEVIK	342
10	352	TINQVID	358
11	395	EVSRAPVVTIMGHVDH	410
12	420	RRTHVAS	426
13	432	ITQHIGAYHV	441
14	469	ATDIVVLVVAA	479
15	484	MPQTVEAIQHAKAAGVPLIVAVN	506
16	549	IDGLLEAILLQAEVLELKAVKQ	570
17	572	MASGVVIE	579
18	586	RGPVATVLVQS	596
19	601	KGDIVLCGQEYGR	613
20	629	GPSIPVEILGLSGVPA	644
21	647	DEATVVR	653
22	670	REVKLAR	676
23	692	GDVALNIVLKADVQGSVEAIADSLTK	717

24	719	STDEVKVNIVGSGV	732
25	737	ETDAVLAAASNAIVVGF	753
26	771	DLRYYSIIYQLIDE	784
27	798	KQEIIGLAEVRDVFKSPKLGAIAGCMVTE	826
28	833	APIRVLRDNVVIY	845
29	856	KDDVAEV	862
30	866	YECGIGV	872
31	876	NDVRVGDQIEVFET	889

Table 1

2.3 MAPPP

This tool is used to find out the type of MHC molecule (MHC 1 or 2) to which the epitope molecule binds.

Mappp results were found to be

Epitope	Position	MHC type	n-mer	Overall score	Cleavage Probability	MHC binding score	Group	
0..897	898	MTQITVKALSEEIGTPVDRL..VRVGDQIEVFETIEIQRTID						
QRKTRSTL	66	HLA_B8	8	0.8500	1.0000	0.7000	n-term. trimmed	
QRKTRSTL	66	HLA_B8	8	0.8500	1.0000	0.7000	c-term. trimmed	
QPRSDEEKL	168	HLA_B_0702	9	0.8429	1.0000	0.6857	n-term. trimmed	
QPRSDEEKL	168	H2_Ld	9	0.8387	1.0000	0.6774	n-term. trimmed	
QPRSDEEKL	168	HLA_B_0702	9	0.8429	1.0000	0.6857	n-term. trimmed	
RRKAEEESR	197	HLA_B_2705	9	0.8514	1.0000	0.7027	c-term. trimmed	
PRGGKAGRK	285	HLA_B_2705	9	0.8514	1.0000	0.7027	c-term. trimmed	
KENELEEAI	376	H2_Kk	9	0.8497	0.9994	0.7000	trimmed twice	
KENELEEAI	376	H2_Kk	9	0.8498	0.9995	0.7000	c-term. trimmed	
ANPDNVKTEL	512	H2_Db	10	0.9545	1.0000	0.9091	n-term.	

							trimmed	
GLLEAILLQA	550	HLA_A_0201	10	0.8528	0.9997	0.7059	same length	
GLLEAILLQA	550	HLA_A_0201	10	0.8529	1.0000	0.7059	c-term. trimmed	
LLQAEVLEL	556	HLA_A_0201	9	0.9028	1.0000	0.8056	n-term. trimmed	
ILLQAEVLEL	555	HLA_A_0201	10	0.9559	1.0000	0.9118	n-term. trimmed	
EVLELKAVK	560	HLA_A3	9	0.8422	0.9868	0.6977	c-term. trimmed	
AIADSLTKL	710	HLA_A_0201	9	0.8916	0.9776	0.8056	same length	
AIADSLTKL	710	HLA_A_0201	9	0.9028	1.0000	0.8056	c-term. trimmed	
DEVKVNIV	721	H2_Kk	8	0.8667	1.0000	0.7333	c-term. trimmed	
RYYSIIYQLI	773	H2_Kd	10	0.9107	1.0000	0.8214	c-term. trimmed	
KRNAPIRVL	830	HLA_B_2705	9	0.8649	1.0000	0.7297	n-term. trimmed	

Table 2

2.4 DISCOVERY STUDIO 2.5

MINIMIZATION OF MHC MOLECULES

The antigenic determinants LEALVEDL was designed

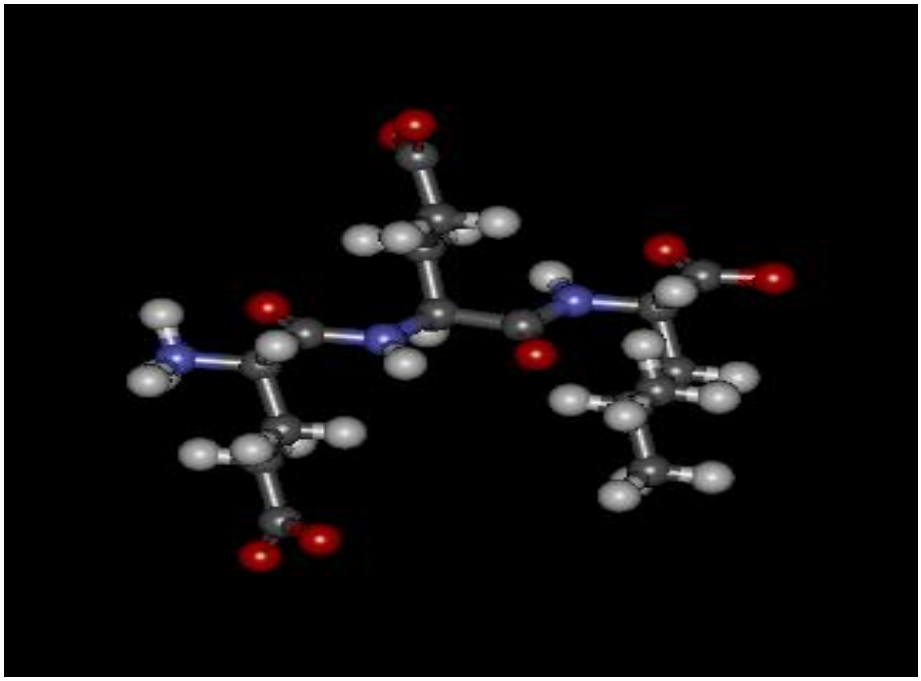

Fig 2. Epitope Molecule V.CHOLERAE

The minimization energy for this molecule is found to be 210.39238 kcal/mol.

The antigenic determinant GPVATVLVQSG was designed

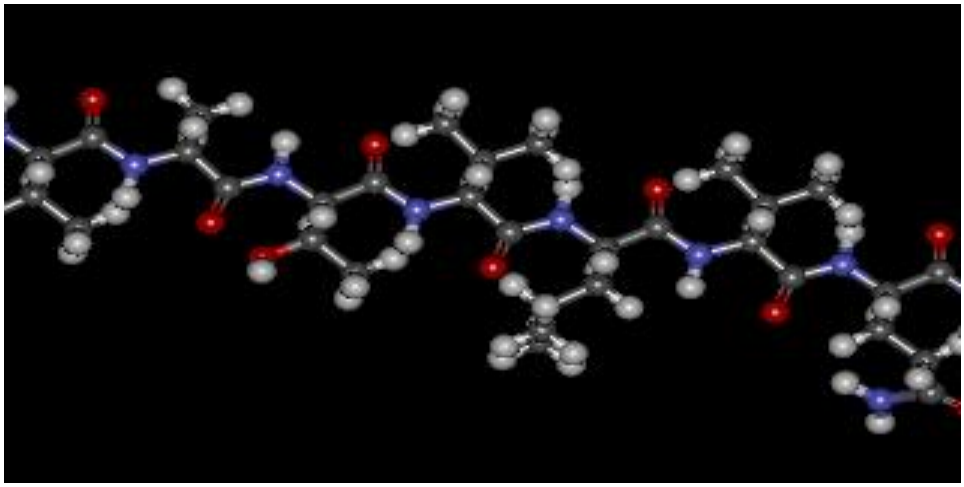

Fig 3. EpitopE Molecule 2, V-CHOLARAE

2.5 Docking

This is mainly used to fit the epitope molecule into the MHC 1 molecule.

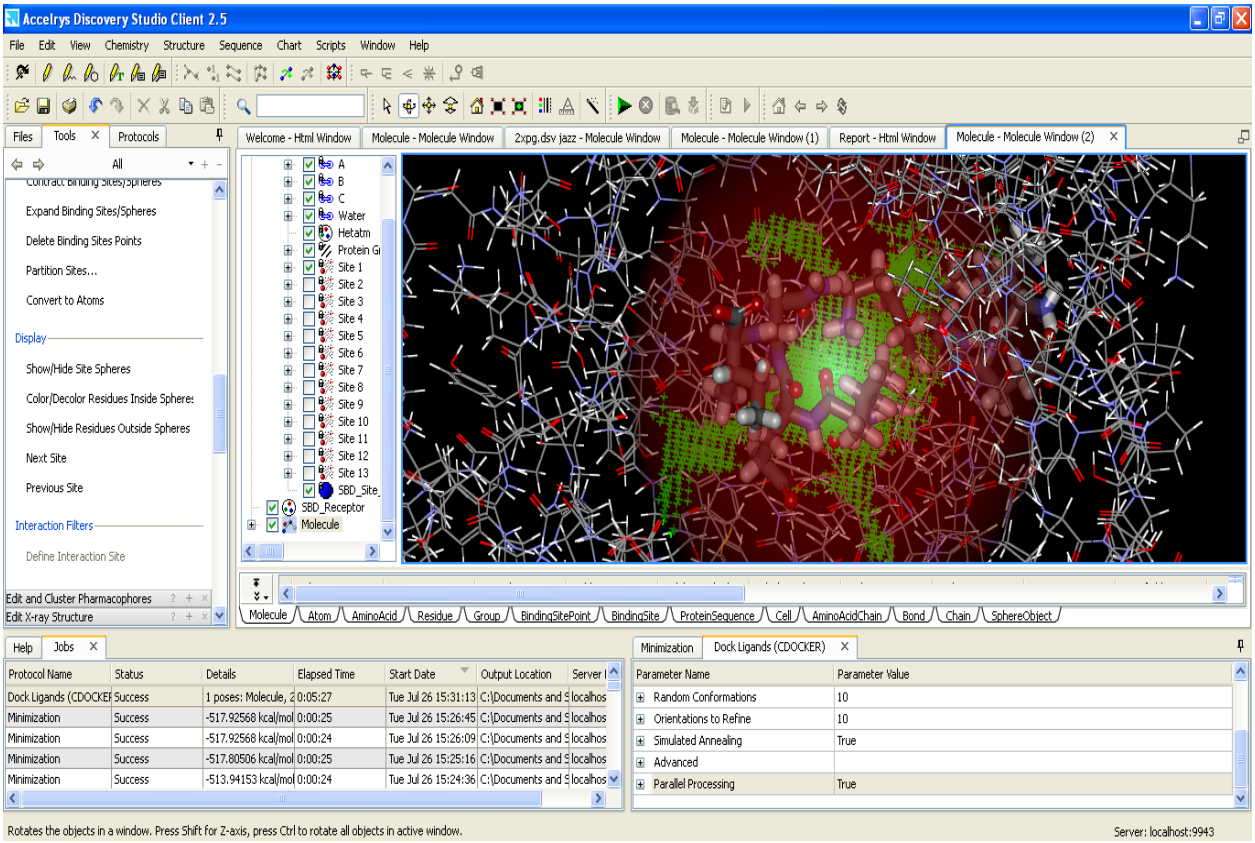

Fig 4. Docked Molecule V.CHOLERAE

INFERENCE : The epitope molecule was docked with MHC I molecule successfully

C docker Energy was found to be 74.1119

C docker Interaction Energy was found to be 41.0479

3. DISCUSSION

Thus from the above result it is found the antigenic determinant with least identity was 'LEALVEDL'. The c docker energy of this molecule was found to be 74.119 and C docker interaction energy was found to be 41.0479. By taking into consideration a vaccine is designed for cholera. The vaccine that is designed can be further sent for clinical trials and if it passes the FDA approval it can be used for curing this disease in future.

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