PREDICTION OF EPITOPE BASED VACCINE CANDIDATES FOR PERIODONTAL DISEASE

A DISSERTATION

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CANDIDATE'S DECLARATION

I hereby certify that the work which is presented in the research work entitled **"Prediction of Epitope based Vaccine candidates for Periodontal disease"** in fulfilment of the requirement for the award of Degree of Masters in Science in Biotechnology and submitted to the Department of biotechnology, Delhi technological university, Delhi is an authentic record of my own work, carried during a period from 7-jan-2021 to 28-may-2021, under the supervision of Dr. Asmita Das.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other university. This work has been communicated in SCI indexed journal with the following details:

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Place: Delhi

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ABSTRACT

Periodontal disease is a multifactorial dental complication that has an impact on the supporting tooth structures like the gingiva, cementum and alveolar bone. Various studies conclude PD is associated with several systemic diseases like cardiovascular, neuronal, autoimmune, and respiratory. It is seen that periodontal disease is also allied with PCOS, infertility, age, obesity, adverse pregnancy outcomes, erectile dysfunction, and diabetes. The individuals suffering from PD are more likely to have dental caries. The initiation is due to dysbiosis of commensal microbes present in the oral cavity releasing a large extent of proinflammatory cytokines including IL-6, TNF-α, and IL-1. The preliminary stage is reversible gingivitis and if proper treatment of gingivitis is not done then it can progress to periodontitis which can lead to alveolar bone loss and is irreversible. There are approximately 700 different bacterial species associated with periodontal disease. This disease immensely affects the daily activities of organisms. There are several risk factors allied with periodontal diseases such as poor oral hygiene, medical diseases, smoking, age, blood group, obesity, orthodontic treatments, heredity, and stress. Several periodontal therapies have been shown to improve the status of PD in individuals. A functionally active vaccine is required for this disease. The proposed study aims to identify vaccine candidates from multiple different species of pathogens involved with periodontal disease. 20 peptides were screened for epitope prediction based on various physicochemical criteria like antigenicity, dependency of the pathogen on the virulence factor. Comprehensive analysis of these antigens revealed that they have several potential B and T-Cell epitopes. 3 epitopes NYFKSQVIFQRLPEI, ASRRLYRGYEALFVP, ELEKAIEMEDLALNP exhibited more than 90% population coverage in the both Indian and Global context. Therefore, this analysis suggests that the

predicted epitopes might be suitable vaccine candidates and can be used for further in vivo and in-vitro studies.

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LIST OF ABBREVIATIONS

S.no	Abbreviation	Full form
1.	Aa	*Aggregatibacter actinomycetemcomitans*
2.	BD	Bipolar disorder
3.	AZ	Alzheimer disease
4.	CHD	Coronary Heart Disease
5.	CAL	Clinical attachment loss
6.	COPD	Chronic obstructive pulmonary disease
7.	CP	Chronic Periodontitis
8.	CRP	C- reactive protein
9.	CSF	Cerebrospinal fluid
10.	PPD	Periodontal Probing Depth
11.	RA	Rheumatoid Arthritis

12.	PD	Periodontal Disease
13.	TNF	Tumor Necrosis Factor
14.	PCOS	Polycystic Ovarian Syndrome
15.	IL	Interleukin
16.	GCF	Gingival Cervical Fluid
17.	ED	Erectile Dysfunction
18.	MMP	Matrix metalloproteinases
19.	LC	Liver Cirrhosis
20.	CVD	Cardiovascular Diseases
21.	HCV	Hepatitis C Virus

CHAPTER 1

INTRODUCTION

1.1 General

Periodontal disease is a set of complex non-communicable disorders that are caused by a diverse range of factors. [1][2] Gingivitis is the gentle form of periodontal disease. It is a location-specific inflammatory condition and is of two types- it can be plaque-induced gingivitis or it can be non- dental biofilm induced. [3][4] Gingivitis is more severe in people who have some genetic abnormality like Down syndrome as compared to genetically healthy individuals. It shows symptoms like redness, edema,[5] and periodontal attachment loss. Acute periodontitis is the 6th most ubiquitous disease worldwide. About 20-50% of the population worldwide is affected by periodontal disease. A study put forward that greater than 47% of Americans who are more than or equal to 30 yrs of age are suffering from chronic periodontitis. The manifestations of the disease include hemorrhage, loss of support for dentition, formation of periodontal pockets, and gingival crevice deepening. In gingivitis, there occurs gingival inflammation without the loss of alveolar bone but in periodontitis, there is a progressive alveolar bone loss which ultimately leads to teeth loss. [6][7]. Due to some reasons like inadequate or infrequent cleaning of the mouth, the deposition of bacterial biofilm occurs which initiates the beginning of this chronic disease. Oral microbiota of different individuals is different varying according to the host genetics, comorbidities, age, or sex,

and thus has different effects. [8][9] The prodrome stage does not occur in the beginning and also there is a very less amount of pain, but as the disease progresses, it becomes severe and shows chronic symptoms [10]. The presence of pathogens is required but is not completely sufficient for the development of periodontitis as the body responds effectively to the bacterial infection and can control the disease. At the site of bacterial infection, various cells like macrophages, neutrophils, monocytes, mast cells are present. Tissue destruction is caused due to the enhanced production of cytokines. [11] It is also observed that some proteins like alkaline phosphatase (ALP), MMP-9, IL-1b, complement factors, fibronectin are higher in the amount in case of gingivitis patient and others like cystatin-S and B are lower in concentration as opposed to that in healthy individuals. [12] Matrix metalloproteinases (MMP-8, 9) degrade the extracellular matrix and perform a major role in the immune response. The cell migration at the inflammatory site is through MMP's. [13]Therefore, for determining the periodontal status, CAL measurement is considered as the most appropriate way as it is based upon a fixed reference point i.e. The cemento-enamel junction. [14][15] Chronic periodontitis (CP) induces bacteremia. It is the entry of pathogenic bacteria from disease sites into the bloodstream during daily routine activities like brushing, chewing, biting, flossing, etc. [16][12] Due to the occurrence of bacteremia or septicemia the circulating bacterial species, their components, and other pro-inflammatory molecules reach to the systemic circulation and hence further amplify the initial inflammation. [17] An increase in B-regulatory cells among PD patients indicates the disease progression and is a potential link between PD and the systemic inflammatory process. [18]

1.2 REVIEW OF LITERATURE

1.2.1 PD and PCOS

A meta-analysis study showed that PCOS patients have almost 28% increased chances to develop PD with moderate heterogeneity as compared to controls. Also, the results showed that women's accompanied with PD have almost 46% more possibilities of having PCOS with complete homogeneity. [19] Systemic inflammation and insulin resistance are common links associating with PD and PCOS. [14] Oxidative stress is a connecting link between PD and other metabolic syndromes. As oxidative stress increases, it contributes to insulin resistance and other features of PCOS. SD Nair et al studied women suffering from PCOS and revealed that the existence of PD was higher in patients with PCOS than without. [20]

1.2.2 PD and Pregnancy Complications

There are two mechanisms linking PD with unpropitious pregnancy results. The first is the passing of PD pathogens to the fetoplacental unit and the second is due to the effect of inflammatory mediators produced by the host in the response to PD pathogens on the fetoplacental unit. [21][22] Research revealed that 6 pathogenic bacteria namely, *P.gingivalis, Treponema denticola, F.nucleatum, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Prevotella intermedia* were present in placenta samples. Also, the aggregate of *Treponema denticola* and *Fusobacterium nucleatum* were higher in threatened preterm labor (TPL) patients than in healthy groups. [22][21] The level of inflammatory mediators in amniotic fluid increase and thus cause rupture of amniotic sac membrane, cervical dilation, uterine contraction, and delivery. Elevated CRP levels activate the

complement system leading to the release of cytokines and hence premature breakdown of the fetoplacental membrane. [23]

1.2.3 PD and Cardiovascular Diseases

The PD pathogens have been discovered in the atherosclerotic plaques hence suggesting that PD affects the initiation and progression of cardiovascular disease. [13] Nitrous oxide regulates platelet aggregation & expression of adhesion molecule. The PD pathogens inhibit nitric oxide and this is the primary mechanism for atherosclerosis. [24]Bacteremia results in direct vascular injury which then contributes to the disease and hence leads to host inflammatory reactions which act as another mechanism connecting CVD & PD. [11][25] The process of atherosclerotic development is elevated by the secretion of cytokines which then causes the stimulation of toll-like receptors (TLRs) and the release of MMP s. [11]

1.2.4 PD and Diabetes:

Diabetes and PD share a two-way relationship. [26] The risk and severity of having inflammatory PD are increased by diabetes and also the PD disease can intensify insulin resistance and hence affect glycemic control. Both diabetes mellitus and PD share a common feature of inflammation. [27] Clinical studies provide evidence to support the fact that amplified levels of systemic markers in gingival tissues of people with inadequately controlled diabetes play a role in the elevated amount of tissue destruction. [28][29]. As proven by several studies, the substantial confounding factors in this bidirectional

relationship include sex, C- related protein, age, WBC count, body mass index (BMI), hypertension, smoking, and several others. [26]

1.2.5 PD and Neuronal diseases

Defects like aphasia (loss of speech abilities), agnosia (loss of sensory processing), and apraxia (loss of motor skills) are some of the indications of AZ disease. [30] A study confirmed the appearance of *P.gingivalis* lipopolysaccharides in AZ patients, confirming the conjecture that *P.gingivalis* infection is related to the severity of AZ including altered cytokine profiles, inflammasome activation, and microglial activation. It was observed that the gingipains (secreted by *P.gingivalis*) had greater immunoreactivity in AZ patients as compared to those in non- AZ controls. These are neurotoxic & exert deleterious effects on tau which is necessary for normal neuronal functioning. *P. gingivalis* can lead to AZ progression in several other ways. They can enter the brain & spread through several pathways that involve direct passage through the blood-brain barrier.

1.2.6 PD and Respiratory disorders

The aspiration of oral pathogens, polysaccharides, and released enzymes result in the inflammation of respiratory airways. The downregulation of inflammatory cytokines and secretion of protective antibodies is done by IL-10. The connection between PD and asthma can be due to the interconnection between IL-10 reduction and the polymorphisms linked with its gene. People suffering from asthma and having low levels of IL-10 have worsened PD conditions. Therefore, the treatment methodologies for PD should involve procedures that amend the inflammatory mechanism of the respiratory tract. [31] Holtfreter et al.found a

connection among decreased respiratory function and chronic periodontitis through systemic inflammation. CP plays a role in the accumulative systemic inflammatory burden. Due to this, there is a decrease in spirometric lung volumes.

1.2.7 PD and Autoimmune diseases

Even though rheumatoid arthritis (RA) is an ailment of joints and PD is of the mouth, still, they have many things in common because both of them include chronic inflammation which results in bone erosion and the breakdown of connective tissue. [32] In PD, antibodies to host components mainly collagen are reported. Peptidyl arginine deaminase (PAD) which is produced by *Porphyromonas gingivalis* has been diagnosed as a susceptibility factor for RA. [33] The pathogen has a unique citrullating capacity of RA autoantigens and foreign microbial antigens. Along with *Porphyromonas gingivalis*, a specific immunodominant role is played by other pathogens like *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*. [34]

1.2.8 PD and Aging

Aging is a process at the cellular level which causes degenerative changes leading to multiple diseases that can be infectious, autoimmune, or inflammatory. Earlier aging has been assumed to be associated with periodontal disease but certain studies doubt this association. PD mainly affects the elderly population because they are prone to additional risk factors like the presence of chronic diseases, and hence can alter the microenvironment of gingiva and the development of periodontal disease. [2] The presence of PD in the aging population shows immune dysregulation associated with age. [35]

1.2.9 PD and Obesity

Obesity is linked to a risk of greater than 20 medical conditions such as diabetes mellitus, PD, CVD, dyslipidemia, hypertension. [36] It is the 2nd most common factor related to increasing PD infection after smoking. [2] It affects the periodontal attachment apparatus by exerting influence on periodontal inflammation. A two-way relationship has been recognized between PD and obesity. [37] Adipocytes release pro-inflammatory cytokines, leptin, resistin, adipokines, adiponectin, plasminogen activator inhibitor- 1, and also generate oxidative stress. [2][38]The increased systemic inflammatory response and oxidative stress contribute to the progression of PD.[2] Genco et al.concluded that BMI increase is directly proportional to CAL. Hence, a clear association was shown where the increase in inflammatory markers can directly worsen the periodontal conditions. [24]

1.2.10 PD and Erectile dysfunction

It has been frequently seen that periodontitis has a significant role in the pathogenesis of impotency. As PD is correlated with both localized and systemic increases in inflammation, it is hypothesized that this proinflammatory state of PD develops endothelial dysfunction and the typical cause of ED is vascular diseases. [39] The two diseases CP and ED have many similar systemic risk factors like age, CVD, smoking, diabetes. Vlachopoulos et al. described that the widespread presence of ED in patients with CVD was 47% which was greater than the expected 24% present in the common population. [40] As there is concluded evidence of the association between CP and CHD, the vasculogenic ED is regarded as a warning sign of cardiovascular heart diseases. It is believed that endothelial dysfunction instigated by CP &

atherosclerosis is demonstrated first in smaller penile vasculature and then into larger coronaries and great vessels. [41]

1.2.11 PD and Blood group antigens

Very less amount of research has been conducted to understand the mechanism linking periodontal diseases with ABO blood group antigens. Kaslick et al. described the link allying aggressive periodontitis and ABO blood grouping. Studies infer that blood groups A and O are directed more towards periodontal disease. The blood group O leaned towards the periodontitis group and blood group A towards gingivitis, whereas the AB blood group depicted a healthy population. These findings suggest a possible genetic basis as discussed by Roberts. Demir et al. discovered that there are differences in the rates of colonization of periodontal pathogens inducing the periodontal disease by the different ABO blood groups. [42]

1.2.12 PD and Liver diseases

Hepatitis C virus infections can lead to oral health problems like PD or cancer, and these are the most frequent liver diseases (87.2%) followed by liver cirrhosis (25.5%). [43][44] Several studies have shown that the periodontal health of patients having HCV-associated liver diseases is poor than those of controls. Patients with viral liver diseases have several oral hygiene problems including reduced salivary flow, elicitation of PD by IR, prolonged bleeding, etc. [45]Yumiko Nagao and Takeshi Tanigawa concluded that patients suffering from liver cirrhosis have high levels of red-complex bacteria and are at a greater threat of

periodontal disease development. A considerable amount of CAL and spontaneous bacterial PD is a characteristic of LC patients. [43]The lipopolysaccharides released via *P.gingivalis* infection are known to induce liver inflammation. [2] It is examined that the mechanisms relating to chronic HCV infection and PD status are insulin resistance, immunological dysfunction, lack of proper dental care, and chronic inflammatory status. The released inflammatory cytokines are related to PD triggering and progression. The GCF fluid flow increases during the PD and hence virus can immigrate easily into the GCF from the bloodstream & then into saliva. A study found viral RNA & Anti- HCV Ab within the GCF samples which are derived from HCV infected patients. The connection of inflammatory markers found in GCF and HCV infection needs to be assessed. [44]

1.3 TREATMENT:

The removal of plaque and supragingival calculus through root planing and scaling is the most frequent method of PD therapy. The local drug delivery systems can provide a constant and undeviating supply of drugs on the target site at the highest concentration. The systemic delivery system can have adverse side effects; it also does not remove bacteria from GCF but is capable of eliminating them from the subgingival microbiota. For the GCF, a higher dosage of the drug is required and thus leading to the evolution of resistant bacterial strains. Hence, the local delivery system has an advantage over the systemic delivery system and thus also has less toxic effects. For the efficient treatment of PD, nanoscale intrapocket nanofibres are beneficial delivery systems. [46] Although several treatments have been developed in the past still they can not redevelop the lost tooth tissues. But still, the use of DMPSCs has some

limitations like ethical problems as the pulp is extracted from a completely healthy tooth for trying to treat a tooth that is damaged. Also, the number of DPMSCs decreases with age. [47] As such, there is no committed vaccine against periodontal disease. The development of a vaccine against periodontal disease is highly important. *Treponema denticola* is an oral spirochete and possesses a number of disease-causing factors like chymotrypsin-like protease, dentilisin, major outer sheath protein (MSP), etc. The adhesion to mucosal surfaces and pathogenesis of *T. denticola* is mediated through the MSP. The fragment F3 (PerioVax3) is the most potent fragment and the application of antisera against fragment F3 inhibited the bonding of *T. denticola* to the surface and also restricted the detachment of human gingival fibroblasts upon the exposure of the pathogen. A study raised antisera against the polypeptides in the rabbit. The data from the study suggested that the PerioVax3 fragment carries antigenic determinants that trigger the humoral immunity against the pathogen. [48]

CHAPTER 2

MATERIALS AND METHOD

2.1. Database and Servers Used-

- National Centre for Biotechnology Information (NCBI)
- UniProt
- PubMed
- IEDB
- BLASTp

2.2 Method-

- **Retrieval of pathogenic organisms associated with PD from literature-**

  The list of pathogenic organisms most commonly associated with periodontal disease has been retrieved from PubMed server. The names of pathogens were easily available.[49]

- **Study of virulence factors produced by pathogens-**

  A number of proteins produced by the listed pathogenic organisms which have a role in the deveoplment or continuation of the periodontal disease has been derived. From UniProt, the sequences of all the virulence factors were obtained.

- **B-cell Epitope Prediction-**

  Immnoglobulins recognize specific sections from the pathogenic organisms and these regions are referred to as B-cell epitopes. The immunoglobulins present inside the body bind with these epitopes from the antigen. They are of two types- linear and conformational epitopes.[50] Linear epitopes are those in which the amino acids are present in a single line and the conformational epitopes are those where the amino acids are folded into a structure. Here the B-cell epitopes included are linear or continuous epitopes. The BepiPred server from IEDB is used for this analysis.

- **T-cell epitope prediction-**

  It is used for identifying the smallest peptides which are present in the antigen and are able to activate either T-helper or T-cytotoxic cells.[51] The T-cytotoxic and T-helper prediction has been done using Tepi Tool server.

- **Population Coverage Analysis-**

  The T- cells recognize peptides presented by MHC complex. The MHC complex and the pathogen derived epitope combine with each other and this depend upon if the individual possess a particular epitope or not. Those individuals that possess MHC molecules specific to a particular epitope will show immune response.[52]

CHAPTER 3

RESULT AND DISCUSSION

3.1 SELECTION OF PATHOGENS:

Literature provides a list of pathogens that have a major role in the initiation and progression of periodontal disease. *Porphyromonas gingivalis, Campylobacter rectus, Fusobacterium nucleatum, Eikenella corrodens, Treponema denticola* are the most common pathogens involved.[53] Retrieval of names of pathogenic organisms playing role in the initiation and progression of periodontal disease has been done. This is the list of some pathogens and among them *Porphyromonas gingivalis, Treponema denticola, and Tanerella forsythia, Fusobacterium nucleatum* are named as red complex bacteria.

Table 3.1- LIST OF PATHOGENS

S.NO	Name of the pathogen
1.	*Porphyromonas gingivalis*
2.	*Mycoplasma arginini*
3.	*Mycobacterium tuberculosis*
4.	*Chlamydia pneumoniae*
5.	*Rhodococcus erythropolis*
6.	*Streptococcus oralis*
7.	*Eikinella corrodens*
8.	*Fusobacterium nucleatum*
9.	*Treponema denticola*
10.	*Tannerella forsythia*
11.	*Aggregatibacter actinomycetemcomitans*
12.	*Prevotella intermedia*
13.	*Actinomyces odontolyticus*
14.	*Veillonella parvula*
15.	*Campylobacter rectus*

3.2 Selection of Virulence factors

The pathogens listed above produce a wide range of virulence factors which are responsible for the initiation and progression of the disease. A list of virulence factors is prepared.

Table 3.2- LIST OF VIRULENCE FACTORS

Name of the Peridontopathogenic organism	Virulence Factor
Porphyromonas gingivalis	• ATP-dependent Clp protease proteolytic subunit • Putative OppC • Putative OppB • Serine protease (Dentilisin) • Hemolysin I • OpdB • Protein-glutamate methylesterase/protein-glutamine glutaminase • CheXdomain-containing protein

	• Chemotaxis protein CheA • Prolyl endopeptidase • Membrane lipoprotein TmpC, putative
Aggrebacter acetomycitans	• Leukotoxin • Cytolethal distending toxin subunit • Leukotoxin-activating lysine-acyltransferase • Autotransporter adhesin • Collagen-binding adhesin autotransporter EmaA • 60 kDa chaperonin • Chaperone protein DnaJ

	• Chaperone protein HtpG • Leukotoxin export protein LtxD • dTDP-4-dehydrorhamnose reductase • DNA mismatch repair protein MutS • Karilysin • Bacterial group 2 Ig-like protein • Dipeptidase

Table 3.2- LIST OF VIRULENCE FACTORS (continued)

	Virulence factors
	• Peptidase S1 domain-containing

Aggrebacter acetomycitans	protein
	•
	• Exo-alpha-sialidase
	• Sialidase
	• Peptidase_S8 domain-containing protein
	• OmpA family protein
	• Cell division protein FtsZ
	• Serine hydroxymethyltransferase
	• Methylglyoxal synthase
	• Beta-N-acetylhexosaminidase
	• Hemagglutinin
	• Dipeptidyl-peptidase

Name of the Peridontopathogenic organism	**Virulence Factor**
	• Peptidase S1 domain-containing protein • Exo-alpha-sialidase

Tannerella forsythia	• Sialidase • Peptidase_S8 domain-containing protein • OmpA family protein • Cell division protein FtsZ • Serine hydroxymethyltransferase • Methylglyoxal synthase • Beta-N-acetylhexosaminidase • Hemagglutinin

The fasta sequences of the various virulence factors involved were obtained from Uniprot and saved.

3.3 SELECTION OF ANTIGENS-

A total of 20 antigens has been selected for epitope predicion based on the role in the development of periodontal disease and also on the basis of diversity between them. [54]–[59]

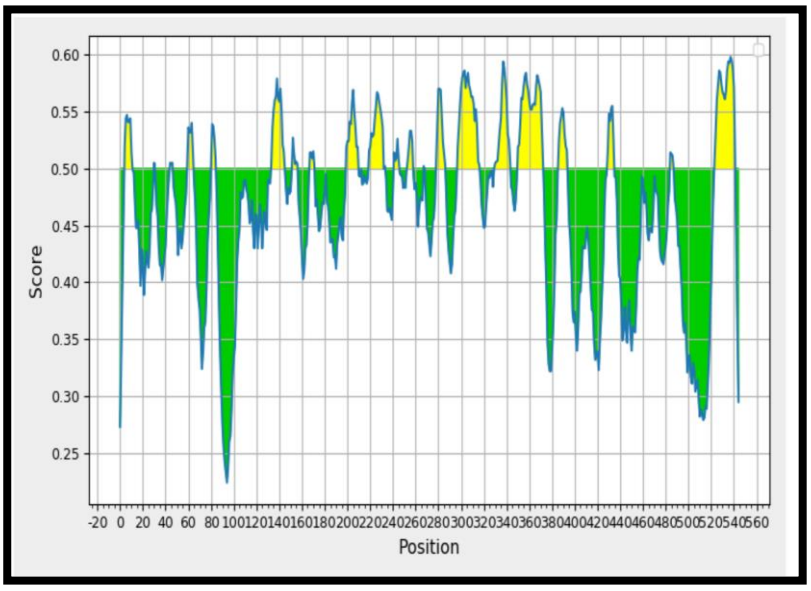
Table 3.3- LIST OF SELECTED ANTIGENS

S.No	Virulence Factor	Name of the Pathogen
1.	60 kDa chaperonin	*Porphyromonas gingivalis*
2.	60 kDa chaperonin 2	*Mycobacterium tuberculosis*
3.	60 kDa chaperonin	*Chlamydia pneumoniae*
4.	Sialidase, putative	*Porphyromonas gingivalis*
5.	LPS-assembly protein LptD	*Campylobacter rectus*
6.	Arginine deiminase	*Mycoplasma arginini*
7.	Type IV pilin	*Eikenella corrodens*
8.	Glyceraldehyde-3-phosphate	*Streptococcus gordonii*

	dehydrogenase	
9.	Glyceraldehyde-3-phosphate dehydrogenase	*Streptococcus oralis*
10.	UDP-3-O-acylglucosamine N-acyltransferase	*Prevotella nigrescens*
11.	Hemagglutinin	*Tannerella forsythia*
12.	Methylglyoxal synthase	*Tannerella forsythia*
13.	M10A family KLIKK metalloprotease karilysin	*Tannerella forsythia*
14.	Sialidase, GH35 family	*Tannerella forsythia*
15.	Chaperone protein DnaJ	*Aggregatibacter actinomycetemcomitans*
16.	Cytolethal distending toxin subunit A	*Aggregatibacter actinomycetemcomitans*
17.	Hemolysin III	*Treponema denticola*
18.	Serine protease (Dentilisin)	*Treponema denticola*
19.	Proteasome subunit alpha 2	*Rhodococcus erythropolis*
20.	LysR family transcriptional regulator	*Fusobacterium nucleatum*

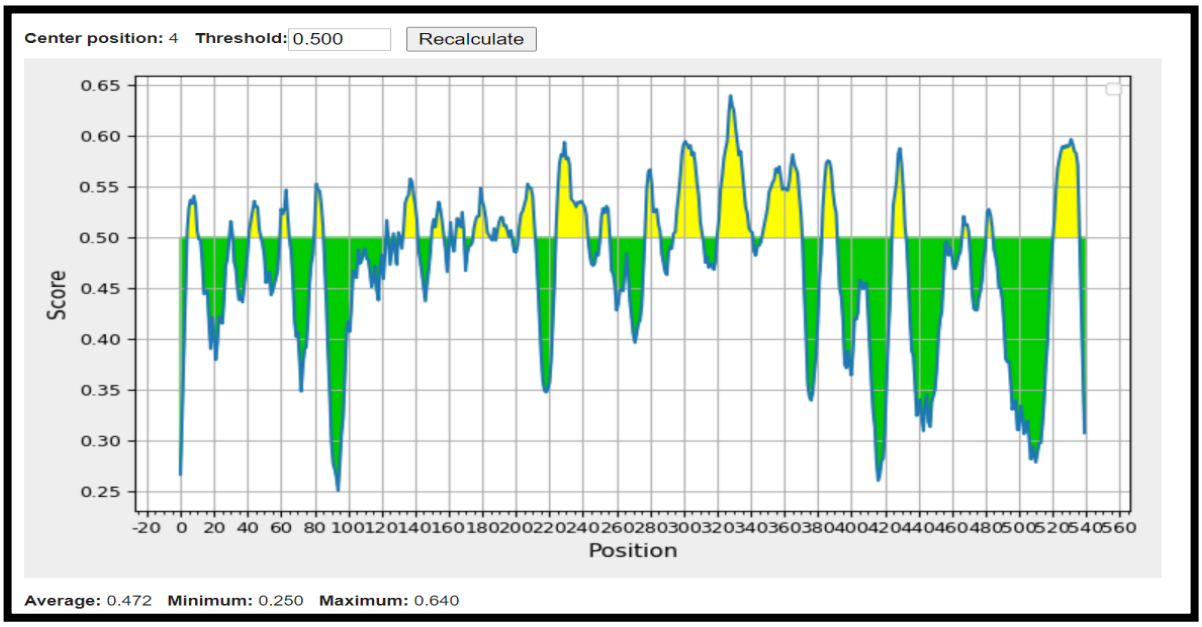
3.4 B-cell Epitope Prediction

B-cell epitope prediction has been done using the BepiPRed tool. It shows the epitopes which are present in the antigen as yellow in the graph and helps in presdicting the linear epitopes. The threshold was set as 0.500. The obtained results show that all the antigens have around 15-25 B-cell epitopes, hence there is high probability of B-cell epitopes in the peptide. The epitopes with the maximum peptide length have been choosen for T-cell epitope prediction because of 2 reasons. The reason is that the linear B-cell epitopes are confirmational and hence they might not be able to phusiologically bind to the antibody.



No.	Start	End	Peptide	Length
1	5	11	IKFDMES	7
2	31	31	G	1
3	45	47	APH	3
4	60	65	ELECPF	6
5	81	85	NDDAG	5
6	134	145	AKEVGDDFQKIE	12
7	153	157	NGDEN	5
8	168	172	KVKKE	5
9	200	210	ISPYFVTNTDK	11
10	220	232	ILIYDKKISVLKE	13
11	234	234	L	1
12	242	246	QTGKP	5
13	254	258	IDSEA	5
14	268	268	R	1
15	279	287	PGFGDRRKA	9
16	298	317	GTVISEETGLKLENATMDML	20
17	331	344	TIVNGAGNKEGIAS	14
18	351	373	AQIENTTSDYDREKLQERLAKLA	23
19	387	394	VEMKEKKD	8
20	430	435	KGENED	6
21	485	487	FEN	3
22	524	542	KKEDNPAAPAMPGGMGGMG	19

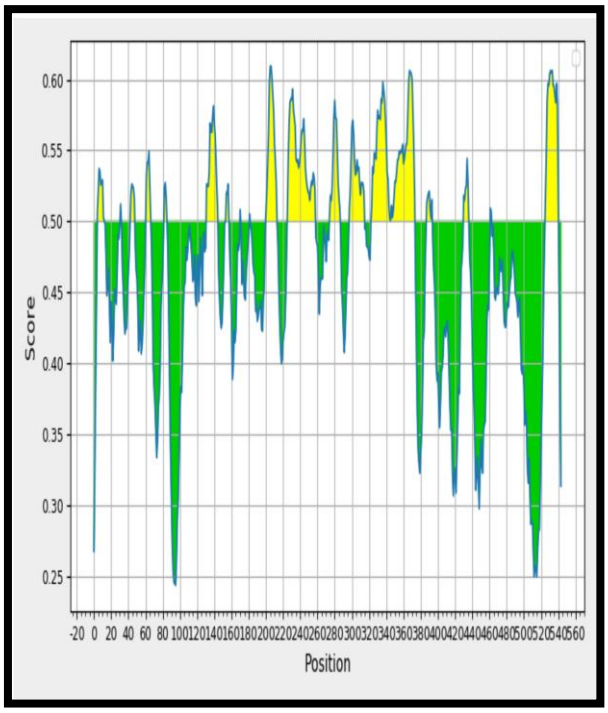
Fig.3.1- B- Cell Epitopes of *Porphyromonas gingivalis* for 60 kDa chaperonin peptides were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	11	IAYDEEA	7
2	31	32	GP	2
3	42	49	KWGAPTIT	8
4	61	65	LEDPY	5
5	81	86	DDVAGD	6
6	124	124	E	1
7	128	128	E	1
8	131	132	LK	2
9	134	142	AKEVETKEQ	9
10	151	158	AGDQSIGD	8
11	162	162	E	1
12	165	170	DKVGNE	6
13	176	185	EESNTFGLQL	10
14	188	188	T	1
15	190	196	GMRFDKG	7
16	198	198	I	1
17	203	212	VTDPERQEAV	10
18	225	243	VSTVKDLLPLLEKVIGAGK	19
19	252	257	VEGEAL	6
20	278	287	GFGDRRKAML	10
21	295	312	GGQVISEEVGLTLENADL	18
22	321	342	VVVTKDETTIVEGAGDTDAIAG	22
23	348	371	RQEIENSDSDYDREKLQERLAKLA	24
24	384	392	EVELKERKH	9
25	426	433	ELKLEGDE	8
26	467	471	RNLPA	5
27	482	485	YEDL	4
28	522	537	PEKEKASVPGGGDMGG	16

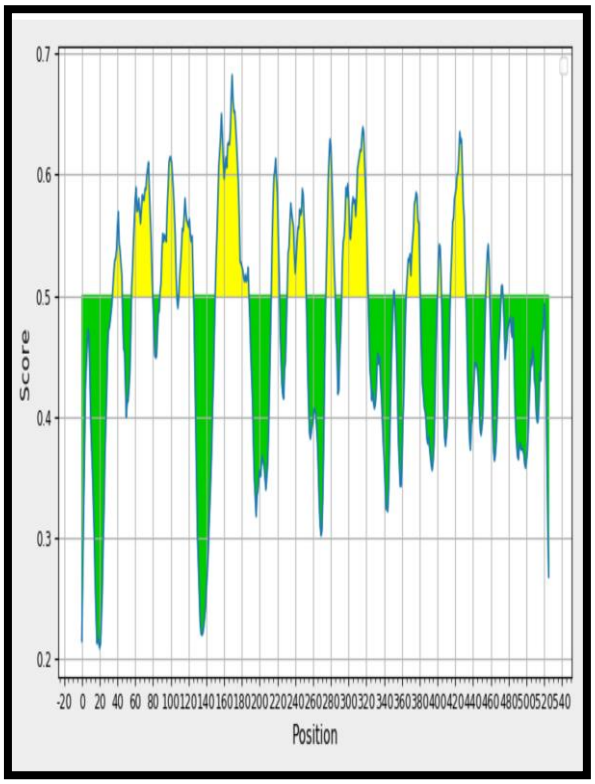
Fig.3.2- B- Cell Epitopes of *Mycobacterium tuberculosis* for 60 kDa chaperonin 2 peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	12	NIKYNEEA	8
2	32	32	G	1
3	44	48	FGSPQ	5
4	62	67	LEDKHE	6
5	83	85	DKA	3
6	132	145	KKISKPVQHHKEIA	14
7	155	158	DSEI	4
8	171	171	K	1
9	182	182	G	1
10	201	213	SSYFSTNPETQEC	13
11	226	258	KISGIKDFLPVLQQVAESGRPLLIIAEEIEGEA	33
12	275	287	AVKAPGFGDRRKA	13
13	298	315	GQLVSEELGMKLENTTLA	18
14	324	374	IVTKEDTTIVEGLGNKPDIQARCDNIKKQIEDSTSDYDKEKLQERLAKLSG	51
15	388	394	EMKEKKD	7
16	431	436	PMLANE	6
17	462	463	KE	2
18	526	541	IPEEKSSSAPAMPSAG	16

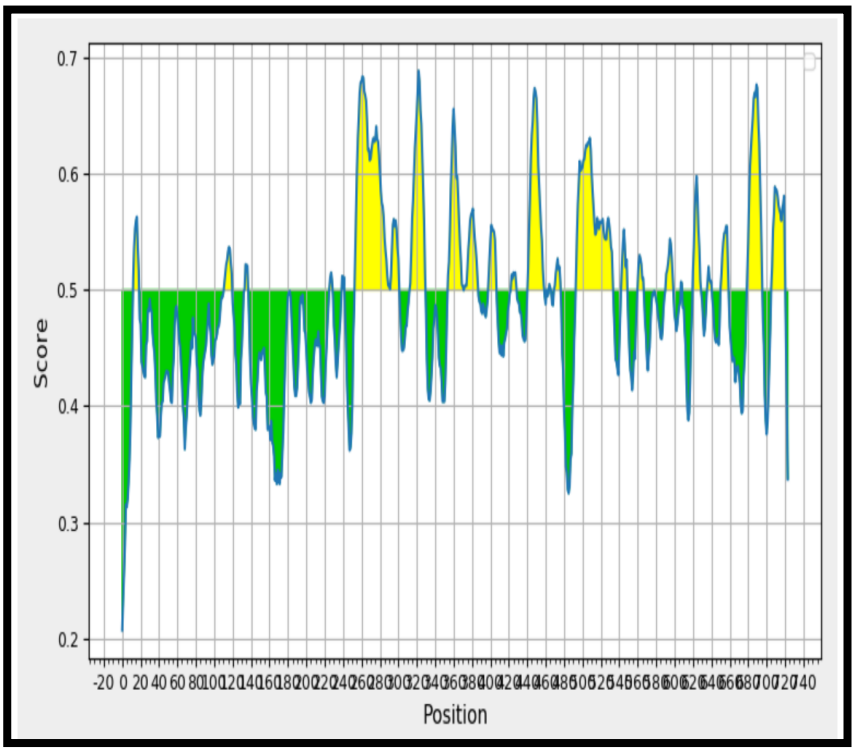
Fig.3.3.B- Cell Epitope of *Chlamydia pneumoniae* for 60 kDa chaperonin peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	36	46	WGDSHGVAPNQ	11
2	57	80	SESLPPGAKQIRIGFSLPKETEEK	24
3	89	107	SDSLAVRDLPDYKGRVSYD	19
4	111	126	ISKEDRTTALSADSVA	16
5	151	188	ARVEEVAVDGRPLPLKELSPASRRLYRGYEALFVPGDG	38
6	214	223	KYNQTDLPED	10
7	232	253	TDGGKSWSDPRIIVQGEGRNHG	22
8	276	286	GLWQSTPDRPQ	11
9	294	323	RDEGLTWSPPRDITHFIFGKDCADPGRSRW	30
10	352	352	Q	1
11	366	381	EGDTWQLSDCAYRRGD	16
12	401	405	GRQES	5
13	416	432	DGLTWERAKQFEGIHDP	17
14	456	459	GPDG	4
15	473	474	GR	2

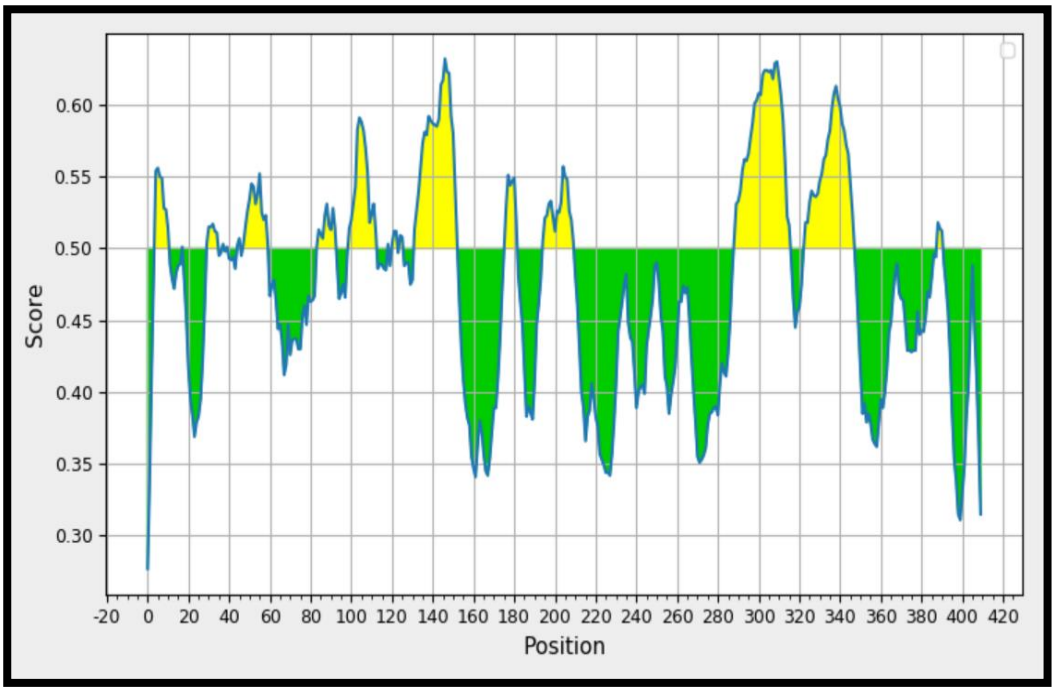
Fig-3.4 B-Cell epitopes of *Porphyromonas gingivalis* for Sialidase peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	13	19	AFSCLAV	7
2	112	120	QSDESNSTS	9
3	134	137	NVQN	4
4	226	229	KRGA	4
5	240	242	SPY	3
6	254	302	DTESYRQKQTKKNSLRLPLKNKTHKGAEVKYERDRLVKHLISGDLQEGL	49
7	314	330	YINLKKRSTGSDDNPLV	17
8	355	371	TSKIGSPNENKDTLQEY	17
9	373	387	SFQYHKFTDSFILPN	15
10	399	407	YTRKIGVKA	9
11	423	429	ADDYLKF	7
12	442	459	YSKKIYRPTGDEDRSANY	18
13	465	466	KF	2
14	470	477	TDLAKAYE	8
15	494	534	YNKGDLPDRYETAEDDGNVYIYDTLGRKYQSFINPQHTRDE	41
16	543	549	FFNENGS	7
17	561	567	YTKENKR	7
18	591	599	SHVNKYFEK	9
19	608	609	HD	2
20	621	629	RKNAQEKQN	9
21	637	641	VELPH	5
22	651	659	YDLERNYNK	9
23	680	696	EELEPTTTTSGTAAKKS	17

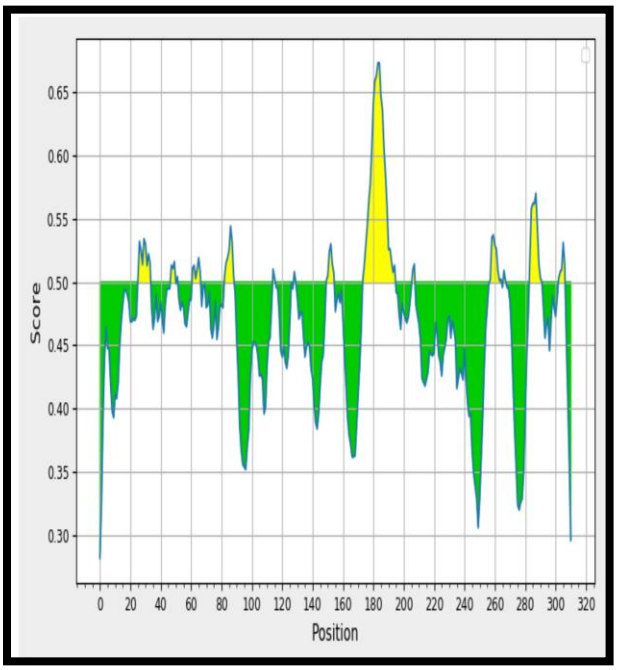
Fig 3.5 B-cell epitopes of *Campylobacter rectus* for LPS-assembly protein LptD peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	11	DSKFKGI	7
2	18	18	G	1
3	30	35	REIDYI	6
4	38	38	A	1
5	40	40	L	1
6	45	46	FS	2
7	48	60	ILESHDARKEHKQ	13
8	84	93	DLASQEAKDK	10
9	100	113	EDSEPVLSEEHKVV	14
10	119	119	K	1
11	121	123	KKT	3
12	125	126	RE	2
13	132	153	MAGITKYDLGIEADHELIVDPM	22
14	177	182	YKVRQR	6
15	195	210	PKLINTPWYYDPSLKL	16
16	289	316	NDVFKFWDYDLVNGGAEPQPVENGLPLE	28
17	324	348	NKKPVLIPIAGEGASQMEIERETHF	25
18	389	391	LSL	3

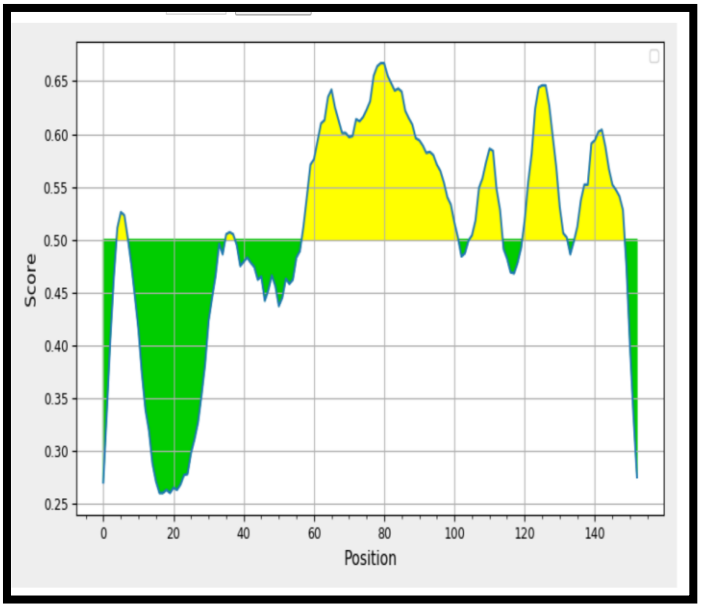
Fig- 3.6. B-Cell epitopes of *Mycoplasma arginini* for Arginine deiminase peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	26	34	YVSQPALSQ	9
2	48	50	FYR	3
3	52	52	K	1
4	62	67	AEFIKE	6
5	83	89	KLLQYKK	7
6	115	116	QK	2
7	129	129	S	1
8	150	155	IYNSNL	6
9	174	195	IKALTIAREQEIIQHGVSLSAL	22
10	207	208	FK	2
11	258	265	NSKQLEFF	8
12	267	268	IK	2
13	284	292	ILKYIDLAQ	9
14	303	307	KNQQV	5

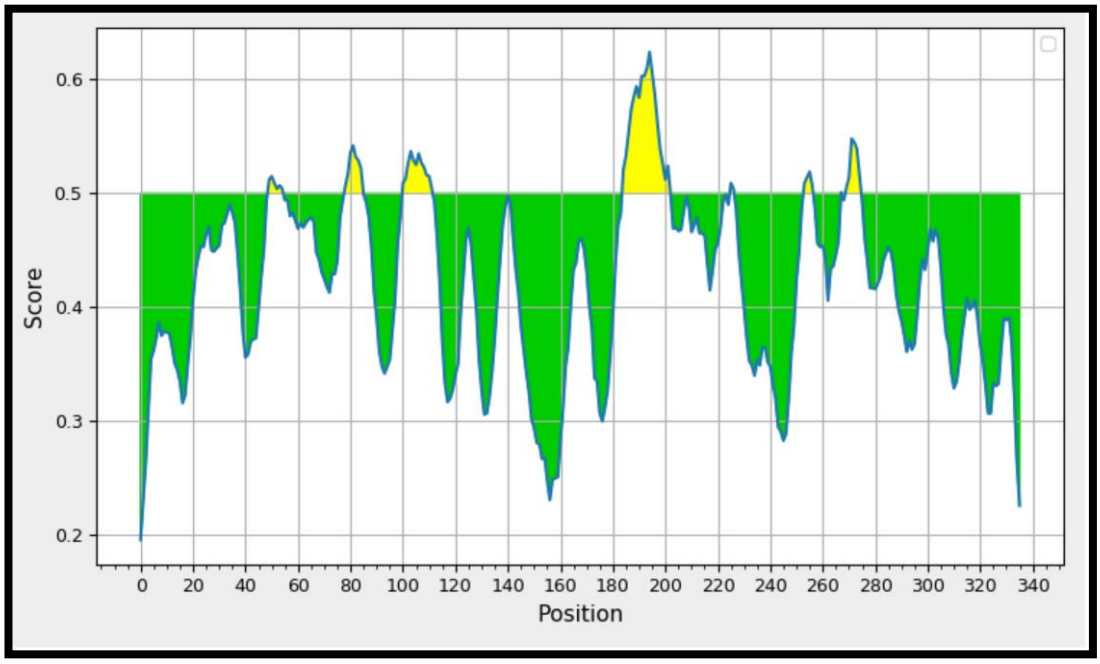
Fig 3.7. B-Cell epitopes of *Fusobacterium nucleatum* for LysR family transcriptional regulator peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	8	VQKG	4
2	36	38	TAR	3
3	58	102	WRADRGSFPNAAALVAGNVIFDSANGLDGKYFAPGGVTVTPDTGA	45
4	106	114	AFDAGANAG	9
5	121	133	PTVIAASGQISTW	13
6	136	149	APGAANGIETRRLP	14

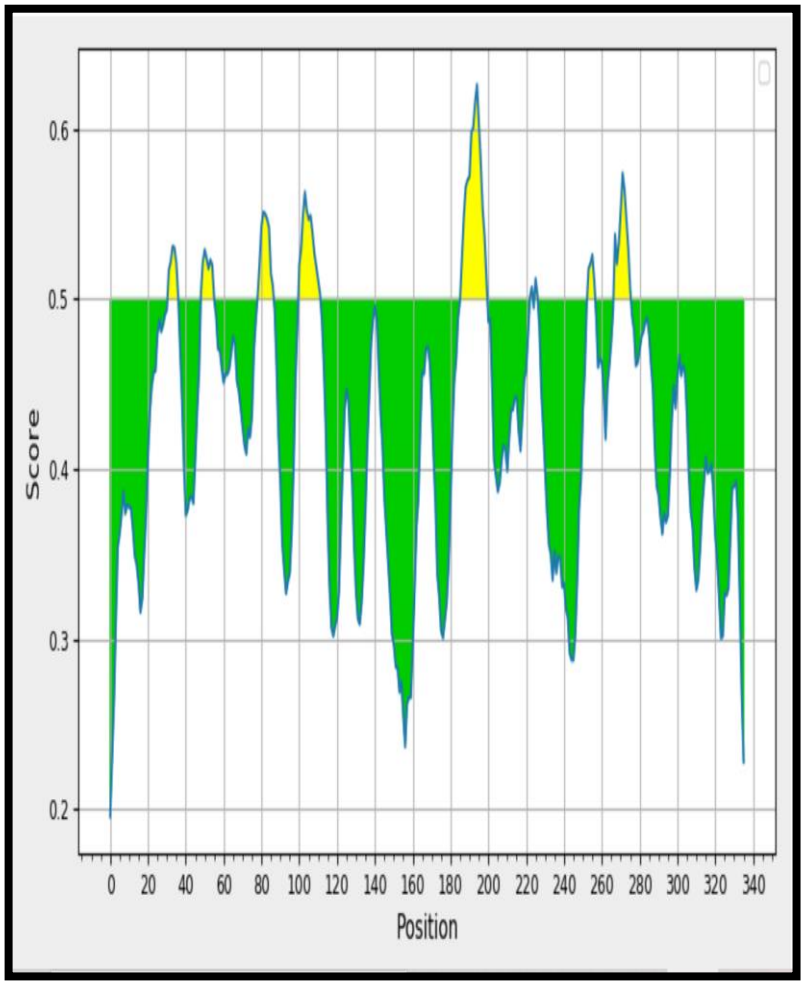
Fig 3.8. B-Cell epitopes of *Eikenella corrodens* for Type IV pilin peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	50	55	TQGRFD	6
2	79	85	DPENIDW	7
3	101	112	ATKAAAEKHLHA	12
4	185	202	QMILDGPHRKGDLRRARA	18
5	226	227	NG	2
6	254	257	VTVD	4
7	268	268	N	1
8	270	275	SYGYTE	6

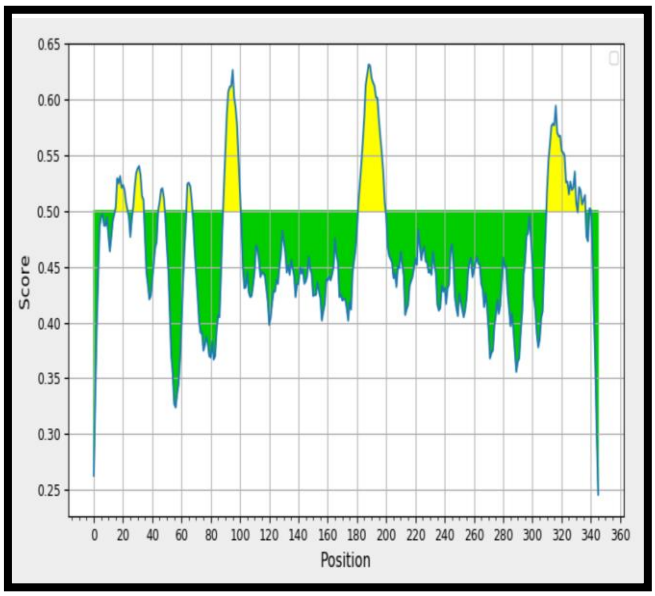
Fig. 3.9 B-Cell Epitopes of *Streptococcus gordonii* for Glyceraldehyde-3-phosphate dehydrogenase peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	32	37	INDLTD	6
2	49	56	TTQGRFDG	8
3	79	87	DPEQIDWAT	9
4	101	112	AKKAAAEKHLHA	12
5	187	200	ILDGPHRGGDLRRA	14
6	223	224	PE	2
7	226	227	NG	2
8	254	257	VTVD	4
9	268	276	NESYGYTED	9

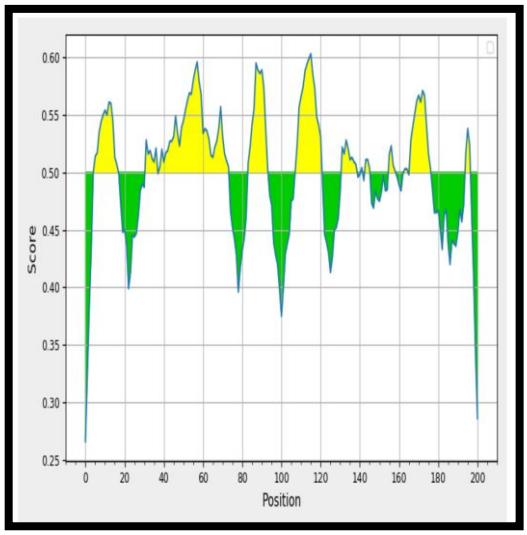
Fig 3.10. B-cell epitopes of *Streptococcus* oralis for Glyceraldehyde-3-phosphate dehydrogenase peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	16	24	IEGDEEATI	9
2	28	35	AKIEEGKH	8
3	45	49	KYTHH	5
4	65	68	KIEK	4
5	90	101	LYESMKPRKQGI	12
6	182	200	DGFGFAPNAETNSYDKIPQ	19
7	311	331	EPRNYFKSQVIFQRLPEIYKQ	21
8	333	337	DALQK	5
9	340	341	DE	2

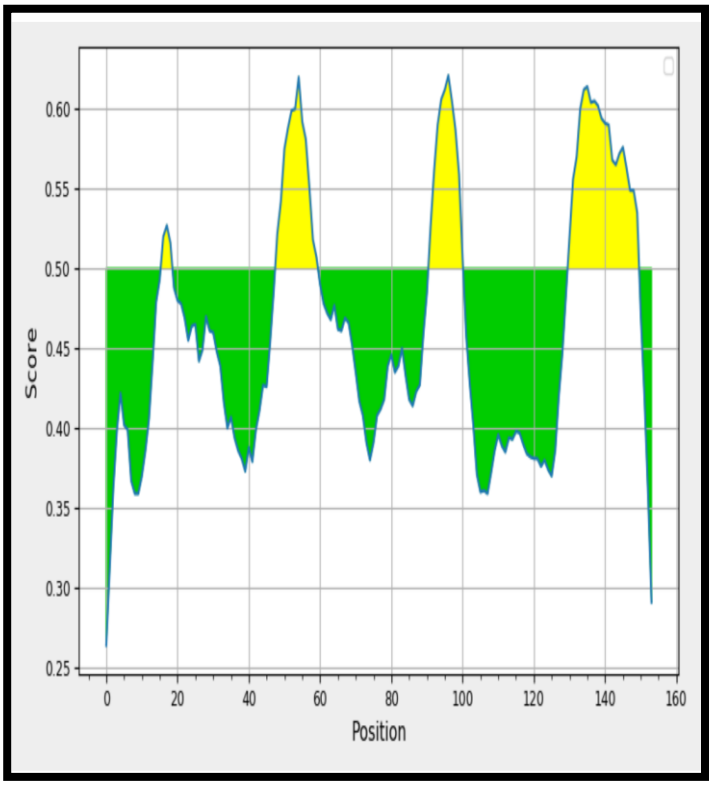
Fig.3.11 B-Cell epitopes of *Tannerella forsythia* for Hemagglutinin peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	6	17	FSFSRLQNLEHY	12
2	32	37	IAKLNP	6
3	39	74	LPELEKAIEMEDLALNPPVANELTPQVIALDEERDR	36
4	84	94	RSYAFDEDSQL	11
5	108	121	YGNVIRMNYDKETA	14
6	132	139	GENIRPLV	8
7	142	142	L	1
8	144	146	VTA	3
9	156	159	KAFA	4
10	164	165	RR	2
11	167	177	STDQRGKYDVK	11
12	195	197	DSI	3

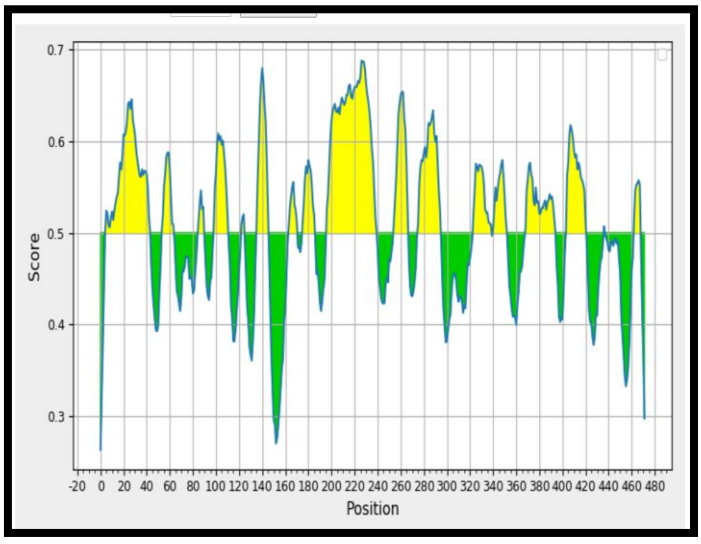
Fig.3.12. B-Cell epitopes of *Tannerella forsythia* for Methylglyoxal synthase peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	17	19	KVD	3
2	49	60	AFERRGIEYSNI	12
3	92	101	DLNPQPHEAD	10
4	131	150	LNDDDAYEPMEPKYAPFNRE	20

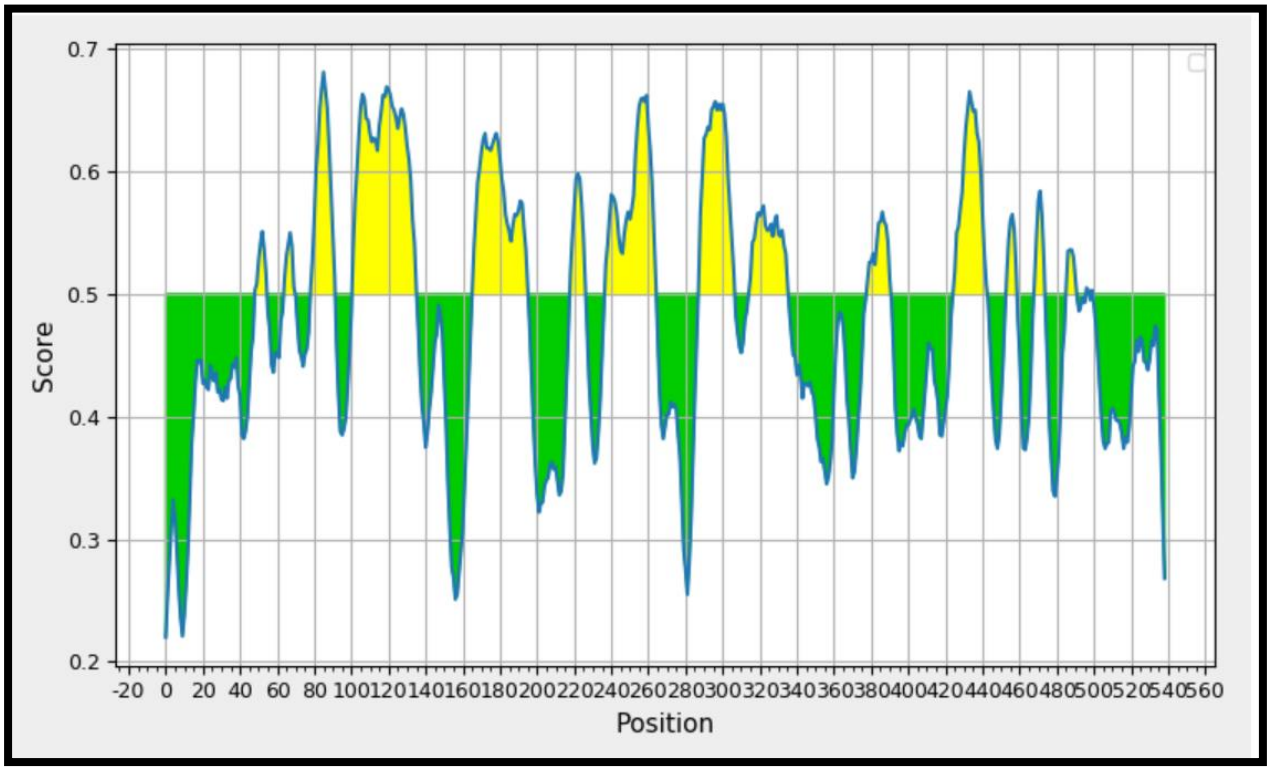
Fig.3.13 B-cell epitopes of *Tannerella forsythia* for karilysin peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	44	ILLFFLSTIAIFKVYSQRLYDNGPLTGDNNYVLQGSKWNK	40
2	54	64	SSSHLTTTERE	11
3	86	90	YNPNQ	5
4	99	111	KGNHGDGYPFDGN	13
5	123	125	PAG	3
6	136	146	DDENWSINGSG	11
7	164	171	EHSNVSSA	8
8	176	186	YYTGIKRQLDN	11
9	197	240	GYPFSISGPTSVCNQATYTIENLPSGATVQWSVSNPNIATINSS	44
10	255	267	RATINNSSVALTP	13
11	276	295	ISQDITLTVESLNSNGTLCT	20
12	324	339	GWQIAHHPGDNGIYAD	16
13	341	352	FIVTVIPLSPLP	12
14	369	394	TWKEVQIPAVSCSRTISPFTLSPNPA	26
15	404	421	ETDEVSGLSVLSTDRSTY	18
16	437	437	T	1
17	463	468	QTYTQK	6

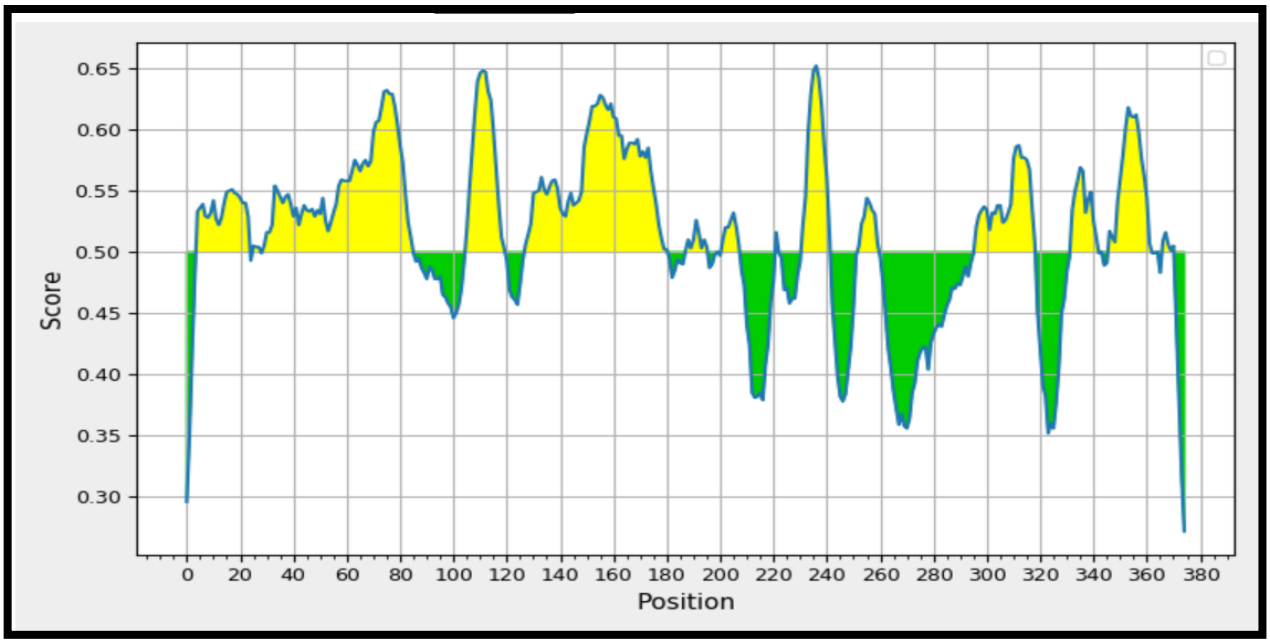
Fig.3.14 B-Cell epitopes of *Tannerella forsythia* for Sialidase peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	49	55	ATKGDVL	7
2	65	70	EDKLSE	6
3	79	92	AGTEAGTKGRSRFA	14
4	102	136	IRNTRSANPSYSVRQDEVTTVANTLTLKTRQPMVK	35
5	167	196	NDKPAVIAGEQAAVRRMGIGVRHAGDDGSA	30
6	219	227	YNNSVDLQE	9
7	238	265	DKGQTWEPMRIAMSFGETDGLPSGQNGV	28
8	288	307	GMGNARAWTNSMPGMTPDET	20
9	316	336	TDDGRTWSEPINITSQVKDPS	21
10	379	391	GETWHIHQPARTN	13
11	426	443	GKSWTEHSSNRSALPESI	18
12	454	459	KDNIIG	6
13	469	474	NTTEGR	6
14	486	491	GVTWLP	6
15	497	498	LD	2
16	500	500	E	1

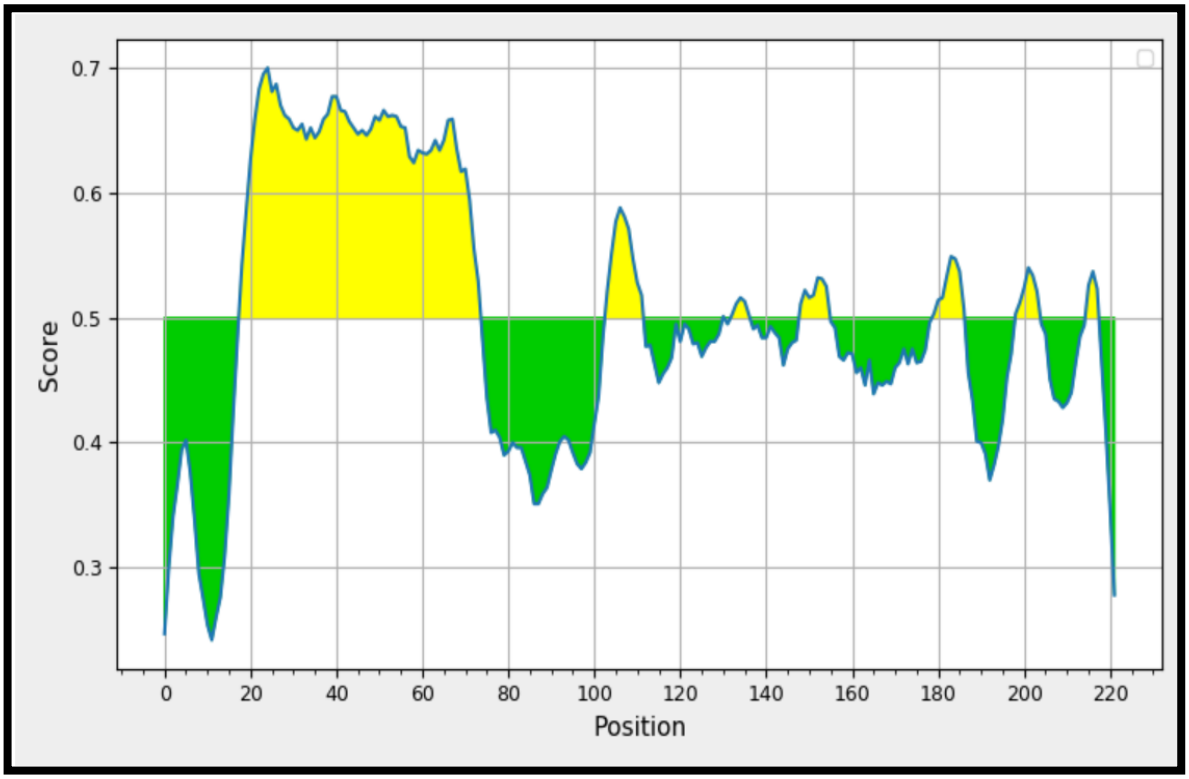
Fig.3.15 B-Cell Epitope of *Aggregatibacter actinomycetemcomitans* for Chaperone protein DnaJ peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	24	DYYELLGISRSADEKEIKRA	20
2	26	28	KKL	3
3	30	85	MQYHPDRTKGDKEKEEKFKEIQEAYEVLNDKEKRAAYDQYGHAAFEQGTGFGGGSF	56
4	106	120	GRGRQRVVRGDDLRY	15
5	128	181	EAVKGTTKDIKIHTLAPCDTCHGTGAEAGSKVETCPHCHGAGRLRRQQGFFVTE	54
6	188	196	HGTGKKIEK	9
7	200	200	T	1
8	202	208	HGDGRVN	7
9	222	222	D	1
10	232	242	EGAAGENGAPA	11
11	253	260	EHHIFERD	8
12	297	319	ETQTGKLFRMRGKGVTSTRSGYA	23
13	333	342	KLNEEQKELL	10
14	344	344	K	1
15	347	362	ESLEGQTKQRPKSSSF	16
16	365	365	G	1
17	367	371	KRFFD	5

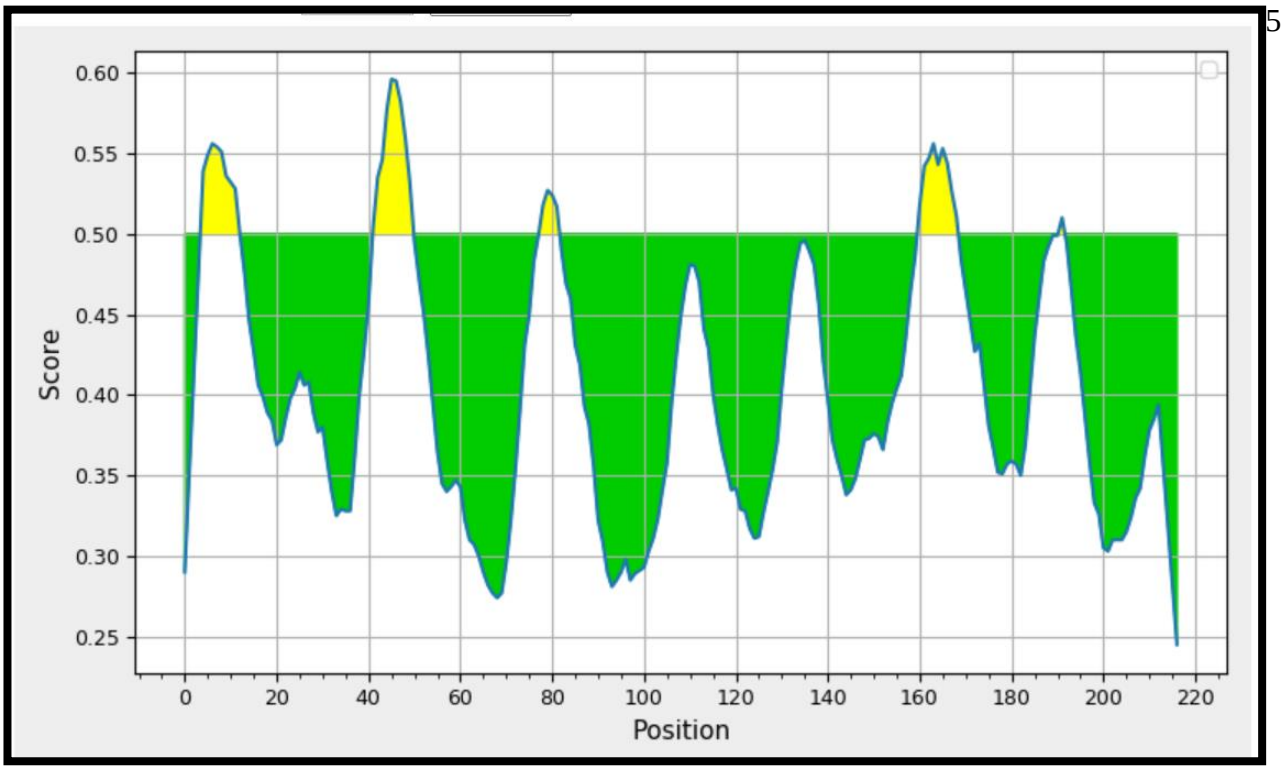
Fig.3.16.B-cell epitopes of *Aggregatibacter actinomycetemcomitans* for Chaperone protein DnaJ were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	19	74	NQRMSDYSQPESQSDLAPKSSTTQFQPQPLLSKASSMPLNLLSSSKNGQVSPSEPS	56
2	104	112	IYSQDFGNI	9
3	131	131	Q	1
4	133	137	LGTCI	5
5	149	155	CSLDKLA	7
6	180	187	TYNPVSPT	8
7	199	204	GATEPL	6
8	216	218	LEA	3

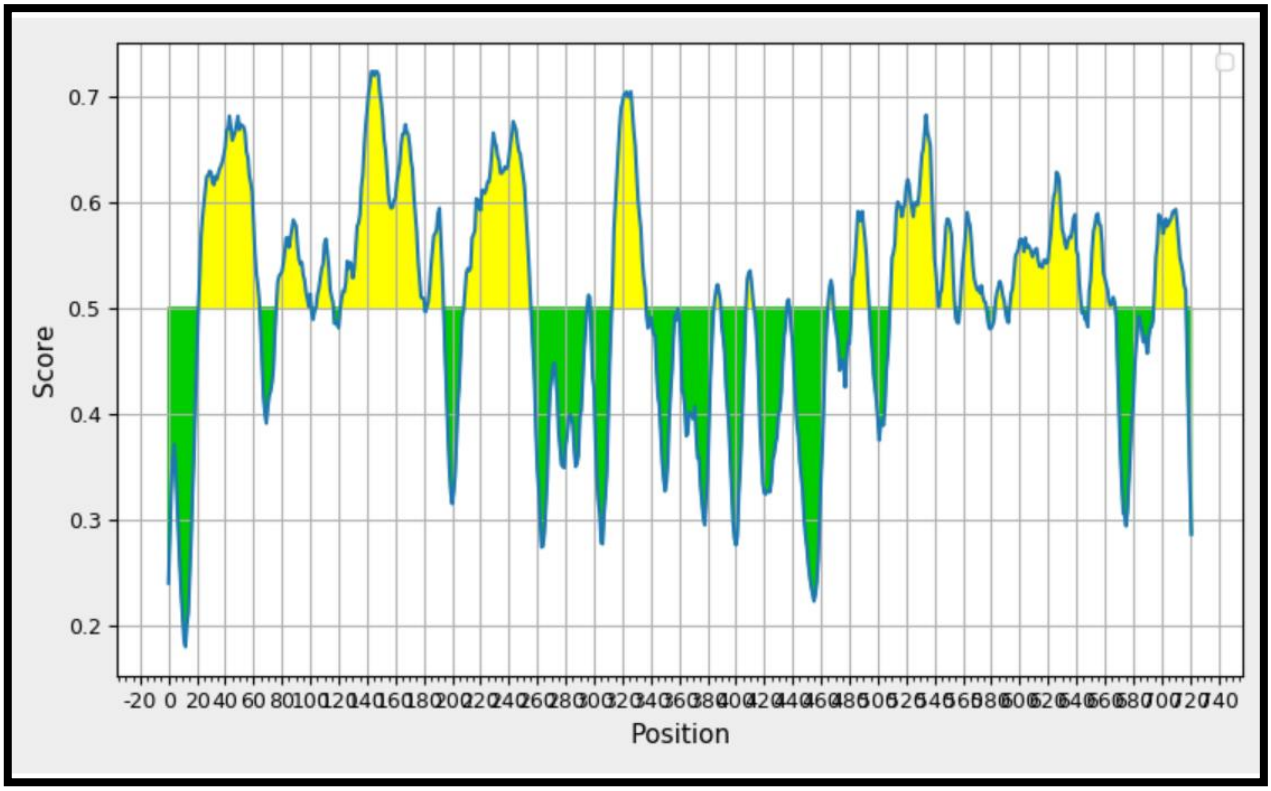
Fig.3.17.B-cell epitopes of Aggregatibacter actinomycetemcomitans for Cytolethal Subunit A peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	5	13	KIKRRYSIG	9
2	42	50	RYAPPDLKA	9
3	79	82	KAKK	4
4	161	169	KEQLPLLSF	9
5	192	192	K	1

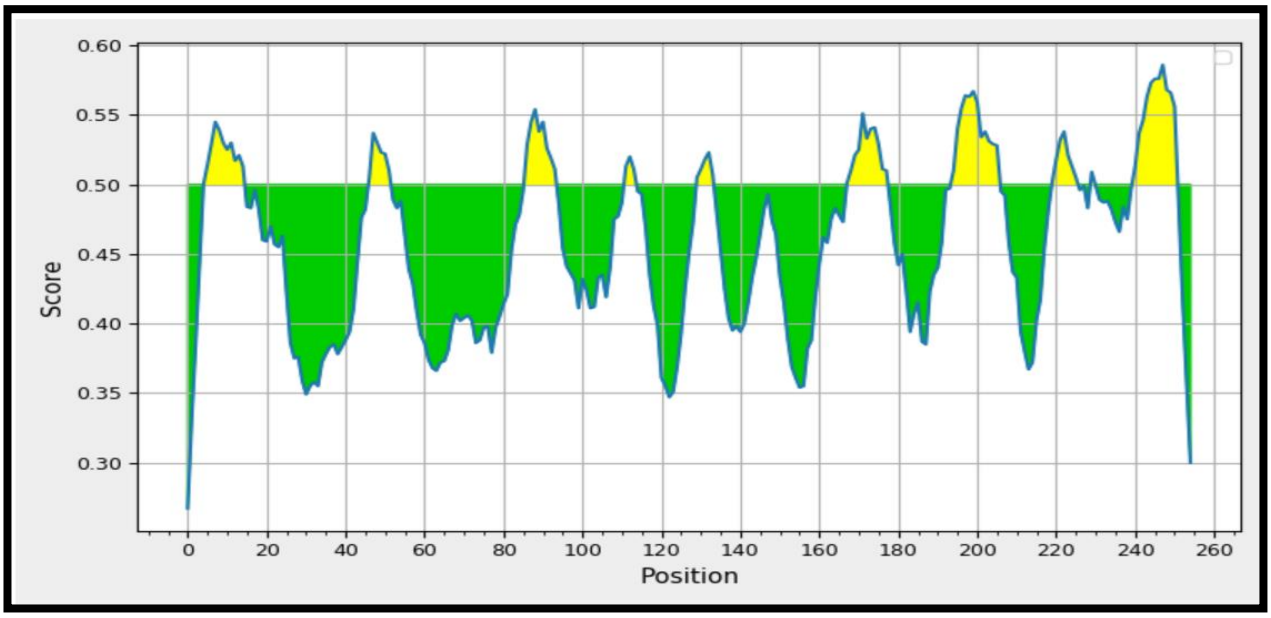
Fig 3.18. B-cell epitopes of *Treponema denticola* for Hemolysin III peptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	23	65	MNTAVIGNKDNGTVLNGYTGGSGNSSSIELPNGANYKPDDKDI	43
2	77	101	GFDKTLFEKKGFIVEGNISLTDTGF	25
3	105	117	YLNKKGDNKKNLL	13
4	123	181	EGVLSAEHDYKVVEPDGSKAPNQNDSPVDPSNAGTYGLTDGNYLDDPEANNADYGLSIT	59
5	183	194	ALRAYKEIGYGD	12
6	210	256	HKDFKDENGESIVLYAKSCATDGTGGTYIGDGNPFTEIPIGLNWDKG	47
7	296	298	GSG	3
8	314	337	VKILRKPKANRSVAENNALPSYLQ	24
9	386	390	RYTAA	5
10	408	413	DKKVHF	6
11	437	438	ED	2
12	466	469	AHKI	4
13	483	494	DKVDGATDFTDS	12
14	510	555	KDGNIPAPNDIYTEAEVTVTVKNNDGHSNDIVPCKITLVDEETHAP	46
15	559	577	VAGTGSNPPAFKGLIKGRS	19
16	584	590	FLGSSSN	7
17	594	644	TAQDVDNPITIQFNKKIVWVSTVPNLHYNSGDDDTDTRIMVYKADASGNLD	51
18	650	668	SIVDYDQDLLDTTCFEAET	19
19	696	718	RTPLNSNGCKYRRYGKKSQQCFN	23

Fig.3.19. B-cell epitpes of *Treponema denticola* for dentilisin pptide were determined



Predicted peptides:

No.	Start	End	Peptide	Length
1	6	15	YASAEQIMRD	10
2	47	52	SRALHK	6
3	87	94	YSYDRRDV	8
4	112	114	TEQ	3
5	130	134	FGSST	5
6	168	178	RESYQRDLDLE	11
7	195	206	PAGTTEAEPRTL	12
8	220	226	RPRRAFK	7
9	230	230	G	1
10	241	251	AATEDAPPANG	11

Fig 3.20. B-cell epitpes of *Rhodococcus erythropolis* for Proteasome subunit alpha 2 were determined

3.5 T-cell Epitope Prediction-

List of peptides along with the alleles have been obtained for every sequence for both T-Helper and T-Cytotoxic cells.

- **T-helper Cell Predictions:**

Peptide sequences along with the alles have been obtained for every sequence added. A threshold of 11 repetions has been selected for population coverage analysis. The T-helper cells bind with epitopes presented by the MHC-Class II.

Table 3.4- List of predicted T-cell epitopes

Seq #	Peptide st	Peptide en	Peptide sequence	Consensus	Allele
3	18	32	SGRPLLIIAEEIEGE	1.6	HLA-DQA1*03:01/DQB1*03:02
3	16	30	AESGRPLLIIAEEIE	0.98	HLA-DQA1*04:01/DQB1*04:02
3	4	18	GIKDFLPVLQQVAES	5.4	HLA-DQA1*04:01/DQB1*04:02
3	19	33	GRPLLIIAEEIEGEA	1.6	HLA-DQA1*05:01/DQB1*02:01
3	3	17	SGIKDFLPVLQQVAE	9.4	HLA-DRB1*08:02
3	6	20	KDFLPVLQQVAESGR	7.5	HLA-DRB4*01:01
4	21	35	ASRRLYRGYEALFVP	6.2	HLA-DPA1*01:03,HLA-DPB1*02:01
4	21	35	ASRRLYRGYEALFVP	1.8	HLA-DPA1*02:01,HLA-DPB1*05:01
4	24	38	RLYRGYEALFVPGDG	5.9	HLA-DQA1*01:01,HLA-DQB1*05:01
4	22	36	SRRLYRGYEALFVPG	5.7	HLA-DQA1*05:01,HLA-DQB1*02:01
4	1	15	ARVEEVAVDGRPLPL	5.4	HLA-DRB1*03:01
4	23	37	RRLYRGYEALFVPGD	4	HLA-DRB1*04:05
4	20	34	PASRRLYRGYEALFV	2.4	HLA-DRB1*09:01
4	13	27	LPLKELSPASRRLYR	8.3	HLA-DRB1*11:01
4	21	35	ASRRLYRGYEALFVP	1.4	HLA-DRB1*15:01
4	20	34	PASRRLYRGYEALFV	6.7	HLA-DRB3*01:01

- **T-Cytotoxic Predictions-**

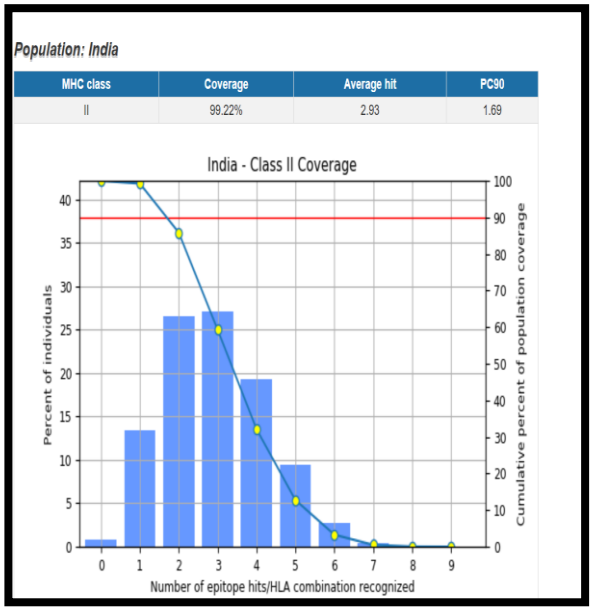
Among them the epitopes with the longest peptide length have been choosen for T - cell prediction.Epitopes binding with Class-I MHC have been predicted. A list of peptides along with the alleles has been obtained for every sequence and then used for population coverage analysis.

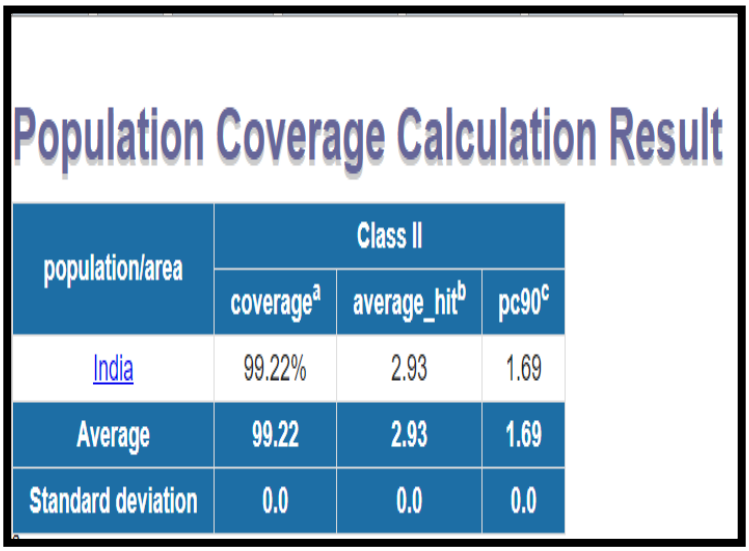
Table- 3.5 T-cytoxic epitope prediction

Seq #	Peptide st	Peptide en	Peptide	Percentile	Allele
17	17	25	APKSSTTQF	0.01	HLA-B*07:02
17	17	25	APKSSTTQF	0.08	HLA-B*35:01
17	23	31	TQFQPQPLL	0.09	HLA-A*02:06
17	17	25	APKSSTTQF	0.14	HLA-B*53:01
17	17	25	APKSSTTQF	0.2	HLA-B*08:01
17	23	31	TQFQPQPLL	0.21	HLA-B*15:01
17	23	31	TQFQPQPLL	0.22	HLA-B*40:01
17	11	19	ESQSDLAPK	0.22	HLA-A*68:01
17	23	31	TQFQPQPLL	0.25	HLA-A*32:01
17	33	41	KASSMPLNL	0.3	HLA-B*58:01
17	33	41	KASSMPLNL	0.35	HLA-A*32:01
17	23	31	TQFQPQPLL	0.36	HLA-A*02:01
17	25	33	FQPQPLLSK	0.46	HLA-A*11:01
17	31	39	LSKASSMPL	0.47	HLA-A*30:01
17	26	34	QPQPLLSKA	0.53	HLA-B*07:02
17	17	25	APKSSTTQF	0.61	HLA-A*26:01
17	22	30	TTQFQPQPL	0.64	HLA-A*68:02
17	25	33	FQPQPLLSK	0.65	HLA-A*03:01
17	26	34	QPQPLLSKA	0.68	HLA-B*51:01
17	23	31	TQFQPQPLL	0.7	HLA-A*02:03
17	33	41	KASSMPLNL	0.76	HLA-B*57:01

3.5 POPULATION COVERAGE

- This method calculates the fraction of individuals predicted to respond to a given epitope on the basis of MHC binding. It is the selection of the set of epitopes which yields the best immune response in a given population. The results show that the epitopes have a coverage of 99.57% in India and a total coverage of 98.51% globally. This means that most of the Indian population is going to have this class-2 HLA epitope. Also in this case, the Indian coverage is 99.2% and World is 98.17% .The epitopes are very well presented by the MHC-CLASS 2 in Indian population and the global coverage is also giving percentage above 90. So it means that these proteins show greatest potential for experimental immunogenicity analysis. They have several potential T cell and B cell epitopes. The epitopes have been determined on the basis of their binding ability with maximum number of HLA alleles along with highest population coverage rate values for the geographical area studied. The predicted epitopes show a coverage of above 90% for both Indian and global population.



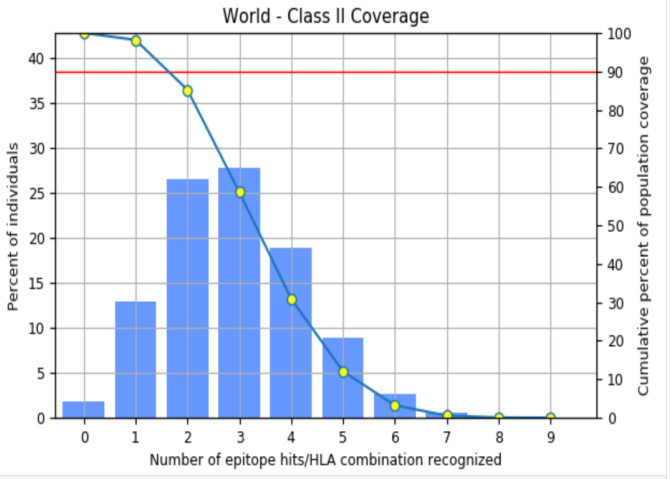


Population Coverage Calculation Result

population/area	Class II		
	coverage[a]	average_hit[b]	pc90[c]
World	98.17%	2.89	1.63
Average	98.17	2.89	1.63
Standard deviation	0.0	0.0	0.0

Population: World

MHC class	Coverage	Average hit	PC90
II	98.17%	2.89	1.63



World - Class II Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	1.83	100.0
1	12.96	98.17
2	26.57	85.21
3	27.73	58.65
4	18.84	30.91
5	8.84	12.07
6	2.7	3.24
7	0.49	0.54
8	0.05	0.05
9	0.0	0.0

India - Class II Coverage

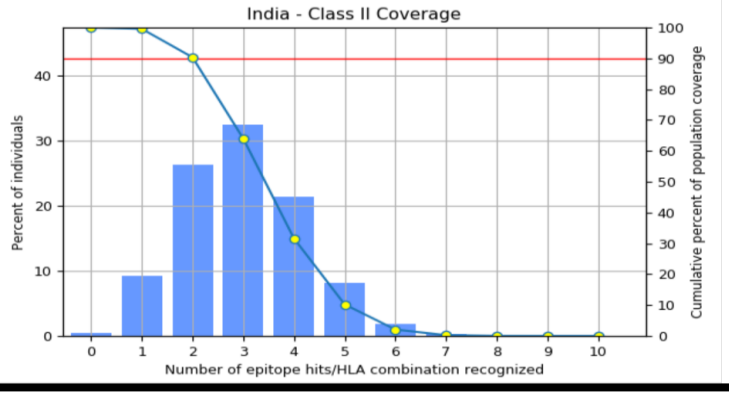
Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	0.78	100.0
1	13.4	99.22
2	26.55	85.83
3	27.16	59.27
4	19.36	32.11
5	9.46	12.76
6	2.8	3.3
7	0.46	0.5
8	0.04	0.04
9	0.0	0.0

Fig 3.21- Indian and Global Population coverage for epitope EPRNYFKSQVIFQRL

population/area	Class II		
	coverage[a]	average_hit[b]	pc90[c]
India	99.57%	2.98	2.01
Average	**99.57**	**2.98**	**2.01**
Standard deviation	**0.0**	**0.0**	**0.0**

Population: India

MHC class	Coverage	Average hit	PC90
II	99.57%	2.98	2.01



India - Class II Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	0.43	100.0
1	9.23	99.57
2	26.34	90.34
3	32.39	64.0
4	21.38	31.61
5	8.16	10.23
6	1.82	2.07
7	0.23	0.25
8	0.02	0.02
9	0.0	0.0
10	0.0	0.0

World - Class II Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	1.49	100.0
1	11.28	98.51
2	26.68	87.23
3	30.74	60.55
4	19.97	29.81
5	7.74	9.84
6	1.82	2.1
7	0.26	0.28
8	0.02	0.02
9	0.0	0.0
10	0.0	0.0

population/area	Class II		
	coverage[a]	average_hit[b]	pc90[c]
World	98.51%	2.88	1.75
Average	98.51	2.88	1.75
Standard deviation	0.0	0.0	0.0

Population: World

MHC class	Coverage	Average hit	PC90
II	98.51%	2.88	1.75

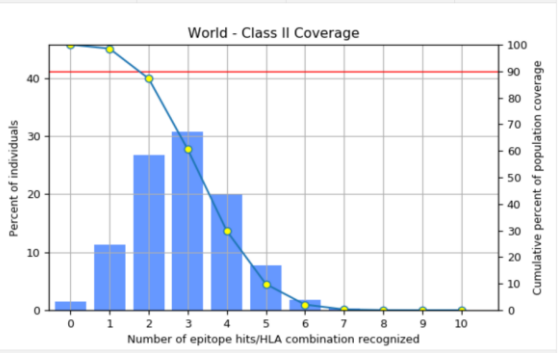


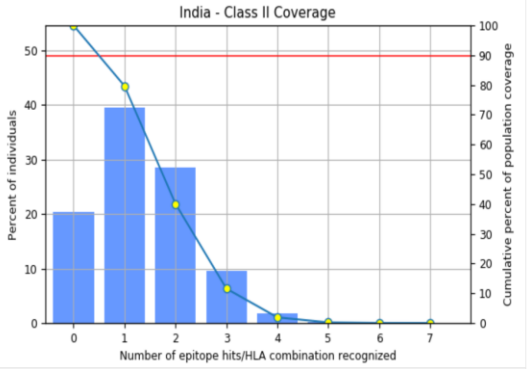
Fig 3.22- Indian and Global Population coverage for epitope ASRRLYRGYEALFVP

population/area	Class II		
	coverage[a]	average_hit[b]	pc90[c]
India	79.65%	1.33	0.49
Average	**79.65**	**1.33**	**0.49**
Standard deviation	**0.0**	**0.0**	**0.0**

[a] projected population coverage
[b] average number of epitope hits / HLA combinations recognized by the population
[c] minimum number of epitope hits / HLA combinations recognized by 90% of the population

Population: India

MHC class	Coverage	Average hit	PC90
II	79.65%	1.33	0.49



India - Class II Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	20.35	100.0
1	39.58	79.65
2	28.51	40.07
3	9.65	11.55
4	1.71	1.91
5	0.19	0.2
6	0.01	0.01
7	0.0	0.0

population/area	Class II		
	coverage[a]	average_hit[b]	pc90[c]
World	89.89%	1.85	0.99
Average	**89.89**	**1.85**	**0.99**
Standard deviation	**0.0**	**0.0**	**0.0**

[a] projected population coverage
[b] average number of epitope hits / HLA combinations recognized by the population
[c] minimum number of epitope hits / HLA combinations recognized by 90% of the population

World - Class II Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	10.11	100.0
1	30.01	89.89
2	33.7	59.87
3	18.8	26.17
4	6.02	7.37
5	1.21	1.36
6	0.14	0.14
7	0.01	0.01

Population: World

MHC class	Coverage	Average hit	PC90
II	89.89%	1.85	0.99

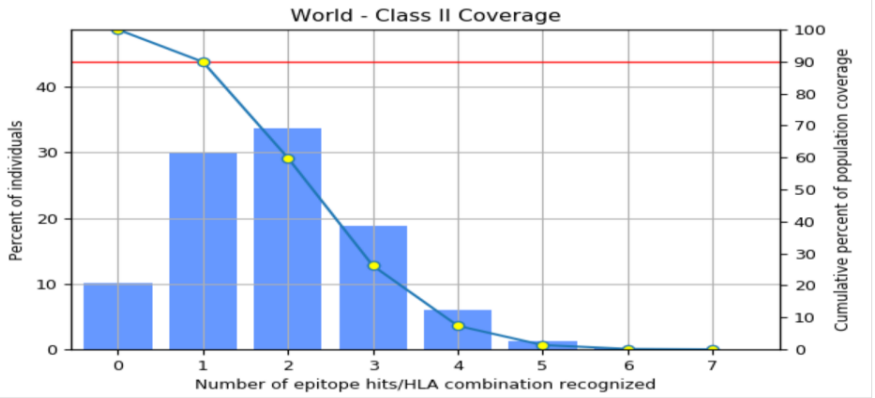


Fig 3.22- Indian and Global Population coverage for epitope GYPFSISGPTSVCNQ

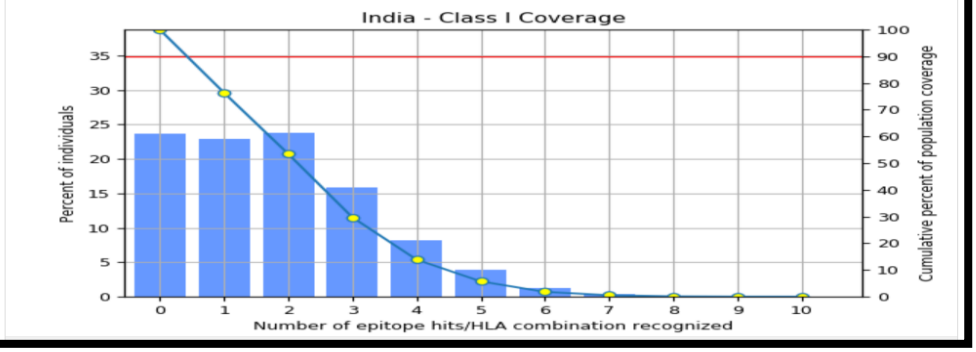
Population Coverage Calculation Result

population/area	Class I		
	coverage[a]	average_hit[b]	pc90[c]
India	76.36%	1.82	0.42
Average	**76.36**	**1.82**	**0.42**
Standard deviation	**0.0**	**0.0**	**0.0**

[a] projected population coverage
[b] average number of epitope hits / HLA combinations recognized by the population
[c] minimum number of epitope hits / HLA combinations recognized by 90% of the population

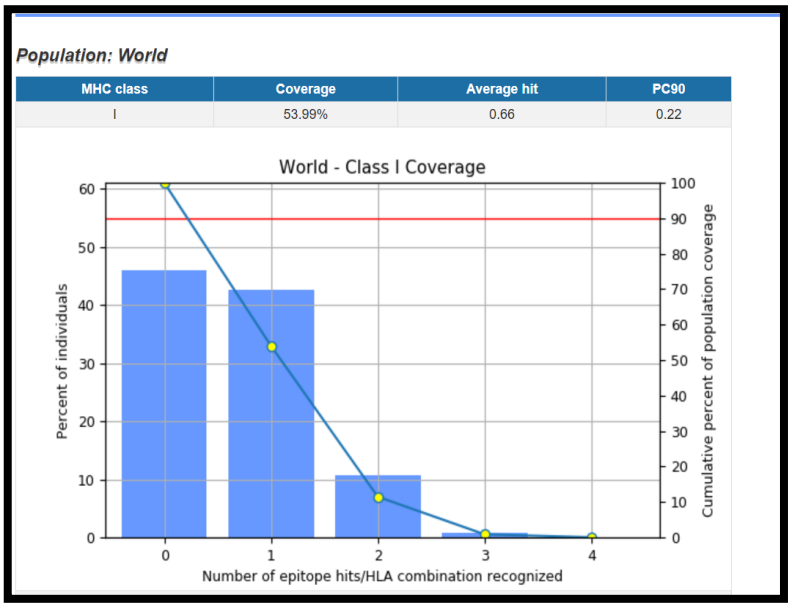
Population: India

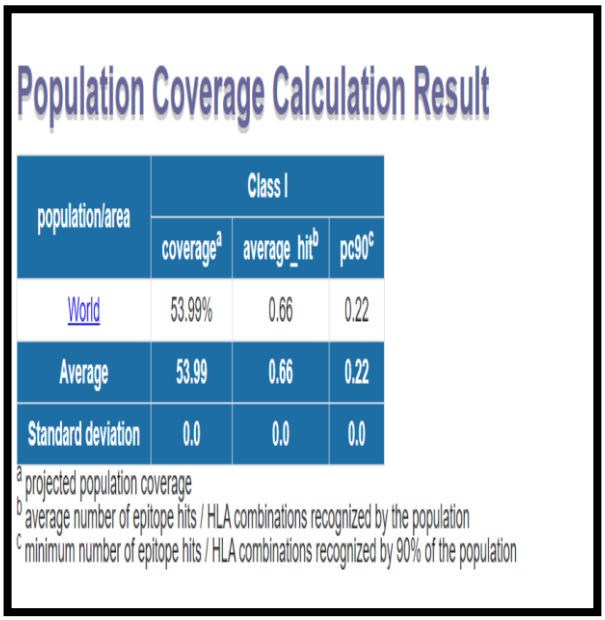
MHC class	Coverage	Average hit	PC90
I	76.36%	1.82	0.42

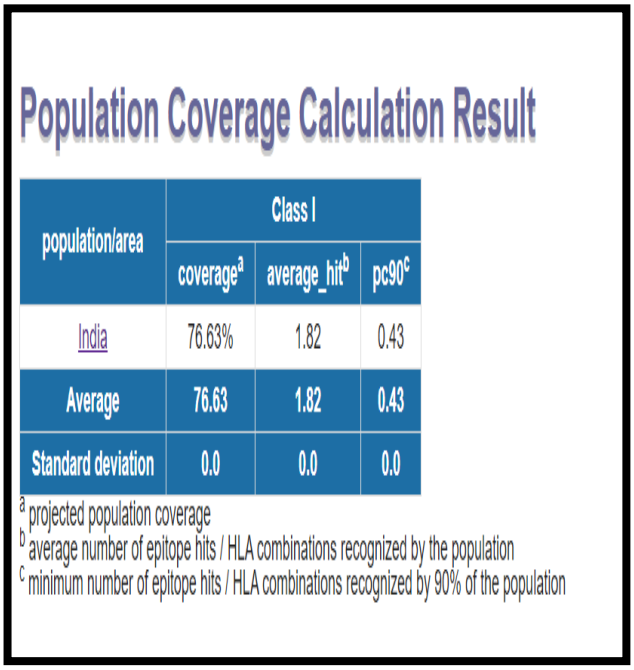


India - Class I Coverage

Number of epitope hits / HLA combinations recognized	Percent of individuals	Cumulative percent of population coverage
0	23.64	100.0
1	22.9	76.36
2	23.77	53.46
3	15.82	29.7
4	8.16	13.88
5	3.93	5.71
6	1.26	1.79
7	0.42	0.53
8	0.09	0.11
9	0.02	0.02
10	0.0	0.0







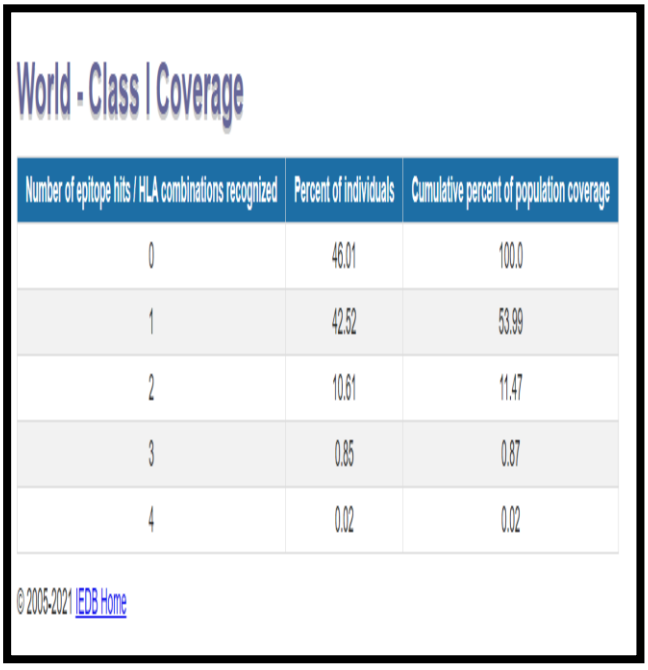


Fig 3.23- Indian and Global Population coverage for epitope APKSSTTQF

3.6 CONCLUSION –

Periodontal disease causes the activation of both humoral and cellular immune response but the main reason for the progression of disease is the inflammation caused due to the presence of periodontal pathogens. The PD pathogens have an immense impact on overall health either directly or indirectly. In the past few years the use of epitope based vaccines has been increased and they serve as an important factor from the protection of infectious diseases. In this study, 3 epitopes NYFKSQVIFQRLPEI, ASRRLYRGYEALFVP, ELEKAIEMEDLALNP were found to give both B and T-cell stimulation and also showed a population coverage of above 90% in both Indian and Global context. Epitopes were predicted from multiple bacteria such that the predicted vaccine candidates could cross react with multiple species of bacteria commonly associated with periodontal diseases. All predicted epitopes were tested for their cross reactivity with human antigens to eliminate the possibility of autoreactivity. The studies showed that all the 3 epitopes predicted were capable of potent humoral and CMI response and were not cross reacting with human epitopes and hence lacked potential to initiate autoimmune response. In the presented study, the capability of MHC haplotype in epitope presentation was extensively studied in both Indian and global context, so as to ascertain the effectiveness of such a vaccine candidate in global context since HLA supertypes are known to alter immune potentiation. The T- cell epitopes were predicted within the B-cell epitopes and hence these epitopes can be considered as better vaccine candidates and can be expressed with recombinant proteins for further studies to assess immunogenecity and protection.

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