Prediction of vaccine candidates for dental caries using Immunoinformatic

A PROJECT REPORT

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE
OF
MASTER OF TECHNOLOGY
IN
BIOINFORMATICS

Submitted by:

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CANDIDATE'S DECLARATION

I, Harsh Yadav, 2K18/BIO/06 of M.Tech (Bioinformatics), hereby declare that the project Dissertation titled **"Prediction of vaccine candidates for dental caries using Immunoinformatics"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirements for the award of the degree of Master of Technology, is original and not copied from any source with proper citation. This work has not previously formed the basis for the award of the Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Harsh yadav

Place: Delhi **Harsh Yadav**

Date: 29/06/2020 (2K18/BIO/06)

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CERTIFICATE

I hereby certify that the Project Dissertation titled **"Prediction of vaccine candidates for dental caries using Immunoinformatics"** which is submitted by Harsh Yadav (2K18/BIO/06), Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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ACKNOWLEDGEMENT

I would like to express my heartfelt gratitude to all those who have contributed directly or indirectly towards obtaining completion of this project. I am grateful to my esteemed supervisor, **Dr. Asmita Das (Assistant Professor)**, who has guided me through thick and thin. I would also like to acknowledge the many helpful comments received from my Teachers of the biotechnology and bioinformatics department.

I am obliged to all those who provided reviews & suggestion for improving the result and the topics covered in my project, and extend my apologies to anyone if I may have failed to mention.

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LIST OF ABBREVIATIONS

S.mutans	*Streptococcus mutans*
HLA	Human Leukocyte Antigen
GI	GenInfo Identifier
NCBI	National Centre for Biotechnology Information
UniProtKB	UniProt KnowledgeBase
HOMD	Human Oral Microbiome Database
BCPREDS	B-cell Epitope PREDiction Server
MHC	Major Histocompatibilty Complex
IgG	Immunoglobulin G
IgE	Immunoglobulin E
IgA	Immunoglobulin A
CPP	Casein phosphopeptide
CPP-ACP	Casein phosphopeptide-amorphous calcium phosphate
GTF	Glucosyltransferase
GBP	Glucan Binding Protein
VIP	Virulence Associated Immnumodulatory Extracellular Protein
AAP	Amino acid pair

CONTENTS

Prediction of vaccine candidates for dental caries using Immunoinformatics.

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1. ABSTRACT

Despite the existing preventive measures, dental caries stays one of the most pervasive infection in human. Epidemiological studies imply that there is a recent increase in dental caries in children. Conventionally, *Streptococcus mutans* has been considered as the earliest colonizer of cavity and the main causing agent of dental caries. However, there are reports where other microbes have resulted in dental caries even in not presence of *S.mutans*. These studies support the poly-microbial nature of this disease and the use of immunization strategies using vaccine targets shared by various pathogens associated with the procedure of tooth decay. Till date, almost all the efforts put in to immunize people against caries have targeted *S.mutans* only. Only a few studies have tried to develop vaccines targeting both *S.mutans* and *S.sobrinus* with little or no success. This study, aimed at preventing dental caries, provides the basis for identification of vaccine candidates for developing a dental caries vaccine that can elicit both B and T Cell mediated immune response against multiple cariogenic microorganisms. A novel strategy has been utilized to Anticipate such antigenic B-cell epitopes which contain T-cell epitopes also.

Keywords: Dental caries, *Streptococcus mutans*, B-cell, epitope, T-cell, HLA alleles, cariogens, antigenicity, vaccine, immune response.

2. INTRODUCTION.

DENTAL CARIES

Dental caries is the aftereffect of localize destruction of vulnerable dental rough tissues by acidic side-effects based upon bacterial maturation of dietary starches. It is one of the most widely recognized avertable pediatric infections. Thinking about the US populace, 90% of youths and youthful grown-ups have been determined to have dental caries and 94% of dentate grown-ups have clinical history of treated or untreated coronal caries. It is a disease which results in the damage of whole tooth as it progresses. Dental caries is the result of complex interactions between both the host, his/her diets and the microflora on the tooth surface limited when factor that leads to the dissolution of inorganic and destruction of organic matter of the tooth.

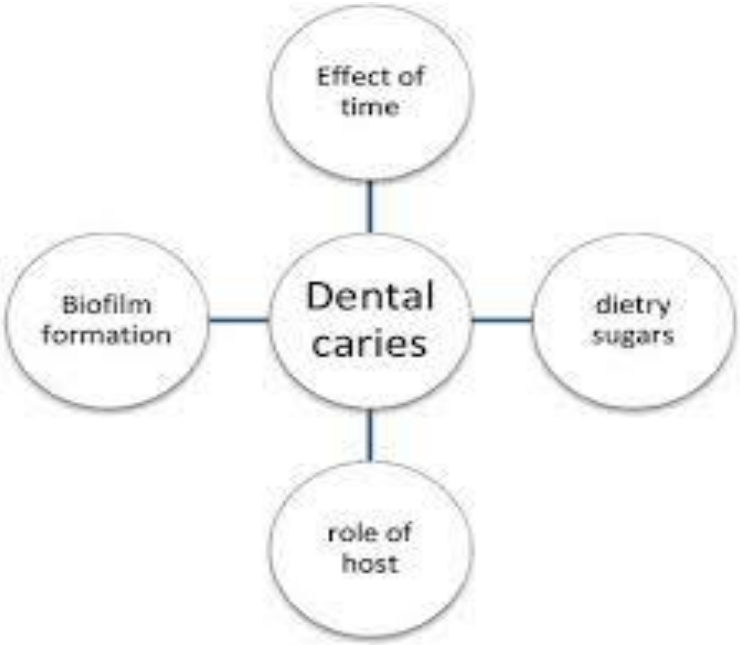


Fig.2.1: Showing the factors responsible for dental caries.

3. REVIEW OF LITERATURE

3.1 MICROORGANISM ASSOCIATED WITH DENTAL CARIES.

Previously, S. mutans has been embroiled as a causative microorganism of dental caries. S. mutans represents 7 species takeout from human and animals; Streptococcus sobrinus, Streptococcus cricetus, Streptococcus sobrinus, Streptococcus rattus, Streptococcus downey, S. mutans, and Streptococcus ferus Streptococcus sobrinus. S. mutans and Streptococcus macacae are solely isolated from human and S. mutans is the predominant species in which some are Acid producers which are gernally consider as Dental caries inhibitor and few are related with Acid tolerant which are consider as grow in caries. Acid-tolerant streptococcal isolates included Streptococcus oralis, Streptococcus sanguis, Streptococcus constellatus, Streptococcus intermedius, Streptococcus mitis, Streptococcus anginosus, Streptococcus gordonii and Streptococcus salivarius which are usaaly grow in caries.

In further studies, other microorganisms capable of initiating caries were also identified. There are specific group of microbes that are most acid producers in case of dental caries such as Streptococcus mutan, Actinomyces viscosus and Lactobacillus acidophilus Actinomyces viscosus and also there is some groups that are acid tolerants S. oralis, Streptococcus anginosus , S. sanguinis and Streptococcus mitis. Elevated levels of S. Veillonella, S. parasanguinis, S. sobrinus parasanguinis and salivarius have been associated with caries even in the absence of S. mutans in the subjects. Krithika et al. identified Streptococcus mutans, Actinomyces viscosus, and Lactobacillus acidophilus as the main causing species involved in the dental caries. S.constellatus, Bifidobacterium, Lactobacillus fermentum, S.parasanguinis, S.salivarius, Actinomyces gerencseriae, Veillonella and S.mutans have been associated with childhood caries. Scientific literature is replete with studies demonstrating the cariogenic potential of microorganisms other than S.mutans.

These studies prove the existence of multiple pathogens in the causing of dental caries and suggest that a strategy targeting multiple microorganisms, i.e., mixed-bacterial approach is needed to prevent caries.

3.2 INITIATION AND PROGRESSION OF CARIES

The adherence of bacteria on tooth surface produces dental plaque. Dental plaque is a pale yellow colored biofilm which develops naturally on the teeth. Develop dental plaque is an unpredictable multispecies biofilm that develops on the tooth surface and is inserted in a defensive grid and bacterial polymers (polysaccharides, proteins, and DNA) emitted by the cells. Bacteria in the biofilm (dental plaque) utilize dietary carbohydrates to produce organic acid as metabolized by product. These acids cause a decrease in local pH and when the pH falls under a critical value, demineralization of the tooth tissue occur.

Specifically speaking, the formation of lactate by the acidogenic oral microflora causes demineralization of calcium and phosphate present in the valuable precious stone sort of hydroxyapatite, which involves the enamels of the tooth. At the point when the recurrence and rate of acid formation surpasses the normal re-mineralization movement of the teeth, demineralization happens and brings about the resulting movement of cavitations, gave the pH stays underneath a critical estimation of roughly 5.5–5.3 for an adequate measure of time.

In the demineralization method, the organic acid produced by bacteria spread into the tooths surface through the water into the hydroxyapatite crystals. When a susceptible site, formed due to impurities like carbonates in the hydroxyapatite crystals, comes in contact with these diffused acids, dissolution of phosphate and calcium into the surrounded aqueous phase between the crystal occurs (Featherstone JD, 2004). When the diffusion of calcium, carbonates and phosphates carbonates out of the tooth occurs without proper re-mineralization, cavitation takes place (Featherstone JD, 2008).

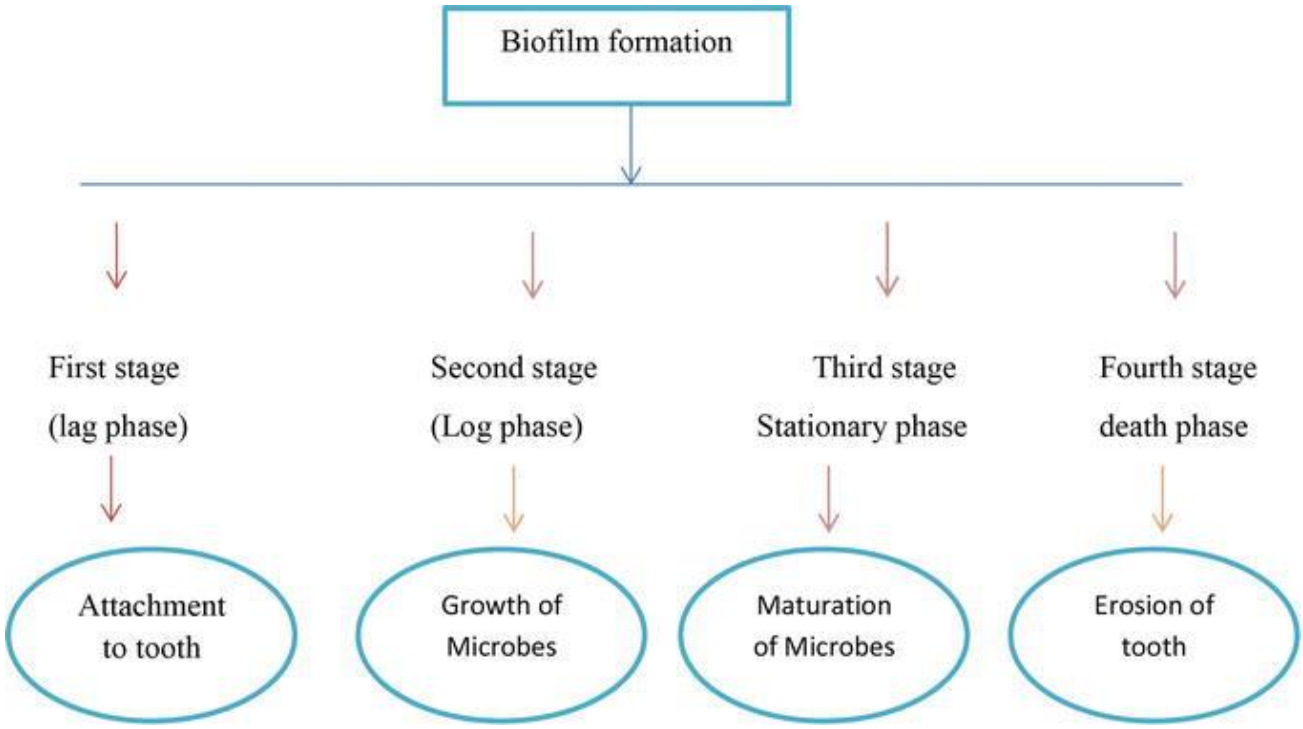


Fig.3.2.1: showing formation of biofilm

Demineralization and re-mineralization occur simultaneously in the oral cavity. The progress in development of dental caries depends on the balance between the process of demineralization and re-mineralization. Hence, any factor that can maintain this balance towards re-mineralization can be utilized to combat dental caries

3.3 PREVENTION OF DENTAL CARIES

A number of aids like fluoride gel and varnish, chlorhexidine and sealant are available for preventing dental caries (Rozier RG, 2001)**.** These aids can be grouped into physical and medical aids.

3.3.1 Physical Aids

Physical aids help in mechanical debridement of tooth surface. These include toothbrush, dental floss, ultrasonic for cleaning tooth surface, dentifrices etc (Daly *et al*., 2009).

3.3.2 Medical Aids

Medicals aids help in prevention of plaque build-up, decrease bacterial count and shift the equilibrium towards re-mineralization.

The available medical aids to control dental caries can be classified into two groups: one that prevent demineralization of tooth and the other one that promote the re- mineralization process (Chen *et al*., 2004).

a) Therapeutics to prevent the demineralization of tooth

This category of therapeutics utilizes strategies involving: killing of bacteria, preventing their attachment to the tooth surface or detaching them. This category includes chemoprophylactics agents, antimicrobials peptides, sugar substitutes, vaccine , probiotic and replacement therapies.

Chemoprophylactic agents include antibiotics like penicillin, cationic, anionic and non- ionic agents, plant extracts such as sanguinaria extract. For chemoprophylactic agents to be effective, it is necessary to maintain their minimum inhibitory concentration in the oral cavity because the concentration of a drug decreases almost immediately after the delivery of drug. Further, the chemoprophylactic agents do not provide protection for all the strains of a particular microorganism.

Though antimicrobial peptides (Brogden *et al*., 2005) have resolved this problem, but their use is limited due to the difficulty in synthesizing these peptides. These peptides are also susceptible to proteolytic cleavage, which may make them ineffective. (Marr *et al*.,2006**).**

As vaccines do not need to be maintained in minimum inhibitory concentrations like chemoprophylactic agents and utilize body's own defense mechanism to

prevent a disease, they are better candidates for preventing demineralization of tooth surface.

In oral cavity, there are some bacteria which are beneficial for preventing dental caries. One strategy is the reduction in growth of pathogenic microorganisms and the promotion of growth of beneficial bacteria, i.e., probiotics.

A novel strategy is replacement therapy which is based on the concept of replacing pathogenic strains of microorganisms with non-pathogenic ones. A recombinant *S.mutans* strain BCS3-L1 incapable of metabolizing fermentable sugars has been developed (Hillman *et al*., 2000).

b) Therapeutics to promote the remineralization process.

The remineralization process is an inorganic chemistry process in which the phosphate and calcium from saliva are recrystallized on the remnants of crystals on the dentine or enamel surface. If fluoride is incorporated within the crystal during remineralization, the mineral formed is much more resistant to acids than the original enamel or dentine mineral (Featherstone JD, 1999).
This fact is supported by the marked decrease in the levels of dental caries in individuals using fluoride-containing toothpastes (Nabi *et al*., 1990). This may be reason for incorporation of fluoride salts in water and mouthwashes. The salivary fluoride concentration is 0.02ppm, but the currently used fluoride delivery methods are not able to deliver this much amount of fluorine. A device

was developed maintaining the salivary concentration of fluoride. This device called "glass devices" dissolved slow when it comes in contact with saliva and releases fluorides (Pessan *et al*., 2008). Further, Xu *et al*. developed a dicalcium phosphate anhydrous (DCPA) nanocomposite capable of slowly releasing $CaPO_4$ as a restoration material. In 2010, another nanocomposite based on CaF_2 was developed.

This nanocomposite has greater fluoride releasing and stress bearing capabilities (Xu *et al.*,2010).

3.4 IMMUNE RESPONSE IN ORAL CAVITY

In the oral cavity, humoral immune response is predominant. The major immunoglobulin present in saliva is secretory (IgA). Saliva has also present IgM and IgG in the gingival sulcular fluids. Lymphocytes, neutrophils macrophages and macrophages, which are the components of cell mediated immunity, are also present in gingival sulcus. The following immune mechanisms are responsible for eliciting immune responses in oral cavity:

- Agglutination: Antibodies in saliva may interact with bacterial cell surface receptors. S- IgAs do not activate complement system but if IgG interacts with the antigens, complement system may be activated. The antibodies in saliva may prevent interaction of bacteria with teeth by specifically binding to bacterial surface receptors. Further, antibodies may inhibit the activity of enzymes like glucosyltransferase and prevent plaque formation.

- Sensitization of B-cells: If the gut associated lymphoid tissue (GALT) is immunized, sensitization of B-cells occurs and these sensitized B-cells then move to the salivary glands, which in turn secrete IgA. The secreted IgA may then prevent bacterial adhesion to the tooth surface.

- Cell-mediated immune responses may be elicited in animals after immunization with *S. mutans*, these responses may modify the humoral response via helper and suppressor functions of T-cells and may also cause gingival inflammation but they are not known to play a important roles in the immunology of caries.

3.5 Anti Carries Vaccine.

It stimulates the immune system of the host. It may stimulate humoral or cell-mediated immune response. Vaccines are prepared in a two from that can be live modified organisms, extracted cellular fractions, inactivated or killed organisms.

3.6 VACCINE CANDIDATES IN S MUTANS

Cell surface proteins or substances of *S. mutans* have been used as vaccines in a number of studies. These cell surfaces substances include adhesins, GTFs, GBP and dextranase. Most of the recent experimental studies for finding a vaccine against *S.mutans* have been utilizing these cell surface proteins as vaccine candidates.

Adhesins: Adhesins (Antigen I/II, P1 and Spa-a from *S.mutans* and *S.sobrinus* have been purified and used for vaccine preparation. Antigens I/II are present in both the culture super natant and on *S. mutans* cell surface. An antibody specific for the Antigen I/II molecule or to its salivary binding domain was successful in blocking the adherences of *S. mutans* to saliva-coated hydroxyapatites. Synthetic peptide comprising of residues 301-319 of Antigen I/II was effective in reducing tooth colonization by *S.mutans* (Smith DJ,2002).

- GLUCOSYLTRANSFERASE

S. mutans has the following three forms of glucosyltransferases (GTFs):

- GTF-I
- GTF-S-I
- GTF-S

The genes encoding GTF--I, GTF--SI, AND GTF--S are called the *gtf-b, gtf-c, and gtf-d* genes. *Streptococcus sobrinus* also induces a water insoluble glucan-

synthesizing enzyme *gtf-s*. *S.mutans* and *Streptococcus sobrinus* both synthesize a number of GTFs (Luo *et al*., 1988).

Dextranases: Dextran, is degraded by the enzyme dextranase produced by *S.mutans*. Due to dextranase, *S.mutans* has the capability of invading early dental plaque. Dextranase when it is use as an antigen, can prevent organism in earlier phase of dental plaque (Krithika *et al*., 2004).

Some other antigens have also been explored for designing a vaccine against dental caries. These antigens include the virulence-associated immunomodulatory extracellular proteins (VIP), secreted by *S.mutans* and *S.sobrinus*. These VIPs evade the host immune system by inducing the production of IL-10, which suppresses the host's immune system response against bacteria. Vaccines developed from VIPs have been able to induce immune-neutralization of VIP induced immunomodulatory effects (Gomes *et al*., 2009). Vaccines have also developed using Glucans. Glucans have been found to be less antigenic.

3.7 VACCINE DEVELOPMENT APPROACHES

3.7.1 Immunoinformatics based approach.

Recently, a study predicting B-cell and T-cell vaccine candidates from *S.mutans* GtfD using Immunoinformatics was published (Bower *et al*., 2014). In another study, the antigenic potential of the catalytic regions (CAT) and *glucan-binding domains* (GBD) of glucosyltransferase B (GtfB) from Streptococcus mutans has been evaluated using in- silico approaches followed by in-vivo and in-vitro experiments (Hoshino *et al*., 2011).

3.7.2 Laboratory (Wet-lab) based approach.

- ANIMAL STUDIES

Most of the studies evaluating the effectiveness of dental caries vaccines use rats and monkeys as animal models. The use of purified components of

S.mutans is still limited. GTF, when used as a vaccine provided protection against dental caries but for getting positive results 5-15 injections of GTF are required. Whereas cell wall antigen, Antigen I/II utilizes only one subcutaneous injection with adjuvant. The presence of s-IgA has been correlate with a reduce impact of the caries vaccine (Bowen WH, 2002).

- HUMAN STUDIES

The possibility of prevent dental caries by vaccine has been instituted due to it infectious nature. The idea is that immunization with *S.mutans* could induce an immune responses that can prevents the colonization of surfaces of teeth by *S.mutans* which can surely prevent dental caries. Administration of vaccine at the age of 6 months (before the eruption of deciduous/ primary/ milk teeth) prevent the caries in childrens who display the highest cases of caries. Thereafter, booster doses can be given at regular intervals. Immunization could be done using existing delivery systems.

The effects of immunization with *S.mutans* or *S.sobrinus* proteins in humans have been documented by few studies (Smith *et al*., 1987). These small-scale human trials of dental caries vaccines have reported increase in levels of S-IgA. Oral administration of GTF from *S. sobrinus* merged with aluminum phosphates in capsule form to fourteen subject results in an increase in salivary IgA Ab response when merged with an aluminum based adjuvants (Smith *et al*., 1987).

In another study, GTF from *S.sobrinus* considered topicall over the lower lip of younger adult stimulates local Ab formation in the salivary gland and result into delay oral re-colonization with S.mutans (Smith *et al*., 1990). Levels of salivary antibodies were also elevated when same preparation were

considered intranasal by topical case to the tonsils, either in associated in liposome or in soluble form (Li *et al*., 2003).

Despite the abundance of experimental evidences for the effectiveness of dental caries vaccine, not a single one is yet available for human use. Also, all these studies are oriented to the protection of colonization of oral cavity by S.mutans or S.sobrinus. None of the studies considers the polymicrobial nature of dental caries (Belda-Ferre et al., 2012; Kleinberg I., 2002). In the current research , an trial has been performed to design vaccines keeping in view the polymicrobial nature of this disease.

4. METHODOLOGY

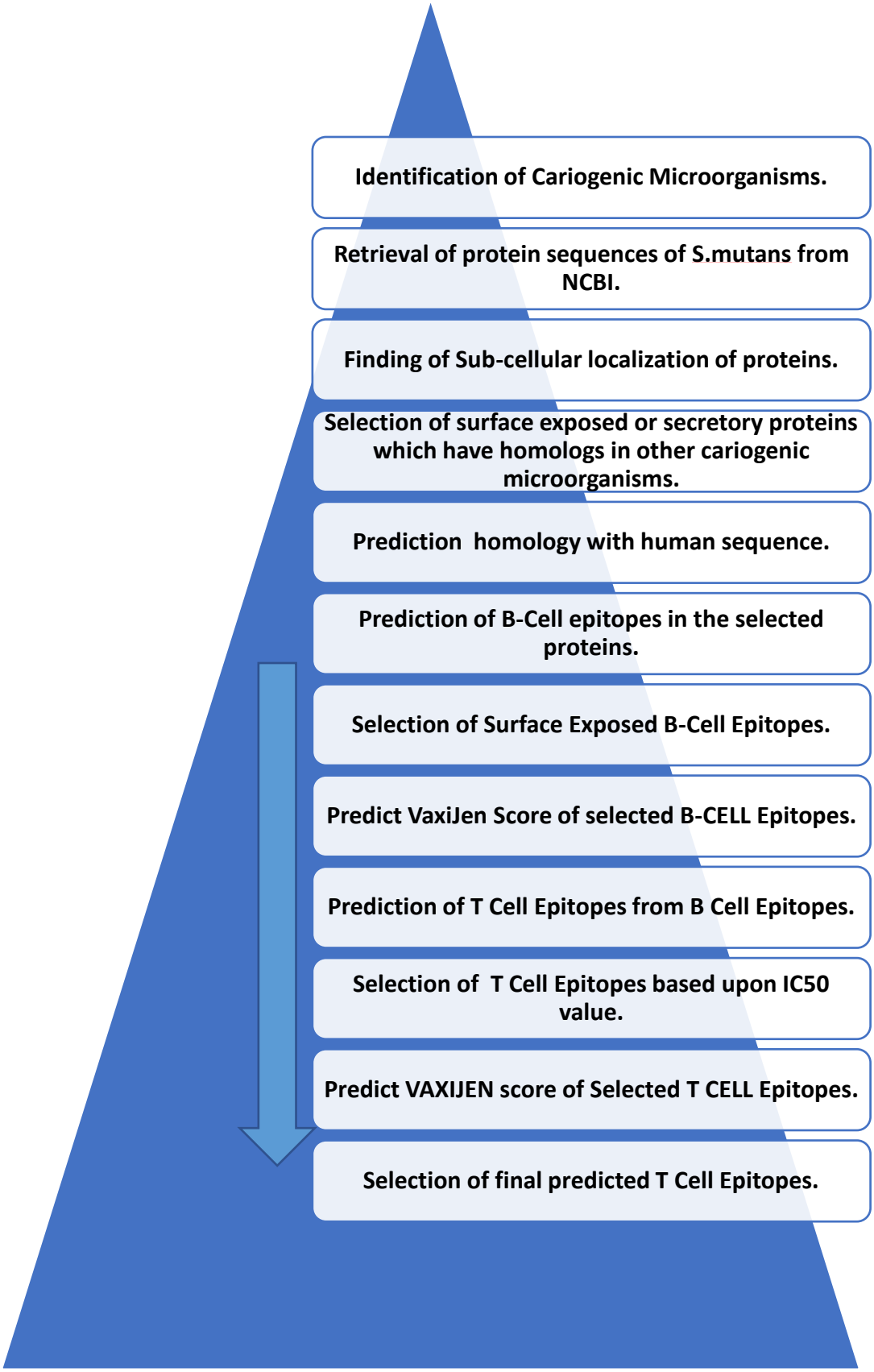
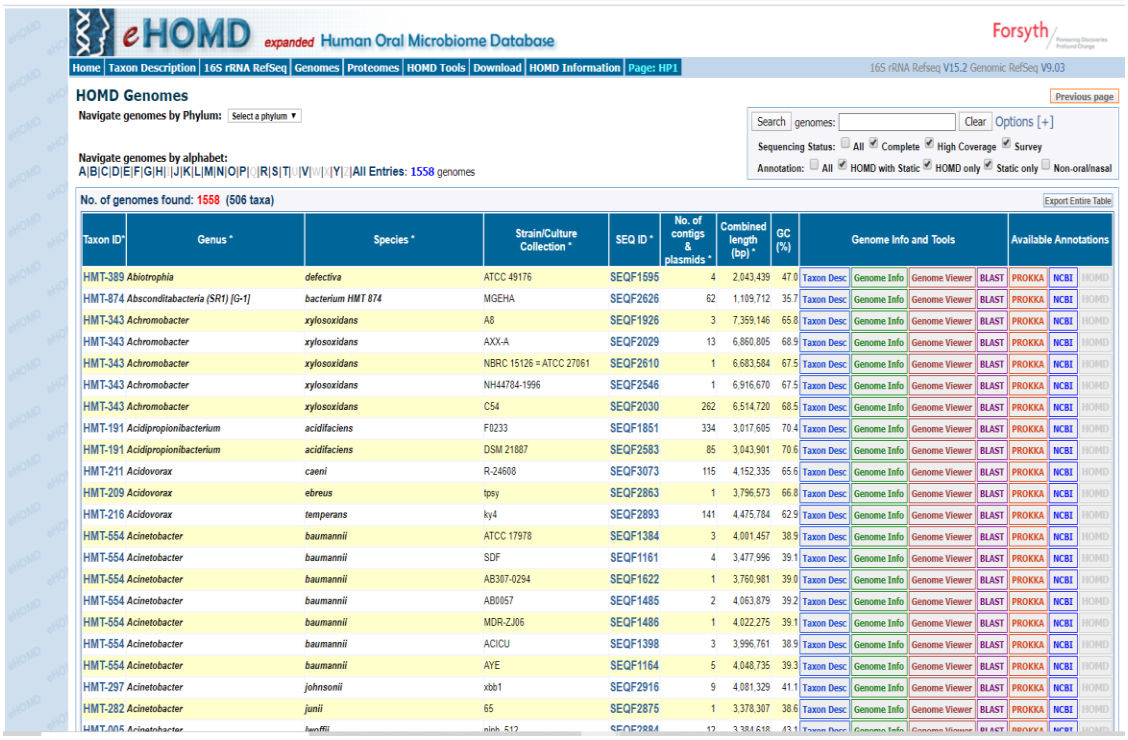


Fig.4.1: Showing the project workflo

4.1 **Identification of cariogenic microorganisms.**

Human Oral Microbiome Database (HOMD) (available at: http://www.homd..org/) gives a list of microorganism present in the oral cavities. Literature search was performed to select microorganisms associated with dental caries.



eHOMD expanded Human Oral Microbiome Database — Forsyth

Home | Taxon Description | 16S rRNA RefSeq | Genomes | Proteomes | HOMD Tools | Download | HOMD Information | Page: HP1 — 16S rRNA Refseq V15.2 Genomic RefSeq V9.03

HOMD Genomes

Navigate genomes by Phylum: Select a phylum

Navigate genomes by alphabet: A|B|C|D|E|F|G|H|I|J|K|L|M|N|O|P|Q|R|S|T|U|V|W|X|Y|Z|All Entries: 1558 genomes

Search genomes: Clear Options [+]

Sequencing Status: All Complete High Coverage Survey

Annotation: All HOMD with Static HOMD only Static only Non-oral/nasal

Previous page

No. of genomes found: 1558 (506 taxa) Export Entire Table

Taxon ID*	Genus *	Species *	Strain/Culture Collection *	SEQ ID *	No. of contigs & plasmids *	Combined length (bp) *	GC (%)	Genome Info and Tools	Available Annotations
HMT-389	*Abiotrophia*	*defectiva*	ATCC 49176	SEQF1595	4	2,043,439	47.0	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-874	*Absconditabacteria (SR1) [G-1]*	*bacterium HMT 874*	MGEHA	SEQF2626	62	1,109,712	35.7	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-343	*Achromobacter*	*xylosoxidans*	A8	SEQF1926	3	7,359,146	65.8	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-343	*Achromobacter*	*xylosoxidans*	AXX-A	SEQF2029	13	6,860,805	68.9	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-343	*Achromobacter*	*xylosoxidans*	NBRC 15126 = ATCC 27061	SEQF2610	1	6,683,584	67.5	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-343	*Achromobacter*	*xylosoxidans*	NH44784-1996	SEQF2546	1	6,916,670	67.5	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-343	*Achromobacter*	*xylosoxidans*	C54	SEQF2030	262	6,514,720	68.5	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-191	*Acidipropionibacterium*	*acidifaciens*	F0233	SEQF1851	334	3,017,605	70.4	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-191	*Acidipropionibacterium*	*acidifaciens*	DSM 21887	SEQF2583	85	3,043,901	70.6	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-211	*Acidovorax*	*caeni*	R-24608	SEQF3073	115	4,152,335	65.6	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-209	*Acidovorax*	*ebreus*	tpsy	SEQF2863	1	3,796,573	66.8	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-216	*Acidovorax*	*temperans*	ky4	SEQF2893	141	4,475,784	62.9	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-554	*Acinetobacter*	*baumannii*	ATCC 17978	SEQF1384	3	4,001,457	38.9	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-554	*Acinetobacter*	*baumannii*	SDF	SEQF1161	4	3,477,996	39.1	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-554	*Acinetobacter*	*baumannii*	AB307-0294	SEQF1622	1	3,760,981	39.0	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-554	*Acinetobacter*	*baumannii*	AB0057	SEQF1485	2	4,063,879	39.2	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-554	*Acinetobacter*	*baumannii*	MDR-ZJ06	SEQF1486	1	4,022,275	39.1	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-554	*Acinetobacter*	*baumannii*	ACICU	SEQF1398	3	3,996,761	38.9	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-554	*Acinetobacter*	*baumannii*	AYE	SEQF1164	5	4,048,735	39.3	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-297	*Acinetobacter*	*johnsonii*	xbb1	SEQF2916	9	4,081,329	41.1	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-282	*Acinetobacter*	*junii*	65	SEQF2875	1	3,378,307	38.6	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD
HMT-005	*Acinetobacter*	*lwoffii*	ninh_512	SEQF2884	12	3,384,618	43.1	Taxon Desc Genome Info Genome Viewer BLAST	PROKKA NCBI HOMD

Fig.4.1.1: Showing Human Oral Microbial taxa with annotated genes

As S.mutans is the fundamental etiological specialist in dental caries, so we ensured that every vaccine candidate in this investigation must evoke immnune reaction against S.mutans. Consequently, the genome of S. mutans strain of UA159 was taken as the reference genome; S. mutans strain UA159 is the first S.mutans genome to be sequenced (Song *et al.*, 2013).

4.2 **Retrieval of Proteins Sequences of S.mutans from NCBI.**

NCBI's GENOME database (http://www.ncbi.nlm.nih.gov/genome/) was searched with the keyword "*Streptococcus mutans*".

Since proteins(peptides) which surface uncovered or emitted by the cell are conceivably immunogenic, the proteins which are restricted either on surface or discharged by S.mutans were chosen. This progression was performed by foreseeing sub-cell restriction of all considerable number of proteins.

4.3 **Prediction of Sub-cellular localization of Proteins.**

The tools that are used to find the sub-cellular location of *S.mutans* proteins retrieved from NCBI are:

- PSORTb (http://www.psort.org/psortb/)
- CELLO (http://cello.life.nctu.edu.tw/)
- Gpos-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/Gpos-multi/) were used to find the sub-cellular location of S.Mutans proteins retrieved from NCBI.

Protein localization was predicted using 3 different servers, so as to minimize false positives in the result.

4.3.1 **Protein Localization prediction by PSORTb**.

PSORTb, an SVM based classifier, predicts the localization score of a protein in four different locations, namely cytoplasmic, cytoplasmic membrane, cell wall, extracellular (Yu *et al.*, 2010). The location having the highest score is the predicted localization of a protein.

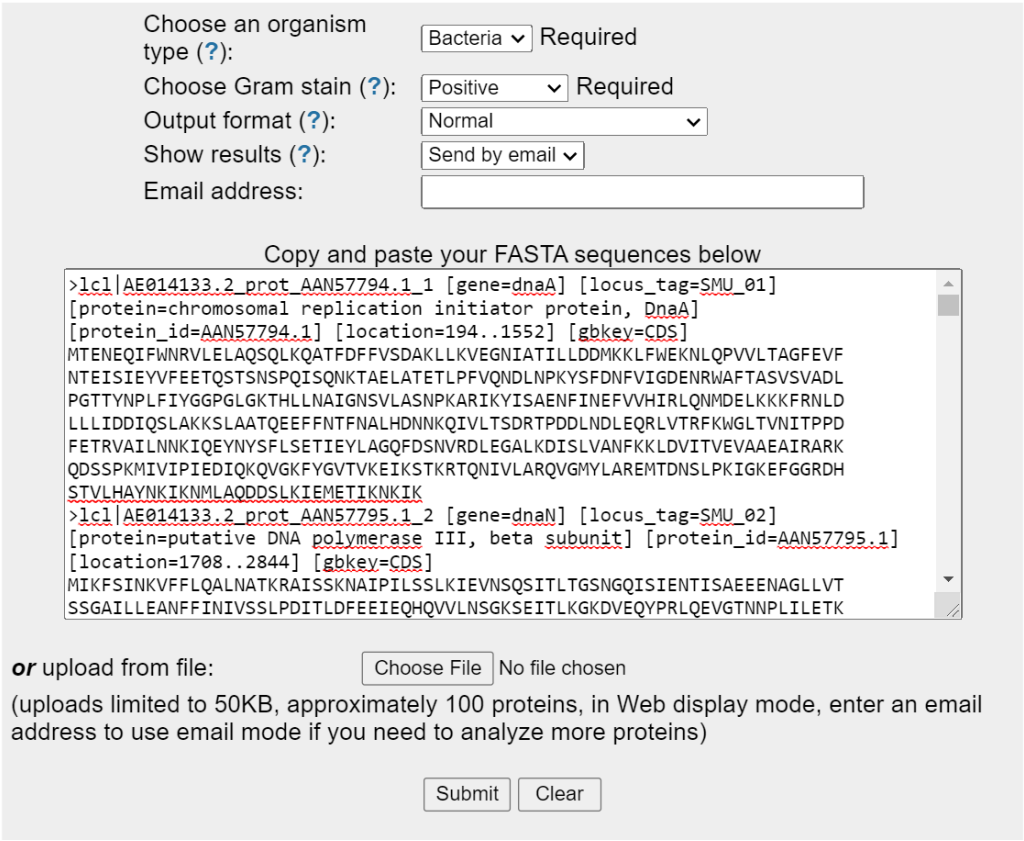


Figure 4.3.1: Showing protein sequence submission in PSORTb.

4.3.2 Protein Localization prediction by CELLO

CELLO is a multi-class SVM grouping framework. CELLO utilizes 4 sorts of arrangement coding plans: the amino acid structure, the di-peptide creation, the apportioned amino acid states and the grouping sythesis dependent on the physical and chemical properties of amino acids.

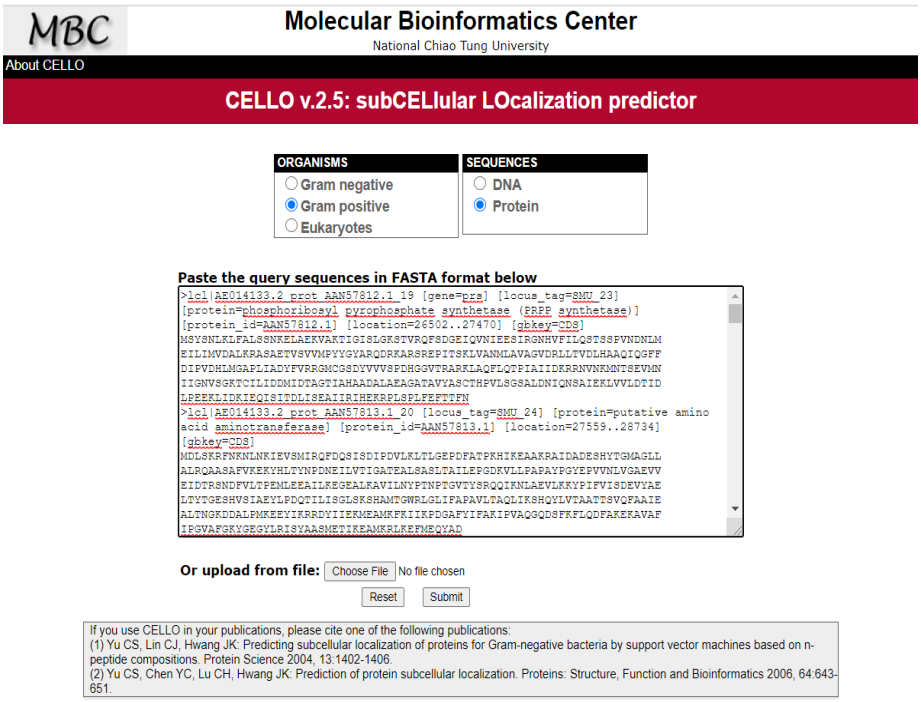


Figure 4.3.2: Showing protein sequence submission in CELLO.

4.3.3 Protein Localization prediction by Gpos-mPLoc

Gpos-mPLoc predicts the sub-cellular localization of Gram positive bacteria of protein by merging the data of genes and also the sequential evolution and functional domain data (Shen et al., 2009).

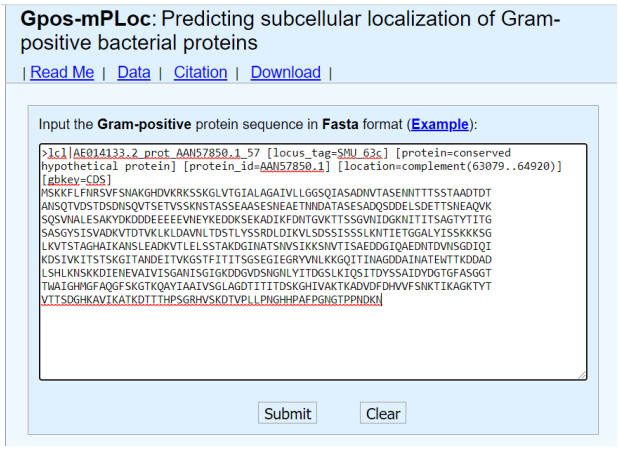


Figure 4.3.3: Showing protein sequence submission in Gpos-mPLoc.

Proteins which do not have homologs in other cariogenic microorganisms are of no significance in this study as the principal aim of the study is to ensure broad spectrum effectiveness of vaccine. Also the proteins which have homologs in humans cannot be used as vaccines due to cross-reactivity. Consequently, only the proteins which are conseved in cariogenic microorganisms and do not have homologs in humans were selected.

4.4 **Selection of surface exposed or secretory proteins which have homologs in other cariogenic microorganisms.**

Selection of S.mutans proteins having regions conserved in other cariogenic microorganisms. To predict vaccine candidates which can elicit immune response against a number of microorganisms, the selected antigens should be well conserved in all the cariogenic microorganisms. To select such conserved antigens, the homolog's of S.mutans proteins selected in Step 3 were predicted in other cariogenic bacteria.

• Blast search (available at: http://www.ncbi.nlm.nih.gov/BLAST) was perform using the BLASTP program to find homolog's of the selected S.Mutans proteins in the microorganisms

This step served to remove the S.Mutans proteins which do not have homologs in other cariogenic microorganisms and thus, are not suitable as vaccine targets for other cariogenic microorganisms, in the initial phase of this study.

4.5 **Prediction of homologs in humans.**

- To avoid autoimmunity and induce strong immunity, predicted antigens must not have sequence similarity to host (e.g., human) proteins.
- This step ensured that none of the selected proteins of *S.mutans* has similarity to the human proteins. Hence, no cross-reactivity should be observed for the vaccine candidates derived

from these proteins. **Since the major immunological response in oral cavity is of humoral type, the B-cell epitopes were find in all selected proteins.**

4.6 **Prediction of B-CELL Epitopes in the selected Proteins.**

- ABCpred tool was used to find the linear B-cell epitopes (20 aa long) in S.mutans proteins.
- The point of ABCpred server is to find out B cell epitope in an antigen grouping, ANS. This is the principal server created dependent on recurrent neural system (based upon machine method) utilizing fix length pattern.
- The protein sequence of each protein selected in pervious step was taken as an input and following parameters were selected
    - Window length to use for prediction of fixed length epitope.
    - Threshold set to 0.51.
    - Overlapping filter On because we wanted to predict non over lapping region.

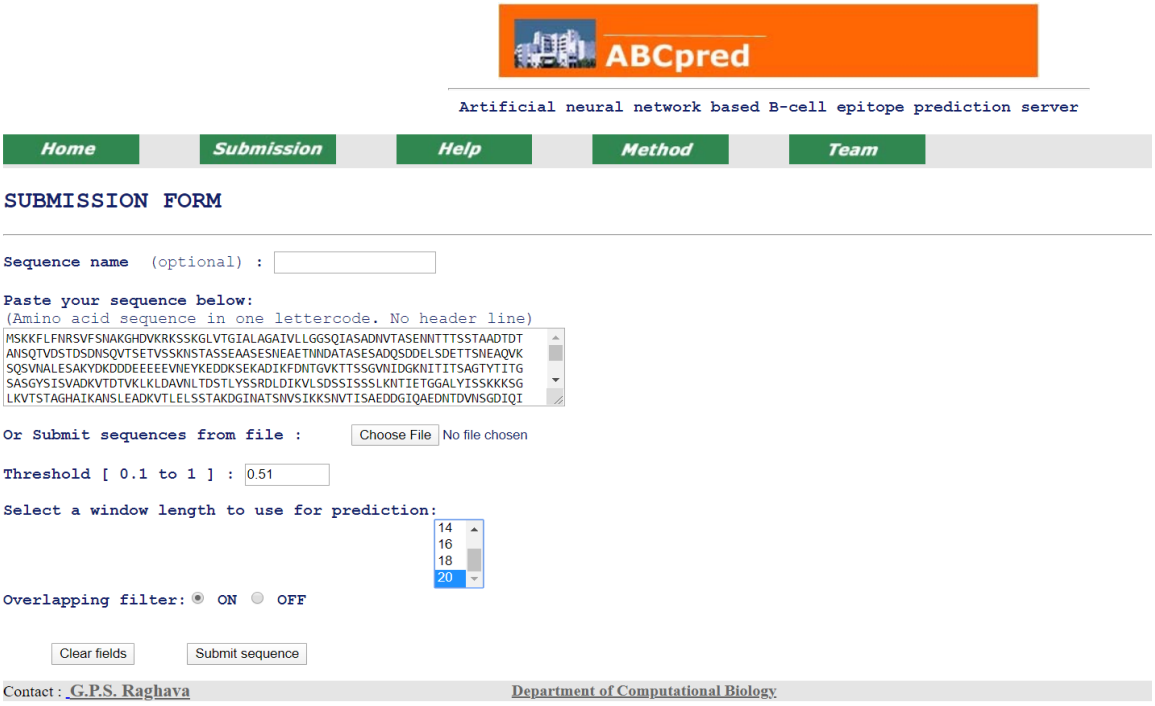


Fig 4.6.1: showing sequence submission in ABCpred.

Antibodies cannot bind the B-cell epitopes lying in the transmembrane regions. Hence, B-cell epitopes lying in the transmembrane regions cannot be used as vaccine candidates.

4.7 **Selection of Surface Exposed B CELL Epitopes.**

This step is useful because Antibodies cannot bind the B-cell epitope lying in the transmembrane regions. Hence, B-cell epitopes lying in the transmembrane regions cannot be used as vaccine candidates.

By the use of tool TMHMM server v. 2.0 the residues of a protein lying in the transmembrane region (helices) along with the residues lying inside and outside the cell. The B-cell epitopes present only in the surface exposed part or region (outside the cell) were selected.

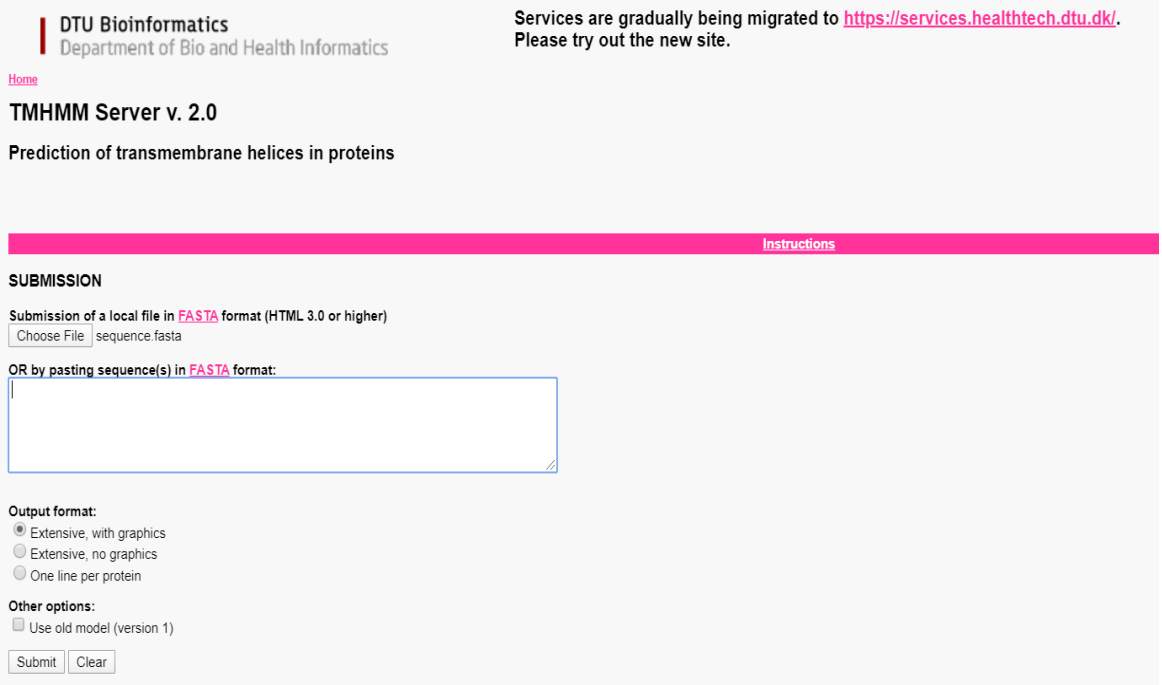


Fig.4.7.1: Showing sequence submission in TMHMM Server v. 2.0

4.8 **Predict VaxiJen Score of selected B-CELL Epitopes.**

- The antigenicity of remaining B-cell epitopes was find out by using VaxiJen server (http://www.ddg-pharmfac.net/VaxiJen/VaxiJen/VaxiJen.html).
- VaxiJen find out the antigenicity of an amino acid sequence is totally based on the physicochemicals property of protein without resource to arrangement.
- The default threshold value for a bacterial amino acid sequence to be anitgenic is 0.4.
- Epitope sequence in plain format was given as input in VaxiJen selecting "bacteria" as the target organism.

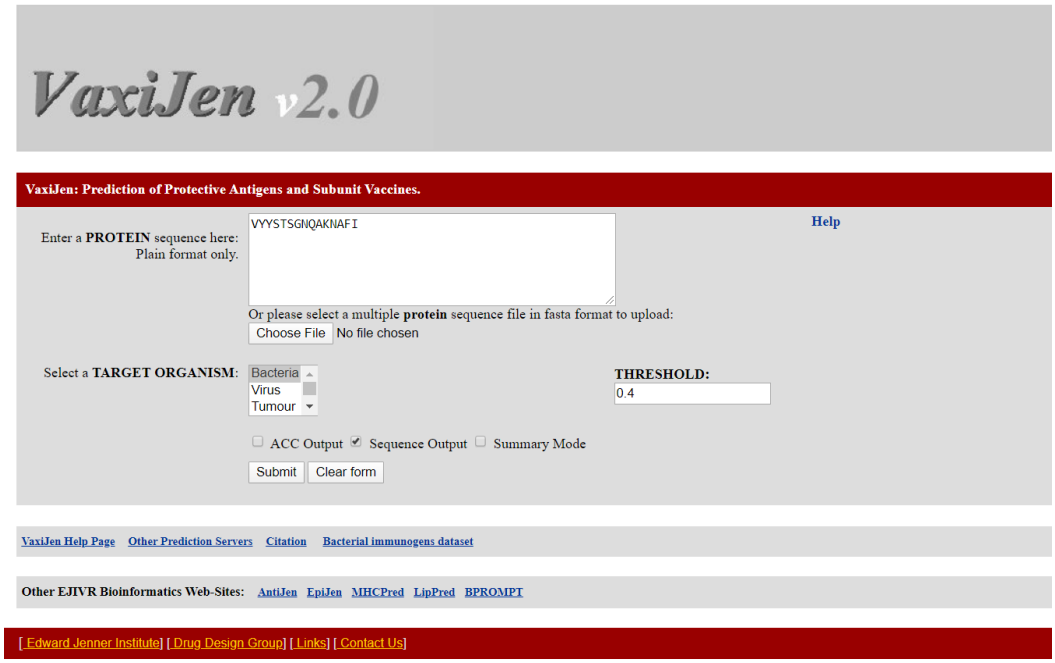


Fig.4.8.1: Showing sequence submission in VaxiJen.

CD4+ T-cells can recognize the antigenic peptides presented by antigen-presenting cells and activate the B-cells, which produce IgG antibody. Hence, CD4+ T-cells activation plays a very important role in eliciting immune response in oral cavity.

Not every administered vaccine is capable of eliciting immune response as it may

be degraded by proteases. To expand the odds of evoking an invulnerable reaction by an immunization, B-cell epitopes have been chosen in a way that, T cell epitope can predict from there.

As 15 amino acid long T-cell epitopes are efficient in stimulating CD4+ T-cells, the B-cell epitopes having >14 consecutive amino acid residues conserved in more than 2 cariogens have been used in this step.

4.9 **Prediction of T CELL Epitopes from B CELL Epitopes.**

The Immune Epitope Database Analysis Resource (IEDB). This site gives a combination of tools that helps in prediction or analysis of immune epitopes.

Net MHCII server 2.3 was used to find the binding of T-cell epitopes to HLA-DR alleles.

The T Cell epitopes predicted from the B cell Epitopes by using following parameters:

- Select species (human, HLA-DR).
- Prediction method Net MHCII 2.3
- length of epitopes (15).
- Select B cell Epitope as an input.

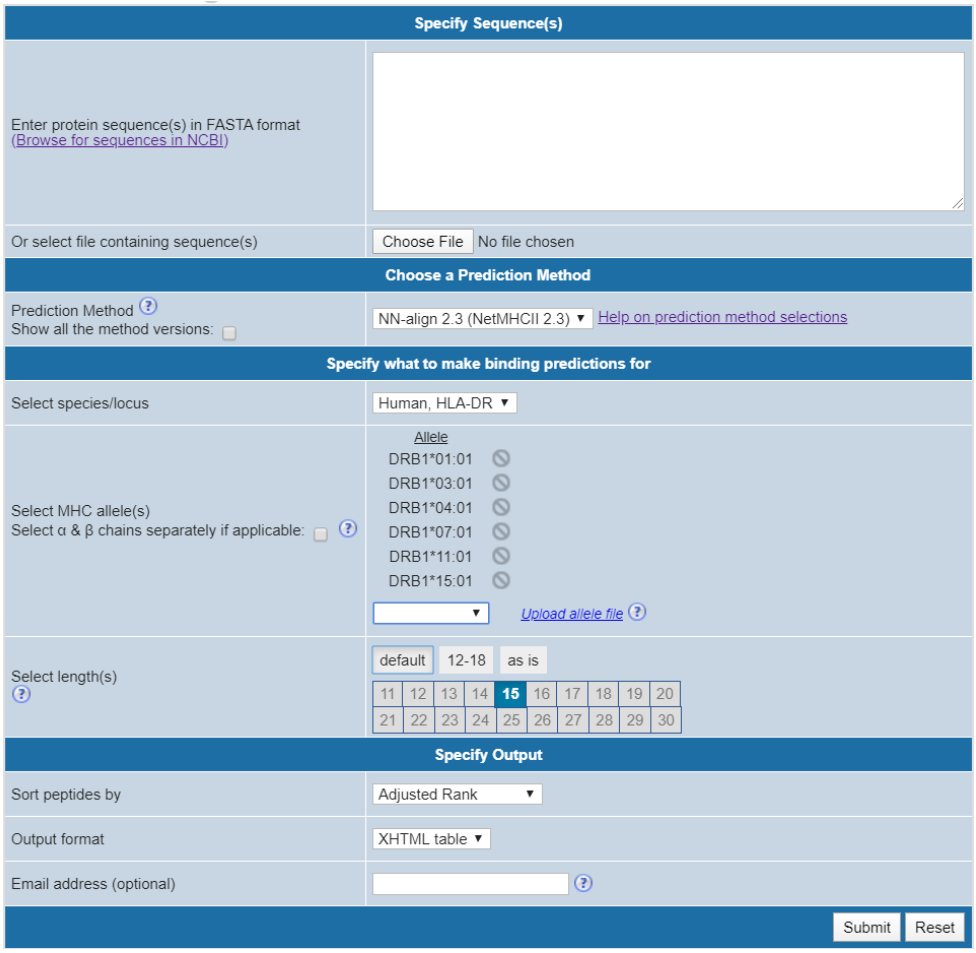


Fig 4.9.1: Showing sequence submission in IEDB

The next step is the selection of antigenic T-cell epitopes which bind to the maximum number of HLA-DR allele. HLA-DRBI*0101, HLA-DRBI*1501, HLA- DRBI*0401, HLA-DRBi*0701, HLA-DRBI*0401 and HLA-DRBI*0301 are the very frequently occur allele in the human population. Therefore, T-cell epitopes binding to these most frequently occurring alleles have been selected so as to ensure maximum population coverage. HLA-DRBI*0101 is the commonest bound allele, therefore the epitopes interacting with this allele should produce better antigenic responses.

4.10 **Prediction of T-CELL Epitopes.**

Selection of the T cell epitopes on the basis of these following criteria.

- Binding to HLA -DRB1 with IC_{50} value < 100Nm.
- The antigenicity of each T-cell epitope was predicting by using VaxiJen 2.0.
- Having VaxiJen antigenic score > 0.4 were consider as probable antigen and choose for next step.
- Binding to max. number of alleles among HLA-DR allele listed on NetMHCIIpan server 2.0 with IC50 value<100nM were selected.

5 Results.

5.1 Identification of cariogenic microorganisms.

Human Oral Microbiome Database (HOMD) (available at: http://www.homd.org/) provides a list of microorganism found in the oral cavities. Literature search was performed to select microorganisms associated with dental caries.

From literature, 52 microorganisms (including gram positive as well as gram-negative bacteria) were found to be cariogenic that have been listed in Appendix I.

5.2 Retrieval of protein sequences of S.mutans from NCBI.

- As *S.mutans* UA159 genome is the first fully sequenced *S.mutans* genome (Song *et al*., 2013), more information is available about this genome compared to the other strains of *S.mutans*.
- 1962 amino acid sequences encoding the entire proteome of *S.mutans* UA159 were retrieved from NCBI

5.3 Prediction of Sub-Cellular Localizations of Proteins.

The entire arrangement of protein of S.mutans was then screened by means of various protein localization finding tools in order to mine out the proteins which could act as antigens, i.e., the proteins/peptides that are either surface exposed (present on the cell divider) or emitted by the cell.

- Protein localization was predicted using 3 different servers, so as to minimize false positives in the result.

- there are total 1962 protein, out of which 36 proteins were find out to be secreted or surface exposed by all the 3 servers.

SNo.	Protein name	Localization
1.	exo-beta-D-fructosidase; fructanase FruA	Cell wall
2.	exo-beta-D-fructosidase FruB	Cell wall
3.	transfer protein	Cell wall
4.	cell surface antigen SpaP	Cell wall
5.	hypothetical protein SMU_1091	Cell wall
6.	glucan-binding protein GbpC	Cell wall
7.	hypothetical protein SMU_984	Cell wall
8.	cell wall-associated protein WapA	Cell wall
9.	cell wall protein, WapE	Cell wall
10.	thioredoxin family protein	Cell wall
11.	dextranase	Cell wall
12.	hypothetical protein SMU_2147c	Cell wall
13.		Extracellular

	peptidoglycan hydrolase	
14.	bacteriocin peptide	Extracellular
15.	prolyl dipeptidyl peptidase	Extracellular
16.	hypothetical protein SMU_616	Extracellular
17.	autolysin; amidase	Extracellular
18.	glucan-binding protein D	Extracellular
19.	hypothetical protein SMU_629	Extracellular
20.	putative autolysin; amidase	Extracellular
21.	hypothetical protein SMU_836	Extracellular
22.	glucosyltransferase-1	Extracellular
23.	hypothetical protein SMU_963c	Extracellular
24.	putative transposase fragment SMU_1024	Extracellular
25.	glucosyltransferase-S	Extracellular
26.	glucosyltransferase-I	Extracellular
27.		Extracellular

	glucosyltransferase-SI	
28.	Glucan 1,4-alpha-maltohexaosidase	Extracellular
29.	hypothetical protein SMU_1752c	Extracellular
30.	hypothetical protein SMU_1882c	Extracellular
31.	competence stimulating peptide	Extracellular
32.	beta-D-fructosyltransferase	Extracellular
33.	hypothetical protein SMU_2048	Extracellular
34.	hypothetical protein SMU_2076	Extracellular
35.	glucan-binding protein GbpA	Extracellular
36.	hypothetical protein SMU_2146c	Extracellular

Table. 5.3.1: Showing Proteins predicted to be either extracellular or localized in cell wall by all the three servers.

5.4 **Selection of surface exposed or secretory proteins which have homologs in other cariogenic microorganisms.**

- This step served to remove the S.Mutans proteins which do not have homologs in other cariogenic microorganisms and thus, are not suitable as vaccine targets for other cariogenic microorganisms, in the initial phase of this study.
- Using BlastP, only 16 proteins of *S.mutans* out of 36 proteins were found to have

regions conserved in other cariogens also.

S.No.	Protein name	Localization
1	hypothetical protein SMU_63c	Cellwall
2	exo-beta-D-fructosidase; fructanase FruA	Cellwall
3	exo-beta-D-fructosidase FruB	Cellwall
4	transfer protein	Cellwall
5	cell surface antigen SpaP	Cellwall
6	glucan-binding protein GbpC	Cellwall
7	cell wall protein, WapE	Cellwall
8	dextranase	Cellwall
9	hypothetical protein SMU_616	Extracellular
10	glucan-binding protein D	Extracellular
11	hypothetical protein SMU_836	Extracellular
12	glucosyltransferase-S	Extracellular
13		Extracellul

	glucosyltransferase-I	ar
14	glucosyltransferase-SI	Extracellul ar
15	beta-D-fructosyltransferase	Extracellul ar
16	glucan-binding protein GbpA	Extracellul ar

TABLE 5.4.1: Showing the proteins having homologs in other cariogens.

5.5 Prediction of homologs in humans.

- Using BlastP, no human homologs were found for the proteins.

- This step ensured that none of the selected proteins of *S.mutans* has similarity to the human proteins. Hence, no cross-reactivity should be observed for the vaccine candidates derived from these proteins.

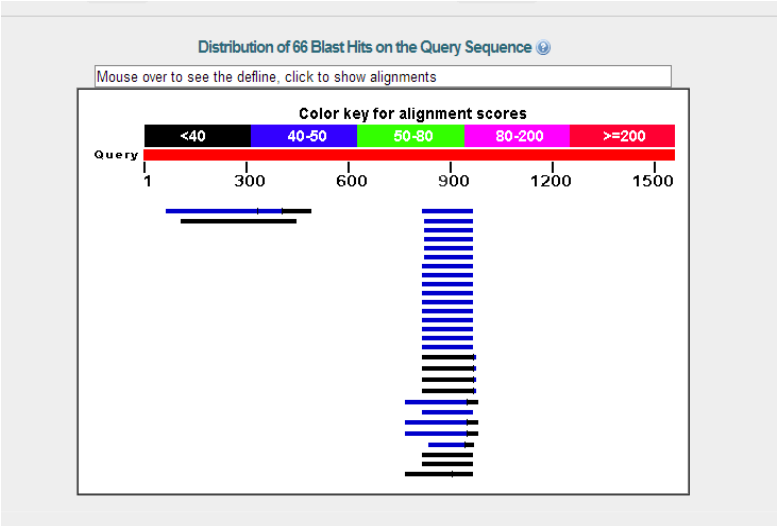


Figure 5.5.1: Showing BLAST hits when *S.mutans* proteins were blasted against human genome (taxid 9606).

5.6 **Prediction of B CELL Epitopes in the selected proteins**.

- ABCpred server was used to find out the linear B-cell epitopes (which ussally contain 20 aa long) in S.mutans proteins.

- ABCpred predicted a total of 1111 B-cell epitopes in the 16 proteins.

Antibodies cannot bind the B-cell epitopes lying in the transmembrane regions. Hence, B-cell epitopes lying in the transmembrane regions cannot be used as vaccine candidates.

5.7 **Selection of surface Exposed B CELL Epitopes**.

The surface exposed B-cell epitopes were selected on the basis of:

- **Transmembrane topology**

Based on TM-HMM results listed in Table 3. shows that there are 10 B-cell epitopes were removed because they were present in the transmembrane regions.

PROTEIN GI	**TM-HMM PREDICTION RESULTS**		
	INSIDE	**HELIX**	**OUTSIDE**
hypothetical protein SMU_63c			1-613
exo-beta-D-fructosidase; fructanase FruA	1-19	20-39	40-1423
exo-beta-D-fructosidase Fru B			1-519
transfer protein	1-16	17-39	40-365
cell surface antigen SpaP	1558 – 1562	1538 -1557	1-1537
glucan-binding protein GbpC			1-583
cell wall protein, WapE	1-12; 501-507	13-35; 483-500	36-482

dextranase	845-850	827-844	1-826
glucan-binding protein D			1-726
hypothetical protein SMU_836			1-544
glucosyltransferase-S			1-1462
glucosyltransferase-I			1-1476
glucosyltransferase-SI			1-1455
beta-D-fructosyltransferase	1--12; 795	13-35; 777-794	36-776
glucan-binding protein GbpA	1-16	17-39	40-565

Table 5.7.1: Showing result of TM-HMM.

5.8 **Predict VaxiJen Score of selected B-CELL Epitopes.**

- Based on VaxiJen scores, 369 B-cell epitopes have antigenicity score below the set threshold of 0.4.
- On the basis of prediction total number of 369 epitopes were rejected.

In this step, 372 B-cell epitopes Shows in table 4. (three present in the transmembrane region and 69 non- antigenic) were rejected. The remaining epitopes were checked for conservancy in the next step.

NAME	B CELL EPITOPE	Vaxijen	transmembrane region remov
hypothetical protein SMU_63c	51	7	0
exo-beta-D-fructosidase; fructanase FruA	114	43	0
exo-beta-D-fructosidase FruB	45	19	0
transfer protein	27	6	0
cell surface antigen SpaP	127	31	1
glucan-binding protein GbpC	51	13	0
cell wall protein, WapE	42	15	3
dextranase	70	32	0
hypothetical protein SMU_616	5	1	0
glucan-binding protein D	58	14	0
hypothetical protein SMU_836	44	14	0
glucosyltransferase-S	119	50	0
glucosyltransferase-I	117	38	0
glucosyltransferase-SI	126	55	0
beta-D-fructosyltransferase	68	20	3
glucan-binding protein GbpA	47	11	3

Table 5.8.1: Showing number of B-CELL Epitope, Non-Probable Antigen and Transmembrane region.

5.9 Prediction of T-CELL Epitopes from B CELL Epitopes.

The Immune Epitope Database Analysis Resource (IEDB). This site gives a combination of tools that helps in prediction or analysis of immune epitopes.

- NetMHCII server 2.3 was used to find the binding of T-cell epitopes to HLA-DR alleles.
- T cell Epitopes predicted from IEDB software.

Allele	#	Start	End	Length	Core Sequence	Peptide Sequence	IC50	Percentile Rank	Adjusted rank
HLA-DRB1*04:01	12	1	15	15	LKLDAVNLT	VKLKLDAVNLTDSTL	10.40	0.03	0.03
HLA-DRB1*04:01	12	2	16	15	LKLDAVNLT	KLKLDAVNLTDSTLY	15.20	0.13	0.13
HLA-DRB1*04:01	38	6	20	15	FASGGTTWA	DGTGFASGGTTWAIG	16.00	0.14	0.14
HLA-DRB1*04:01	38	5	19	15	FASGGTTWA	YDGTGFASGGTTWAI	20.00	0.31	0.31
HLA-DRB1*04:01	23	6	20	15	YITDGSLKI	SNGNLYITDGSLKIQ	21.80	0.35	0.35
HLA-DRB1*04:01	40	1	15	15	YITDGSLKI	NLYITDGSLKIQSIT	22.00	0.36	0.36
HLA-DRB1*04:01	37	1	15	15	VLSDSSISS	DIKVLSDSSISSSLK	23.80	0.45	0.45
HLA-DRB1*04:01	37	2	16	15	VLSDSSISS	IKVLSDSSISSSLKN	26.20	0.53	0.53
HLA-DRB1*11:01	34	3	17	15	YVNLKKGQI	GRYVNLKKGQITINA	8.30	0.55	0.55
HLA-DRB1*11:01	34	2	16	15	YVNLKKGQI	EGRYVNLKKGQITIN	8.40	0.55	0.55
HLA-DRB1*11:01	34	1	15	15	YVNLKKGQI	IEGRYVNLKKGQITI	8.90	0.62	0.62
HLA-DRB1*04:01	40	2	16	15	YITDGSLKI	LYITDGSLKIQSITD	36.20	0.97	0.97
HLA-DRB1*03:01	31	1	15	15	LEADKVTLE	NSLEADKVTLELSST	29.50	1.10	1.10
HLA-DRB1*04:01	26	1	15	15	FITITSGSE	GSTFITITSGSEGIE	41.70	1.20	1.20
HLA-DRB1*04:01	37	3	17	15	VLSDSSISS	KVLSDSSISSSLKNT	39.40	1.20	1.20
HLA-DRB1*04:01	23	5	19	15	YITDGSLKI	DSNGNLYITDGSLKI	44.10	1.30	1.30
HLA-DRB1*04:01	38	4	18	15	FASGGTTWA	DYDGTGFASGGTTWA	44.40	1.30	1.30
HLA-DRB1*04:01	36	4	18	15	IASADNVTA	GSQIASADNVTASEN	46.50	1.50	1.50
HLA-DRB1*04:01	36	3	17	15	IASADNVTA	GGSQIASADNVTASE	50.50	1.70	1.70
HLA-DRB1*04:01	12	3	17	15	LKLDAVNLT	LKLDAVNLTDSTLYS	51.80	1.80	1.80
HLA-DRB1*03:01	37	1	15	15	VLSDSSISS	DIKVLSDSSISSSLK	43.10	1.80	1.80
HLA-DRB1*03:01	12	1	15	15	LKLDAVNLT	VKLKLDAVNLTDSTL	49.70	2	2
HLA-DRB1*03:01	37	2	16	15	VLSDSSISS	IKVLSDSSISSSLKN	49.60	2	2
HLA-DRB1*11:01	34	4	18	15	YVNLKKGQI	RYVNLKKGQITINAG	21.30	2.10	2.10
HLA-DRB1*07:01	7	4	18	15	VTSTAGHAI	KKSGLKVTSTAGHAI	19.60	2.20	2.20
HLA-DRB1*04:01	26	2	16	15	FITITSGSE	STFITITSGSEGIEG	58.90	2.20	2.20
HLA-DRB1*07:01	7	5	19	15	VTSTAGHAI	KSGLKVTSTAGHAIK	20.60	2.30	2.30
HLA-DRB1*04:01	36	5	19	15	IASADNVTA	SQIASADNVTASENN	65.70	2.60	2.60
HLA-DRB1*04:01	36	2	16	15	IASADNVTA	LGGSQIASADNVTAS	72.60	2.80	2.80
HLA-DRB1*07:01	7	6	20	15	VTSTAGHAI	SGLKVTSTAGHAIKA	25.20	2.90	2.90
HLA-DRB1*03:01	31	2	16	15	LEADKVTLE	SLEADKVTLELSSTA	71.80	3.20	3.20
HLA-DRB1*04:01	25	1	15	15	IKKSNVTIS	VSIKKSNVTISAEDD	81.40	3.30	3.30

Fig 5.9.1: Showing result of T cell Epitope predicted by Net MHCII server 2.3

By using this result, we eliminate the T cell epitope on the basis of their IC_{50}<100nM. We have selected only those T cell epitopes which contain IC_{50} less than 100Nm.

5.10 Selection of T-Cell Epitopes.

- Out of these T-cell epitopes, 84 epitopes bind to HLA-DRB1 with IC_{50}<100nM are

selected.

B CELL EPITOPE NUMBER	T CELL EPITOPE	HLA-DRB1*01:01	HLA-DRB1*03:01	HLA-DRB1*04:01	HLA-DRB1*07:01	HLA-DRB1*11:01	HLA-DRB1*15:01
12	VKLKLDAVNLTDSTL	71.7	49.7	10.4			
28	NKTIKAGKTYTVTTS	74.3			41.7		95.5
11	ANDGVLKWVLSRGGR				71.4	65.5	76.6
29	GRTVTLTYRINVHRR				38.3	78.3	
48	NQWFMVLAGGPLRIY	7.3			25.7	52.2	86.8
53	GELASIVRVKVSHIE	38.1			10.7	48.7	
53	KGELASIVRVKVSHI	55.5			93.9	19.5	
55	GNVLHLTAVKKGKLT	29.7			43.2	60.1	91.9
55	KGNVLHLTAVKKGKL	25.4			8.8	42.2	
55	NVLHLTAVKKGKLTI	35.4			73.6	24.7	
65	DDQYHHIKVTKTKNS	25.4			26.9	88.7	
65	DQYHHIKVTKTKNSI	24.1			91.6	21.6	
65	QYHHIKVTKTKNSII	24			7	47.4	
65	YHHIKVTKTKNSIII	50.3			6.8	71.5	
19	DPYIWYDSNSKRLMM	52.2		63.7	21.7		
5	HSQELSLKFQANAAT	46.3		50.8			80.9
1	LSLKFQANAATLNGH	5.6		13.7			66.3
19	PYIWYDSNSKRLMMY	40.4	96.7	86.2	23.4		
19	RDPYIWYDSNSKRLM	76		49.5	24.8		
22	TYSMVKLSTASDMDI	29.2			28.2	95.6	
SNaP							
47	AITIKFKEAFLRSVS	56.4				92.6	38.7
90	AYQKALAAYQAELKR	8.4		81.8			42.8
56	ETTSFVLVDPLPSGY	8.3		48.7	90.3		
47	GAITIKFKEAFLRSV	86.9			82.7		35.4
9	GIDLKIVSPMVVKKQ	86.9			38		43.3
71	GKKPNIWYSLNGKIR	84.6			29		57.3
72	GQTIPLNTVFNYRLI	92.7			49.1		80.7
9	IDLKIVSPMVVKKQM	48.9			42.4		60.6
47	ITIKFKEAFLRSVSI	25.1			45		44
47	KFKEAFLRSVSIDSA	10.9		69.6	43		
50	KKTYGFRKSKISKTL	62.4			25.2	62.5	
57	KNGMIYATDTLNFRQ		95.3	26.7	78.3		
9	KTGIDLKIVSPMVVK	50.4			28.9		55.4
50	KTYGFRKSKISKTLC	64.3			32.3	78.9	
72	PLNTVFNYRLIGGII	40.8			39.7		54.2
90	QAAYQKALAAYQAEL	5.7		30.2			83.7
9	TGIDLKIVSPMVVKK	39.4			32.4		41.8
56	TSFVLVDPLPSGYQF	7.7		46.4		83.2	
56	TTSFVLVDPLPSGYQ	7.1		41	98.1	84.9	
DEXTRANSE PRECURSOR							
35	AAAGGYHMSLAALAN	39.9			24.2	52.8	
35	AAGGYHMSLAALANP	3.6			31.9	41.1	
35	AAIAAAGGYHMSLAA	17.5			75.6		27.4
35	AIAAAGGYHMSLAAL	20.5			96.9		49.4
PROTEIN D							
4	AAALKALKGQPMWLI	5.7			68.9		26.2
4	AALKALKGQPMWLIH	7.4			79.1		24.4
31	DINIPLLASNVARLT	8.8			61.9		38.5
31	INIPLLASNVARLTE	8.9			77.6		41
22	MTLDMGVAYPNYFAA	33.6	95.8				69.3
31	TDINIPLLASNVARL	9.7			54.3		47.6
SMU 836							
28	KGFKIGTVPKVGAIA	13.1		49.7		62.5	
27	LNQIVHYQPSAVRIT	19.7			74.2		11
27	NQIVHYQPSAVRITA	14.6			81.4		14.6
27	QLNQIVHYQPSAVRI	33.5			82.5		12
28	STVAVKGFKIGTVPK	24.1			22.1		46.2
28	TVAVKGFKIGTVPKV	25.9			27.5		65.9
28	VKGFKIGTVPKVGAI	12.8		43.7		82.6	
GLYCOSYLTRANSFERASE - S							
3	NWYYFGSDGVAVTGS	29.3		52.5	51.2		
6	QIAYLNYMNQQGLGT	70.3		77.2			84.8
45	SEVQTVIAKIIKAQI	95.2			89.4	46.2	
3	WYYFGSDGVAVTGSQ	42.4		59.3	77.8		
GLYCO - I							
40	RLSLLFSLAKPLNQR	6.2		45.3	4.9	11.5	50.3
40	SLLFSLAKPLNQRSG			60.7	7	11.5	
59	TVNKDIVTTRSNLYK		50.2	88.4	20.8		
GLYCO - SI							
47	FKLRKVKKRWVTVSV				77.3	9.1	94
37	GANYYFLSNGIQLRN	3.8		28.6	18.6		30.4
33	GQRLYFKSNGVQAKG	67.4	74.4				
56	GQRLYFKSNGVQAKG	12.7	19.3		86.5		79
47	KKVRFKLRKVKKRWV	55.8			53.2	4.5	93.5
47	KVRFKLRKVKKRWVT	62			61.9	5	
48	LLKARIKYVSGGQAM	38.3				85.6	66.4
37	NGANYYFLSNGIQLR	4.8		39.8	16		31.1
47	RFKLRKVKKRWVTVS	91			72.8	6.4	
47	VRFKLRKVKKRWVTV	82.3			63.9	5.6	
57	VYYSTSGNQAKNAFI	30.5		95	90.6		
BETA -D Glyco							
41	AIPYFNAKAIKNMKA	19.5			76.4	40.5	69.7
37	ATYSYYAVPVAGSSD	19.1		65.9		63.7	
41	IPYFNAKAIKNMKAA	22.2			97.6	32.7	
41	RYAIPYFNAKAIKNM	43.5			60.8		25
37	TYSYYAVPVAGSSDT	21.3		74.1		54.4	
41	YAIPYFNAKAIKNMK	27.1			71	55.2	39.3

Fig. 5.10: Showing T Cell Epitope bind to HLA-DRB1 with IC_{50}<100nM are selected.

5.10.1 Predict VAXIJEN score of Selected T CELL Epitopes.

- There are 79 Epitopes having VaxiJen antigenic score > 0.4 were consider as probable antigen and choose for next step.
- In this Fig. 5, pink region donates having VaxiJen score <0.4

B CELL EPITOPE	T CELL EPITOPE	HLA-DRB1*01:01	HLA-DRB1*03:01	HLA-DRB1*04:01	HLA-DRB1*07:01	HLA-DRB1*11:01	HLA-DRB1*15:01	vaxiJEn Score of t cell
VKLKLDAVNLTDSTLYSSRD	VKLKLDAVNLTDSTL	71.7	49.7	10.4				**0.9516**
NKTIKAGKTYTVTTSDGHKA	NKTIKAGKTYTVTTS	74.3			41.7		95.5	**0.8585**
MYPIVANDGVLKWVLSRGGR	ANDGVLKWVLSRGGR				71.4	65.5	76.6	**0.4502**
SFGRTVTLTYRINVHRRQND	GRTVTLTYRINVHRR				43.2	60.1	91.9	**1.1097**
VFHWNNQWFMVLAGGPLRIY	NQWFMVLAGGPLRIY	7.3			26.9	88.7		**0.7163**
KGELASIVRVKVSHIETNDA	GELASIVRVKVSHIE	38.1			10.7	48.7		0.3193
KGELASIVRVKVSHIETNDA	KGELASIVRVKVSHI	55.5			8.8	42.2		0.2638
SQKGNVLHLTAVKKGKLTIT	GNVLHLTAVKKGKLT	29.7			93.9	19.5		0.3335
SQKGNVLHLTAVKKGKLTIT	KGNVLHLTAVKKGKL	25.4			73.6	24.7		0.1562
SQKGNVLHLTAVKKGKLTIT	NVLHLTAVKKGKLTI	35.4			91.6	21.6		0.21
INDDQYHHIKVTKTKNSIII	DDQYHHIKVTKTKNS	25.4			38.3	78.3		**0.8325**
INDDQYHHIKVTKTKNSIII	DQYHHIKVTKTKNSI	24.1			25.7	52.2	86.8	**0.5302**
INDDQYHHIKVTKTKNSIII	QYHHIKVTKTKNSII	24			7	47.4		**0.6149**
INDDQYHHIKVTKTKNSIII	YHHIKVTKTKNSIII	50.3			6.8	71.5		**0.5627**
LSLKFQANAATLNGHIRLIF	LSLKFQANAATLNGH	5.6		13.7			66.3	**1.543**
SVSRWHSQELSLKFQANAAT	HSQELSLKFQANAAT	46.3		50.8			80.9	**1.446**
QNARDPYIWYDSNSKRLMMY	DPYIWYDSNSKRLMM	52.2		63.7	21.7			**0.4945**
QNARDPYIWYDSNSKRLMMY	PYIWYDSNSKRLMMY	40.4	96.7	86.2	23.4			**0.6024**
QNARDPYIWYDSNSKRLMMY	RDPYIWYDSNSKRLM	76		49.5	24.8			0.3815
TYSMVKLSTASDMDIVIETS	TYSMVKLSTASDMDI	29.2			28.2	95.6		**0.563**
Snap								
KTGIDLKIVSPMVVKKQMGQ	GIDLKIVSPMVVKKQ	86.9			38		43.3	**1.4774**
KTGIDLKIVSPMVVKKQMGQ	IDLKIVSPMVVKKQM	48.9			42.4		60.6	**1.1779**
KTGIDLKIVSPMVVKKQMGQ	KTGIDLKIVSPMVVK	50.4			28.9		55.4	**1.7139**
KTGIDLKIVSPMVVKKQMGQ	TGIDLKIVSPMVVKK	39.4			32.4		41.8	**1.4851**
GAITIKFKEAFLRSVSIDSA	AITIKFKEAFLRSVS	56.4				92.6	38.7	**0.7164**
GAITIKFKEAFLRSVSIDSA	GAITIKFKEAFLRSV	86.9			82.7		35.4	**0.5951**
GAITIKFKEAFLRSVSIDSA	ITIKFKEAFLRSVSI	25.1			45		44	**0.8251**
GAITIKFKEAFLRSVSIDSA	KFKEAFLRSVSIDSA	10.9		69.6	43			**0.5477**
KVKKTYGFRKSKISKTLCGA	KKTYGFRKSKISKTL	62.4			25.2	62.5		**0.498**
KVKKTYGFRKSKISKTLCGA	KTYGFRKSKISKTLC	64.3			32.3	78.9		**0.5295**
ETTSFVLVDPLPSGYQFNPE	ETTSFVLVDPLPSGY	8.3		48.7	90.3			**0.405**
ETTSFVLVDPLPSGYQFNPE	TSFVLVDPLPSGYQF	7.7		46.4		83.2		0.3617
ETTSFVLVDPLPSGYQFNPE	TTSFVLVDPLPSGYQ	7.1		41	98.1	84.9		**0.4201**
SIGEKNGMIYATDTLNFRQG	KNGMIYATDTLNFRQ		95.3	26.7	78.3			0.3941
MAIETGKKPNIWYSLNGKIR	GKKPNIWYSLNGKIR	84.6			29		57.3	**0.8174**
DGQTIPLNTVFNYRLIGGII	GQTIPLNTVFNYRLI	92.7			49.1		80.7	**0.9696**
DGQTIPLNTVFNYRLIGGII	PLNTVFNYRLIGGII	40.8			39.7		54.2	0.3289
ANQAAYQKALAAYQAELKRV	AYQKALAAYQAELKR	8.4		81.8			42.8	0.3352
ANQAAYQKALAAYQAELKRV	QAAYQKALAAYQAEL	5.7		30.2			83.7	**0.4631**
DEXTRANSE PRECURSOR								
TAAIAAAGGYHMSLAALANP	AAAGGYHMSLAALAN	39.9			24.2	52.8		**0.6877**
TAAIAAAGGYHMSLAALANP	AAGGYHMSLAALANP	3.6			31.9	41.1		**0.6247**
TAAIAAAGGYHMSLAALANP	AAIAAAGGYHMSLAA	17.5			75.6		27.4	**0.7208**
TAAIAAAGGYHMSLAALANP	AIAAAGGYHMSLAAL	20.5			96.9		49.4	**0.6582**
PROTEIN D								
NQITAAALKALKGQPMWLIH	AAALKALKGQPMWLI	5.7			68.9		26.2	**1.0215**
NQITAAALKALKGQPMWLIH	AALKALKGQPMWLIH	7.4			79.1		24.4	**0.9573**
SNGGGMTLDMGVAYPNYFAA	MTLDMGVAYPNYFAA	33.6	95.8				69.3	**0.6466**
EVGTDINIPLLASNVARLTE	DINIPLLASNVARLT	8.8			61.9		38.5	**0.8298**
EVGTDINIPLLASNVARLTE	INIPLLASNVARLTE	8.9			77.6		41	**0.6526**
EVGTDINIPLLASNVARLTE	TDINIPLLASNVARL	9.7			54.3		47.6	**0.7254**
ASMU 836								
ANQQLNQIVHYQPSAVRITA	LNQIVHYQPSAVRIT	19.7			74.2		11	**0.4194**
ANQQLNQIVHYQPSAVRITA	NQIVHYQPSAVRITA	14.6			81.4		14.6	**0.5308**
ANQQLNQIVHYQPSAVRITA	QLNQIVHYQPSAVRI	33.5			82.5		12	**0.4674**
STVAVKGFKIGTVPKVGAIA	KGFKIGTVPKVGAIA	13.1		49.7		62.5		**0.1478**
STVAVKGFKIGTVPKVGAIA	STVAVKGFKIGTVPK	24.1			22.1		46.2	**0.6961**
STVAVKGFKIGTVPKVGAIA	TVAVKGFKIGTVPKV	25.9			27.5		65.9	**0.7427**
STVAVKGFKIGTVPKVGAIA	VKGFKIGTVPKVGAI	12.8		43.7		82.6		0.3601
GLYCOSYLTRANSFERASE - S								
NWYYFGSDGVAVTGSQTIAG	NWYYFGSDGVAVTGS	29.3		52.5	51.2			**0.9**
NWYYFGSDGVAVTGSQTIAG	WYYFGSDGVAVTGSQ	42.4		59.3	77.8			**1.1037**
TQIAYLNYMNQQGLGTGENY	QIAYLNYMNQQGLGT	70.3		77.2			84.8	**0.5802**
IRAHDSEVQTVIAKIIKAQI	SEVQTVIAKIIKAQI	95.2			89.4	46.2		0.214
GLYCO - I								
LRLSLLFSLAKPLNQRSGMN	RLSLLFSLAKPLNQR	6.2		45.3	4.9	11.5	50.3	0.5408
LRLSLLFSLAKPLNQRSGMN	SLLFSLAKPLNQRSG			60.7	7	11.5		**0.4764**
DTSIDTVNKDIVTTRSNLYK	TVNKDIVTTRSNLYK		50.2	88.4	20.8			**0.5408**
GLYCOSYL - SI								
TVTFNGQRLYFKPNGVQAKG	GQRLYFKSNGVQAKG	67.4	74.4					**1.0292**
AQSINGANYYFLSNGIQLRN	GANYYFLSNGIQLRN	3.8		28.6	18.6		30.4	0.7682
AQSINGANYYFLSNGIQLRN	NGANYYFLSNGIQLR	4.8		39.8	16		31.1	0.9517
EKKVRFKLRKVKKRWVTVSV	FKLRKVKKRWVTVSV				77.3	9.1	94	0.3783
EKKVRFKLRKVKKRWVTVSV	KKVRFKLRKVKKRWV	55.8			53.2	4.5	93.5	0.7271
EKKVRFKLRKVKKRWVTVSV	KVRFKLRKVKKRWVT	62			61.9	5		**0.7096**
EKKVRFKLRKVKKRWVTVSV	RFKLRKVKKRWVTVS	91			72.8	6.4		**0.6227**

Fig.5.10.1: Showing Predicted VaxiJen Score (Antigenic Score).

5.10.2 **Selection of final predicted T CELL Epitopes**.

Based on below criteria we finally predicted the T CELL Epitopes:

- Binding to HLA-DRB1 with IC_{50} value < 100Nm.
- The antigenicity of each T-cell epitope was predicted using VaxiJen 2.0.
- Having VaxiJen antigenic score > 0.4 were consider as probable antigen and choose for next step.
- Binding to max. number of alleles among HLA-DR alleles listed on NetMHCIIpan server 2.0 with IC50 value<100nM were selected.
- The T-cell epitopes RLSLLFSLAKPLNQR, GANYYFLSNGIQLRN, NGANYYFLSNGIQLR, KKVRFKLRKVKKRWV, AIPYFNAKAIKNMKA, YAIPYFNAKAIKNMK were selected.

B CELL EPITOPE	T CELL EPITOPE	HLA-DRB1*01:01	HLA-DRB1*03:01	HLA-DRB1*04:01	HLA-DRB1*07:01	HLA-DRB1*11:01	HLA-DRB1*15:01	vaxiJEn Score of t cell
LRLSLLFSLAKPLNQRSGMN	RLSLLFSLAKPLNQR	6.2		45.3	4.9	11.5	50.3	0.5408
AQSINGANYYFLSNGIQLRN	GANYYFLSNGIQLRN	3.8		28.6	18.6		30.4	0.7682
AQSINGANYYFLSNGIQLRN	NGANYYFLSNGIQLR	4.8		39.8	16		31.1	0.9517
EKKVRFKLRKVKKRWVTVSV	KKVRFKLRKVKKRWV	55.8			53.2	4.5	93.5	0.7271
RYAIPYFNAKAIKNMKAATT	AIPYFNAKAIKNMKA	19.5			76.4	40.5	69.7	0.5229
RYAIPYFNAKAIKNMKAATT	YAIPYFNAKAIKNMK	27.1			71	55.2	39.3	0.5694

Fig. 5.10.2: FINAL PREDICTED T Cell Epitopes

6 Conclusion.

This study adopts a strategy that targets multiple microorganisms associated with the causation of dental caries. Till date, vaccines for preventing dental caries target only *S.mutans* and not for other microorganisms which either initiate or help in the progression of dental caries. In this study, an attempt has been made to target caries initiating as well as microorganisms associated with progression of caries using a single vaccine. Advances in sequence based technique and the methods accessible for analyzing the sequences information have enabled prediction of most likely vaccine candidate from the protein sequences. For targeting multiple microorganisms along with the major etiological agent *S.mutans*, vaccine candidates that are conserved in a number of microorganisms have been predicted.

This study, directed towards the identification of vaccine candidates for dental caries has resulted in the prediction of probable epitopes that could be used to elicit immune response against a number of microorganisms growing in a biofilm. LRLSLLFSLAKPLNQRSGMN, AQSINGANYYFLSNGIQLRN, EKKVRFKLRKVKKRWVTVSV, RYAIPYFNAKAIKNMKAATT has been recognized as an antigen that can be used as a vaccine against cariogenic microorganism.

As the role of CD8+ cell mediated immunity is not prominent in oral cavity (Setia *et al*., 2012) and the major immunological response is due to salivary IgA and the IgG antibody produced by the B-cells activated by CD4+ T cell, epitope prediction has been restricted to B-cell and MHC class II branch of antigen presentation only. Also, such linear B-cell epitopes have been predicted from which MHC-class II restrict T-cell epitopes can be derived. This kind of epitopes have higher odds of electing immune response regardless of whether the B-cell epitope isn't perceived by the immune responses, there are chances that T Cell epitope will evoke immune reaction in the host.

The predicted T-cell epitopes have been selected based on the criteria:

(a) antigenicity of the epitope should be >0.4 (VaxiJen).

(b) must bind to HLA-DRB1*0101 with IC_{50} value < 100nM.

(c) bind to max. number of HLA-DR allele with IC_{50} < 100nM. This criterion helps in the selection of T-cell epitopes which will cover the maximum HLA-DR allele diversity while binding to the most frequently occurring alleles.

In the selected vaccine candidate, the best 6 T cell epitopes predicted on the basis used to select T cell Epitopes RLSLLFSLAKPLNQR, GANYYFLSNGIQLRN, NGANYYFLSNGIQLR, KKVRFKLRKVKKRWV, AIPYFNAKAIKNMKA, YAIPYFNAKAIKNMK were selected.

7 Discussion and Future Perspective.

S.mutans has been identified as the main etiological agent in dental caries (Loesche WJ, 1986). However, the oral cavity is inhabited by a large number of microorganisms which produce acids by utilizing sugars and decrease the pH of oral cavity. This decrease in pH for a prolonged time results in creating an imbalance between the demineralization and re- mineralization of tooth surface (Fehr VD, 1965). As the sugar acids produced by bacteria are responsible for cavitations of tooth enamel and dentine, the best strategy for preventing and controlling dental caries would be to inhibit all the acid producing bacteria.

Most of the studies aimed at preventing dental caries target S.mutans but various studies have shown that bacteria other than S.mutans can also cause dental caries as they produce acids and thus, promote demineralization of tooth surface. Further, once a biofilm has been established it is not necessary that only S.mutans will be responsible for demineralization of tooth surface. In view of this, a strategy targeting most of the acid producing bacteria will be more apt for preventing a disease like dental caries which is cause by a number of bacteria (Kleinberg I., 2002).

This study adopts a strategy that targets multiple microorganisms associated with the causation of dental caries. Till date, vaccines for preventing dental caries target only S.mutans and not other microorganisms which either initiate or help in the progression of dental caries. In this study, an attempt has been made to target caries initiating as well as microorganisms associated with progression of caries using a single vaccine. For targeting multiple microorganisms along with the major etiological agent S.mutans, vaccine

candidates that are conserved in a number of microorganisms have been predicted.

As a plethora of microorganisms have been associated with dental caries, the first and foremost step for targeting multiple microorganisms associated with the causation of dental caries was to identify the microorganisms that have to be targeted. Using literature search, 70 microorganisms were selected as probable caries associated microorganisms. These may be responsible for initiation (S.mutans, S.sobrinus) or progression (Lactobacilli and others) of dental caries.

An antigenic peptide conserved in a number of microorganisms growing in a biofilm can be used as a vaccine to target all those microorganisms. For a protein to be used as a vaccine it should either be secreted or should be present on the outer side of the cell, i.e, it should be surface-exposed. So, for assessing the vaccine like character of a protein, the first and foremost step is the elucidation of its sub-cellular localization. Though a number of bioinformatics tools are available for localization prediction but most of them perform well for gram negative bacteria. To validate the sub-cellular localization of proteins instead of one, three localization prediction tools have been used, namely PSORTb, CELLO and Gpos- mPLoc. The proteins predicted as extracellular/secreted or cell wall components by all the three servers have been considered in this study. This decreases the rate of false positives in the result. Another important consideration for a protein to act as a vaccine candidate in humans is that it should not have homolog's in humans, otherwise the vaccine will either be a poor immunogen. it will result in auto immunity (Wilson et al., 2000; Weber et al., 2009). Out of the selected S.mutans proteins, no protein showed a significant level of homology when blasted with the human genome.

As the role of CD8+ cell mediated immunity is not prominent in oral cavity (Setia et al., 2012) and the major immunological response is due to salivary IgA and the IgG antibody produced by the B-cells activated by CD4+ T cell, epitope prediction has been restricted to B-cell and MHC class II branch of antigen presentation only. Also, such linear B-cell epitopes have been predicted from which MHC-class II restricted T-cell epitopes can be derived. This type of epitopes have contains higher chances of eliciting immune responses because even if the B-cell epitope is not recognized by the immune system and there are

chance that T- cell epitope will elicit immune response in the host.

ABCpred server was used to find out the linear B-cell epitopes (which ussally contain 20 amino acids long) in S.mutans proteins. This software predicted a total of 1111 B-cell epitopes in the 16 proteins. Antibodies cannot bind the B-cell epitopes lying in the transmembrane regions. Hence, B-cell epitopes lying in the transmembrane regions cannot be used as vaccine candidates. The surface exposed B-cell epitopes were selected on the basis of Transmembrane topology. On the basis of this software there are 10 B-cell epitopes were removed because they were present in the transmembrane regions. Now we can also eliminate B-cell epitopes on the basis of their antigenicity score. By using tool VaxiJen scores, 369 B-cell epitopes have antigenicity score below the set threshold of 0.4. On the basis of prediction total number of 369 epitopes were rejected.

CD4+ T-cells can recognize the antigenic peptides presented by antigen-presenting cells and activate the B-cells, which produce IgG antibody. Hence, CD4+ T-cells activation plays a very important role in eliciting immune response in oral cavity. Not every administered vaccine is capable of eliciting immune response as it may be degraded by proteases. To expand the odds of evoking an invulnerable reaction by an immunization, B-cell epitopes have been chosen in a way that, T cell epitope can predict from there. Net MHCII server 2.3 was used to find the binding of T-cell epitopes to HLA-DR alleles. By utilize this result, we eliminate the T cell epitope on the basis of their IC_{50}<100nM. We have selected only those T cell epitopes which contain IC_{50} less than 100Nm. Out of these T-cell epitopes, 84 epitopes bind to HLA-DRB1 with IC_{50}<100nM are selected. Now again Predict VAXIJEN score of Selected T CELL Epitopes. There are 79 Epitopes having VaxiJen antigenic score > 0.4 were consider as probable antigen and choose for further step.

The next step is the selection of antigenic T-cell epitopes which bind to the maximum number of HLA-DR alleles. HLA-DRBI*0101, HLA-DRBI*1501, HLA-DRBI*0401, HLA-DRBI*0701, HLA-DRBI*0401 and HLA-DRBI*0301 are the most frequently occurring alleles in the human population. Therefore, T-cell epitopes

binding to these most frequently occurring alleles have been selected so as to ensure maximum population coverage. HLA-DRB1*0101 is the commonest bound allele, therefore the epitopes interacting with this allele should produce better antigenic responses.

Bind to max. number of alleles among HLA-DR alleles listed on Net MHCII pan server 2.0 with IC50 value<100nM were selected. The T-cell epitopes, were selected.

In the selected vaccine candidate, the best 6 T cell epitopes predicted on the basis used to select T cell Epitopes RLSLLFSLAKPLNQR, GANYYFLSNGIQLRN, NGANYYFLSNGIQLR, KKVRFKLRKVKKRWV, AIPYFNAKAIKNMKA, YAIPYFNAKAIKNMK were selected. Which are used as vaccine candidates for Dental caries causing microorganism.

Future Perspective.

Until the emergence of field of Bioinformatics, wet lab experiments were the sole source for identifying targets that can be used as vaccines. Bioinformatics gave rise to the field called Immunoinformatics, which allows for the selection of probable vaccines in-silico, thereby saving time and money required to perform wet lab experiments. Immunoinformatics considerably decreases the number of putative targets to be explored for their effectiveness as vaccines in animal models. This study has applied the various tools available for identifying vaccine targets to predict probable vaccine candidates against dental caries.

As dental caries is a poly-microbial disease, a great visulaization of the mechanisms involved in adhesion and signaling and the interactions between the microbes will aid in the determination of the role of known virulence determinants and the factors which are of utmost importance. For combating polymicrobial diseases, factors like microbe-microbe interactions, host-microbe pathogenic mechanisms, host-immunity mediated, antimicrobial defenses and environmental factor need to be considered. So, the future studies should be focused on the study of biofilms in vivo in order to gain deeper insights

into complex dynamics within the microbial populations and their interactions with the host.

The composition of microbial consortia in oral cavity differs from person to person and different microbes have been shown to initiate and develop dental caries. In view of this observation, a vaccine targeting a number of cariogenic microbes present in the oral biofilms can cover a much larger percentage of human population. This study predicted a vaccine candidate that can potentially be used as a vaccine against 6 cariogens. For targeting more caries associated microbes, these microbes can be split into several group relay on the level of homology between their genomes and then studies similar to the present one can be conducted to identify vaccine targets for each group. Vaccine targets for each group may then be formulated into a single vaccine by protein engineering. The engineered protein will present multiple epitopes on its surface, thereby eliciting immune response to multiple microbes. This approach can further be extrapolated to prevent multiple diseases also, e.g., a multi-epitope vaccine for dental caries may also include epitopes specific for the microbes responsible for other oral diseases like periodontal diseases and halitosis. Further, other diseases like urinary tract infections, endocarditis, and infections in cystic fibrosis, associated with the formation of biofilms can also be targeted using the same strategy.

The immunogenic potential of vaccine candidates predicted in-silico needs to be validated in animal models and humans. These studies are helpful in deciding the right time for immunization, the route of administration, the adjuvant to be used and other factors affecting the efficacy of a vaccine.

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APPENDIX I

LIST OF MICROORGANISMS ASSOCIATED WITH THE CAUSATION OF DENTAL CARIES

1. *Actinomyces viscosus (taxid:1656)*
2. *Acinetobacter baumannii (taxid:470)*
3. *Actinobaculum sp. oral taxon 183 (taxid:712888)*
4. *Actinomyces georgiae (taxid:52768)*
5. *Actinomyces gerencseriae (taxid:52769)*
6. *Actinomyces naeslundii (taxid:1655)*
7. *Actinomyces odontolyticus (taxid:1660)*
8. *Atopobium parvulum (taxid:1382)*
9. *Bacteroidetes bacterium oral taxon 272 (taxid:651591)*
10. *Bacteroidetes bacterium oral taxon 274 (taxid:712899)*
11. *Bifidobacterium dentium (taxid:1689)*
12. *Campylobacter gracilis (taxid:824)*
13. *Capnocytophaga ochracea (taxid:1018)*
14. *Corynebacterium diphtheriae (taxid:1717)*
15. *Dialister invisus (taxid:218538)*
16. *Enterococcus faecalis (taxid:1351)*
17. *Eubacterium alactolyticum (taxid:113287)*
18. *Granulicatella adiacens (taxid:46124)*
19. *Haemophilus influenzae (taxid:727)*
20. *Haemophilus parainfluenzae (taxid:729)*
21. *Lactobacillus acidophilus (taxid:1579)*
22. *Lactobacillus brevis (taxid:1580)*
23. *Lactobacillus casei (taxid:1582)*
24. *Lactobacillus fermentum (taxid:1613)*
25. *Lactobacillus gasseri (taxid:1596)*
26. *Lactobacillus jensenii (taxid:109790)*
27. *Lactobacillus johnsonii (taxid:33959)*
28. *Lactobacillus paracasei subsp. paracasei (taxid:47714)*
29. *Lactobacillus plantarum (taxid:1590)*
30. *Lactobacillus rhamnosus (taxid:47715)*
31. *Lactobacillus salivarius (taxid:1624)*
32. *Lactobacillus vaginalis (taxid:1633)*
33. *Neisseria mucosa (taxid:488)*
34. *Parvimonas micra (taxid:33033)*
35. *Prevotella multisaccharivorax (taxid:310514)*

36. Prevotella intermedia / Prevotella nigrescens-like organism (PINLO) (taxid:60133)
37. Propionibacterium acnes (taxid:1747)
38. Propionibacterium avidum (taxid:33010)
39. Rothia dentocariosa (taxid:2047)
40. Scardovia inopinata (taxid:78259)
41. Scardovia wiggsiae (taxid:230143)
42. Staphylococcus aureus (taxid:1280)
43. Streptococcus agalactiae (taxid:1311)
44. Streptococcus constellatus (taxid:76860)
45. Streptococcus downei (taxid:1317)
46. Streptococcus intermedius (taxid:1338)
Streptococcus parasanguinis (taxid:1318)
47. Streptococcus pyogenes (taxid:1314)
48. Streptococcus salivarius (taxid:1304)
49. Streptococcus sobrinus (taxid:1310)
50. Streptococcus vestibularis (taxid:1343)
51. Veillonella atypica (taxid:39777)
52. Veillonella parvula (taxid:29466)