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IMMUNOPROTEOMICS APPROACH FOR SYNTHETIC VACCINE DEVELOPMENT FORM *STREPTOCOCCUS DYSGALACTIAE SUBSP. EQUISIMILIS*

GOMASE V.S.* and CHITLANGE N.R.

School of Technology, S.R.T.M. University, Sub-Centre, Latur, 413531, MS, India

***Corresponding Author** gomase.viren@gmail.com

ABSTRACT

Streptococcus dysgalactiae subsp. equisimilis are the causative organisms of infections with Beta-hemolytic group C and G streptococci. Peptide fragments of antigen protein can be used to select nonamers for use in rational vaccine design and to increase the understanding of roles of the immune system in infectious diseases. Analysis shows MHC class II binding peptides of antigen protein from *Streptococcus dysgalactiae* are important determinant for protection of host form bacterial infection. In this assay, we used PSSM and SVM algorithms for antigen design and predicted the binding affinity of bacterial protein having 200 amino acids, which shows 192 nonamers. Binding ability prediction of antigen peptides to major histocompatibility complex (MHC) class I & II molecules is important in vaccine development from *Streptococcus dysgalactiae*.

KEYWORDS

Antigen protein, epitope, PSSM, SVM, MHC, peptide vaccine

Abbreviations: *Goldman, Engelberg and Steitz, (GES); major histocompatibility complex, (MHC); Position Specific Scoring Matrices, (PSSMs); Support Vector Machine, (SVM)*

INTRODUCTION

Streptococcus dysgalactiae subsp. equisimilis are the causative organisms of infections with Beta-hemolytic group C and G streptococci (GCGS) causes nasal and throat secretions, tonsils, and vaginal and preputial secretions.[1, 2]. *Streptococcus dysgalactiae* bacterial peptides are most suitable for subunit vaccine development because with single epitope, the immune response can be generated in large population. This approach is based on the phenomenon of cross-protection, whereby host infected with a mild strain of bacteria is protected against a more severe strain of the same bacteria. The phenotype of the resistant transgenic hosts includes fewer centers of initial infection, a delay in symptom development, and low accumulation. Antigen protein from *Streptococcus dysgalactiae* is necessary for new paradigm of synthetic vaccine development and target validation [3-5].

Methodology

In this research work antigenic epitopes of antigen protein from *Streptococcus dysgalactiae* is determined using the Gomase in 2007, Hopp and Woods, Welling, Parker and Protrusion Index (Thornton) antigenicity [6-8]. The major histocompatibility complex (MHC) peptide binding of antigen protein is predicted using neural networks trained on C terminals of known epitopes. In analysis predicted MHC/peptide binding of antigen protein is a log-transformed value related to the IC50 values in nM units. Rankpep predicts peptide binders to MHCI and MHCII molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). Support Vector Machine (SVM) based method for prediction of promiscuous MHC class II binding peptides. SVM has been trained on the binary input of single amino acid sequence [9-14]. In addition, we predict those MHC ligands from whose C-terminal end is likely to be the result of proteosomal cleavage [15].

RESULTS AND INTERPRETATIONS

We found binding of peptides to a number of different alleles using Position Specific Scoring Matrix. A antigen protein sequence is 200 residues long, having antigenic MHC binding peptides. MHC molecules are cell surface glycoproteins, which take active part in host immune reactions and involvement of MHC class-I and MHC II in response to almost all antigens. PSSM based server predict the peptide binders to MHCI molecules of antigen protein sequence are as 11mer_H2_Db, 10mer_H2_Db, 9mer_H2_Db, 8mer_H2_Db and also peptide binders to MHCII molecules of antigen protein sequence as I_Ab.p, I_Ad.p, analysis found antigenic epitopes region in putative antigen protein (Table 1). We also found the SVM based MHCII-IAb peptide regions; MHCII-IAd peptide regions; MHCII-IAg7 peptide regions and MHCII- RT1.B peptide regions, which represented predicted binders from bacterial antigen protein (Table 2). The predicted binding affinity is normalized by the 1% fractil. We describe an improved method for predicting linear epitopes (Table 2). The region of maximal hydrophilicity is likely to be an antigenic site, having hydrophobic characteristics, because terminal regions of antigen protein is solvent accessible and unstructured, antibodies against those regions are also likely to recognize the native protein (Fig. 1, 2, 5). It was shown that a antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Fig. 3, 4). Predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design .

Table 1.

PSSM based prediction of MHC ligands, from whose C-terminal end are proteosomal cleavage sites

MHC-I	POS.	N	Sequence	C	MW (Da)	Score	% OPT.
8mer_H2_Db	153	PSL	GNTSGQTY	FYY	808.79	20.44	38.94 %
8mer_H2_Db	156	GNT	SGQTYFYY	HPL	1010.08	15.942	30.37 %
8mer_H2_Db	176	KLY	KNGGNIYY	SRE	909.99	11.918	22.70 %
8mer_H2_Db	155	LGN	TSGQTYFY	YHP	948.0	11.223	21.38 %
9mer_H2_Db	186	YSR	EVHFNLYLI	ELM	1129.33	15.564	30.90 %
9mer_H2_Db	14	LLI	FLLIFYQVL	VIR	1137.44	15.222	30.22 %
9mer_H2_Db	173	SGG	KLYKNGGNI	YYS	988.14	12.865	25.54 %
9mer_H2_Db	189	EVH	FNLYLIELM	SLF	1137.41	12.858	25.53 %
10mer_H2_Db	116	TAV	QAYNFGTAYI	DYV	1129.24	12.849	21.83 %

10mer_H2_Db	115	WTA	VQAYNFGTAY	IDY	1115.21	10.18	17.30 %
10mer_H2_Db	88	DSQ	SSIEHGVSLL	SHN	1023.16	7.893	13.41 %
10mer_H2_Db	32	HRV	LAYKPMVEKT	LAE	1161.42	6.211	10.55 %
11mer_H2_Db	115	WTA	VQAYNFGTAYI	DYV	1228.37	34.112	42.91 %
11mer_H2_Db	172	ISG	GKLYKNGGNIY	YSR	1208.37	15.496	19.49 %
11mer_H2_Db	156	GNT	SGQTYFYYHPL	ALI	1357.5	12.337	15.52 %
11mer_H2_Db	176	KLY	KNGGNIYYSRE	VHF	1282.38	11.549	14.53 %

Table 2.

SVM based prediction of promiscuous MHC class II binding peptides from capsid protein

ALLELE	Sequence	Residue No	Peptide Score
I-Ab	RVLAYKPMV	30	1.171
I-Ab	PLATTYSKT	138	0.959
I-Ab	LATTYSKTV	139	0.882
I-Ab	VLAYKPMVE	31	0.880
I-Ad	QAYNFGTAY	116	0.761
I-Ad	GGKLYKNGG	171	0.696
I-Ad	LIFLLIFYQ	12	0.678
I-Ad	VCALLIFLL	8	0.589
I-Ag7	SKTVVAPSL	144	1.760
I-Ag7	YIDYVADHG	124	1.619
I-Ag7	TYFYYHPLA	159	1.583
I-Ag7	GGQNTIPLA	132	1.479
RT1.B	DTKANVDLV	46	0.760
RT1.B	TDSQSSIEH	84	0.677
RT1.B	TYFYYHPLA	159	0.647
RT1.B	WTAVQAYNF	112	0.600

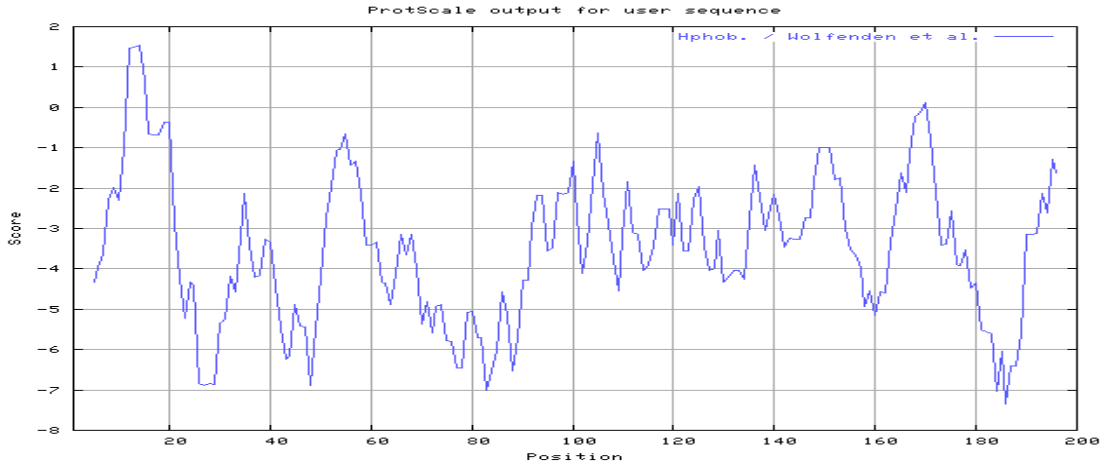

Fig1. ***Antigenicity plot of capsid protein by Welling, et al., scale***

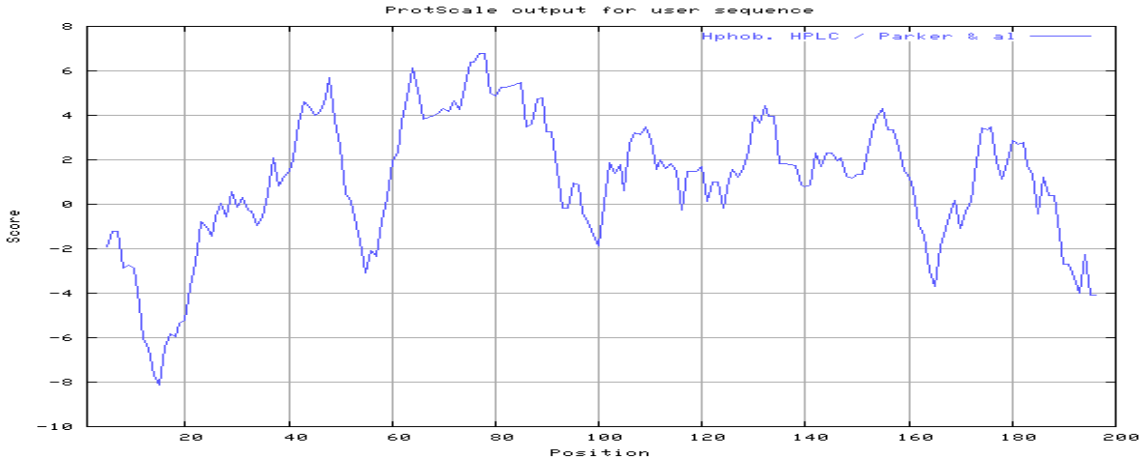

Fig.2. ***Antigenicity plot of capsid protein by HPLC / Parker, et al., scale***

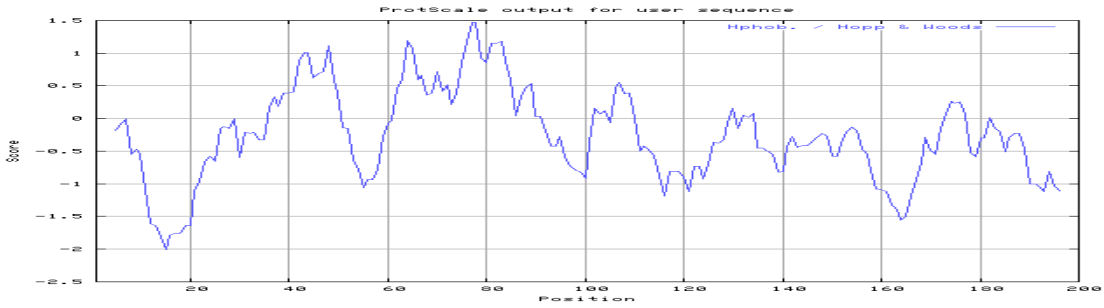

Fig 3. ***Hydrophobicity plot of capsid protein by Hopp & Woods, et al., scale***

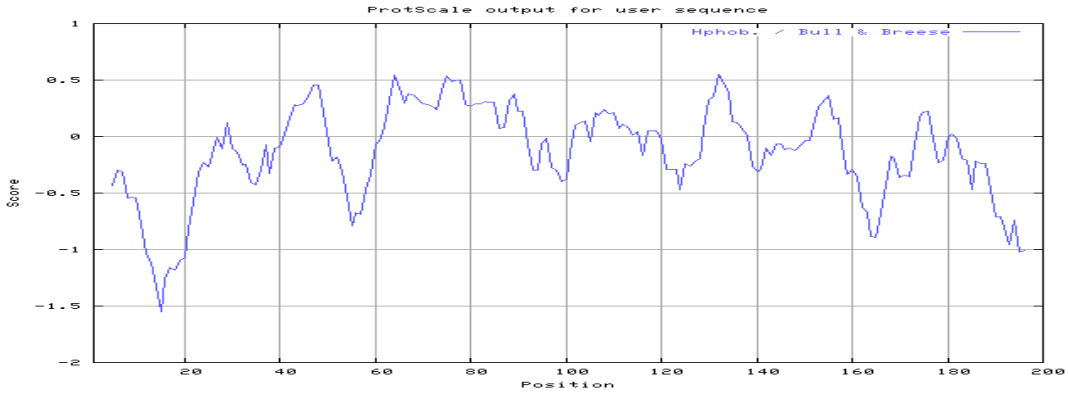

Fig 4.***Hydrophobicity plot of capsid protein by Bull & Breese scale***

CONCLUSION

A antigen protein from *Streptococcus dysgalactiae* peptide nonamers are from a set of aligned peptides known to bind to a given MHC molecule as the predictor of MHC-peptide binding. MHCII molecules bind peptides in similar yet different modes and alignments of MHCII-ligands were obtained to be consistent with the binding mode of the peptides to their MHC class, this means the increase in affinity of MHC binding peptides may result in enhancement of immunogenicity of bacterial antigen protein. These predicted of bacterial protein antigenic peptides to MHC class molecules are important in vaccine development from *Streptococcus dysgalactiae*.

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