ISSN: 0975-766X
Research Article
Available Online through
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ANTIGEN PROTEIN FROM XYLELLA FASTIDIOSA: NEW PARADIGM OF SYNTHETIC VACCINE DEVELOPMENT

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Received on 14-07-2010 ***Accepted on 29-07-2010***

Abstract

Xylella fastidiosa is a pathogenic bacterium that infects plants, causing a variety of diseases in over 100 plant species. 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 pathogenic diseases. Analysis shows MHC class II binding peptides of antigen protein from *Xylella fastidiosa* are important determinant for protection of host form infection. In this assay, we used PSSM and SVM algorithms for antigen design and predicted the binding affinity of antigen protein having 790 amino acids, which shows 782 nonamers. Binding ability prediction of antigen peptides to major histocompatibility complex (MHC) class I & II molecules is important in vaccine development from *Xylella fastidiosa*.

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)

I. Introduction

Xylella fastidiosa is a Gammaproteobacteria that infects plants, causing a variety of diseases in over 100 plant species, including grapevine, citrus, almonds, coffee, and many other species of economic importance.including phoney peach disease, oleander leaf scorch and Pierce's disease. [1, 2]. *Xylella fastidiosa* baccterial 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 a plant infected with a mild strain of bacterium is protected against a more severe strain of the same bacterium. The phenotype of the resistant transgenic hosts includes fewer centers of initial bacterial infection, a delay in symptom development, and low bacterial accumulation. Antigen protein from *Xylella fastidiosa* is necessary for new paradigm of synthetic vaccine development and target validation [3-5].

II. Methodology

In this research work antigenic epitopes of antigen protein from *Xylella fastidiosa* 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].

III. Results and Interpretations

We found binding of peptides to a number of different alleles using Position Specific Scoring Matrix. A antigen protein sequence is 790 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). 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	428	PLQ	GVETGCMI	GYH	790.94	21.838	41.60 %
8mer_H2_Db	213	VEL	SIVDPCTL	LGG	828.98	20.229	38.54 %
8mer_H2_Db	228	RVY	QWAKGRYI	FFA	980.17	19.86	37.83 %
8mer_H2_Db	611	SEL	PPGAPPDI	DGM	744.86	18.36	34.98 %
8mer_H2_Db	335	TDN	PAQRRIFY	TSL	1032.23	15.213	28.98 %
8mer_H2_Db	691	YAV	DPVSGIYV	IGS	830.94	14.831	28.25 %
8mer_H2_Db	56	FIG	SSGHGHTF	PGA	810.82	14.348	27.33 %
8mer_H2_Db	422	DGV	GIWPLQGV	ETG	828.01	13.272	25.28 %
8mer_H2_Db	765	GAQ	PNLDFGTH	PQD	881.94	11.625	22.15 %
8mer_H2_Db	82	TDN	AVWDACSG	YHA	766.86	10.884	20.73 %
8mer_H2_Db	547	LEN	GAWATPFD	PRA	822.91	10.854	20.68 %
8mer_H2_Db	492	VDE	SVSRTLEY	AYD	936.04	10.377	19.77 %
8mer_H2_Db	562	EHL	KQWHDFTE	CNA	1049.14	9.847	18.76 %
8mer_H2_Db	628	YAH	GNEPSHHI	AYL	871.91	9.809	18.69 %
8mer_H2_Db	196	LSH	GMQDNPTT	PPR	844.88	9.69	18.46 %
8mer_H2_Db	130	LTP	GPLNAPET	GYR	779.85	9.643	18.37 %
9mer_H2_Db	374	TLP	AGYHNYSTY	SLW	1057.09	17.098	33.95 %
9mer_H2_Db	733	YVQ	ALRWNGSPM	TCA	990.18	17.082	33.92 %
9mer_H2_Db	90	CSG	YHASNGSIM	GFS	961.06	14.796	29.38 %
9mer_H2_Db	613	LPP	GAPPDIDGM	VGQ	853.95	14.438	28.67 %

9mer_H2_Db	501	EYA	YDDWACAHL	ATA	1052.17	13.171	26.15 %
9mer_H2_Db	553	ATP	FDPRALEHL	KQW	1079.24	12.248	24.32 %
9mer_H2_Db	592	LFG	GADHFAAKL	DAL	911.03	11.859	23.55 %
9mer_H2_Db	249	ARA	QLYSNDAPL	TAG	1002.1	11.259	22.35 %
9mer_H2_Db	666	GRD	GLSGNEDCG	QMS	832.84	11.079	22.00 %
9mer_H2_Db	227	RRV	YQWAKGRYI	FFA	1143.35	10.151	20.15 %
9mer_H2_Db	762	FEM	GAQPNLDFG	THP	899.96	9.875	19.61 %
9mer_H2_Db	588	GYM	ALFGGADHF	AAK	916.01	9.395	18.65 %
9mer_H2_Db	643	VYA	GQAYKTQAM	VRR	979.11	9.297	18.46 %
9mer_H2_Db	212	EVE	LSIVDPCTL	LGG	942.14	8.598	17.07 %
9mer_H2_Db	753	ELA	GGGTLEFEM	GAQ	922.02	8.448	16.77 %
9mer_H2_Db	625	VGQ	YAHGNEPSH	HIA	993.01	8.258	16.40 %
10mer_H2_Db	374	TLP	AGYHNYSTYS	LWD	1144.17	26.565	45.13 %
10mer_H2_Db	296	SAA	NALANLNAEL	PDF	1024.14	24.988	42.45 %
10mer_H2_Db	542	FMQ	PRLENGAWAT	PFD	1073.21	18.653	31.69 %
10mer_H2_Db	591	ALF	GGADHFAAKL	DAL	968.08	16.005	27.19 %
10mer_H2_Db	129	KLT	PGPLNAPETG	YRQ	934.02	14.89	25.30 %
10mer_H2_Db	665	DGR	DGLSGNEDCG	QMS	947.93	14.749	25.06 %
10mer_H2_Db	249	ARA	QLYSNDAPLT	AGT	1103.2	14.569	24.75 %
10mer_H2_Db	629	AHG	NEPSHHIAYL	YVY	1162.28	13.443	22.84 %
10mer_H2_Db	658	LLR	EQYHDGRDGL	SGN	1171.2	13.338	22.66 %
10mer_H2_Db	445	ALA	EGYTKGFTGI	DYA	1054.16	12.931	21.97 %
10mer_H2_Db	108	LSG	TGIGDMLDIL	LMP	1029.21	11.486	19.51 %
10mer_H2_Db	605	ALF	SALSELPPGA	PPD	923.05	11.11	18.88 %
10mer_H2_Db	41	IDT	QAQADLTRYV	DVF	1146.27	10.898	18.52 %
10mer_H2_Db	698	GIY	VIGSPLFPYA	ELD	1045.26	10.737	18.24 %
10mer_H2_Db	441	HSA	VALAEGYTKG	FTG	990.12	10.127	17.21 %
10mer_H2_Db	94	HAS	NGSIMGFSHT	HLS	1032.13	10.105	17.17 %
11mer_H2_Db	724	ARH	TSASNVYVQAL	RWN	1134.25	20.238	25.46 %
11mer_H2_Db	374	TLP	AGYHNYSTYSL	WDT	1257.33	19.253	24.22 %
11mer_H2_Db	444	VAL	AEGYTKGFTGI	DYA	1125.24	16.568	20.84 %
11mer_H2_Db	231	QWA	KGRYIFFAMRL	SRP	1383.73	15.765	19.83 %
11mer_H2_Db	87	WDA	CSGYHASNGSI	MGF	1077.14	10.694	13.45 %
11mer_H2_Db	465	HYR	KRAMQDHAHGL	RYY	1245.42	9.876	12.42 %
11mer_H2_Db	590	MAL	FGGADHFAAKL	DAL	1115.26	9.311	11.71 %
11mer_H2_Db	636	HHI	AYLYVYAGQAY	KTQ	1263.43	8.635	10.86 %
11mer_H2_Db	265	RQV	QGVCLKAALHF	PDA	1168.42	7.928	9.97 %
11mer_H2_Db	427	WPL	QGVETGCMIGY	HSA	1139.3	7.14	8.98 %
11mer_H2_Db	441	HSA	VALAEGYTKGF	TGI	1137.3	6.743	8.48 %
11mer_H2_Db	512	LAT	AAGAHSEARVL	RAR	1063.19	6.624	8.33 %
11mer_H2_Db	498	RTL	EYAYDDWACAH	LAT	1302.39	6.409	8.06 %
11mer_H2_Db	259	PLT	AGTRQVQGVCL	KAA	1113.29	5.796	7.29 %
11mer_H2_Db	578	ATF	LNQHDVYGYMA	LFG	1292.43	5.349	6.73 %
11mer_H2_Db	665	DGR	DGLSGNEDCGQ	MSA	1076.06	5.335	6.71 %

Table 2- SVM based prediction of promiscuous MHC class II binding peptides from antigen protein.

ALLELE	Sequence	Residue No	Peptide Score
I-Ab	PTTPPRLRE	201	1.439
I-Ab	YAAAWPHYR	456	1.232
I-Ab	RALAHPRAN	24	1.157
I-Ab	PLTAGTRQV	256	1.138
I-Ad	FIGSSGHGH	53	0.882
I-Ad	GSSGHGHTF	55	0.852
I-Ad	IAYLYVYAG	635	0.849
I-Ad	GIGDMLDIL	109	0.764
I-Ag7	GIDYAAAWP	453	2.137
I-Ag7	EAVAAWENA	316	2.004
I-Ag7	FPDAGEAPL	275	2.003
I-Ag7	IDYAAAWPH	454	1.929
RT1.B	DTQAQADLT	39	2.020
RT1.B	ATAAGAHSE	510	1.092
RT1.B	LYSNDAPLT	250	0.997
RT1.B	AWQATFLNQ	572	0.940

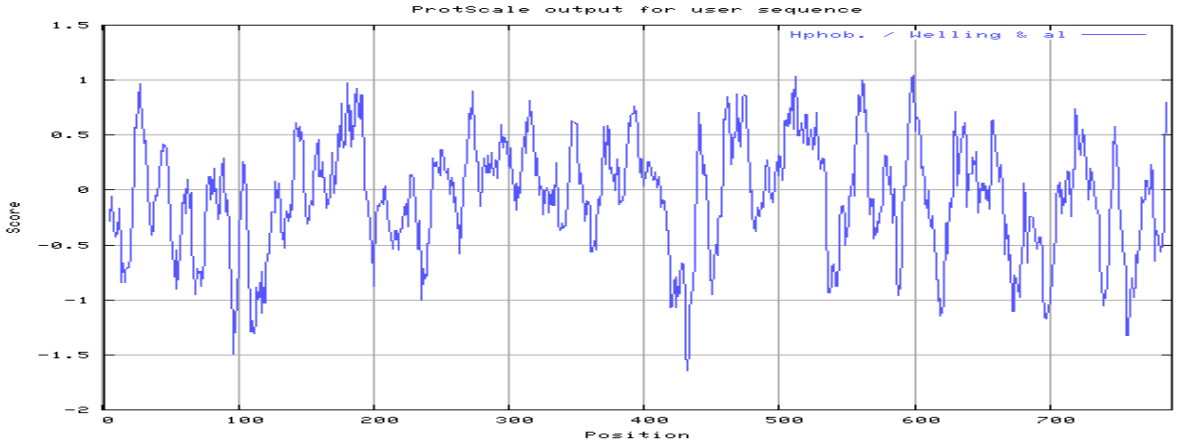

Fig. 1- Antigenicity plot of antigen protein by Welling, et al., scale

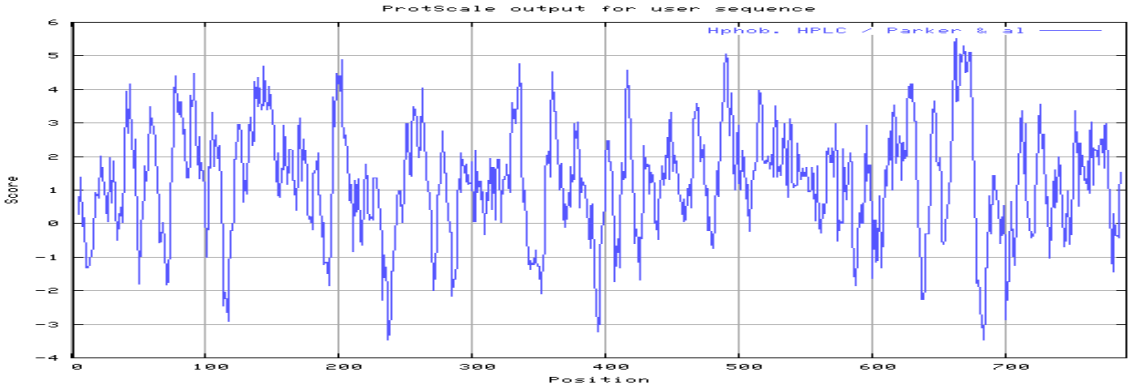

Fig. 2- Antigenicity plot of antigen protein by HPLC / Parker, et al., scale

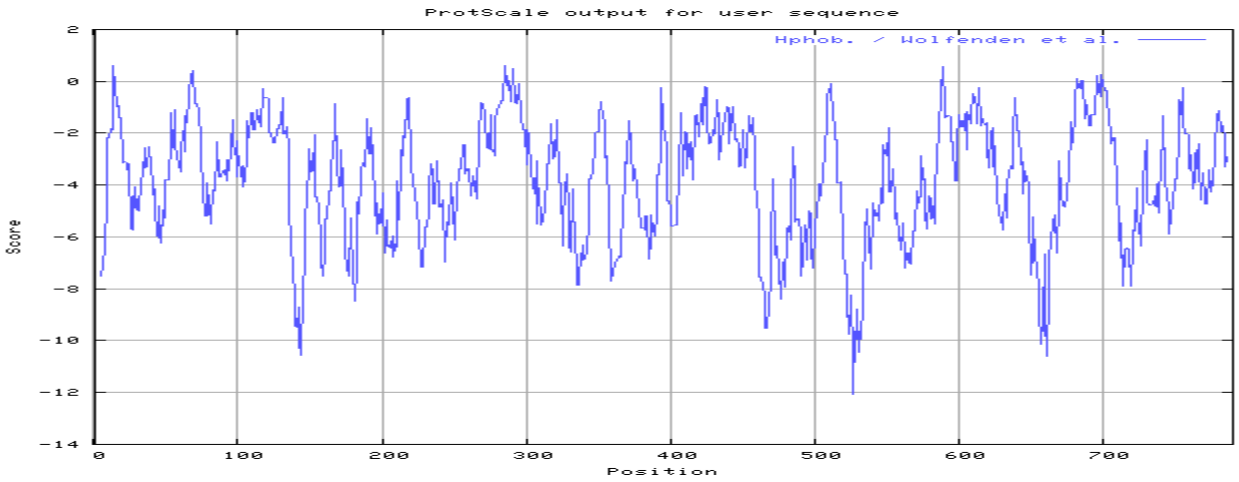

Fig. 3- Hydrophobicity plot of antigen protein by Wolfenden, et al., scale

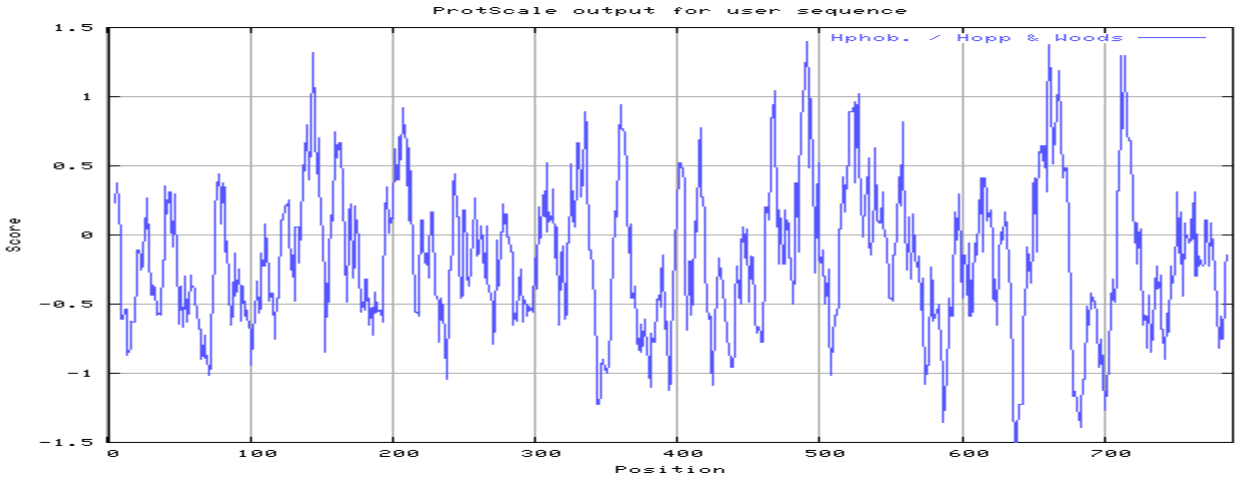

Fig. 4-Hydrophobicity plot of antigen protein by Bull & Breese scale

IV. Conclusion

A antigen protein from *Xylella fastidiosa* 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 *Xylella fastidiosa*.

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