***Eimeria acervulina* analysis for binding peptides using protein profiling for target validation**

Gomase V.S.¹, Kapoor R.A.², Ladak S.S.²

¹School of Technology, S.R.T.M. University, Sub-Centre, Latur, 413512, India

²Padmashree Dr. D.Y. Patil University, Navi Mumbai, 400614, India

Abstract- *Eimeria acervulina* is a species of *Eimeria* that causes coccidiosis in older poultry. Lesions are limited to anterior or first third of the small intestine. 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 *Eimeria acervulina* are important determinant for protection of host from parasitic infection. In this assay, we used PSSM and SVM algorithms for antigen design and predicted the binding affinity of antigen protein having 299 amino acids, which shows 291 nonamers. Binding ability prediction of antigen peptides to major histocompatibility complex (MHC) class I & II molecules is important in vaccine development from *Eimeria acervulina*.

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

Eimeria acervulina is a species of *Eimeria* that causes coccidiosis in older poultry. Lesions are limited to anterior or first third of the small intestine [1, 2]. *Eimeria acervulina* antigen 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 infected with a mild strain and is protected against a more severe strain of the same. The phenotype of the resistant transgenic hosts includes fewer centers of initial infection, a delay in symptom development, and low accumulation. Antigen protein from *Eimeria acervulina* 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 *Eimeria acervulina* is determined using the Gomase in 2007, Hopp and Woods, Bull & Breeze, Eisenberg, Chou & Fasman, and Levitt 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. MHC2Pred predicts peptide binders to MHC I and MHC II 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. An antigen protein sequence is 299

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 MHC I molecules of antigen protein sequence are as 11mer_H2_Db, 10mer_H2_Db, 9mer_H2_Db, 8mer_H2_Db and also peptide binders to MHC II 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 MHC II-IAb peptide regions; MHC II-IAd peptide regions; MHC II-IAG7 peptide regions and MHC II-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. 1-10). It was shown that a antigen protein is hydrophobic in nature and contains segments of low complexity and high-predicted flexibility (Fig. 4, 5). Predicted antigenic fragments can bind to MHC molecule is the first bottlenecks in vaccine design.

IV. Conclusion

An antigen protein from *Eimeria acervulina* peptide nonamers are from a set of aligned peptides known to bind to a given MHC molecule as the predictor of MHC-peptide binding. MHC II molecules bind peptides in similar yet different modes and alignments of MHC II-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 antigen protein antigenic peptides to MHC class

molecules are important in vaccine development from *Eimeria acervulina*

V. References

- [1] Allen P. C. and Fetterer R. H. (2002) *Clinical Microbiology Reviews*, Vol. 15, No. 1, 58-65.
- [2] Arai K. I., Lee F., Miyajima A., Miyatake S., Arai N. and Yokota T. (1990) *Annu. Rev. Biochem.* 59:783-836.
- [3] Joshi J. Tang, Kuzma M., Wagner J., Mookerjee B., Filicko J., Carabasi M., Flomenberg N., Flomenberg P. (2009) *J. Gen Virol.*, 90(Pt 1), 84-94.
- [4] McDonald D., Stockwin L., Matzow T., Blair Zajdel M.E., Blair G.E. (1999) *Gene Ther.*, 6(9),1512-9.
- [5] Gomase V.S., Kale K.V. and Shyamkumar K. (2008) *J. of Proteomics & Bioinformatics*, 1 (4), 188- 205, 2008.
- [6] Gomase V.S., Kale K.V., Chikhale N.J., Changbhale S.S. (2007) *Curr. Drug Discov. Technol.*, 4(2),117-1215.
- [7] Gomase V.S., Kale K.V. (2008) *Int. J. of Applied Computing*, 1(1), 39-46.
- [8] Gomase V.S., Kale K.V. (2008) *Int. J. of Applied Computing*, 1(1), 33-38.
- [9] Gomase V.S., Kale K.V. (2008) *Int. J. of Information Retrieval*, 1(1), 11-15.
- [10] Gomase V.S., Kale K.V., Shyamkumar K. and Shankar S. (2008) *ICETET 2008, IEEE Computer Society in IEEE Xplore, Los Alamitos, California*, 629-634.
- [11] Gomase V.S., Tandale J.P., Patil S.A., Kale K.V. (2006) *14th International Conference on Advance Computing & Communication, Published by IEEE Computer Society in IEEE Xplore USA* 614-615.
- [12] Reche P.A., Glutting J.P., Reinherz E.L. (2002) *Hum Immun.*, 63, 701-709.
- [13] Buus S., et al. (2003) *Tissue Antigens*, 62, 378-384.
- [14] Nielsen M., et al. (2003) *Protein Sci.*, 12, 1007-1017.
- [15] Bhasin M. and Raghava G.P. (2005) *Nucleic Acids Research*, 33, W202-207.

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	29	SGG	GSRNPRNV	PSL	880.96	16.638	31.70 %
8mer_H2_Db	240	QVG	KVVNCRRV	GDM	955.18	15.342	29.23 %
8mer_H2_Db	212	GQD	VTGNFRET	RAN	904.97	13.125	25.00 %
8mer_H2_Db	147	VDF	GSNLCRQL	CQM	872.01	13.087	24.93 %
8mer_H2_Db	205	QGH	IVFNGQDV	TGN	872.97	11.814	22.51 %
8mer_H2_Db	172	ATL	PNGEAVTL	PGS	781.86	11.772	22.43 %
9mer_H2_Db	31	GGG	RNPRNVPSL	HYF	1034.19	14.901	29.59 %
9mer_H2_Db	169	SVQ	ATLPNGEAV	TLP	852.94	12.849	25.51 %
9mer_H2_Db	46	ISV	NHQCNFSSF	SWS	1065.13	12.593	25.00 %
9mer_H2_Db	132	PSR	FGVDLVDCL	KVE	962.13	12.374	24.57 %
9mer_H2_Db	42	LHY	FISVNHQCN	FSS	1043.16	12.029	23.88 %
9mer_H2_Db	239	SQV	GKVVNCRRV	GDM	1012.23	10.142	20.14 %
10mer_H2_Db	46	ISV	NHQCNFSSFS	WSV	1152.21	15.72	26.71 %
10mer_H2_Db	127	FKP	SAPSRFGVDL	VDC	1030.16	12.455	21.16 %
10mer_H2_Db	49	NHQ	CNFSSFSWSV	NTR	1122.26	10.879	18.48 %
10mer_H2_Db	204	DQG	HIVFNGQDVT	GNF	1111.21	10.442	17.74 %
10mer_H2_Db	170	VQA	TLPNGEAVTL	PGS	996.12	9.781	16.62 %
10mer_H2_Db	31	GGG	RNPRNVPSLH	YFI	1171.33	9.391	15.96 %
11mer_H2_Db	218	NFR	ETRANFRFTKL	LDI	1364.57	11.235	14.13 %
11mer_H2_Db	105	VLR	RSALKNAVMW	PEN	1263.51	7.985	10.04 %
11mer_H2_Db	239	SQV	GKVVNCRRVGD	MHV	1184.37	7.816	9.83 %
11mer_H2_Db	202	FGD	QGHIVFNGQDV	TGN	1195.29	6.213	7.82 %
11mer_H2_Db	169	SVQ	ATLPNGEAVTL	PGS	1067.2	5.98	7.52 %
11mer_H2_Db	140	VDC	LKVEVDFGSNL	CRQ	1202.37	3.418	4.30 %

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

MHC ALLELE	Rank	Sequence	Residue No.	Peptide Score
I-Ab	1	GLTSRFNKH	2	0.869
I-Ab	2	RMTTNLGRN	271	0.815
I-Ab	3	PVLRRSALE	101	0.744
I-Ab	4	VVTWGSVPK	280	0.737
I-Ad	1	SESLVSVAT	13	0.702
I-Ad	2	HAGSLPVLR	96	0.698
I-Ad	3	PGSKEGAFF	67	0.633
I-Ad	4	GSNSVQATL	163	0.627
I-Ag7	1	EGAAFAAST	71	1.792
I-Ag7	2	KEGAFAAAS	70	1.561
I-Ag7	3	RETRANFRF	217	1.548
I-Ag7	4	QPFADHEEL	82	1.489
RT1.B	1	AFAASTRTQ	74	1.220
RT1.B	2	LDIQSAASQ	229	0.868
RT1.B	3	RTQPFADHE	80	0.839
RT1.B	4	RFTKLLDIQ	224	0.618

Fig.1-Hydrophobicity plot of antigen protein by Hopp & Woods scale

Fig. 2- Hydrophobicity plot of antigen protein by Eisenberg, et al., scale

Fig. 3-Hydrophobicity plot of antigen protein by Bull & Breese scale

Fig. 4- Beta Sheet plot of antigen protein by Chou & Fasman., scale

Fig. 5- Beta Sheet plot of antigen protein by Levitt, scale

Fig.6-Hydrophobicity plot of antigen protein by Hphob. / Abraham & Leo, scale

Fig.7-Hydrophobicity plot of antigen protein by Chothia, scale

Fig.8-Hydrophobicity plot of antigen protein by Hphob. / Miyazawa et al., scale

Fig.9-Hydrophobicity plot of antigen protein by Manavalan et al., scale

Fig.10-Hydrophobicity plot of antigen protein by Fauchere et al., scale