Detection of *Clavibacter michiganensis* in Infected Tomato with the Help of Immunoinformatics Technique

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ABSTRACT

Objective: *Clavibacter michiganensis* is an aerobic non-sporulating gram-positive plant pathogenic actinomycete that currently founds the only species within the genus Clavibacter. This microorganism causes considerable economic losses worldwide by detrimental effect on tomatoes and potatoes. *Clavibacter michiganensis* subsp. michiganensis is the causative agent of bacterial wilt and canker of tomato (Lycopersicon esculentum Mill.) The causal agent of bacterial wilt and canker of tomato survives in or on seeds for up to 8 months. This is also found in microbial populations commonly found on the teat skin of dairy cows. Clavibacter must be omitted from the farming environment by proper detection procedures in plant seeds for shake of human beings. An expansin protein present in *Clavibacter michiganensis* (PDB ID 4JJO) is a predicted antigen for human being. This study therefore aims to detect specific B cell as well T cell epitopes present in antigenic protein as immunodiagnotic tool against this pathogen. Materials and Methods: By analyzing the crystal structure of this antigen, we can predict linear and non-linear epitopes present on the antigen using Bepipred and ABCpred server. Several characteristics e.g. surface accessibility, hydrophilicity, flexibility and beta-turn of the predicted epitopes are calculated. T cell epitopes are primarily selected by using NetCTLv1.2 server. By this process interacting major histocompatibility complex (MHC) super types are identified for predicted T cell epitopes. Conclusion: The epitope which interacts with the highest numbers of MHC class I and class II molecules is chosen as the best epitope candidate for this antigen protein. This immunoinformatics study will help us to reduce, cost and labour for developing a monoclonal antibody against the antigenic protein of *Clavibacter michiganensis*.

Keywords: Immunoinformatics, *Clavibacter michiganensis*, B cell epitope, T cell epitope, Monoclonal antibody design, Epitope mapping, Molecular docking.

INTRODUCTION

Clavibacter michiganensis subsp. michiganensis is the contributing agent of bacterial wilt and canker of tomato (Lycopersicon esculentum Mill.) reductions in the yield of tomato[1,2,3]. The bacterium *Clavibacter michiganensis* subsp. michiganesis causes bacterial wilt and crancker in tomato is difficult to detect, hampering the diagnosis of this disease. Not only that among the vast diversity of the microbial community on dairy cow teat skin, this specific microorganism is also present prominently. Teat skin has been marked as the first pool of microbial diversity that can be found in milk during milking[4]. Thus, this plant pathogenic microorganism comes in contact with human being through cow milk. Infected tomato with *Clavibacter michiganesis* transmits this microorganism through seeds[1]. To prevent the growth of tomato with infected seeds immunodiagonsis of this microrganism using specific monoclonal antibody is appropriate decision. For both vaccine design and monoclonal antibody preparation B cell and T cell epitope identification are basic steps in modern immunology.

Polyclonal antibody using heat killer whole bacteria against this microorganism is already available commercially[5]. By using monoclonal antibody against this microorganism helps us to detect asymptotic seeds containing this microorganism. During monoclonal antibody preparation, the very first step is epitope mapping using immunoinformatics. An expansin protein which is present in *Clavibacter michiganensis* (PDB ID 4JJO) is a predicted antigen for human being[6]. This study therefore aims to identify specific B cell epitope and T cell epitope to produce monoclonal antibody against this pathogen which is helpful to prevent and detection of this plant pathogenic microorganism.

MATERIALS AND METHODS

Retrieval of 3D structure of expansin protein and its antigenicity prediction
Crystal structure of apo-clavibacter Michiganensis expansin protein is recovered from RSCB protein data bank with PDB ID 4JJO[7]. Antigenicity of this protein is predicted by VaxiJen v 2.0, an online prediction server[8]
Non-linear B cell epitope prediction
By using Discotope[9], a conformational epitope prediction method, non-linear B cell epitopes are predicted calculating their contact distances and solvent accessible surface area of the residues of peptides.
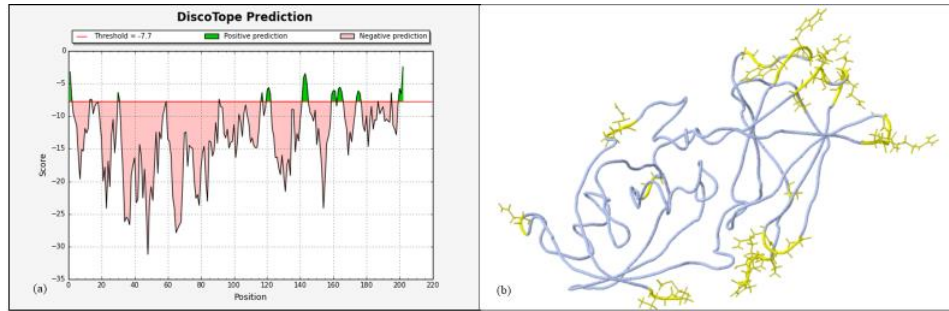*Linear B cell epitope prediction*

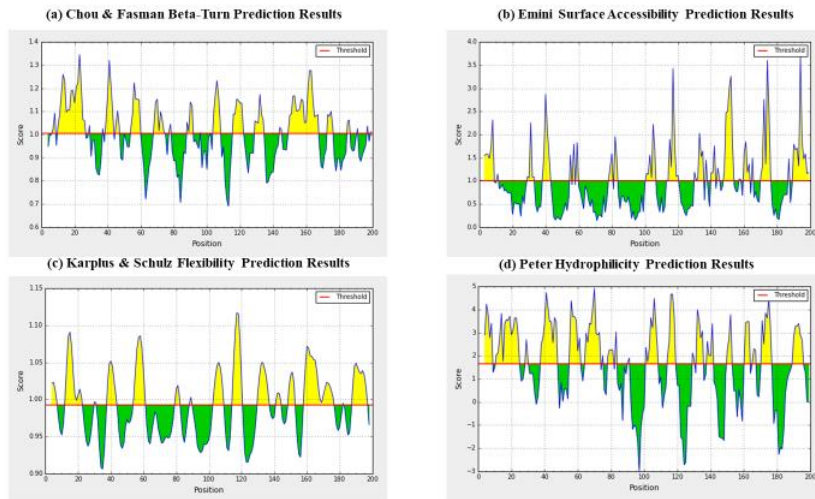Figure 1: predicted Non-linear epitopes for expansin protein.

Figure 2: Various prediction results for expansin protein.

Table 1: BepiPred linear epitope prediction for B cell epitope.

No.	Start	End	BepiPred linear epitope prediction for B cell epitope	Length
1	1	32	MTTHGRATHYSLGQGNTIANGNCSMPAVPADR	32
2	35	35	V	1
3	37	47	VSSPEYSGAAA	11
4	53	64	DVTGPKGTVRVQ	12
5	68	71	QCHG	4
6	79	79	L	1
7	82	82	E	1
8	84	85	FR	2
9	89	89	D	1
10	100	122	VTVRDPAGPTVAIRVKEGSSRWW	23
11	132	136	NRIDR	5
12	139	142	IQAG	4
13	145	177	WLPLTRTDYGYWVTPSPIQDGPLTVKVTDQYGR	33
14	185	198	RMAPGEIQRTASRF	14

Various physico- chemical properties e.g. hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity and antigenic propensity of peptides chains have been calculated to identify the locations of linear epitopes of an antigenic protein[10]. Thus, different tools from IEDB (www.iedb.org), including the classical propensity scale methods such as Kolaskar and Tongaonkar antigenicity scale[11], Emini surface accessibility prediction[12], Parker hydrophilicity prediction[13], Karplus and Schulz flexibilty prediction[14], Bepipred linear epitope prediction[15] and Chou and Fashman beta turn prediction tool[16] are used to predict linear or continous B cell epitopes of expansin protein in *Clavibacter michiganensis*. With the help of graphical findings and prediction scores the most probable B cell epitope of that antigenic protein can be identified. BepiPred prediction method combines Hidden Markov model and propensity scale method to predict score and identification of B cell epitopes of antigenic protein[15].

T cell epitope prediction

MHC I T- cell epitope prediction

T-cell epitopes for MHC-I binding for allergenic expansin proteins of bacterial origin are selected by NetCTLv1.2[17] where the epitope prediction is limited to various MHC I super types with 0.45 weighted on C terminal cleavage, 0.50 weighted on TAP transport

Table 2: Various prediction scores for peptide VRDPAGP.

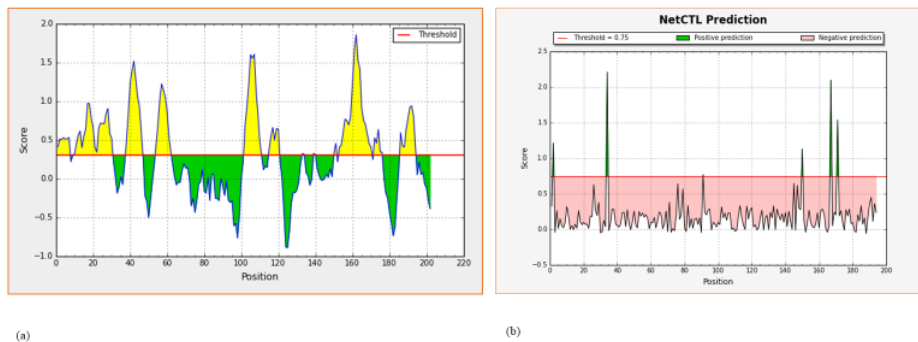| B cell epitope	Chou and Fashman beta turn score for each residue (threshold=1.006)	Emini surface accessibility score for each residue (threshold=1.000)	Karplus and Schulz flexibility score for each residue (threshold=0.993)	Parker hydrophilicity score for each residue (threshold=1.654)	Kolaskar and Tongaonkar antigenicity score for each residue (threshold=1.036)
V	1.068	1.159	0.984	2.65	1.059
R	1.077	1.337	1.01	2.21	1.064
D	1.059	1.232	1.031	2.92	1.039
P	1.059	1.232	1.046	2.92	1.039
A	1.029	0.862	1.05	2.61	1.054
G	1.026	0.814	1.043	2.18	1.031
P	1.026	0.814	1.026	2.18	1.031

Figure 3: Graphical representation of (a) BepiPred prediction results and (b) NetCTL prediction result.

Table 3: Result for interacting MHC I super types prediction from NetCTLv1.2 server.

Sl no.	Peptide sequences	Start position	Interacting MHC I super types
1.	TTHGRATHY	2	A1,A3,A26,B8,B27,B58,B62
2.	YVAVSSPEY	34	A1, A2, A3, A24, A26, B7, B8, B27, B39, B44, B58, B62
3.	LTVKVTDQY	167	A1, A3, A26, B8, B58, B62
4.	NAGIIPISY	91	A1, B58, B62
5.	WLRLTRDY	145	A1, A26, B8, B62

Table 4: Prediction scores for selected peptide sequences.

Sl no.	Peptide sequences	Start position	Predicted MHC binding affinity	C terminal cleavage affinity	Tap transport efficiency	Prediction Score
1.	TTHGRATHY	2	0.1350	0.9991	2.9480	1.2139
2.	YVAVSSPEY	34	0.2807	0.9970	3.0960	2.2107
3.	NAGIIPISY	91	0.0711	0.9979	2.7460	0.7700
4.	LTVKVTDQY	167	0.2664	0.9811	2.8400	2.0985
5.	VTDQYGRAV	171	0.2180	0.2984	0.2800	1.5386

efficiency with threshold value 2 for epitope identification. Selected epitopes are further analysed by neural network based method to predict the affinities for predicted MHC binding and C terminal cleavage. Similarly, TAP transport efficiency and overall prediction score for primarily selected epitopes are calculated [17].

Docking study of B cell and T cell epitopes
For docking studies, the T cell epitope [34]YVAVSSPEY[42] and B cell epitope [102]VRDPAGP[108] are selected and subjected to PEP-FOLD server[18,19] for 3D structure formation. To identify the molecular interactions with specific HLA protein and immunoglobulin E for respective epitopes, docking studies are performed using PepDock[20] and ClusPro[21] respectively.

RESULTS

Antigenicity prediction for expansin protein
Overall antigenicity prediction score is 0.5025, which implies that expansin protein present in *Clavibacter Michiganensis* is probably antigenic in nature.

Non-linear B cell epitope prediction
Seven non-linear epitopes with prediction score above the threshold value 7.7 are shown graphically in Figure 1(a) and three-dimensionally in Figure 1(b).

Linear B cell epitope prediction
BepiPred prediction method[15] guesses that the peptide sequences from 1-32, 37-47,53-64, 68-71, 100-122, 132-136, 139-142, 145-177 and 185-198 amino acids are able to induce the immune responses as B cell epitopes (Table 1). Graphical representation of the prediction results is shown in Figure 3(a).
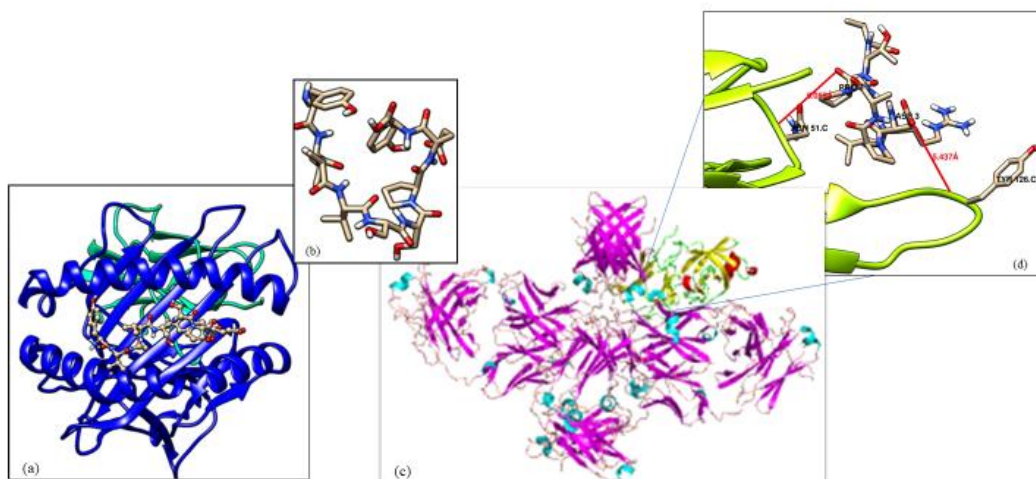
Figure 4: Docking structures of T cell and B cell epitopes, Docking structure of T cell epitope with MHC I molecule 3D Structure of [34]YVAVSSPEY[42] epitope, Docking structure of IgE with 4JJO protein, H bonding interaction of B cell epitope [102]VRDPAGP[108] with Heavy chain of IgE.

Predicted scores for Chou and Fashman beta turn[16], Emini surface accessibility[12], Karplus and Schulz flexibility[14] and Parker hydrophilicity[13], along with their threshold values for every amino acid residues present in expansin protein are exhibited in Figure 2 (a), (b), (c) and (d) respectively.

All physico chemical parameters which are mentioned above for peptide [102]VRDPAGP[108] in tabular form along with their threshold values are shown in Table 1. Similarly, Kolaskar and Tongaonkar antigenicity prediction scores[11] for each residue also shows that the specific peptide is antigenic in nature. So, considering the following parameters such as hydrophilicity, flexibility, accessibility, beta turns, exposed surface, polarity and antigenic propensity of antigenic protein, it can be concluded that the antigenic determinant [102]VRDPAGP[108] is selected as the best B cell epitope for this protein.

T cell epitope prediction

Graphical representation of overall prediction score using NetCTLv1.2[17] for each amino acid residues of expansin protein along with threshold value is shown in Figure 3(b).

Among the five peptide sequences, [34]YVAVSSPEY[42] peptide sequence is selected as most probable T cell epitope for the antigenic protein present in *Clavibacter michiganensis* on the basis of its interactions with highest number of alleles (Table 3). Furthermore, overall prediction score is also highest for this epitope among the five peptide sequences (Table 4). This epitope has IC_{50} value of 4.43 nm with specific HLA-A*29.02 allelic protein.

Docking study of B cell and T cell epitopes

The T cell epitope [34]YVAVSSPEY[42] is selected on the basis of its interactions with large number of alleles and lowest IC50 value (4.43 nm) with HLA-A*29:02 MHC I allele. Similarly, [102]VRDPAGP[108] is designated as most probable B cell epitope of expansin protein present in *Clavibacter michiganensis*. Docking studies are performed with these two epitopes with human HLA class MHC I molecule (PDB ID 5EU3) and immunoglobulin protein E (PDB ID 4J4P) respectively

(Figure 4). B cell epitope interacts with immunoglobulin E through two hydrogen bonding interactions involving atoms of Asp and Pro amino acid residues present in B cell epitope.

DISCUSSION

Clavibacter michiganesis infected tomato seeds and plants do not show visible symptoms of the disease[1,2]. But due to bacterial cranker in tomato 20-46% yield losses have been observed all-over the world[3]. So, specific detection method for this infection for example molecular biology based as well as immunological methods have been investigated earier with various experimental results[22,23]. Though these methods are specific, but time comsuming in nature.

In order to prepare monoclonal antibody to detect *Clavibacter michiganesis* in infected seeds of tomato, peptide sequences[34]YVAVSSPEY[42] and [102]VRDPAGP[108] are identified using bioinformatics tools. These two peptide sequences are found as most suitable T cell and B cell epitopes present in antigenic protein expansin. After experimental verification, these two epitopes can be used to produce monoclonal antibody against plant pathogenic microorganism *Clavibacter michiganesis*. By using monoclonal antibody technique, infected seeds of bacterial wilt and cranker can be detected and reduction in tomato production can be prevented in near future.

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