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A structural insight towards identify specific epitopes of phytoplasma diseases

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ABSTRACT
The aim of this work was to predict the 3D structure of the phytoplasma SecA protein and also to compare its epitopes and different bacteria's antigenic sites for identity the specific epitopes. Phytoplasma SecA protein was modeled by Modeler 9v8 program and validated using the Anolea, Qmean and PROCHECK, which showed that 91.3% similarity of residues observed in the most favored region and overall quality factor of the model identified to be 76.95%. The modeled protein was submitted PMDB (Protein Model Database) for Public access. The PMDB id is PM0077063. By employing the CEP server, it was found that of the residues were 21 conformational epitopes and 9 sequential epitopes. The secondary structure elements i.e., helix, Extended strand and coils were predicted with GORIV program. 11 Specific epitopes were identified based on the comparison to selected bacterial species. This will pioneer the attempt to predict the 3D structure and specific epitopes of the phytopasma secA protein. Which ultimately lead to efficient diagnosis and development of novel control methods for Phytoplasma diseases.

KEYWORDS: *Comparative modeling, Epitopes, Phytoplasma, SecA.*

1. INTRODUCTION
Phytoplasmas are wall-less bacteria and known to cause several plant diseases worldwide. So far 28 groups of phytoplasma have been classified (Nejat *et al*., 2010). The SecA is an essential phytoplasmal protein for ATP translocation from the host and there is no SecA analogue in human or animals (Pohlschröder *et al*., 1997). Therefore, SecA is a viable candidate immunogen for production of antibodies that react with many different phytoplasmas and helps to diagnose by ELISA (Economou 1999). Due to lack of specific antigen, there is no efficient diagnosis of phytoplasma diseases. Antigen is a substance stimulating antibody production when introduced into the body. The identification of the regions of interaction between an antigen (Ag) and an antibody (Ab) is one of the most interesting problems in molecular immunology. The most remarkable feature of antigen–antibody interactions is the high affinity and strict specificity of antibodies for their antigens. It is known that antibodies recognize the unique conformations and spatial locations on the surface of antigens (Regenmortel 1998). The Antigen has epitopes which are responsible for specificity of the antigen. Epitopes are of two types, namely, sequential (when Ab binds to a contiguous stretch of amino acid residues that are linked by peptide bond) and conformational (when Ab binds to non-contiguous residues, brought together by folding of polypeptide chain) (Regenmortel 1996; Regenmortel and Pellequer 1994). So the predictions of sequential and conformational epitopes are necessary to synthesize specific antigen for phytoplasma diagnosis. Sequence and the knowledge of the 3D structure of the phytoplasma SecA protein are pre–requisite for epitope prediction. At a halt, there is no experimental structure available for phytoplasma SecA. Here, we made the first attempt to predict the 3D structure of phytoplasma SecA, which will be beneficial to the researchers for producing specific antibody for diagnosis of phytoplasma, and this may be a powerful candidature for new antibiotic discovery for phytoplasmal diseases.

2. MATERIALS AND METHODS
2.1 Three Dimensional structure prediction and Validation
The knowledge-based 3D structure of the protein was predicted by comparative modeling method. The phytoplasma SecA protein sequence (Q2NJH2) was retrieved from swiss prot database. The phytoplasma SecA sequence was BLAST (Altschul *et al*., 1990) against the sequence from PDB database. The appropriate template *Bacillus subtilis* Preprotein translocase secA subunit (1TF5)

was selected and found with 48% identities with target sequence (**Fig. 1**). Comparative modeling was carried out by using modeler 9v8 (Sali and Blundell 1993). The energy minimization of the model protein was performed with help of SPDB Viewer (Schwede *et al*., 2003). The quality of the model structure was validated by PROCHECK program (Laskowski *et al*., 1993), Anolea (Melo and Feytmans 1998), Qmean (Benkert *et al*., 2009) and Errat (Colovos and Yeates 1993).

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 Score =  779 bits (2011),  Expect = 0.0, Method: Compositional matrix adjust.
 Identities = 399/839 (48%), Positives = 564/839 (67%), Gaps = 35/839 (4%)

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Sbjct  1    MFNFLKKIFNSSKKALRKARTIANKVQNLEAQIALLDDKDFATKTAELKKLFQEGKTLNQ  60

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            LL EA+A+ +EA++RVTG+ P+ VQ++G V LH GNIAEMKTGEGKTLT+ +P YLNAL+
Sbjct  61   LLPEAYALAKEATKRVTGLTPYYVQILGAVILHQGNIAEMKTGEGKTLTAIMPAYLNALS  120

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Sbjct  121  GNAVHIVTVNEYLAKREFEGSIGDVFRFLGMTVGLNTKDKDQTQKQQAYLCDVLYTTNSE  180

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            LGFDYLRDNM +    +V +RP  +A++DEVDSILIDEARTPLIIS    ++  LY +A
Sbjct  181  LGFDYLRDNMEIEASNLVMKRPYSYAIVDEVDSILIDEARTPLIISQSVKETKNLYKEAQ  240

Query  242  AFVRTLKAEKDYTYDIKTKAVQLTEEGMTKAEKAFGIDNLFDVKHVALNHHINQALKAHV  301
             FVRTLK    Y  +++TK ++LTEEG+TKAE  F IDNL++++H +L HH+  ALKA
Sbjct  241  RFVRTLK-NSHYLIELETKTIELTEEGITKAENFFQIDNLYNIEHASLLHHVKNALKAAF  299

Query  302  AMQKDVDYVVE--DGQVVIVDSFTGRLMKGRRYSEGLHQAIEAKEGLEIQNESMTLATIT  359
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Sbjct  300  TMHKDKDYLVDYKDGQVLIIDQFTGRALPGRQFSDGLHQALEAKEGLLIKKETSIGATIT  359

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Sbjct  360  YQNFFRLYQKLSGMTGTAKTEEDEFRDIYNMEVIEIPTNVPMIRIDEPDFIFVSLKEKYD  419

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Sbjct  480  KNAVTIATNMAGRGTDIRLGEGVKELGGLSVLGTERHESRRIDNQLRGRAGRQGDPGYSR  539

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Sbjct  540  FFISSEDELAQRFGGTRIEKIISLLQKIS-DSETKTSSKMVTKFFTKIQKKVESSNFDYR  598

Query  597  KQLLQYDDVLRQQREVIYKQRFEVIDSENLREIVENMIKSSLERAIAAYTPREELPEEWK  656
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Sbjct  599  KYLLKYDDILRIQREIIYNQRKEILVSDKVEQIVQDLMKKTLNKAI--FTHFTNKPNKCQ  656

Query  657  LDGLVDLINTTYLDEGALEKSDI--FGKEP-----DEMLELIMDRIITKYNEKEEQFGK-  708
               L+  +   +  +  ++      P     D   +    ++      +++ F K
Sbjct  657  TQALITFLENKFFPKQTFDLEEVQELCNNPKTNSLDSFQQYSFQKVKDILQSQKDFFVKD  716

Query  709  -EQMREFE---KVIVLRAVDSKWMDHIDAMDQLRQGIHLRAYAQTNPLREYQMEGFAMFE  764
             E+ + F    K I L+ +D+ +  HI+ M  LRQGI   +Y Q +   EYQ EG  +F
Sbjct  717  PEKAQYFAKGLKWITLKIIDNYYQRHINDMSSLRQGIGFVSYGQQDSFIEYQKEGQVLFN  776

Query  765  HMIESIEDEVAKFVMKAEIENNLEREEVVQGQTTAHQ----PQEGDDNKKAKKAPVRKV  819
            +MI  I +++   ++K    ++         QT   Q      +  D++ +KK   RKV
Sbjct  777  NMITKIANDITATILKFSFADSF--------QTPPKQKVFFKNDSSDDESSKKRRTRKV  827
```

Fig. 1. Sequence alignment between modeled protein (query) and template (subject).

2.2 Secondary Structure prediction

The GOR IV algorithm (Garnier *et al*., 1996) was used to predict the secondary structural elements of Phytoplasma SecA protein.

2.3 Epitope prediction

CEP (Conformational Epitope Prediction) server (Kulkarni-Kale *et al*., 2005) was used to predict the Epitopes from Phytoplasma SecA. Three-dimensional structure of the phytoplasma SecA was used as an input to predict Conformational epitopes and Sequential epitopes. The conformational epitopes has been predicted using the accessibility of residues and spatial distance cut-off to predict antigenic determinants

2.4 Identification of specific epitopes

The SecA protein sequence of *Acholeplasma, Bacillus, Streptococcus,* and *Clostridium* were retrieved from NCBI database, and multiple sequence alignment was performed with phytoplasma SecA using ClustalX program (Thompson *et al*., 1997). The non-conserved regions were selected and compared with predicted epitope.

3. RESULTS AND DISCUSSION

3.1 Three-dimensional structure prediction and validation

The predicted 3D structure of phytoplasma SecA is shown in (**Fig.2**). So far there is no 3D structure for phytoplasma SecA. So without the knowledge of 3D structure of the protein, it is impossible to predict the conformational epitopes. The comparative modeling is a method that helps to predict the 3D structure of the protein by exit crystallography structure. Kolaskar and Kulkarni-Kale (1999) proposed Knowledge-based 3D structure of protein was necessity to predict the conformational epitopes.

The refinement of model was done by Anolea and Qmean (**Fig 3 & 4**). The predicted model structure has been validated by Ramachandran plot and it reveals the quality of the model (**Fig. 5**). The ideal structure has over 90% of residues present in favored region (Morris *et al*., 1992). Here, our structure has 91.3% residues that lie in the most favored region; 6.7 % of the residues lie in the additional allowed region, and 0.6% of residues lie in the precluded region. The overall quality of the model is identified to be 76.95%. So the predicted structure can be submitted to Protein Model DataBase (Castrignano *et al*., 2006) for public access. The PMDB id is PM0077063.

Fig. 2. 3D structure of modeled protein

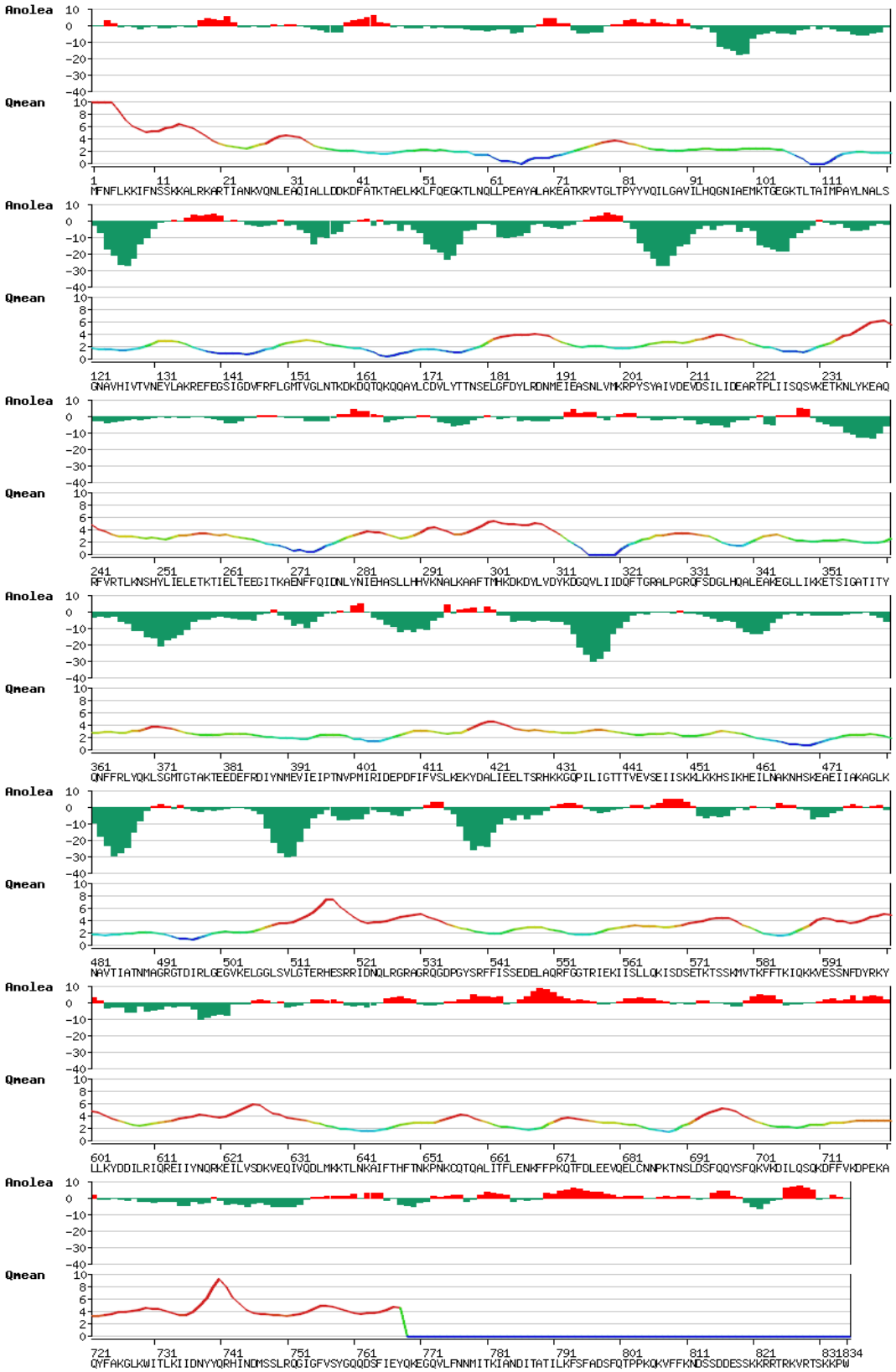


Fig. 3. Anolea and Qmean plot of modeled protein

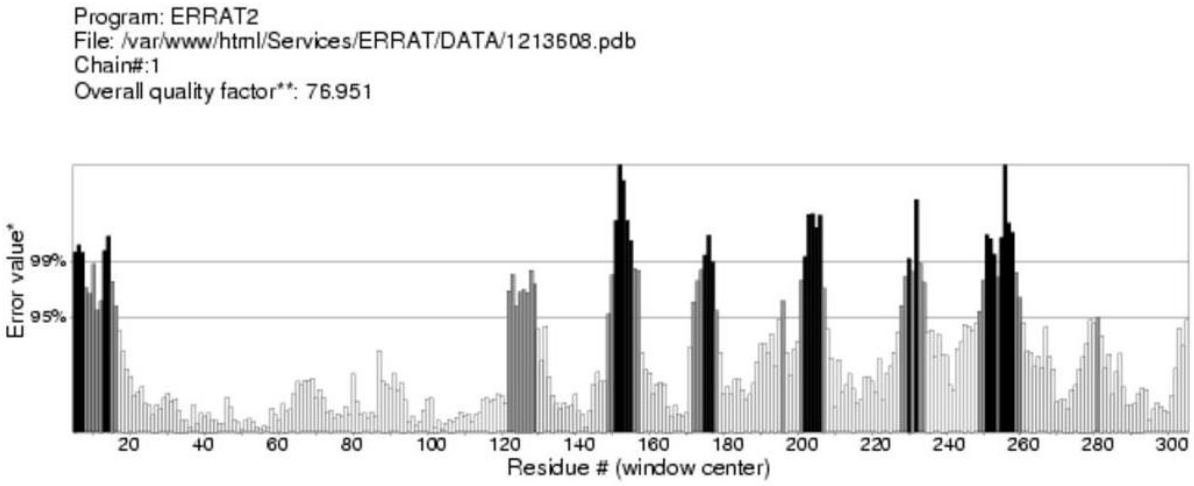


Fig. 4. Quality factor analysis of modeled protein by ERRAT.

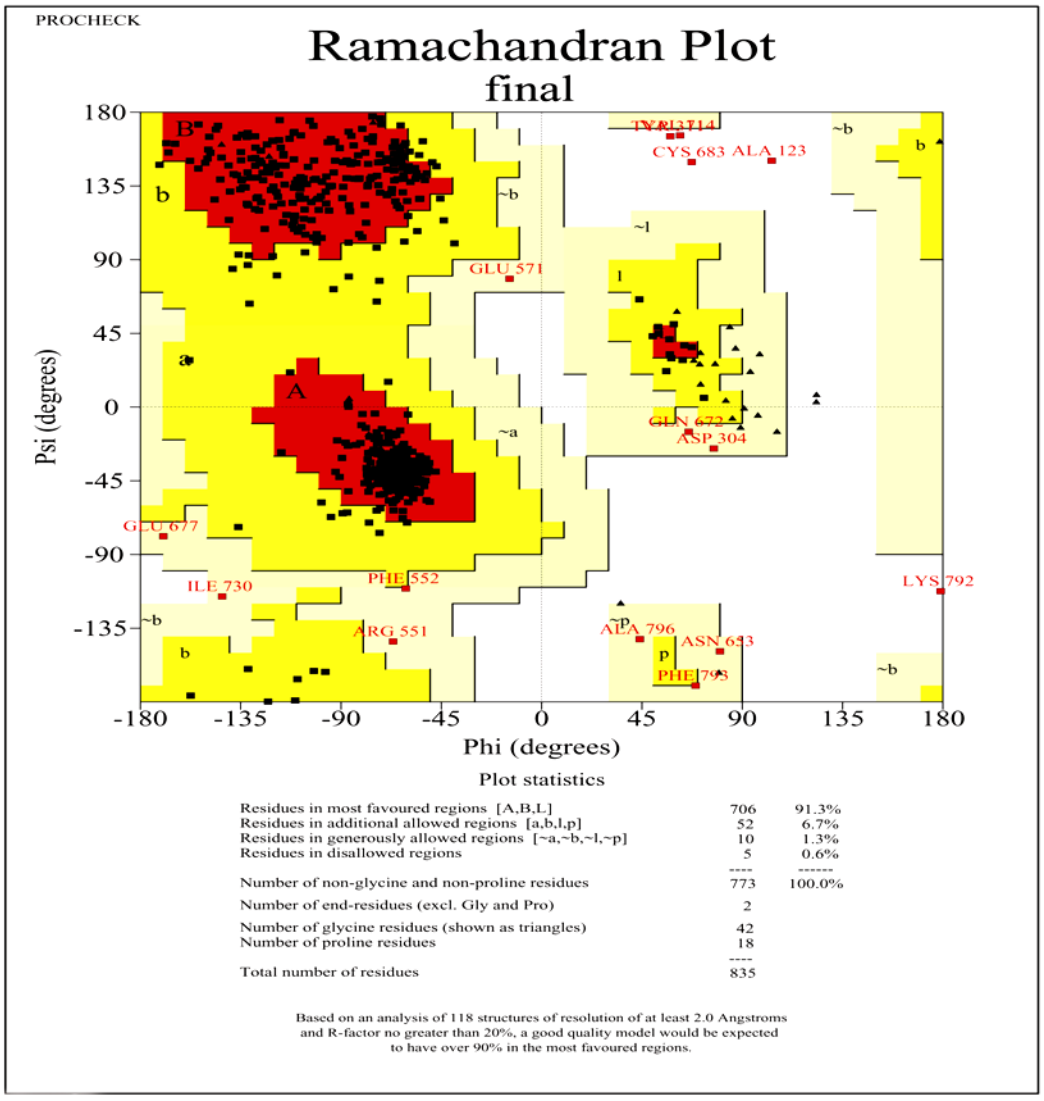
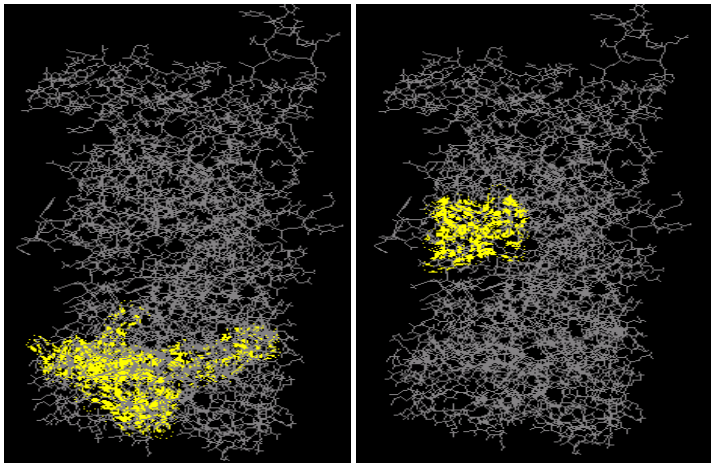


Fig. 5. Ramachandran plot for modeled protein



a) Conformational epitope **b) Sequential epitope**

Fig. 6. Graphical view of predicted epitopes of phytoplasma SecA

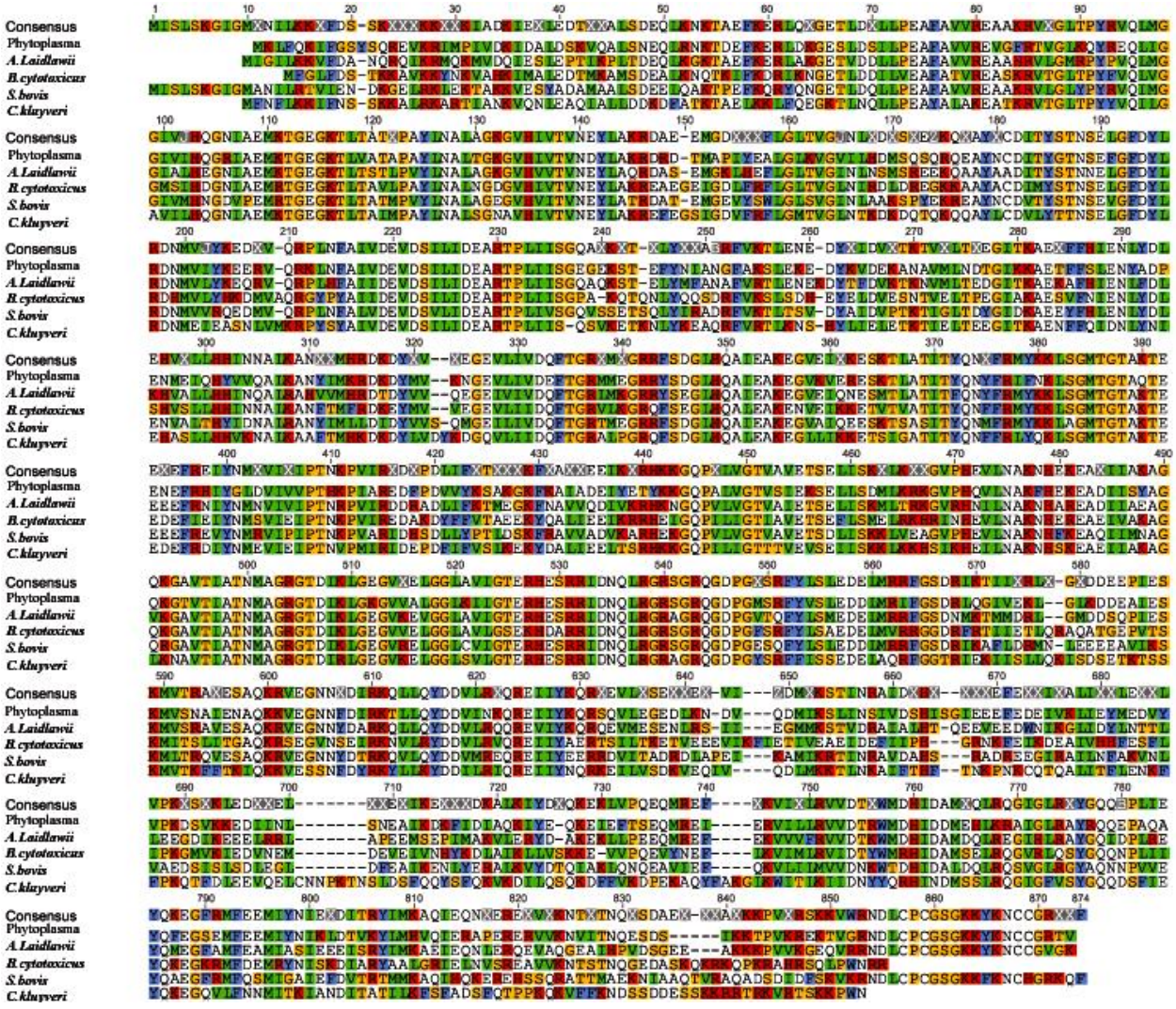


Fig. 7. Multiple sequence alignment of phytoplasma SecA with a protein sequence of different bacteria SecA.

3.2 Secondary Structure prediction

Predicted Secondary structure elements of Phytoplasma SecA protein is shown in table 1. Most of the residues have Alpha helix (55.21%), followed by a random coil (31.98%) and extended strand (12.81%). The secondary structure based on Garnier algorithm provides additional information about the possible sequence accessibility. The secondary structure prediction is to provide the location of alpha helices and beta strands within a protein or protein family. Residue conformational propensities, sequence edge effects, moments of hydrophobicity, position of insertions and deletions in aligned homologous sequence, moments of conservation, auto-correlation, residue ratios, secondary structure feedback effects, and filtering are the important concepts involved in the secondary structure prediction (Robson and Garnier 1993).

3.3 Epitope Prediction

Totally 30 Epitopes were predicted from Phytoplasma SecA Protein in which 21 were conformational epitopes (**Table 2**) and 9 were sequential epitopes (**Table 3**). We predicted the B-cell epitopes from phytoplasma SecA, because the B-cell epitopes are accessible, hydrophilic regions and majority of them are capable of neutralization. Antibodies produced by B cells recognize the intact antigen in its native conformation. The epitopes recognized by T cells are products of processed or partially degraded proteins that are bound to MHC molecules and are usually amphipathic (i.e., alternating hydrophobic and hydrophilic) regions. But the B- cell epitopes can be contiguous / Sequential (Hopp and Woods 1981; Saha *et al*., 2005). Target protein residues with more than or equal to 30% ASA (Accessible Surface Area) were considered as accessible residues. Residues with accessibility less than 25% were shown in lower case and also secondary structural properties were compared with the predicted epitopes. The specificity of the sequential epitopes (SE) is determined by the sequence of subunits (e.g. amino acids). On the other hand, specificity of conformational epitopes (CE) depends on the spatial folding or conformation of the contributing individual sequential epitopes (Regenmortel and Dispersion 1998). Our mechanism compared the predicted epitopes with the secondary structural elements.

Table 1. Predicted secondary structure elements of phytoplasma SecA.

Types of secondary structure	No of Elements	Percentage of elements
Alpha helix	461	55.21
3_{10} helix	0	0
Pi helix	0	0
Beta bridge	0	0
Extended strand	107	12.81
Beta turn	0	0
Bend region	0	0
Random coil	267	31.98
Ambigous states	0	0
Other states	0	0

Table 2. Conformational epitopes of phytoplasma SecA and Secondary structure elements

No.	Position	Conformational epitopes	Types of secondary structure
1	1 - 20	MFNFLKKIFNSSKKALRKaR	CCCCCHHHHHHHHHHHHHHH
2	24 – 40	NKvQNLEAQiALIDDKD	HHHHHHHHHHHHHHHHH
3	74 – 82	KRVTGLTpY	CEECCCCCC
4	158 – 196	KDkDQTQkQQ	CCCHHHHHHH
5	217 – 238	DEaRTPlIiSQSVKETKNLyKE	HHCCCCHHHHHCHHHHHHHHH
6	302 – 305	HKDK	HCCC
7	308 – 315	LVDYKDGQ	EECCCCCC
8	320 – 334	DQFTGRALPGRQfSD	ECCCCCCCCCCCCHH
9	394 – 407	EiPtNVPMIrIDEP	EECCCCCCEEECCC
10	412 – 415	VSLK	HHHH
11	453 – 461	KKHSiKhEI	HHCCHHHHH
12	465 – 469	KNHSK	HCCHH
13	550 – 556	QRFGgTR	HHCCCCC
14	559 – 585	KIiSLLQKiSDSETKtSSKMvTKFfTK	HHHHHHHCCCCCCCCCCCHHHHHHHHH
15	592 - 600	SSNFDYrKY	CCCHHHHHH
16	645 – 659	FThFTNKPNKCqTQA	EEEECCCCCCCCHHH
17	671 – 674	KQTF	CCCC
18	677 – 692	EEVQeLCNNPKTNsLD	HHHHHCCCCCCCCCC
19	711 – 718	DFfVKDPE	HHHCCCHH
20	799 – 815	FQTPPKQKVFFKNDSsD	CCCCCCCEEEEECCCCC
21	821 – 835	KRRTRKvRTSKKPWN	HHHEEEEEEEEECEE

(C = Random coil**, H** = Alpha helix **and E** = Extended sheet**)**

Table 3. Sequential epitopes of phytoplasma SecA

No	Position	Sequential epitope	Elements
1	45 – 60	AElKKLfQEGKTlNQ	HHHHHHHHHHHHHHH
2	120 – 122	SGN	CCC
3	139 – 141	EGS	CCC
4	190 – 196	MeIEASN	HHHHHHH
5	244 – 249	RTlKNS	HHHCCC
6	252 – 261	LIELETKTiE	HHHHHHCCHH
7	441 – 443	TVE	CHH
8	479 – 481	LKN	CCC
9	758 – 762	YGQQD	CCCCC

Table 4: Specific epitopes for phytoplasma diagnosis identified from phytoplasma SecA

Sl.No	Position	Specific epitopes for phytoplasma
1	1 - 20	MFNFLKKIFNSSKKALRKaR
2	24 – 40	NKvQNLEAQiALlDDKD
3	139 – 141	EGS
4	158 – 196	KDkDQTQkQQ
5	252 – 261	LIELETKTiE
6	412 – 415	VSLK
7	479 – 481	LKN
8	671 – 674	KQTF
9	677 – 692	EEVQeLCNNPKTNsLD
10	711 – 718	DFfVKDPE
11	799 – 815	FQTPPKQKVFFKNDSsD

The exit algorithms employ propensity values of amino acid properties (hydrophilicity, accessibility, and flexibility) and the accuracy of the algorithms 35 to 75% only. But the CEP server algorithm has 75% accuracy (Kulkarni-Kale *et al*., 2005). Twenty-one conformational epitopes and 9 sequential epitopes were predicted from phytoplasma SecA protein by using this algorithm. The graphical views of a few predicted epitopes are shown in (**Fig. 6**). The yellow colored region represents the epitope of the phytoplasma SecA.

3.4 Identification of specific epitopes

Multiple sequence alignment of the Phytoplasma SecA and other four bacteria show the conserved and non-conserved region (**Fig. 7**). The predicted epitopes that lie in the non-conserved region are considered as specific epitopes. Out of the 30 Epitopes, 11 epitopes are specific for Phytoplasma, and they are shown in table 4. The phytoplasma 16s rRNA region is closely related to Bacillus 16s rRNA and uncultured 16s region. The *Bacillus* and the other bacteria are naturally associated with plants, and so the diagnosis of phytoplasma remains a challenge due to cross–reactivity in ELISA. The antibody reacts with other bacteria hence giving out false results. The predicted epitopes are specific for phytoplasma and they have no similarity with other bacteria. The predicted epitopes may show the way to phytoplasma disease diagnosis.

4. CONCLUSION

The 3D structure of Phytoplasma SecA protein is important to predict the conformational epitopes. There is no experimental structure for Phytoplasma SecA. So cost-effective, time-consumed knowledge-based 3D structure insights will help to predict the conformational epitopes. The specific epitopes are used to avoid the cross-reactivity and provides the effective information regarding diagnosis. Over the past one decade, there is no effective control method for phytoplasmal diseases. Our 3D structure is a powerful candidate and gives an insight into epitopes for efficient diagnosis and imminent in the development of novel control method for phytoplasma diseases. In the future, these epitopes may be used for synthesis in wet lab practice.

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