**International Journal of Advanced Biotechnology and Research(IJBR)**
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-6, Issue-3, 2015, pp354-359
http://www.bipublication.com

**Research Article**

# *In silico* prediction of pilW protein of *Xanthomonas axonopodis* pv. *Punicae* for future drug target

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[Received-20/05/2015, Accepted-03/06/2015, Published-25/07/2015]

**ABSTRACT:**

*Xanthomonas axonopodis* pv. *punicae* is Gram negative plant pathogen it has host specific to the pomegranate which leads to cause bacterial blight disease. It is become a serious threat to pomegranate cultivation and also to the tremendous export potential such cultivation represents because of the effect of its disease on the quality and quantity of the fruit. PilW is an outer membrane pilin protein acts as an antigenic due to it serve as various biological function such as adhesion, signaling, protein secretion, competence and biofilm development etc. The main purpose of this investigation is to find out drug to block the pilW protein mechanism involved in disease development. The Primary protein sequence analysis of PilW was carried out using Protparam tool. SOPMA was used for the prediction of secondary structure of protein. Swiss model server was used to predict the 3D structure from X-ray crystal structure (2VQ2) as a template. The model quality was checked using PROCHECK. The TPR_15 is conserved domain was identified using Pfam. The fidoximycin shows minimum energy value (-360.32) on docking study with pilW protein. So the fidoximycin may lead as drug for treat bacterial blight disease on pomegranate

**Keywords**: *X.axonopodis* pv. *punicae*, drug, PilW, fidoximycin.

## [I] INTRODUCTION

*Xanthomonas axonopodis* pv. *punicae* gram negative plant pathogen belong to the family of xanthomonads. Is one of the various host-specific pathovars of phytopathogenic was first reported in India on pomegranate [7]. It has now emerged as a destructive pathogen in pomegranate-cultivating areas of India, the biggest producer of pomegranates in the world. The *xanthomonas axonopodis* pv. *Punicae* (also called Telya) causes bacterial blight disease on pomegranate fruit. About 60 to 80 percent crop loss due to these bacteria attack. *X. axonopodis* pv. *punicae* has become a grave threat to pomegranate cultivation and also to the terrific export potential such cultivation represents because of the effect of its disease on the quality and quantity of the fruit. As India is an area where *X. axonopodis* pv. *punicae* infections are presently endemic, the

species is also a potential threat to pomegranate-growing areas of the world where such infections are not endemic. It is the causative agent of bacterial blight on pomegranate. The pilW is the outer membrane protein of *X. axonopodis* pv. *punicae* is required for pilus stability, pilus functions such as adherence to plant cells, also for signaling, protein synthesis and adhesion. Members of this family contain copies of the TPR (Tetratrico Peptide Repeat) domain. The Tetratrico Peptide Repeat region (TPR) is a structural motif present in a wide range of proteins. It mediates protein-protein interactions and the assembly of multiprotein complexes. In Humans protein containing Tetratrico Peptide Repeats are involved in a variety of biological processes, such as cell cycle regulation, transcriptional control, mitochondrial and Peroxisomal protein transport, Neurogenesis and Protein folding. In bacteria the Type IV pili (T4P) are adhesive cell surface appendages formed by a broad range of bacterial species and consist of multiprotein Complexes that span the cell envelope. Especially In gram-negative bacteria, T4P are thought to polymerize at the inner membrane and are extruded from the cell through an oligomeric secretin pore located in the outer membrane [1, 12]. The Type IV pili provide motility ability to surface associated switching [14]. The all classes of T4P participate in biofilm development by promoting cell-cell interaction [3].

## [II] MATERIALS AND METHODS

### 2.1 Retrieval of PilW sequence

The PilW protein sequence was retrieved from UniprotKB database [9]. UniprotKB is the central hub for the collection of functional information of proteins.

### 2.2 Primary structure prediction

The primary structure was predicted using ProtParam tool [4]. For physio-chemical characterization, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient [5], instability index [5] aliphatic index and grand average hydropathy (GRAVY) [13] were computed using the Expasy's). Protparam server (http://expasy.org/cgi-bin/protpraram )

### 2.3 Secondary structure prediction

The secondary structure was predicted using FASTA sequence of PilW protein by SOPMA according to the method of Geourjon and Deleage (2006) [8]. It was employed for calculating the seconbil .ibcp. fr /cgibin/ npsa_autodary structural features of the selected protein sequence. http://npsa-pmat.pl?page=/NPSA/npsa_sopma.html

### 2.4 Protein functional sites

InterPro scan and Fingerprint scan are the tools used to predict the signatures and the motif regions in the sequence.

### 2.5 Homology Modeling

The protein sequence was subjected for comparative homology modeling via Swiss model according to the method of Arnold [9] to generate putative 3D model. SWISS-MODEL is fully automated protein structure homology modeling server to make the protein modeling accessible to all biotechnologist. The SWISS MODEL performs the sequence alignments and searches for the putative template protein for generating the 3D model.

### 2.6 Structure validation using PROCHECK

According to the method of Laskowski [9]. PROCHECK checks the stereochemical quality of a protein structure, producing a number of PostScript plots analyzing its overall residue-by residue geometry. It includes PROCHECK NMR for checking the quality of structures solved by NMR. The structure was visualized and analyzed in RasMol.

### 2.6 molecular docking

The Hex is the FFT algorithm for performing macromolecular docking. Hex is an interactive

protein docking and molecular superposition program built by the Dave Ritchie[2].

## [III] RESULT

### 3.1 Protein primary sequence analysis

The primary structure of PilW was predicted using Expasy's ProtParam server (http://expasy.org/cgi-bin/protparam ). The result revealed that PilW had 262 amino acid residues and molecular weight (Da) 27398.8. The maximum number of amino acids present in the sequence was found to be Valine (4.6%) and least was that of Cysteine (1.1%).The total number of negatively charged residues (Asp+Glu) was 28 and the total number of positively charged residues (Arg+Lys) was 33.The isoelectric point pI was 9.13, protein is acidic in nature. The high aliphatic index (84.92).While instability index 41.64. The ground average hydropathicity (GRAVY) is very low -0.239.

### 3.2 Secondary structure prediction of PilW protein

The secondary structure is composed of alpha helix and beta sheets and is predicted by SOPMA as shown in Table-1.The secondary structure prediction was done and random coil was found to be 40.98% followed by extended strand 26.33% **[Figure-1].** The protein 3D structure was built using SWISS-MODEL and the template target alignment wad done**[figure. 2].** The highest identity template was m332 the model quality was checked using PROCHECK. The model quality was obtained about 87.5. %
As shown in **[Figure-3].** The domain analysis was done using pfam and functional domains were obtained shown in **[Figure-4]**.

### 3.3 Protein functional sites

Interpro scan and Fingerprint scan were the tools used to predict the signatures and the motif regions in the protein as shown in the **[Table-2]**.

### 3.4 molecular docking analysis

The molecular docking was performed on the pilW protein i.e. 2VQ2 as a template. The interaction of these protein with fidoxymicin ligand was done. The fidoxymicin binds to the active amino acid of 2VQ2 template with minimum energy value -360.32 kcal $mol^{-1}$.

## [IV] CONCLUSION

In present study, we built the 3 D structure of pilW protein using homology modeling. The protein structure verified to be good quality and being used for docking study. The fidoxymicin show high inhibitory effect on pilW protein. This information would also useful for development of new drug to treat bacterial blight disease.

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| Secondary structure | SOPMA |
|---|---|
| Alpha helix | 64.12 |
| $3_{10}$ helix | 0.00 |
| Pi helix | 0.00 |
| Beta bridge | 0.00 |
| Extended strand | 4.58 |
| Beta turn | 11.07 |
| Bend region | 0.00 |
| Random coil | 20.23 |
| Ambiguous states | 0.00 |
| Other stated | 0.00 |

**Table: 1**. Secondary structure prediction by SOPMA

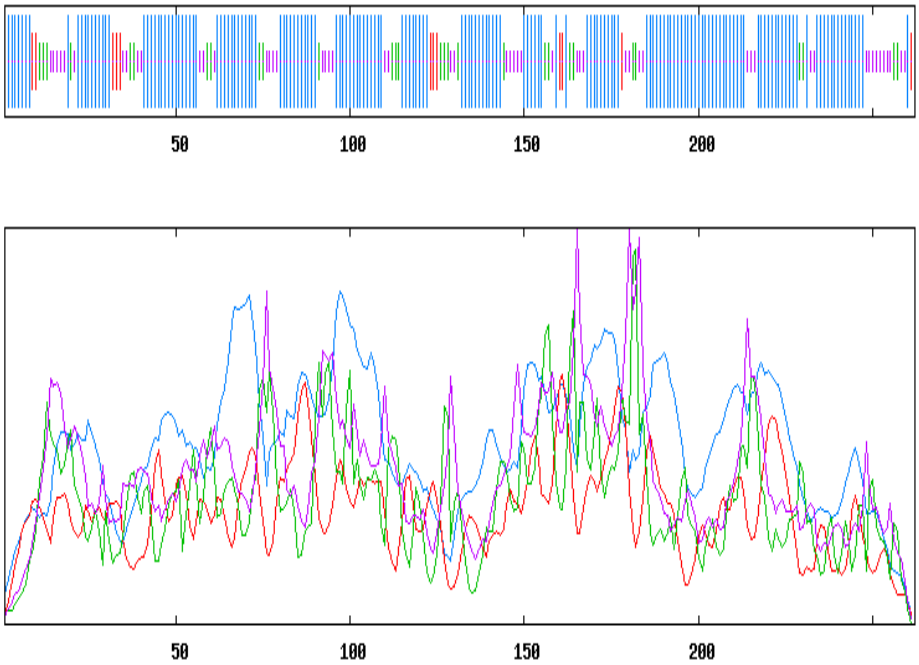


**Figure: 1**.secondary structure prediction by SOPMA

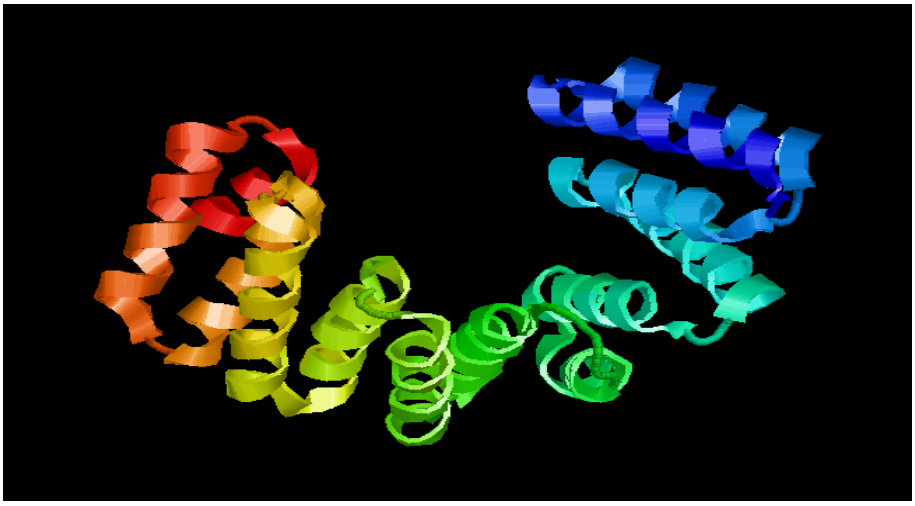

**Figure: 2**. Three dimensional structure of PilW protein (PDB:2VQ2)

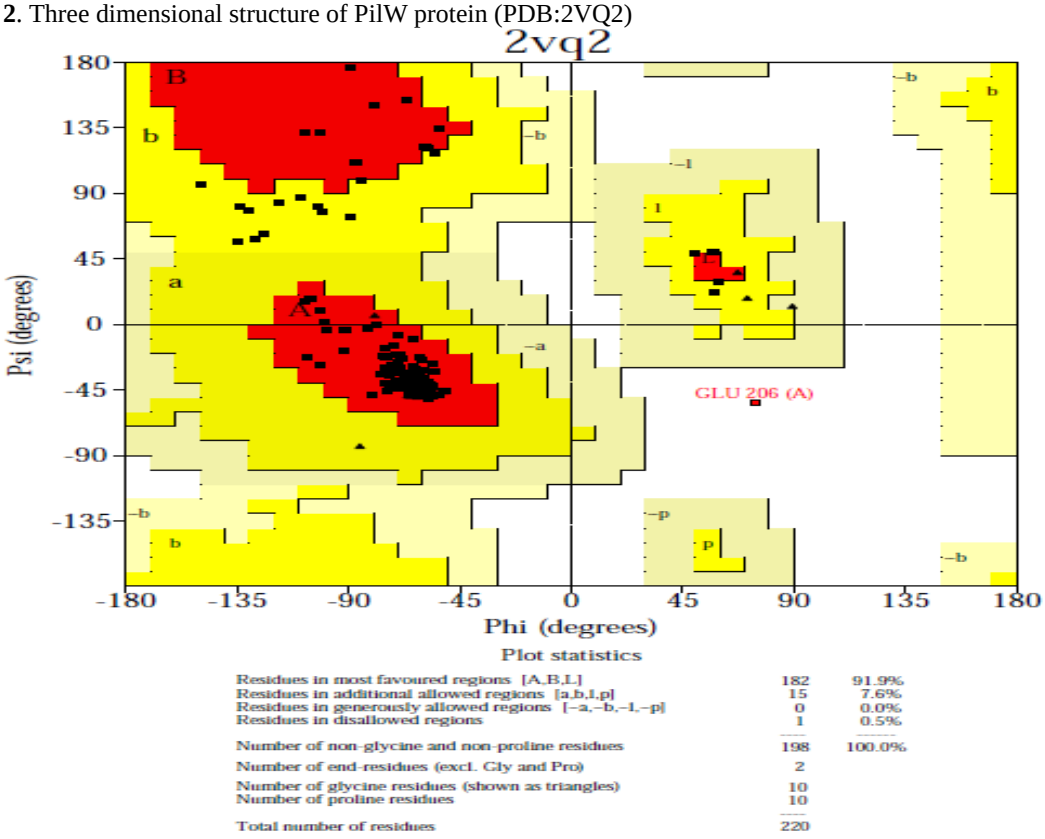


**Figure: 3**. Ramchandran plot PilW protein.


**Figure: 4**. Conserved domain analysis by pfam.

| fingerprint | No. of motifs |
|---|---|
| ANTIFREEZEI | 3 |
| NORNUCRECPTR | 10 |
| INHIBINBB | 8 |
| CHEMTRNSDUCR | 7 |
| ANPHYLATOXNR | 5 |
| PERTACTIN | 11 |
| P2X1RECEPTOR | 10 |
| CAMPKINASE | 5 |
| FLGFLGJ | 13 |
| TCRTETA | 132 |

**Table: 2**. Fingerprint result of PilW protein

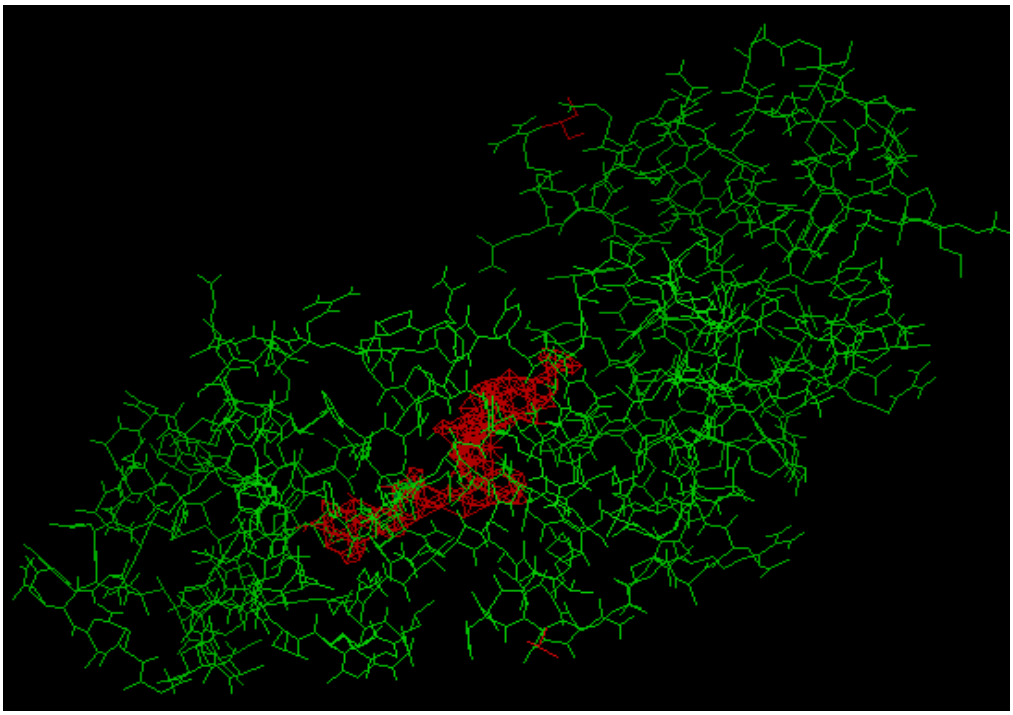

**Figure: 5.** Docking of PilW protein with fidoxymicin