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IN SILICO PREDICTION OF 3D STRUCTURE OF PECTATELYASE FROM *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

Raghavendra Rao B[1], Manojkumar H. Nagasampige[1*], Ramesh K.V[2] and Vikram A[3]

[1]*Department of Life Sciences, Sikkim Manipal University, Syndicate House Manipal-576 104, India*
[2]*Centre for Postgraduate Studies, Sri Bhagawan Mahaveer Jain College, Jayanagar, Bangalore-560011, India*
[3]*Potato Development Centre, NB Department of Agriculture & Aquaculture, Wicklow, NB, Canada*

Introduction

Fusariumoxysporum f. sp. *lycopersici*, is a soil borne plant pathogenic fungus, causes Fusarium wilt specifically in tomato. This disease is of worldwide importance and is particularly severe in countries with warm climate (1).The fungus enters the host roots directly through penetration using hyphae and colonize the cortex by intracellular and intercellular growth. Once it reaches the vascular tissue, the pathogen spreads rapidly upward through the xylem vessels, provoking the characteristic wilt symptoms (2). During root penetration and host plant colonization, *F. oxysporum*secretes an array of enzymes such as pectatelyase, polygalacturonases and xylanases that may contribute to the degradation of the structural barriers constituted by plant cell walls (3, 4, 5, 6, 7, 8). Among these enzymes, Pectatelyase (PL) is most important in breaking the cell wall of host plant and brings about maceration of parenchymatous tissue (9).

Pectatelyases (PL, EC 4.2.2.2), otherwise known as pectatetranseliminases, catalyse the eliminative cleavage of de-esterified pectin, which is a major component of primary cell walls of many higher plants (10). The backbone of pectic polysaccharides is built from blocks of β-1,4 linked polygalactosyluronic acid residues interspersed with regions of alternating galactosyluronic acid and rhamnosyl residues (11). Cleavage of pectin by PL generates oligosaccharides with unsaturated galacturonosyl residues at their non-reducing ends.

The structural details of the enzyme are helpful in developing efficient inhibitors. Even though *F. oxysporum* is a dangerous plant pathogen and PL is one of the potential weapons used by this pathogen to invade the plant system, the structural details of PL are not available in www.rcsb.org.In the absence of experimental data on the structure of PL1 of *F. oxysporum*f.sp.*lycopersici*, homology modeling approach with its ability to derive its reasonable 3D structure based on sequence identity among various proteins of same class, offers reasonable alternative. By considering these points this work was conducted to develop the 3D structure of pectatelyase from *Fusariumoxysporum* f. sp. *Lycopersici*.

Materials and Methods

Sequence search and Analysis

Complete sequence information of PL enzyme (GenBank: AAC64368.1) of *Fusariumoxysporum* f. sp. *lycopersici*retrieved from NCBI (12) was submitted to PSI blast tool (http://www.ebi.ac.uk/Tools/blastpgp/). PSI-BLAST is a tool that produces a position-specific scoring matrix constructed from a multiple alignment of top scoring BLAST responses to a given query sequence. The degree of similarity is given in terms of a scoring parameter called the E-value (13).The conserved amino acid residues in PL protein and in the selected PDB templates were identified by submitting the sequence information to CLUSTAL W (14), a general purpose multiple sequence alignment program for DNA or proteins. All parameters were set at default values. This program produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequence and lines them up so that the identities, similarities and differences can be seen.

Homology modeling, Structure prediction and External Validation

Based on these data homology modeling was done using SWISS-MODEL homology-modeling server (15) (http://swissmodel.expasy.org/workspace).The predicted model for PL 1 of *F. oxysporum* f. sp. *lycopersici* was evaluated using additional structure assessment tools like PROCHECK (16) (Ramachandran plot analysis and G-value), Verify 3D (17), and WHAT_CHECK (18).

Results and Discussion

In the past, several studies have been conducted to understand the 3D structure and function of many proteins from *Fusarium* (19-21).However, 3D structure of PL from *F. oxysporum* f. sp. *lycopersici,* which is considered to be one of the potential weapons used by

* Corresponding Author, *Email*: manojkumar.nagasampige@smudde.edu.in, nagasampige@gmail.com

this pathogen to invade the plant system, has not been determined experimentally. In the present study the authors have attempted to predict the structure of PL. The results pertaining to the tertiary structure prediction of PL enzyme (GenBank: AAC64368.1) of *F. oxysporum* f. sp. *lycopersici* is presented and discussed in this chapter.

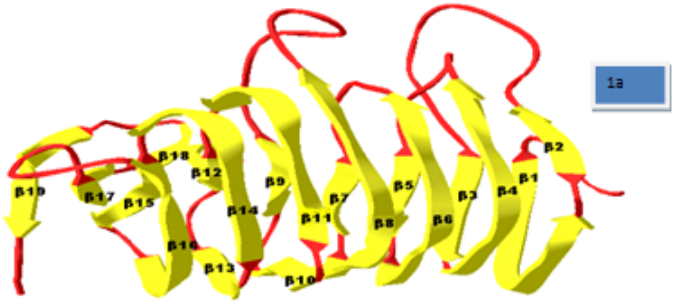
Template search using PSI blast

As the model accuracy is expected to increase with the use of multiple templates (22), the present study focused on generating PL models using several PDB template combinations. One of the main advantages of comparative modeling program is that it can combine information from multiple template structures in two ways. In the first way multiple template structures may be aligned with different domains of the target with little overlap between them wherein the modeling procedure can construct a homology based model of the whole target sequence. In the second waySecond the template structures may be aligned with same part of the targetand themodeling procedure is likely to automatically select the best template (sali).The search for potential templates of PL enzyme (GenBank: AAC64368.1) from*Fusariumoxysporum* f. sp. *lycopersici*using PSI blast tool revealed many PDB templates sharing similar sequence homology. Three of these templates having high sequence identity and better E valuesthan threshold were selected for generating 3D model (Table 1).

Sequence alignment and Model Building

Multiplesequence alignment ofPL using CLUSTAL W tool revealed that Leu^{44},Ala^{48},Asn^{52},Ile^{55},Ala^{57}, Asn^{75},Val^{76},Trp^{78},Asp^{80},Glu^{83},Asp^{84},Ala^{85},Thr^{87},Cys^{71},T hr^{72},Cys^{66},His^{65},Ala^{63},Ala^{57},Ile^{55},Asn^{52},Ala^{48},Leu^{44},Asn^{11} ^{1},Gln^{109},Lys^{106},Asp^{98},Ala^{96},Ala^{93},Gly^{92},Gly^{91},Ile^{89},Thr^{86}, Gly^{169},Gly^{135}, Arg^{132},Lys^{130} andGly^{129} were completely conserved in all the three PDB templates..... 3D models of PL enzyme (GenBank: AAC64368.1) of *Fusariumoxysporum* f. sp. *lycopersici*which were first obtained using different multiple template combinations from the selected three templates. However, only the template 3B4N_A was chosen for model building as it had the lowest E value (2e-7)(Table-1).

Fig.1: a. Clustal W analysis of pectatelyase sequence with other related sequence; b. Homology modeledpectatelyase from*Fusariumoxysporum* f. sp. *Lycopersici*

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pectate_lyase_Fusarium      ----------SAGATSFPTAVPVKGSYDGGMGRFERSPSVCQGQSETGE 39
1EE6_A                      --------------------VPAGQTFDGKGQTYVANPNTLGDGSQAEN 29
3B4N_A                      SNAVTTAPNAGPLRAAKASSECKPGATFENRTVDCGGVTIGTSCPNDSDK 50
3B90_A                      ------------------SECKPGATFENRTVDCGGVTIGTSCPNDSDK 31
                                               :::.        .      .:: :

pectate_lyase_Fusarium      KDAMFILENGATLSNVIIGAS-QAEGVHCK-GTCTLNNVWWADVCEDAVT 87
1EE6_A                      QKPIFRLEAGASLKNVVIGAP-AADGVHCY-GDCTITNVIWEDVGEDALT 77
3B4N_A                      QKPLIILKN-ATVKNLRISASGGADGIHCDSGNCTIENVIWEDICEDAAT 99
3B90_A                      QKPLIILKN-ATVKNLRISASGGADGIHCDSGNCTIENVIWEDICEDAAT 80
                            :..:: *:  *::.*: *.*.  *:*:**  * **: ** * *: *** *

pectate_lyase_Fusarium      LKQTSGTSYINGGGAFHASD-------KIVQFNGRGTVQIKD---FYAED 127
1EE6_A                      LK-SSGTVNISGGAAYKAYD-------KVFQINAAGTINIRN---FRADD 116
3B4N_A                      NN--GKTMTIVGGIAHNAKDGYGGKPDKVLQHNSKNSTTVVKGNFTLTGE 147
3B90_A                      NN--GKTMTIVGGIAHNAKDGYGGKPDKVLQHNSKNSTTVVKGNFTLTGE 128
                             :  . *  * ** *.:* *       *:.* *. .:  : .     : :

pectate_lyase_Fusarium      YGKLVRSCGNCKDNGGPRNVVISGSVA---VDGGVLCGINTNYGDTCKIT 174
1EE6_A                      IGKLVRQNG-----GTTYKVVMM-------VEN---CNISR-VKDAILRT 150
3B4N_A                      HGKLWRSCGDCSNNGGPRFLTVTSATVNGTIDS--IAGVNRNYGDVATIS 195
3B90_A                      HGKLWRSCGDCSNNGGPRFLTVTSATVNGTIDS--IAGVNRNYGDVATIS 176
                             *** *. *     * .  :.:.       ::.   ..:.    *.   :

pectate_lyase_Fusarium      -----SSCQNKGKYCDRYEGNSSG-AEPSKIGSGPDGKYCTTS 211
1EE6_A                      -----DSSTSTGRIVN--------------------TRYSNV- 167
3B4N_A                      GLKIKNYKEGKPPVCEEFKGVVKGQGSTEKYGEKWDTTNCKVS 238
3B90_A                      GLKIKNYKEGKPPVCEEFKGVVKGQGSTEKYGEKWDTTNCKVS 219
                                 .   ..    :                    ...
```

Table 1: Summary of the template sequence profile that was generated at the end of 20th iteration of PSI blast analysis using Pectatelyase (AAC64368) of *Fusariumoxysporum* f. sp. *lycopersici*as query. Threshold PSI blast E-value = 0.001

Sequences with pattern at position and E-value BETTER than threshold		
Template	Score	E- value
pdb|3B4N|A	261	2e-70
pdb|3B90|A	288	2e-60
pdb|1EE6|A	206	5e-54
Sequences with E-value WORSE than threshold		
pdb|2OJU|A	30.1	0.80
pdb|1WCQ|A	27.8	3.8
pdb|1EUT|A	27.8	3.8
pdb|1W8N|A	27.8	4.1
pdb|1QQE|A	27.4	4.2
pdb|2BER|A	27.4	4.5
pdb|2QE7|D	27.4	5.3
pdb|1EUR|A	27.4	5.3
pdb|2BZD|A	27.0	5.8
pdb|1EGZ|A	27.0	6.1

External validation of tertiary structure

Validation of the predicted 3D neurotoxin structures (after loop refinement) by PROCHECK analysis showed that 73.8 % of the residues of PL model were present in the most favoured region followed by 18.6 % in the allowed region, 4.7 % in generously allowed region and 2.9 % in disallowed region of Ramachandran plot. However, Ramachandran plot analysis of the PDB template, 3B4N_A showed that 82.9 % of the residues were present in the most favored region followed by 15.5 % of residues in additional disallowed region, 1.6% in generously allowed region and 0.0 % in disallowed region of Ramachandran plot.

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