View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by AJOL - African Journals Online

http://www.ajol.info/index.php/jrfwe
jfewr ©2019 - jfewr Publications
E-mail:jfewr@yahoo.com
ISBN: 2141 – 1778
Nwogwugwu et al., 2019

This work is licensed under a Creative Commons Attribution

IN SILICO SEQUENCE ANALYSIS, HOMOLOGY MODELING AND FUNCTIONAL ANNOTATION OF PECTATE LYASE ENZYME FROM *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.

Nwogwugwu J. O. [1] Anicet A. Batcho O. [2], Osunlaja A.[1]

[1]Pathology Section, Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria, P.M.B 5054, Jericho, Ibadan, Oyo State, Nigeria
[2]Faculty of Agriculture & Environmental Sciences, Catholic University of the West Africa, Cotonou, Benin
Corresponding author email**: *anicetbatcho1@gmail.com;** 09037432621

ABSTRACT

Protein structure is more evolutionary conserved than a DNA sequence. To gain more insight into the molecular mechanisms of Colletotrichum gloeosporioides pathogenesis, we analyzed a pectate lyase gene sequence at molecular level using bioinformatics approaches. We evaluated the sequence information of pectate lyase enzyme retrieved from NCBI database. We also interpreted its homology modeling, functional annotation. Based on homology modeling, three dimensional (3D) structure of the gene was constructed and interpreted. Several validation tests were computed to check the reliability of 3D structure. We found conserved domains in pectate lyase protein. These conserved domains have significant role for plant pathogens that use a set of pectate lyases as their main virulence factor and to initiate the symbiosis activity in different organism. The study has clear implications to annotate the role of pectate lyase gene and linked proteins associated. More insights into the structure of the gene will lead to annotate the role of this gene in different biological pathways.

Keywords: Pectate lyase, *Colletotrichum gloeosporioides*, sequence information, bioinformatics tools

INTRODUCTION

The genus Colletotrichum represents a group of destructive pathogenic fungi causing a variety of diseases on crop plants (Kanto *et al*. 2014, Sharma *et al*. 2014). They are implicated in the anthracnose disease of many plant genera (Liao *et al*. 2012); a disease symptom marked with characteristic sunken necrotic lesions which could be on any part of the plant and the seedling blight and rot of plant's parts (Diao *et al*. 2015, Agrios 2005). The pathogenesis of this fungus offers it an advantage to be widely distributed and able to infect almost plant species as both wind and rain favors the dispersal of the primary inoculum and its ability to overwinter as saprobes on organic matter and weed species. It has assumed the top ten positions in the list of most important fungi in a recent survey by plant pathologists due to its scientific importance and pathogenic interactions due to its diversity as well as the economic implications on many crop plants (Dean *et al*; Crouch, 2012). About 25 plant diseases has been reported to be caused by different species of *Colletotrichum* in India namely, *C. gloeosporioides*, *C. capsici*, *C. falcatum*, *C. truncatum*, *C. sansevieriae*, *C.acutatum* and *C. coccodes*, *C. gloeosporioides were* found more prevalent anthracnose pathogen (Gautam, 2014). The genus employs diverse mechanisms for colonizing host tissues, surviving both as intracellular hemibiotroph to necrotroph but can also have endophytic or saprobic lifestyles (Jayawardena *et al*., 2016). The pathogens develop a series of specific infection structures, including germ tube, appressoria, intracellular hyphae, and secondary necrotrophic hyphae. *Colletotrichum* species are economically important worldwide; the fungi are highly significant for experimental studies of fungal development, infection processes, host resistance, signal transduction, and the molecular biology of plant pathogen interactions (Gautam, 2014). One of the mechanisms employed by microorganisms to overcome host defenses for plant invasion and

nutrition is by the production of cell wall degrading enzymes and among these are the pectin lyase groups (Lara –Marquez *et al*., 2011). This family shares a conserved structure in a parallel β-helix and has been well characterized in many opportunistic fungi such as *Aspergillus* and *Penicillium* and in the pathogen, *C. gloeosporioides.*

Expression of pectate lyase (pl) from *Colletotrichum gloeosporioides* has been reported to promote fungal pathogenicity on hosts (Yakoby *et al*., 2000). Pectate lyase gene is reported as a pathogenic factor required for the penetration and colonization of host tissues by *Colletotrichum* species. The degradative enzymes of plant cell wall such as pectate lyase are increased during the necrotrophic phase of these species (Medeiros LV, *et al*., 2010). Several molecular studies have identified pectate lyase gene as a virulence factor and its expression being strongly affected by alkalization. But little work was done to study its 3D structure. In this study we try to understand the pathogenic pathways of the ubiquitous and economic important pathogen, *Colletotrichum gloeosporioides* using latest bioinformatics tools to describe functional annotation of pectate lyase based on homology modeling which may help to proffering safe management options against the pathogen.

MATERIALS AND METHODS

Sequence Analysis

The amino acid sequences of pectate lyase gene were retrieved from NCBI data base using primary accession name (http://www.ncbi.nlm.nih.gov/). About 332 amino acid sequences were used for sequence analysis.

Homology Modeling of *C. gloeosporioides*

NCBI BLAST tool with non-redundant database was used to search a similarity-based sequence of pectate lyase gene (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*).

The raw sequences pectate lyase was translated to amino acid sequences using ExPASy translate tool (http://web.expasy.org/translate). The translated sequence was then processed with Swiss-Model tool (*http://swissmodel.expasy.org*) to build 3D structure. The obtained 3D structure was further functionally annotated.

Functional Annotation

The pectate lyase was analyzed for the presence or absence of conserved domains; NCBI Conserved Domains Database (NCBI-CDD) (Marchler *et al*., 2011), Protein families' database (Pfam) (Aranda *et al*., 2010) and InterProScan (Zdobbnov and Apweiler, 2001). NCBI-CDD is a tool for protein annotation having a huge data of multiple sequence alignment to study full length proteins. Pfam database uses Markov models to annotate multiple sequence alignment while InterProScan combines signatures of different proteins native.

RESULTS

The present study describes homology modeling and functional annotation of pectate lyase gene in *C. gloeosporioides*using different bioinformatics tools.

Homology Modeling

Protein 3D structure was constructed using Swiss model (http://swissmodel.expasy.org/) and SURFNET program (Fig.1). The SURFNET program generates surfaces and void regions between surfaces from coordinate data supplied in a PDB file. A total of 218 templates were found to match the target sequence. This list was filtered by heuristic down to 50 and the top templates are: 3vmw.1A; 1bn8.1A; 2bsp1.A; 5amv.1A and 3krg1A. Sequence alignment was performed to build a reliable 3D structure using BLASTP software. Both template and query sequence were aligned using algorithms of multiple sequence alignment. The sequence homology of pectate lyase of *C. gloeosporioides*.

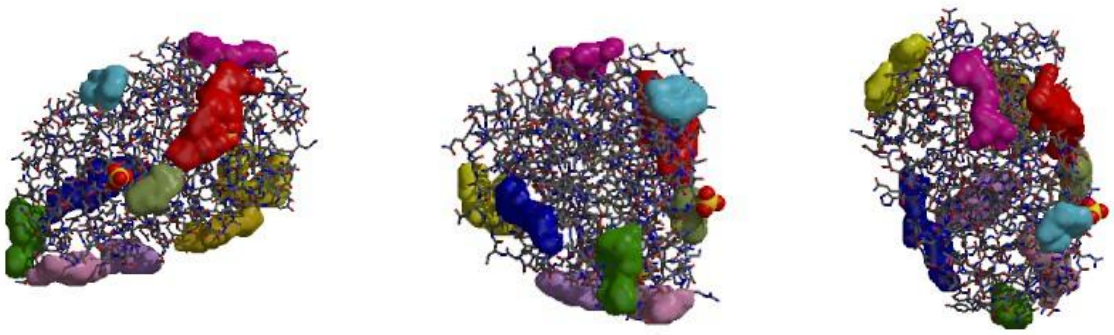
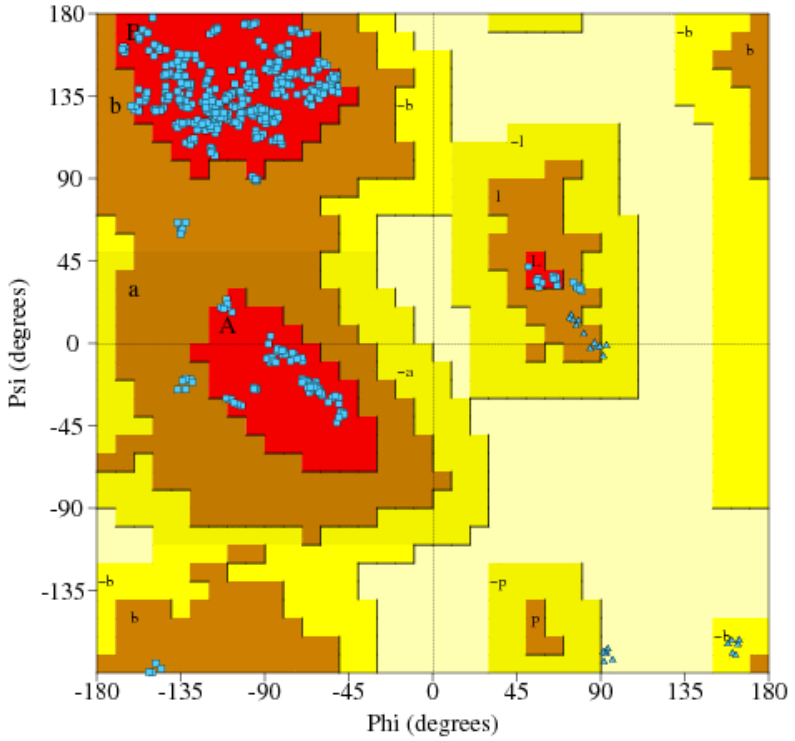


Fig. 1: Predicted 3D structure of pectate lyase and Cleft analysis in PDBSum using SURFNET Program

Functional Annotation

The reliability of *C. gloeosporioides* 3D model was confirmed using Ramachandran plot. The point at which charge on protein becomes zero is called an isoelectric point (PI). The ratio of PI/Mw was estimated by using PROCHECK (*http://www.ebi.ac.uk/thorntonsrv/software/PROCHECK*). The same tool (PROCHECK) was used to construct Ramachandran plot (Fig.2). ProSA was used to calculate Z-score; an indication of the overall model quality and is used to observe whether the input structure lies within the range of scores spotted for native proteins of similar size or not. Z-score for model protein was -4.38 (Fig.3).

The validation of model protein was performed by Ramachandran plot, Ramachandran plot showed 94.2% residues in the most favored region (Fig.2). ProSA-web and ERRAT. PDBsum used Ramachandran plot to check stereochemical quality of 3D structure of template using PROCHECK that analyze residue by residue geometry and overall geometry of the structure. ProSA provides Z-score, an indicative of the overall model quality and is used to observe whether the input structure lies within the range of scores spotted for native proteins of similar size or not. Z-score for model protein was -4.38 indicating that protein is of good quality (Fig.3). The 3D model of pectate lyase revealed different types of structure: Alpha helices, mostly found in cell membranes and play important role in transport, Beta plates sheets which have intracellular localization and Interlopes domain to connect different domains and structures (Fig.4)



		No. of residues	%-tage
Most favoured regions	[A,B,L]	475	94.2%
Additional allowed regions	[a,b,l,p]	29	5.8%
Generously allowed regions	[~a,~b,~l,~p]	0	0.0%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		504	100.0%
End-residues (excl. Gly and Pro)		12	
Glycine residues		24	
Proline residues		24	
Total number of residues		564	

Fig. 2: Structural validation of protein structure via Ramachandran plot

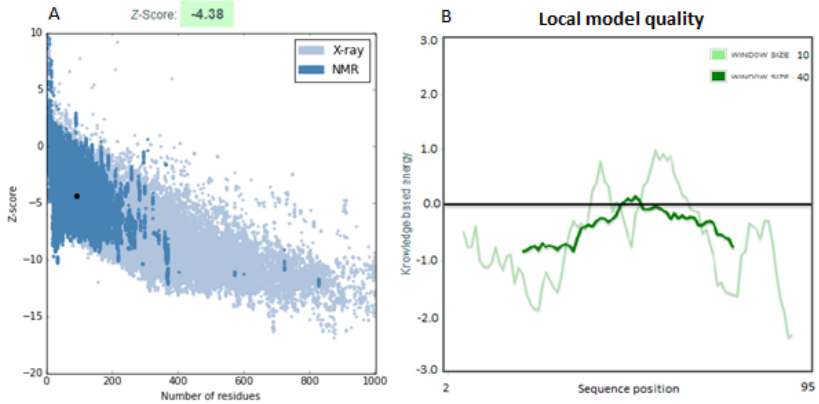
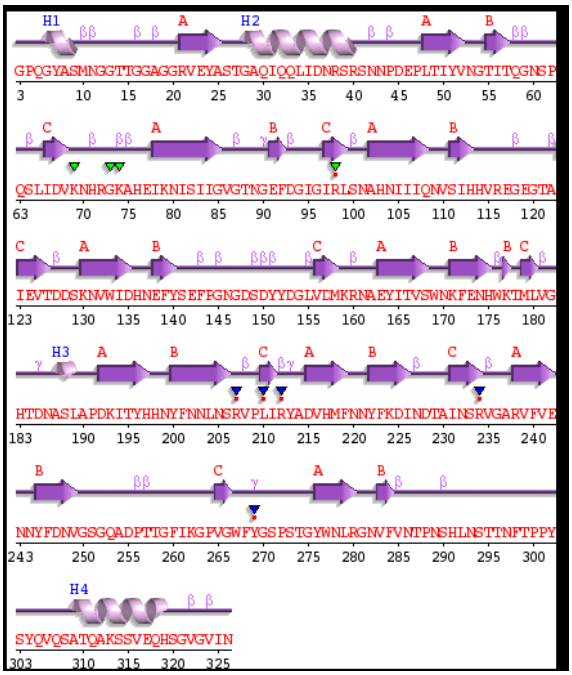


Fig. 3: Z-score of model protein and local model quality determination of pectate lyase 3D structure using ProSA-web



Helices labelled H1, H2, ... and strands by their sheets A, B, ...

Helix Strand

Motifs: β beta turn γ gamma turn

Residue contacts: to ligand

PDB SITE records: AC1 AC2

Fig 4: Secondary structure of protein containing 3 beta sheets, 3 beta hairpins, 2 beta bulges, 8 beta turns, 8 strands and 2 helices generated using PDBsum.

DISCUSSION

Microorganisms produce plant cell wall-degrading enzymes as part of their strategies for plant invasion or plant degradation. Pectinolytic enzymes consist of four classes of enzymes: pectin lyase, polygalacturonase, pectin methylesterase and rhamnogalacturonase. Among pectinolytic enzymes, pectin lyase is the most important in depolymerization of pectin, since it cleaves internal glycosidic bonds of highly methylated pectins, favors pectate, the anion, over pectin, the methyl ester. Many scientists studied the genetic basis of pectic enzymes and its crucial role in microbial plant biomass degradation, such as the saprotrophic/opportunistic *Aspergillus niger* (Kusters-van Someren*et al*., 1991; Kusters-van Someren *et al*., 1992; Harmsen *et al*., 1990; Gysler*et al*., 1990), *A. oryzae* (Kitamoto *et al*., 2001), *P. occitanis* (Trigui-Lahiani and Gargouri, 2007), and the phytopathogenic fungi *Colletotrichium gloeosporioides* (Wei *et al*., 2002). Evidence of the importance of pectate lyase secretion during *Colletotrichum* colonization on fruits has been found in a number of studies: (i) reduced symptom development of a *C. magna* mutant (Wattad et al. 1995); (ii) inhibition of decay following co-inoculation of *C. gloeosporioides* spores (Wattad et al. 1997); (iii) the activity inhibition of pectolytic enzymes by the host flavonoid epicatechin, which correlated with the inhibition of symptom development (Wattad *et al*. 1994); and (iv) a lack of decay development under conditions that are not permissive to pectate lyase secretion (Yakoby *et al*. 2001). These results suggest that pectate lyase is a limiting factor during the early stages of pathogenesis (Wattad *et al*. 1997; Yakoby *et al*. 2000). The present study describes an organized workflow using latest bioinformatics tools to functionally annotate the pectate lyase gene based on homology modeling from *Colletotrichum gloeosporioides*. The functional annotation of pectate lyase was revealed using different web-based tools: NCBI-CDD (*http://www.ncbi.nlm.nih.gov/cdd),* Swiss-model (*http://swissmodel.expasyorg/*).

CONCLUSION

In the current study, we have interpreted the results of homology modeling, functional

annotation of pectate lyase enzyme, and its associated proteins using latest bioinformatics approaches. Conserved domains were found in amino acid sequence of the pectate lyase sequence. These conserved domains have significant role in promoting fungal pathogenicity on hosts of pectate. However, further bioinformatics analysis is obligatory to explain the structural image and functions of these genes and linked proteins in detail.

REFERENCES

Agrios, G. N. (2005). Plant Pathology. 5th Ed. San Diego: Academic Press, USA.

Aranda B.,Achuthan, P., Alam-Faruque, Y., Armean, I.*et al*. (2010). The IntAct molecular interaction database in 2010. *Nucleic Acids Research.* 38:D525–531. Available from: *https://www.researchgate.net/publication/38030302_The_IntAct_molecular_Interaction_dabase_in_2010* [accessed Jul 16 2019].

Crouch, J. A. (2014). *Colletotrichum caudatum*s.l. is a species complex. IMA *Fungus* 5: 17–30.

Dean, R., Van-Kan, J. A. L., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Pietro, D. S., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J. and Foster, G. D. (2012). The Top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* 13: 414–430.

Diao, Y. Z., Zhang, C., Xu, J. P., Lin, D., Liu, L., Mtung'e, O. G. and Liu, X. (2015). Genetic differentiation and recombination among geographic populations of the fungal pathogen *Colletotrichum truncatum* from chili peppers in China. *Evolutionary Applications* 8: 108–118.

Gautam, A.K. (2014). The genera *Colletotrichum*: an incitant of numerous new plant diseases in India. *Journal on New Biological Reports* 3(1): 09 – 21.

Gysler, C., Harmsen, J. A., Kester, H. C., Visser, J. and Heim, J. (1990). Isolation and structure of the pectin lyase D-encoding gene from *Aspergillus niger*. *Gene*. 89:101–108.

Harmsen, J. A., Kusters-van Someren, M. A. andVisser, J. (1990): Cloning and expression of a second *Aspergillus niger* pectin lyase gene (pelA): indications of a pectin lyase gene family in *A. niger*. *Curr Genet*. 18:161–166.

Jayawardena, R. S., Hyde, K. D., Damm, U., Cai, L., Liu, M., Li, X. H., Zhang, W., Zhao, W. S. and Yan, J. Y. (2016). Notes on currently accepted species of *Colletotrichum*. *Mycosphere* 7(8): 1192–1260.

Kanto, T., Uematsu, S., Tsukamoto, T., Moriwaki, J., Yamagishi, N., Usami, T. and Sato, T. (2014). Anthracnose of sweet pepper caused by *Colletotrichum scovillei* in Japan. *Journal of General Plant Pathology* 80(1): 73–78.

Kitamoto, N., Yoshino-Yasuda, S., Ohmiya, K. and Tsukagoshi, N. (2001). A second pectin lyase gene (pel2) from *Aspergillus oryzae* KBN616: its sequence analysis and overexpression, and characterization of the gene products. *J Biosci Bioeng*. 91: 378–381.

Kusters-van Someren, M. A., Harmsen, J. A. M., Kester, H. C. M. andVisser, J.(1991). Structure of the *Aspergillus niger*pelA gene and its expression in *Aspergillus niger* and *Aspergillus nidulans*. *Curr Genet*. 20:293–299.

Kusters-van Someren, M. A., Flipphi, M., de Graaff, L., van den Broeck, H., Kester, H., Hinnen. A., *et al*. (1992). Characterization of the *Aspergillus niger*pelB gene: structure and regulation of expression. *Mol Gen Genet*. 234:113–120.

Lara-Marquez, A., Zavala-Paramo, M. G., Lopez-Romero, E., Calderon-Cortes, N., Lopez-Gomez, R., Conejo-Saucedo, U. and Cano-Camacho, H. (2011). Cloning and characterization of a pectin lyase gene from *Colletotrichum lindemuthianum* and comparative phylogenetic/structural analyses with genes from phytopathogenic and saprophytic/opportunistic microorganisms. *BMC Microbiol*. 11: 260. Doi: 10.1186/1471-2180-11-260.

Liao, C. Y., Chen, M. Y., Chen, Y. K., Wang, T. C., Sheu, Z. M., Kuo, K. C., Chang, P. L., Chung, K. R. and Lee, M. H. (2012). Characterization of three *Colletotrichum acutatum* isolates from *Capsicum spp*. *European Journal of Plant Pathology* 133: 599–608.

Marchler-Bauer, A., Lu, S., Anderson, J. B., Chitsaz, F., Derbyshire, M. K., DeWeese-Scott, C., Fong, J. H., Geer, L. Y., Geer, R. C., Gonzales, N. R., Gwadz, M., Hurwitz, D. I., Jackson, J. D., Ke, Z., Lanczycki, C. J., Lu, F., Marchler, G. H., Mullokandov, M., Omelchenko, M. V., Robertson, C. L., Song, J. S., Thanki, N., Yamashita, R.

A., Zhang, D., Zhang, N., Zheng, C. and Bryant, S. H. (2011). CDD: a Conserved Domain Database for the functional annotation of proteins. *Nucleic Acids Research*. 39: D225-D229.

Medeiros, L. V., Maciel, D. B., Medeiros, V. V., Houllou-Kido, L. M. and Oliveira, N. T. (2010). pelB gene in isolates of *Colletotrichum gloeosporioides* from several hosts. *Genet Mol Res* 9: 661–673.

Sharma, G., Kumar, P. A. and Damodara, S. B. (2014). Infra-specific diversity of *Colletotrichum truncatum* associated with chilli anthracnose in India based on microsatellite marker analysis. *Archives of Phytopathology and Plant Protection* 47: 2509–2523.

Trigui-Lahiani, H. and Gargouri, A. (2007). Cloning, genomic organisation and mRNA expression of a pectin lyase gene from a mutant strain of *Penicillium occitanis*. *Gene*. 388: 54–60.

Wattad, C., Dinoor, A., and Prusky, D. (1994). Purification of pectate lyase produced by *Colletotrichum gloeosporioides* and its inhibition by epicatechin: A possible factor involved in the resistance of unripe avocado fruits to anthracnose. *Mol. Plant-Microbe Interact*. 7:293-297.

Wattad, C., Freeman, S., Dinoor, A., and Prusky, D. (1995). A nonpathogenic mutant of *Colletotrichum magna* is deficient in extracellular secretion of pectate lyase. *Mol. Plant-Microbe Interact*. 8:621-626.

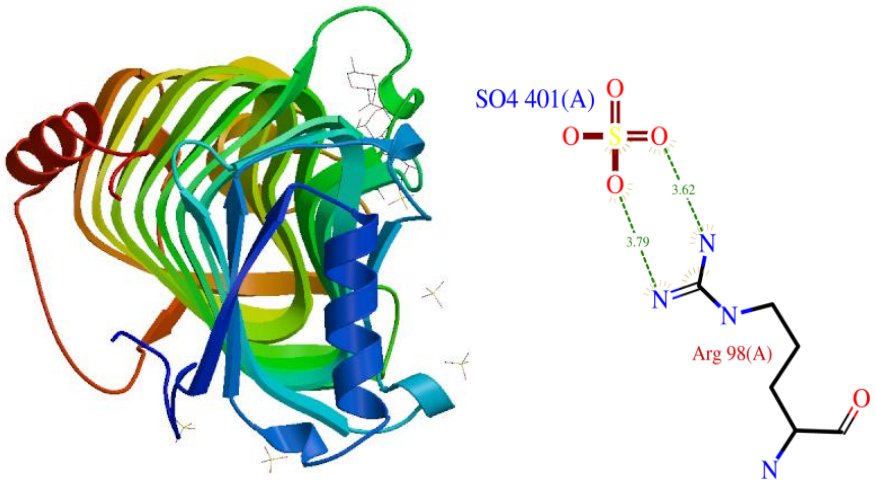
Wattad, C., Kobiler, D., Dinoor, A., and Prusky, D. (1997). Pectate lyase of *Colletotrichum gloeosporioides* attacking avocado fruits: cDNA cloning and involvement in pathogenicity. *Physiol. Mol. Pathol*. 50:197-212.

Wei, Y., Shih, J., Li, J. and Goodwin, P. H. (2002). Two pectin lyase genes, pnl-1 and pnl-2, from *Colletotrichum gloeosporioides* f. sp. *malvae* differ in a cellulose-binding domain and in their expression during infection of *Malvapusilla*. *Microbiol Read Engl*. 148:2149–2157.

Yakoby, N., Kobiler, I., Dinoor, A., and Prusky, D. (2000). pH regulation of pectate lyase secretion modulates the attack of *Colletotrichum gloeosporioides* on avocado fruits. *Appl. Environ. Microbiol*. 66: 1026-1030.

Zdobnov, E. M., Apweiler, R. (2001). InterProScan--an integration platform for the signature-recognition methods in InterPro. *Bioinformatics*.17(9): 847-848.

APPENDIX



Ligand SO4 - Sulfate ion