Spring 5-2019

Fusarium Euwallacea: A Serious Threat to the Native and Ornamental Trees and Shrubs in Southern California

Greg Tyler
Chapman University, gtyler@chapman.edu

Yixing Zheng
Chapman University, zheng129@mail.chapman.edu

Michael Kulinich
Chapman University, kulinich@chapman.edu

Hagop Atamian
Chapman University, atamian@chapman.edu

Fusarium Euwallacea: A Serious Threat to the Native and Ornamental Trees and Shrubs in Southern California

Gregory Tyler, Yixing Zheng, Michael Kulinich, and Hagop S Atamian

CHAPMAN UNIVERSITY

Introduction

Fusarium euwallacea is a fungus that has established symbiotic relationship with the beetle *Euwallacea aff. fornicata*. The beetle bores through the tree bark and into the sapwood making long tunnels in which it spreads the fungus[1]. The beetle feeds on the *F. euwallacea* that grows in these tunnels to survive and reproduce. However, the growth of the fungus is detrimental to the plant as it obstructs water and mineral transport in the plant xylem tissue, resulting in dieback, wilt and mortality of the host tree[1]. Fungi, similar to bacteria, are well known to secrete specialized proteins called effectors into the plant cells to suppress plant immune responses and create a favorable environment for growth and reproduction[2]. The *E. fornicata* beetle and the *F. euwallacea* fungus attack more than 200 plant species. The dieback disease caused by this beetle-fungus complex is emerging as a serious threat to the Southern California's landscape tree species as well as agricultural crops such as avocado, citrus, and olive[3]. The goal of this project is to computationally predict and annotate the candidate effector proteins secreted by *F. euwallacea*. Better understanding of the overall infection process and the role of these individual effectors could help us device new strategies to combat this devastating pathogen.

Methods

•The whole genome shotgun sequences of *F. euwallacea* were downloaded from NCBI and subjected to *de novo* gene prediction using the AUGUSTUS software[4].

•The gene sequences of two closely related species, *F. graminearum* and *F. oxysporum,* were previously predicted and available for download from NCBI.

•The effector proteins from the three species were predicted using the EffectorP 2.0 software[5].

•Orthologous protein sequence families were identified using the OrthoFinder program[6].

• Venn diagram was constructed using Venny 2.1[7]

• Multiple sequence alignments were performed using multalin[8]

Overview of fungal effectors

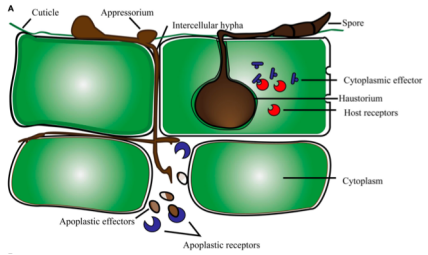

Figure 1: Overview of fungal effector proteins[9]. After penetrating the plant cell, the fungal haustoria release effectors (blue) which interact with the host receptors (red). The intercellular hyphae also release effectors in the apoplast (brown) which interact with the apoplastic receptors.

The number of predicted protein coding genes in three Fusarium species

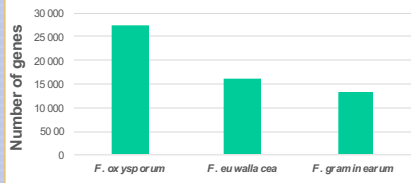

Figure 3: Prediction of the protein coding genes. The y-axis shows the number of proteins predicted and x-axis shows the three fusarium species used in this study.

Key findings

•The *F. Euwallacea* genome is predicted to code for 1297 putative effector proteins compared to 1802 in *F. graminerium* and 3451 in *F. oxysporum.*

•Around 500 predicted effector proteins in *F. Euwallacea* were not assigned to orthologs in the other two closely related fungal species within the genus fusarium. This suggests that some of these proteins could be unique to *F. Euwallacea* and play important roles in it's pathogenicity against wide-range of plant species.

Future directions

- Test the expression of few candidate effector proteins during the infection process using quantitative real-time PCR (qPCR).
- Generate tag fused versions of the most promising effector proteins and identify the target plant proteins that they bind to during the infection process.

Prediction of effector proteins from three fungal pathogens

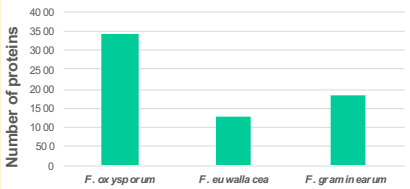

Figure 3: Prediction of effector proteins. The y-axis shows the number of proteins predicted to be effectors and x-axis shows the three fusarium species used in this study.

Orthologous relationship of *F. euwallacea* effectors

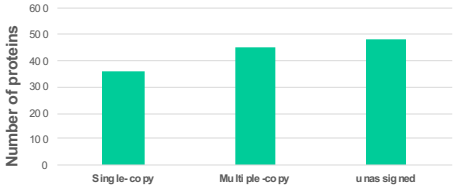

Figure 4: Orthlogous relationship of the predicted *F. euwallaceae* effector proteins with respect to the other fusarium species. Unassigned category refers to proteins that do not show similarity to proteins in the other fusarium species

Overall Statistics

Number of genes	6550
Number of genes in orthogroups	2857
Number of unassigned genes	3693
Percentage of genes in orthogroups	43.6
Percentage of unassigned genes	56.4
Number of orthogroups	980
Number of species-specific orthogroups	0
Mean orthogroup size	2.9
Number of orthogroups with all species present	501
Number of single-copy orthogroups	359

Phylogenetic analysis

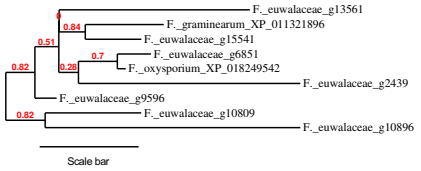

Figure 5: Maximum-likelihood tree showing the phylogenetic relationship among effector sequences from the three fusarium species. Bootstrap values for 100 replicates are indicated in red. Scale bar: number of substitution per site. Members clustered together have a higher probability of serving the same or similar biological functions.

Acknowledgements

Supported by NSF grant# 1758857.

References

1.Freeman S. et al., 2013. Mycologia, 105: 1595–1606. DOI: 10.3852/13-066
2.Presti L. L. et al., 2015. Annu. Rev. Plant Biol. 66:513–45. doi: 10.1146/annurev-arplant-043014-114623.
3.Eskalen, A. et al., 2013. http://sustainability.uci.edu/wp-content/uploads/sites/5/2015/09/01_Eskalen_CurrentStatusPSHB_-FusariumDieback.pdf.
4.Stanke, M. et al., 2006. Nucleic Acids Resources, 34:435–439. doi: 10.1093/nar/gkl200.
5.Sperschneider, J. et al., 2018. Molecular Plant Pathology 19:2094-2110. doi: 10.1111/mpp.12682.
6.Emms, D. & Kelly, S. 2005. Genome Biology 16:157. DOI 10.1186/s13059-015-0721-2
7.Oliveros, J.C. (2007) http://bioinfogp.cnb.csic.es/tools/venny/index.html
8.Corpet, F. 1988. Nucleic Acids Resources 16, 10881-10890
9.Sonah H. et al, 2016. Frontiers in Plant Science doi.org/ 10.3389/fpls.2016.00126