2015-04-519

Detection and disruption of virulence factors associated with *Ophiosphaerella* spp., the causal agents of spring dead spot of bermudagrass

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Start date: 2015
Project duration: 4 years
Total funding: $61,496

Objectives:

1. To produce a genome sequence of *Ophiosphaerella korrae*.
2. To isolate and identify compound(s) secreted by fungi that elicit a necrotic host response.
3. Use a bioinformatics approach to identify the gene(s) that produce the secreted compounds and compare host responses to infection and colonization by wild-type and gene disrupted isolates.

Summary points:

- The genomes (DNA) of three *Ophiosphaerella herpotricha*, five *O. korrae*, and three *O. narmari* isolates were sequenced.
- The transcriptomes (RNA) of two isolates of each species were obtained.
- Protein coding genes of one *O. herpotricha* isolate was validated by mass spectrometry.
- These results include the identification of putative genes of *Ophiosphaerella* involved in pathogenicity and in root cell necrosis by using bioinformatics tools.
- Current efforts are ongoing to identify protein coding genes secreted by the fungi when infecting and colonizing resistant and susceptible hosts.

Summary text:
Bermudagrass is a perennial warm-season grass cultivated extensively in the southern United States. Spring dead spot (SDS) is considered the most devastating disease of bermudagrass where bermudagrass goes dormant in the winter. One of the long-term goals of the Oklahoma State University Bermudagrass Breeding Program is to develop bermudagrass cultivars with resistance to SDS. To achieve this goal the interaction of SDS pathogens and bermudagrass must be understood and much is still unknown. It was previously observed that the same isolate of *Ophiosphaerella* can switch from a disease-causing lifestyle (necrotrophic lifestyle) in susceptible cultivars to an endophytic/symbiotic lifestyle (non-disease) in a resistant cultivar. To continue these investigations, efforts in this research project are focused in identifying the genetics of how *Ophiosphaerella* causes disease and induces root cell necrosis in susceptible bermudagrass cultivars.

The genomes (DNA) of 11 isolates and the transcriptome (RNA) of 6 isolates of *Ophiosphaerella* were sequenced. The sequencing platforms used were Illumina and PacBio for the genome sequencing, and Illumina for transcriptome sequencing. By using bioinformatics tools, the genomes were assembled,

assembly completeness was assessed, the transcriptomes were used as evidence for gene prediction, and then the genes were translated into proteins (protein coding genes, PCG) (Table 1). The function of the PCG of each isolate was predicted by using several bioinformatics tools and/or database searches that have been used in other scientific studies because they provide tailored functions related to pathogenicity and plant-pathogen interactions (Table 2).

In average, less than 30% of the eleven PCG sets had relevant plant-pathogen interaction database matches. The majority of the matches were in categories for a potential role in pathogenicity and virulence. Some of these results were duplicated due to disagreement in the literature/databases for the function of a particular gene. It was also found that approximately 40 genes in *Ophiosphaerella* spp. are potentially involved in developing plant avirulence (stopping infection due to cell death).

The PCG obtained by bioinformatics tools were validated by mass spectrometry. An isolate of *O. herpotricha* was cultivated in liquid media for 15 days in the laboratory. The proteins of the fungal mycelium were extracted and four samples were submitted for mass spectrometry analysis at the OSU Biochemistry and Molecular Biology Core Facility. Each sample was treated with Trypsin (digestion of proteins into peptides) prior to scanning in Orbitrap and Fusion mass spectrometers (Figure 1). The peptides obtained by those machines were compared to the predicted PCG of *O. herpotricha* using MaxQuant software and custom Python scripts. The PCGs that had at least two peptides identified by mass spectrometry were considered validated.

A total of 594 PCG were identified in the Orbitrap, whereas 2,884 PCG were identified by the Fusion spectrometer. The PCGs validated by the Fusion included all PCG validated by the Orbitrap. All PCG validated by the Fusion, were searched against the proteins in the PHI database for determining hypothetical function. There were 1,974 PCG that did not match to any proteins relevant to plant-pathogen interactions, and 431 yielded mixed hypothetical functions (literature disagrees on the ultimate function). There were 472 genes with hypothetical role in disease and seven genes with hypothetical role in plant avirulence.

Concluding remarks:
Potential genes present in the pathogen’s genome that are involved in developing plant avirulence have been identified. One potential gene involved in symbiosis/endophytic lifestyle was also found. These will be investigated further as the tools and databases used are not suited for endophytic interactions. Also, to validate these findings current efforts are ongoing to identify protein coding genes secreted by the fungi when infecting and colonizing resistant and susceptible hosts. Another piece of this puzzle is to study the PCG of the plant. Recently the genomes and transcriptomes of a susceptible cultivar and of a resistant common bermudagrass biotype were sequenced. Bioinformatics pipelines will be used to analyze the genetic information of the plant hosts that will provide answers about the role of plant genes in pathogenicity and symbiosis. Therefore, the current efforts are moving the bermudagrass breeding program at Oklahoma State University closer to the identification of genes responsible for SDS resistance.

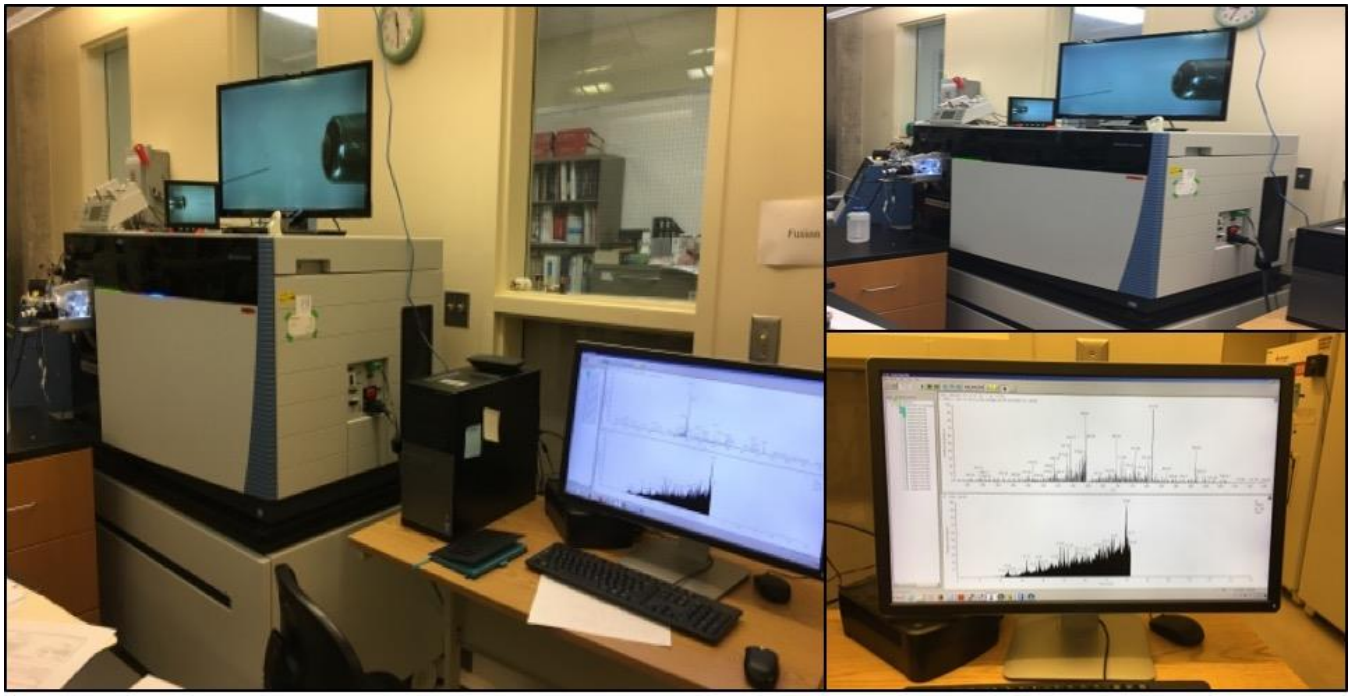

Figure 1. Fusion mass spectrometer at the OSU Biochemistry and Molecular Biology Core Facility (left). A close-up of the ion-source sprayer screen where the samples enter the mass spectrometer (upper right). The computer screen in which the results of the analysis can be followed in real-time (lower right).

Table 1. Genome statistics for three isolates of *Ophiosphaerella herpotricha*, five isolates of *O. korrae*, and three isolates of *O. narmari*.

Species	Isolate	Assembly size	N50 (bp)	Number of contigs	GC-content (%)	Gene count	Gene density (genes/Mbp)	Intergenic bases (Mbp)	Complete & single-copy genes (%)
Ophiosphaerella herpotricha	KS28	66.1	61,683	27,846	41.4	13,901	210.30	45.2	96.6
Ophiosphaerella herpotricha	TX2.5A	67.2	54,308	29,099	41.1	14,001	208.35	45.6	96.9
Ophiosphaerella herpotricha	16FISCC	63.8	50,289	20,526	40.1	13,285	208.23	42.8	97.6
Ophiosphaerella korrae	14BISCC	68.1	37,869	26,403	39.2	13,460	197.65	46.2	96.4
Ophiosphaerella korrae	OW11	67.3	40,856	14,402	38.1	12,576	186.86	47.0	97.0
Ophiosphaerella korrae	TX1.4	72.1	31,766	34,418	41.5	13,880	192.51	50.5	96.5
Ophiosphaerella korrae	KY162	71.4	51,429	13,696	39.2	12,602	176.50	50.8	96.8
Ophiosphaerella korrae	HCW2	71.1	47,026	12,155	38.6	12,615	177.43	50.7	96.6
Ophiosphaerella narmari	BCGCC2	47.0	213,028	7,233	46.5	12,006	255.45	26.7	97.3
Ophiosphaerella narmari	AUS58	47.0	221,777	7,094	46.4	14,091	299.81	25.8	97.6
Ophiosphaerella narmari	ATCC202719	47.7	1,524,584	5,309	45.8	13,384	280.59	26.6	97.3

Table 2. Number of protein coding genes of *Ophiosphaerella* spp. predicted to have a potential role in vitality, pathogenicity, virulence, and plant avirulence.

	O. korrae	O. korrae	O. korrae	O. korrae	O. korrae	O. herpotricha	O. herpotricha	O. herpotricha	O. narmari	O. narmari	O. narmari
Isolate identification	ISCC14B	TX14	OW11	HCW2	KY162	ISCC16F	KS28	TX25A	ATCC201719	AUS58	BCGC-C2
Role in lethal	197	159	146	140	138	148	171	155	151	147	155
Role in pathogenicity	462	434	391	381	391	372	414	415	369	371	379
Role in virulence	1640	1602	1465	1458	1471	1440	1565	1571	1480	1497	1471
Role in plant avirulence	37	44	36	36	37	31	31	32	36	34	33