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Characterization of two ABC transporters from biocontrol and phytopathogenic *Fusarium oxysporum*

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ABSTRACT

Sequence comparisons of ABC transporter genes from two strains of *Fusarium oxysporum* (one biocontrol and one phytopathogenic (f. sp. *lycopersici* Race 1) isolates) to 16 sequences from other fungi among those deposited in GenBank indicated that these genes are well conserved. However, sequences of promoter and 5′ UTR regions of a previously characterized ABC transporter gene FoABC1 differed between 8 phytopathogenic and 11 biocontrol strains of *F. oxysporum*. A phylogenetic analysis of promoter sequences indicated that pathogenic strains were grouped in a single clade, clearly distinct from clades with biocontrol agents. Although sequencing of promoter regions was done on only 20 strains, results suggest that FoABC1 may be regulated differently between phytopathogenic and biocontrol strains of the fungus. In studies on the activity (RNA) of FoABC1 in the presence of the fungicide thiram, one pathogen was down regulated to undetectable levels by a brief exposure to thiram, the other pathogenic strain was not affected, while the one of the two biocontrol strains was up regulated and expression of FoABC1 in the other biocontrol strain was not changed. After a 1 h exposure to thiram, the level of expression of one pathogen remained down regulated to undetectable levels, while the other pathogen and both biocontrol agents were up regulated compared to the time zero control. Additional research is needed to further understand the significance of the genetic difference in promoter regions between phytopathogenic and biocontrol strains for FoABC1.

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1. Introduction

1.1. *Types of* Fusarium oxysporum

F. oxysporum Schlecht. inhabits soils throughout the world. Morphologically identical isolates of this fungus may be saprophytic, phytopathogenic, or biocontrol agents. Pathogenic forms are host specific, and races of the pathogen cause vascular wilts or root and crown rots of many hosts, including a wide range of vegetable and tree crops. Moreover, there are numerous reports on the application of *F. oxysporum* to control pathogenic *F. oxysporum* [1].

Saprophytic, phytopathogenic, or biocontrol agents can currently only be distinguished by plant bioassays. Molecular tools have opened the possibility of discriminating among the various groups without a labor-intensive/time-consuming bioassay. Projects to characterize phytopathogenic *F. oxysporum* molecularly are currently underway. While most previous genetic work on Fusarium has focused on phytopathogenic strains (e.g. [2]), Trouvelot et al. identified a transposable element (Fot1) involved in biocontrol activity of *F. oxysporu*m Fo47 [3]. Other projects will provide complete genome sequences for *Fusarium* graminearum and *Fusarium verticillioides* (http://www.broad.mit.edu).

F. oxysporum strain CS-20 has been shown to reduce incidence of Fusarium wilt of tomato, muskmelon and basil under agricultural production conditions [4–6]. Strain CS-20 functions primarily by inducing resistance in the host plant [7,8].

Previous work on biocontrol of Fusarium wilt of tomato demonstrated that at least 22 genes were expressed differently between two biocontrol strains of *F. oxysporum* and two plant pathogenic ones [9]. Among these 22 genes, two that have strong homology to ABC transporters, are believed to play a role in differentiating between the two types.

1.2. *ABC transporters*

ABC (ATP-binding cassette) transporters are the largest family of membrane proteins that actively transport compounds across the

Abbreviations: ABC transporter, ATP-binding cassette transporter.

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cell membrane [10–13]. Transporters within this family vary in substrate specificity. They actively transport hydrophobic agents in and out of the cell, as well as exporting cytotoxic agents that entered the cell through passive diffusion. The ABC transporter family is subdivided into six topological classes based on the order of four domains within the polypeptide [13–15]. The ability to export hazardous compounds could be beneficial to microbes in the soil environment as well as those facing plant host defense compounds. In some cases, but not others, ABC transporters can be involved in conferring resistance or tolerance to fungicides [13,16]. Two ABC transporters have been shown to play a role in plant pathogenesis of *Fusarium culmorum* (FcABC1) [15], although another ABC transporter did not appear to be involved in pathogenicity on wheat by *F. graminearum* [17]. *Gibberella pulicaris* requires an ABC transporter for virulence on potato and for resistance to phytoalexins (ABC1) [18]. An ABC transporter was identified as a pathogenicity factor in *Magnaporthe grisea* (ABC1) [19], while another was not involved in pathogenicity [20]. Similarly, an ABC transporter from one strain of *Mycosphaerella graminicola* was involved in pathogenicity [21], while an ABC transporter was not needed for pathogenicity in another case [22].

1.3. Purpose

This work was undertaken to further characterize *F. oxysporum* with emphasis on genetic diversity and regulation between pathogenic and biocontrol isolates of *F. oxysporum*.

2. Materials and methods

2.1. Sequencing of FoABC1 and FoABC2

Partial transcript sequences of ABC transporters from the biocontrol fungus *F. oxysporum* strain CS-20 were previously obtained [23]. Activity of these genes is induced by the fungicide thiram [23]. ESTs from a second, not previously reported gene showing high homology to members of the ABC transporter superfamily was also obtained from strain CS-20. To determine the full-length sequence of these two genes, we searched the NCBI Trace Archive Databases with Discontiguous MegaBLAST, comparing our partial transcript sequence against the raw sequence trace files available at NCBI (http://www.ncbi.nlm.nih.gov/blast/tracemb.shtml) from the *F. oxysporum* whole genome shotgun project (WGS) by the Broad Institute. The matching sequence trace files were visually analyzed to remove low quality sections and assembled into contigs using Vector NTI Advance 10. Intron regions were determined by aligning and comparing genomic and cDNA sequences for those genes where both sequences were available. This information was subsequently used to develop specific primers to amplify the full-length sequence of these genes using PrimerQuest software from Integrated DNA Technologies (Skokie, IL). A total of eight primers were used to obtain the complete sequence of both genes.

Total genomic DNA from *F. oxysporum* was extracted with DNeasy Mini Plant Kit (Qiagen, Valencia, CA) following the manufacturer's instructions, except that the DNA was eluted twice with 50 μl. The full-length copies corresponding to EST BM1 24-1 and BM3 54-1 were cloned from both a biocontrol strain (CS-20) and the plant pathogen *F. oxysporum* f. sp. *lycopersici* Race 1 strain 32SK-3 (isolated in Florida and previously described [24]). PCR amplicons obtained were cloned into *Escherichia coli* using Invitrogen's TOPO TA cloning kit (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. Plasmid DNA was extracted and subjected to *Eco*RI digestion to confirm the inserted product's length. Plasmid inserts were sequenced by a primer walking strategy (GeneWiz, South Plainfield, NJ).

2.2. Semi-quantitative PCR expression studies

In order to further understand the role of ABC transporters in *Fusarium*, the expression of FoABC1, FoABC2, and 13 additional ABC transporter genes, 12 of which were from *F. oxysporum*, was studied. The sequence of one ABC transporter gene from *F. verticillioides* was also included. Sequence data on *Fusarium* from The Broad Institute were used to identify ABC transporter genes (http://www.broad.mit.edu/tools/data/seq.html). Twenty-eight entries from their database were considered relevant, and 13 were ultimately used to make specific primers.

All fungi used in this study were originally isolated from two Florida tomato fields [24], except strain CS-20 which was from a Florida watermelon field [7]. Strains were classified as pathogenic, biocontrol or saprophytic relative to their activity on tomato [24], recognizing the possibility that they could be classified differently relative to other plant species. Two biocontrol and two plant pathogenic strains were selected for expression studies. Mycelia of *F. oxysporum* strains CS-20 (biocontrol), 85SK-1 (biocontrol), 34SK-3 (f. sp. *lycopersici* Race 1), and 34SK-6 (f. sp. *lycopersici* Race 1) were grown in potato dextrose broth (PDB; Difco, Sparks, MD) as previously described [23]. Mycelia were separated from the medium and immersed in 100 ml of PDB amended or not amended (3 treatments) before recovering mycelia by filtration and extracting RNA [23]. For the time zero treatments, mycelia filter-collected from starter flasks were placed in PDB (treatment PDB 0), or PDB amended with 25 μg/ml active ingredient thiram (Gustafson, Plano,

Table 1
Genes, primers and PCR conditions used to amplify *Fusarium oxysporum* ABC transporter genes for semi-quantitative studies of gene expression.

Gene	Forward Primer	Reverse Primer	Anneal Temp	PCR Cycles
FOXG_01535.2	TGACTATGACGACGATGATG	TGAAGAGACTCCTTGTATGC	55	40
FOXG_02979.2	GCTTATCGCAACTCTATCG	AGTGGTCGTCTTATACAGG	59	40
FOXG_02985.2	TCCACACTCAACTCACAAC	GCCAACAGACAGCATAGG	55	35
FOXG_03541.2	CCAAGCAGGTAACGGTATC	GTAGTCATCGCCAGTAACG	55	35
FOXG_05904.2	GCACTGAGAAGAACACTGAAG	CATTGAAGCGGCGAATAGC	55	40
FOXG_08610.2	GGTATGTTCGTCTTGATGTTC	GAGATGAGTGCTTCCTTGG	55	40
FOXG_09300.2	AGCAGTGTTGAAGCAGAAG	CGCCGATGAAGAAGTTGG	55	35
FOXG_10675.2	GAGATGTTGGTGGTATTAG	AGACGATGGATGATATAGG	55	40
FOXG_11327.2	ACGAGACAGAGGTTCCAG	TTGCCACAGACTTGATAGC	55	35
FOXG_11989.2	CTCCAACACGCTCAACAC	TACGAATAGGTAAGACGAATCC	55	40
FOXG_15712.2	GGTTCGCTCTTCTACAATC	CTCTTCATCGTGCTTATCG	58	50
FOXG_15760.2	GATACACCAACAGAAGAAC	TTGACTTGAGATACAGAGG	58	40
FVEG_02410.3	CGAGCGTATGAAGTCTGAG	GGTGAAGTAGATGGTGATGG	56	35
FoABC1	GTGCGTAAGTCGGCGGTAAAGG	TCTCCATGAAGGGTTTGGCTTCCT	58	30
FoABC2	TGTATCGACAACCCGGATCCACAA	GAGACGATGTTGCGTGCCATGTTT	59	40
FGACTIN	CAATGGTTCGGGTATGTG	TACCACGCTTGGACTGAG	Any	30–40

Table 2
Isolates of *Fusarium oxysporum* and *F. oxysporum* f. sp. *lycopersici* Race 1 from which promoter regions of FoABC1 and FoAB2 were sequenced.

F. oxysporum isolate name[a]	Type
22SK-9	Biocontrol
107SK-2	Biocontrol
72SK-2	Biocontrol
22SK-10	Biocontrol
72SK-1	Biocontrol
66SK-5	Biocontrol
74SK-11	Biocontrol
74SK-8	Biocontrol
CS-20	Biocontrol
101SK-4	Biocontrol
43SK-1	Biocontrol
32SK-5	Pathogen
34SK-4	Pathogen
32SK-4	Pathogen
32SK-8	Pathogen
1517	Pathogen
34SK-7	Pathogen
32SK-2	Pathogen
32SK-3	Pathogen

[a] Description of isolates and their characterization can be found in [22].

TX) (treatment Thiram 0) and the mycelia were immediately filtered through sterile miracloth, rinsed with sterile water, and frozen in liquid nitrogen. For the third treatment, mycelia were placed in PDB amended with thiram and incubated for 1 h before filtering, rinsing and freezing in liquid nitrogen. Total RNA was extracted from mycelia using the RNeasy Mini Plant Kit (Qiagen, Valencia, CA), following the manufacturer's instructions, except that the RNA was eluted twice with 50 μl of the elution buffer. The 100 μl of sample was then reapplied to the membrane and eluted a third time for maximum recovery. The resulting RNA was treated with DNase (Ambion, Austin, TX) to remove any genomic DNA contamination.

One microgram of RNA was reverse transcribed to cDNA with the Restroscript kit (Ambion, Austin, TX), using random decamers and the manufacturer's instructions. PCR was performed either with Promega Green Master Mix or with Takara Ex Taq (Takara Bio USA, Madison, WI), depending on the primers. 1 μl of a 1:10 dilution of cDNA was used for amplification. Different annealing temperatures and cycle numbers were used depending on the gene of interest (Table 1). The constitutively expressed *Gibberella zeae* (*F. graminearum*) Actin gene (XM_390561) was used as an expression control. PCR products were run on a 1.2% agarose gel and stained with ethidium bromide (CLP, San Diego, CA) at a final concentration of 0.5 μg/ml. At least three separate PCR replicates were performed.

2.3. Restriction digestion

To confirm that each of the genes used in the expression study is different, PCR products were digested with different restriction enzymes and the resulting banding pattern was analyzed. For most of the genes, *Eco*RI, *Hin*dIII, *Bam*HI, and *Bgl*II resulted in conclusive banding patterns. For some genes, *Nco*I, *Xho*I, *Sca*I, and *Eco*RIV were used to resolve inconclusive results from the initial restriction digestions. PCR products were digested overnight at 37 °C, and then run on a 1% agarose gel stained with ethidium bromide.

2.4. Promoter regions

Since phenotypic differences among *F. oxysporum* isolates could be due to gene regulation rather than gene sequence, promoter regions of isolates of *F. oxysporum* were also examined and compared. Isolates for this study were selected from a group of 415 isolates that were previously characterized with respect to pathogenicity (including race) and biocontrol ability [24] (Table 2). The following primers were used to amplify the 5′ UTR of 23 isolates of *F. oxysporum*: FoABC1 P F: CGC CCT TAT CAA CAA CTT CCG TCA; FoABC1 P R: AAG GCA CTT TGA GTC GAG ATG CGA; FoABC2 P F: TCG CAT CTC TCC AAT CTC GCA AGT; FoABC2 P R: AGG TGT TGG TGT ATT GAC GGG CTA.

DNA was amplified using Promega Green Master Mix (Promega, Madison WI). PCR products were cleaned with the Zymoclean Clean & Concentrator (Zymo Research, Orange, CA) prior to sequencing. A total of 40 ng of DNA were used in the sequencing reaction. The products were sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The manufacturer's instructions were followed for the labeling reaction and the reaction purification. Samples were sequenced on an ABI Prism 3700 (Applied Biosystems). Each DNA product was sequenced at least twice in each direction. Sequence data were assembled using the default settings in CLC Combined Workbench, version 3.0.1. Phylogenetic trees were constructed using CLC Main Workbench, Version 4.0.1 by CLC Bio (Aarhus, Denmark).

3. Results

3.1. Characteristics of FoABC1 and FoABC2

The consensus sequences were analyzed using CLC Combined Workbench, version 3.0.1. The gene FoABC1 (from EST BM1 24-1) is a 4327 bp gene consisting of 3 exons (782, 897, and 2543 bp) and 2 introns (49 and 52 bp). FoABC2 (from EST BM3 54-1) is 4571 bp, and also consists of 3 exons (1943, 1755, 771 bp), and two introns

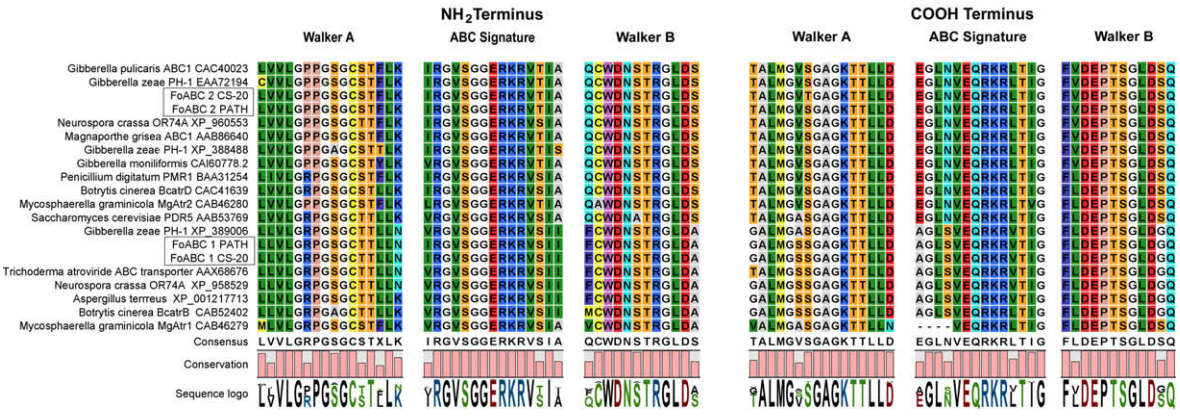

Fig. 1. Alignment of FoABC1, FoABC2, and other fungal ABC transporter genes showing the well-conserved nature of the Walker A, ABC signature, and Walker B residues.

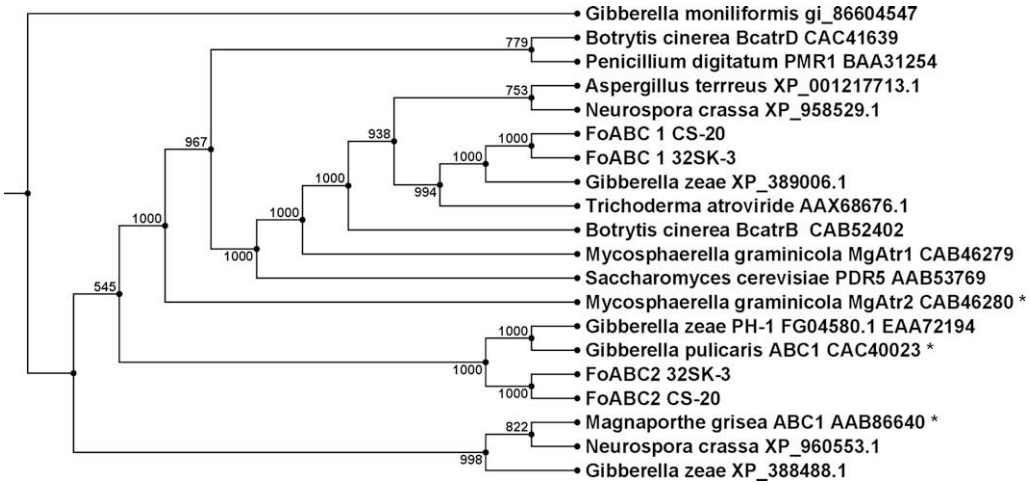

Fig. 2. Neighbor-joining phylogenetic tree of the amino acid sequence of FoABC1, FoABC2 and other related fungal ABC transporters. *Fusarium oxysporum* strain CS-20 is a biocontrol agent used to protect tomato from a wilt disease caused by *F. oxysporum* f. sp. *lycopersici* strain 32SK-3 (Race 1). Numbers on nodes represent 1000 bootstrap replications. Asterisks denote ABC transporter genes needed for pathogenicity [15,18,19,21].

(50 and 48 bp) [23]. The presence of introns was confirmed by comparing PCR amplicons derived from the same set of primers on cDNA and genomic DNA (data not shown). The deduced amino acid sequence of FoABC1 consists of 1407 residues. GenBank accession numbers for the sequence are EU311560 for FoABC1_32SK-3 and EU311561 FoABC1_CS-20. The amino acid sequence of FoABC2 consists of 1489 residues. GenBank accession numbers for the sequence are EU311562 for FoABC2_32SK-3 and EU311563 for FoABC2_CS-20.

3.2. ABC domain characteristics

Both genes contain the conserved domain characteristics of ABC transporters. Both have N and C terminal Walker A motifs, an ABC signature motif, and a Walker B motif (Fig. 1). Analysis of the protein structure using the Transport Classification Database (http://www.tcdb.org/index.php) indicates that both FoABC1 and FoABC2 are members of the ATP-binding Cassette (ABC) Superfamily (3.A.1), specifically the Pleiotropic Drug Resistance (PDR) Family (ABCG). A hydropathy analysis using the Kyte–Doolittle scale (also at http://www.tcdb.org/index.php) shows 11 transmembrane segments for each protein.

3.3. Gene comparison

The amino acids sequences of FoABC1, FoABC2, and 16 other ABC transporters were aligned and compared. A phylogenetic tree calculated from the amino acid sequences of fungal genes close to FoABC1 and FoABC2 shows three clades (Fig. 2). Sequences for FoABC1 and FoABC2 were very similar from *F. oxysporum* strain CS-20 and *F. oxysporum* f. sp. *lycopersici* strain 32SK-3 and all differences were in non-conserved regions.

3.4. Promoter region

The sequenced PCR products of the promoter region of both FoABC1 and FoABC2 were aligned by CLC Combined Workbench, version 3.0.1. Both genes contain a TATAA box (−184 for FoABC1 and −41 for FoABC2) and a CAAT box (−289 for ABC1 and −383 for ABC2). The CAAT promoter regions for pathogens and biocontrol isolates of *F. oxysporum* differed for FoABC1 (Fig. 3). Nine of the 11 biocontrol isolates tested had the consensus sequence of CCAATT, while 7 of the 8 pathogenic isolates tested had the consensus sequence of CCATTT.

The region sequenced contained approximately 850 bp for each of the 19 isolates. The 5′ UTR containing the promoters is generally found within the first 320 bp. The remaining approximately 520 bp is part of the gene, including the transcription start site. Phylogenetic analysis was conducted on all 850 bp of sequence using the neighbor-joining method [25] (Fig. 4). One clade consists solely of pathogenic isolates, while the other clades contain only biocontrol isolates. A similar analysis of the promoter region of FoABC2 shows no such distinction (data not shown).

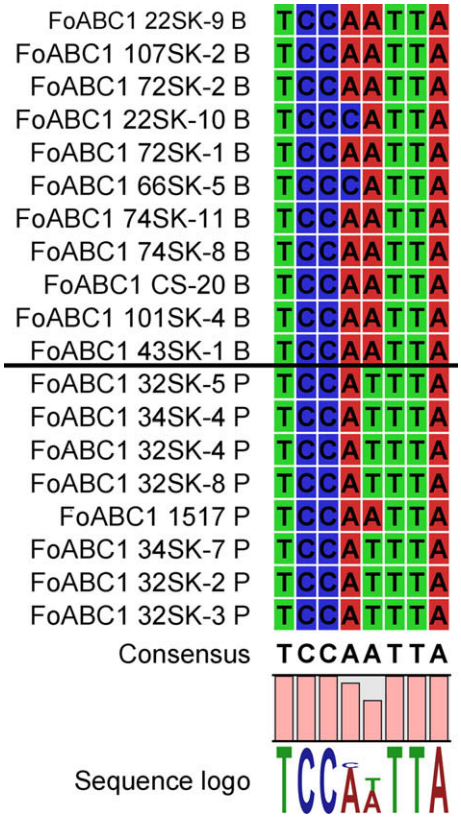

Fig. 3. CAAT box differences between biocontrol (B) and plant pathogenic (P) isolates of *Fusarium oxysporum*.

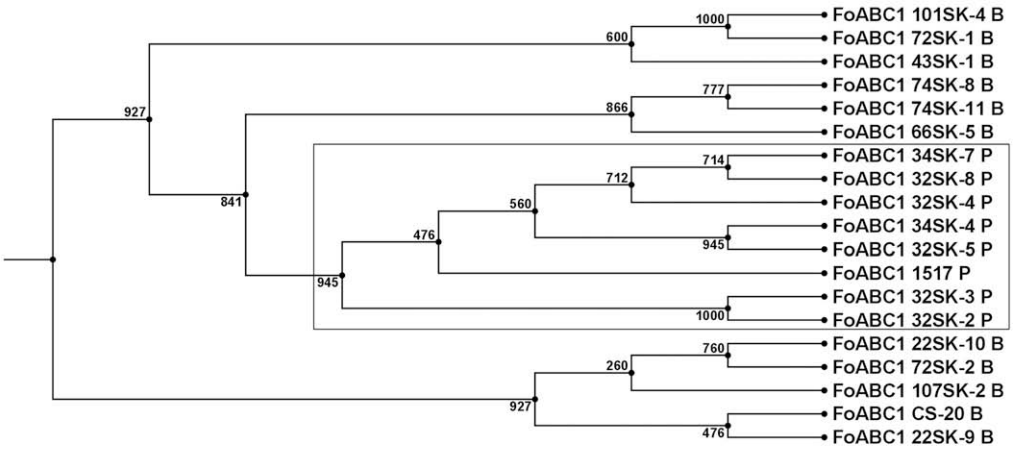

Fig. 4. Neighbor-joining phylogenetic tree of the promoter regions of 19 *Fusarium oxysporum* isolates that are either biocontrol (B) or plant pathogenic (P). Numbers on nodes represent 1000 bootstrap replications. The rectangle surrounds the clade containing only pathogenic strains. Phylogenetic analysis was conducted on 850 bp of sequence. The first approximately 320 bp is the 5′ UTR containing the promoters. The remaining sequence is part of the gene, including the transcription start site.

3.5. Restriction digestion

An analysis of restriction fragments indicated that each Fusarium ABC transporter examined in this study was unique. Although the number of restriction fragments and the sizes of FOXG_08610.2 and FoABC2 appeared similar, the amino acid pair wise alignment only showed a 21.01% similarity between these two genes.

3.6. Expression studies

Results of the semi-quantitative PCR were quantified with the Quantity One software package from BioRad. Each band was analyzed for trace quantity and compared to all other bands of the same replicate. Only bands within a replicate run of PCR were compared. Trace quantity is the quantity of a band as measured by the area under its intensity profile curve (BioRad) relative to the band with the greatest intensity. Ratios of all runs (replicates) of each gene were averaged to obtain the final expression level for each gene (Tables 3 and 4). Table 3 shows the change in expression of 15 Fusarium ABC transporter genes in response to brief exposures of four fungi to 25 μg/ml active ingredient thiram and Table 4 shows the change in expression of these genes in response to a 1 h exposure to thiram. Green highlight denotes increased mean gene expression relative to the 0 h control incubation in PDB for each fungus gene combination. Red shading indicates that expression was reduced.

Table 3
Expression of 15 ABC transporter genes in biocontrol and phytopathogenic isolates of *Fusarium oxysporum* in response to a brief (<1 min) exposure to 25 μg/ml active ingredient of the fungicide thiram.

	CS-20		85SK-1		34SK-3		34SK-6	
	0 PDB	0 THIRAM	0 PDB	0 THIRAM	0 PDB	0 THIRAM	0 PDB	0 THIRAM
FoABC1	0.36	0.53[a]	0.56	0.55	0.69	0.78	0.71	0.00
FoABC2	0.33	0.45	0.43	0.22	0.83	0.48	0.37	0.04
FOXG_01535.2	0.18	0.44	0.78	0.50	0.37	0.68	0.67	0.13
FOXG_02979.2	0.00	0.28	0.00	0.00	0.87	0.88	0.65	0.00
FOXG_02985.2	0.85	0.90	0.63	0.65	0.73	0.74	0.53	0.00
FOXG_03541.2	0.57	0.68	0.63	0.58	0.61	0.49	0.64	0.08
FOXG_05904.2	0.20	0.35	0.80	0.33	0.66	0.98	0.52	0.20
FOXG_08610.2	0.69	0.87	0.69	0.60	0.76	0.62	0.52	0.18
FOXG_09300.2	0.12	0.11	0.23	0.07	0.50	0.64	0.34	0.00
FOXG_10675.2	0.58	0.56	0.00	0.00	0.71	0.61	0.90	0.00
FOXG_11327.2	0.55	0.69	0.55	0.40	0.86	0.93	0.75	0.00
FOXG_11989.2	0.15	0.07	0.00	0.00	0.79	0.98	0.51	0.00
FOXG_15712.2	0.38	0.85	0.18	0.19	0.73	0.71	0.45	0.11
FOXG_15760.2	0.34	0.36	0.12	0.17	0.54	0.26	0.07	0.08
FVEG_02410.3	0.31	0.52	0.60	0.53	0.67	0.52	0.64	0.00
FGActin	0.78	0.82	0.94	0.89	0.88	0.91	0.88	0.60

[a]For each fungus and gene combination, expression after exposure to thiram for one hour is compared with expression in PDB at time 0. Green shading denotes increased expression compared to the control at time 0 and red shading denotes decreased expression.

Table 4
Expression of 15 ABC transporter genes in biocontrol and phytopathogenic isolates of *Fusarium oxysporum* in response to a 1 h exposure to 25 μg/ml active ingredient of the fungicide thiram.

	CS-20		85SK-1		34SK-3		34SK-6	
	0 PDB	1 THIRAM	0 PDB	1 THIRAM	0 PDB	1 THIRAM	0 PDB	1 THIRAM
FoABC1	0.36	0.80[a]	0.56	0.99	0.69	0.87	0.71	0.00
FoABC2	0.33	0.90	0.43	0.20	0.83	0.91	0.37	0.04
FOXG_01535.2	0.18	0.82	0.78	0.82	0.37	0.87	0.67	0.13
FOXG_02979.2	0.00	0.76	0.00	0.00	0.87	0.96	0.65	0.00
FOXG_02985.2	0.85	0.96	0.63	0.60	0.73	0.83	0.53	0.00
FOXG_03541.2	0.57	0.84	0.63	0.73	0.61	0.78	0.64	0.00
FOXG_05904.2	0.20	0.53	0.80	0.58	0.66	0.73	0.52	0.34
FOXG_08610.2	0.69	0.63	0.69	0.49	0.76	0.28	0.52	0.00
FOXG_09300.2	0.12	0.86	0.23	0.83	0.50	1.00	0.34	0.00
FOXG_10675.2	0.58	0.23	0.00	0.00	0.71	0.54	0.90	0.00
FOXG_11327.2	0.55	0.57	0.55	0.36	0.86	0.68	0.75	0.00
FOXG_11989.2	0.15	0.68	0.00	0.00	0.79	0.48	0.51	0.00
FOXG_15712.2	0.38	0.60	0.18	0.21	0.73	0.77	0.45	0.00
FOXG_15760.2	0.34	0.72	0.12	0.26	0.54	0.13	0.07	0.08
FVEG_02410.3	0.31	0.93	0.60	0.91	0.67	0.86	0.64	0.00
FGActin	0.78	0.83	0.94	0.86	0.88	0.88	0.88	0.60

[a]For each fungus and gene combination, expression after exposure to thiram for one hour is compared with expression in PDB at time 0. Green shading denotes increased expression compared to the control at time 0 and red shading denotes decreased expression.

4. Discussion

4.1. Genetic similarities and differences among F. oxysporum ABC transporters

While phytopathogenic, biocontrol and saprophytic strains of *F. oxysporum* are morphologically indistinguishable, they can behave very differently in the presence of certain plants. Results presented here contribute to our knowledge on some of the genetic similarities and differences between phytopathogenic and biocontrol strains of *F. oxysporum* that may contribute to observed phenotypic behavior.

Our data showed similar sequences for two ABC transporter genes from two strains of *F. oxysporum* (one biocontrol and one phytopathogenic isolate; Fig. 2). However, sequences of promoter regions for one of the genes (FoABC1) differed between phytopathogenic and biocontrol strains of *F. oxysporum*. Although sequencing of promoter regions was done on a relatively small number of strains and one exception was noted (Fig. 1), results suggest that FoABC1 may be regulated differently between phytopathogenic and biocontrol strains of the fungus. Data are not sufficient to recommend identification of biocontrol or plant pathogenic strains based on the sequence of the promoter region of FoABC1; however, the data indicate that one difference between biocontrol and plant pathogenic strains may be differences in regulation of otherwise identical genes.

Despite similarities in genetic sequence, there was a diversity of responses in regulation of FoABC1 in the strains of the fungus studied. In studies on the activity (RNA) of FoABC1 in the presence of the fungicide thiram (Table 3), one pathogen was down regulated to undetectable levels by a brief exposure to thiram, the other pathogenic strain was not affected, while the one of the two biocontrol strains was up regulated and expression of FoABC1 in the other biocontrol strain was not changed. After a 1 h exposure to thiram, the level of expression of one pathogen remained down regulated to undetectable levels, while the other pathogen and both biocontrol agents were up regulated compared to the time zero control. Additional research is needed to further understand the significance of the genetic difference in promoter regions between phytopathogenic and biocontrol strains for FoABC1. Studies on regulation of other *F. oxysporum* genes examined, including FoABC2, showed that most appeared to be expressed similarly by phytopathogenic and biocontrol strains (Fig. 1 and data not shown). In all runs of the experiment, the phytopathogenic strain 34SK-6 was down regulated for actin. Down regulation of actin may be considered unusual, but under our conditions this result was consistent.

ABC transporters have been identified in a wide variety of prokaryotic and eukaryotic organisms. Thus, it is not surprising that we found ABC transporters in *F. oxysporum* also. ABC transporters have been linked to pathogenicity for fungi, including *F. culmorum* where they may play a role in overcoming host defense responses [15].

5. Conclusion

Since strains are morphologically identical, identification of genetic differences between the groups would help in early identification of *F. oxysporum* isolates. Identification of genetic markers associated with pathogenicity would benefit plant disease diagnostics, as well as provide a basis for predicting disease potential of soils before planting. Similarly, if traits associated with biocontrol ability can be identified, then this information could be used to screen for new biocontrol agents.

Biocontrol is likely not a simple trait and many genes are likely to be involved. This is an initial step in trying to identify some of traits that set biocontrol *F. oxysporum* apart from phytopathogenic *F. oxysporum*.

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Mention of trade names or commercial products in this article is solely for the purpose of providing scientific information and does

not imply recommendation or endorsement by the U.S. Department of Agriculture.

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