African Journal of Biotechnology Vol. 11(1), pp. 54-79, 3 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2966
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Cloning, *in silico* structural characterization and expression analysis of *MfAtr4,* an ABC transporter from the banana pathogen *Mycosphaerella fijiensis*

Y. Couoh-Uicab[1], I. Islas-Flores[1]*, N. Kantún-Moreno[2], L.-H. Zwiers[3], M. Tzec-Simá[2], S. Peraza-Echeverría[2], L. Brito-Argáez[1], L. Peraza-Echeverría[2], R. Grijalva-Arango[2], A. James[2], C. Rodríguez-García[2] and B. Canto-Canché[2]

[1]Unidad de Bioquímica y Biología Molecular de Plantas, Centro de Investigación Científica de Yucatán A.C., Calle 43 No. 130, Colonia Chuburná de Hidalgo, C.P. 97200, Mérida, Yucatán, México.
[2]Unidad de Biotecnología, Centro de Investigación Científica de Yucatán A.C., Calle 43 No. 130, Colonia Chuburná de Hidalgo, C.P. 97200, Mérida, Yucatán, México.
[3]CBS-KNAW, Fungal Biodiversity Centre, Utrecht, The Netherlands.

Accepted 2 December, 2011

ABC transporters are membrane proteins that use the energy released from the hydrolysis of ATP to drive the transport of compounds across biological membranes. In some plants, pathogenic fungi ABC transporters play a role as virulence factors by mediating the export of plant defense compounds or fungal virulence factors. *Mycosphaerella fijiensis,* the causal agent of black Sigatoka disease in banana, is the main constraint for the banana industry worldwide. So far, little is known about molecular mechanism that it uses to infect the host. In this study, degenerated primers designed from fungal ABC transporters known to be involved in virulence were used to isolate homologs from *M. fijiensis*. Here, we reported the full cloning of *MfAtr4* a putative ortholog of *MgAtr4,* an ABC transporter of the related *Mycosphaerella graminicola* with a function in virulence. Similarities and differences with its presumed ortholog MgAtr4 are described, and the putative function of *MfAtr4* are discussed. Analysis of *MfAtr4* gene expression in field banana samples exhibiting visible symptoms of black Sigatoka disease indicated a higher expression of *MfAtr4* during the first symptomatic stages in comparison to the late necrotrophic phases, suggesting a role for *MfAtr4* in the early stages of pathogenic development of *M. fijiensis*.

Key words: ABC transporters, virulence factors, *MgAtr4* ortholog, *Mycosphaerella fijiensis*, black Sigatoka, *Musa* sp.

INTRODUCTION

The ATP-binding cassette (ABC) protein family constitutes one of the largest and ancient protein families. Currently, more than 10,000 members are known and it is expected that this number increases as new genome sequences become available (Kovalchuk and Driessen, 2010). ABC proteins are present in all organisms, from archaea to higher eukaryotes (Davidson and Maloney, 2007). Most of the ABC proteins characterized are classified as transmembrane proteins involved in the active transport of a broad range of substrates across biological membranes (Higgins, 1992; Laleh et al., 2008). However, to a lesser extent, some ABC proteins act as ion channels or receptors or are involved in ribosome biogenesis (Kovalchuk and Driessen, 2010). Based on the topology and ordering of specific domains normally present within ABC transporters, they can be divided into several subfamilies (ABC-A to ABC-H; Jie et al., 2010).

*Corresponding author. E-mail: islasign@cicy.mx. Tel: +52 (999) 9 42 83 30. Fax: +52 (999) 9 81 39 00.

The structure of typical ABC transporters consists of four core domains, two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). The domains TMD-NBD may be expressed as TMD-NBD in separate polypeptide chains or alternatively, as TMD_2-NBD_2 in multidomain proteins. Based on the number of TMD-NBD domains inside polypeptides, two arrangements are common for eukaryotic ABC transporters. The functional unit is either composed of two "half transporters", each containing its own TMD and NBD, or consists of one large polypeptide chain ("full transporters") that includes all four domains (Del Sorbo et al., 2000; Kovalchuc and Driessen, 2010). ABC proteins containing the NBD but lacking TMDs are generally not involved in membrane transport (Kovalchuc and Driessen, 2010).

In fungi, the most common ABC transporters are the so-called full-size ABC transporters, in which all domains are contained in one polypeptide chain (Del Sorbo et al., 2000). The best characterized examples either belong to the ABC-A (multidrug resistance, MDR) or ABC-G (pleiotropic drug resistance, PDR) protein subfamilies. At the structural level, members of the ABC-A MDR subfamily exhibit the characteristic $(TMD_6\text{-}NBD)_2$ topology, while members of the ABC-G PDR subfamily exhibit the reverse topology $(NBD\text{-}TMD_6)_2$. The TMD impart ligand specificity and the NBDs are responsible for binding and hydrolysis of ATP needed to drive the transport of the substrate against a concentration gradient (Kenneth and Higgins, 2007). Fungal ABC transporters play key roles in many cell vital processes including toxin detoxification, secretion of mating peptides and the transport of a broad variety of substrates ranging from simple ions to complex polypeptides (Jones and George, 2004). These proteins can act as biological export machines (Stergiopoulos et al., 2002; De Waard et al., 2006) providing protection against endogenously produced toxic compounds, (example, secondary metabolites such as mycotoxins) and against exogenous toxic compounds from natural or man-made origin (example fungicides, antibiotics, and plant defense compounds), by preventing their cytoplasmic accumulation (De Waard et al., 2006; Coleman and Mylonakis, 2009).

ABC transporters can be involved in providing protection against fungicides. Characteristic for the involvement of ABC transporters in fungicide resistance is the development of multidrug resistance (MDR). MDR is the simultaneous development of resistance to structurally and functionally unrelated compounds. This phenomenon has originally been described in medicine where it is of great clinical significance. Since the early 1990´s, it was established that an active efflux-mechanism based on the ABC1 (Pgp1 glycoprotein) was preventing the adequate intracellular accumulation of anticancer drugs inside cancerous cells (Gottesman et al., 2002; Szakacs et al., 2006; Nikaido 2009; Kuo et al., 2010). Nowadays, MDR has also been widely described in filamentous fungi both of agricultural and medical relevance. AtrB of *Aspergillus nidulans* mediates resistance to camptothecin and resveratrol, natural toxic metabolites, but additionally AtrB confers resistance to all major classes of fungicides (Andrade et al., 2000). In *Botrytis cinerea*, a fungal pathogen with a broad host range, the ABC transporter BcatrB is upregulated by resveratrol, a grapevine phytoalexin, and also the fungicide fenpiclonil (Schoonbeek et al., 2001). ABC transporters from the wheat pathogen *Mycosphaerella graminicola* have substrates ranging from fungicides, plant secondary metabolites, bacterial antibiotics and fungal mycotoxins (Zwiers et al., 2003).

It has been found that in various fungal pathogens, ABC transporters can play a role in pathogenesis (Kretschmer et al., 2009). The first report was on *Magnaporthe grisea* in which the ABC1 gene, encoding an ABC transporter, was identified in a screening of pathogenicity mutants derived by insertional mutagenesis. Gene-replacement mutants of the ABC1 gene produced a mutant that was arrested in growth early in pathogenesis and unable to detoxify the rice-produced sakuranetin phytoalexin (Urban et al., 1999). Since this report, several papers have correlated the disruption or deletion of particular ABC transporters (especially belonging to the ABC-G subfamily) with a decrease in aggressiveness or loss of pathogenicity. Virulence-related ABC transporters have been described in *Botrytis cinerea* (Schoonbeek et al., 2001), the necrotrophic fungus *Gibberella pulicaris* (Fleibner et al., 2002), the human pathogen *Candida albicans* (Theiss et al., 2002), the wheat pathogen *M. graminicola* (Stergiopoulos et al., 2003) and the causal agent of cereal blight and rot *Fusarium culmorum* (Skov et al., 2004). Recently, Gupta and Chattoo (2008) reported a second ABC transporter called ABC4, required for pathogenesis in *M. grisea*. Both virulence-associated ABC transporters, ABC1 and ABC4, are required during early steps in pathogenesis. *Abc1* mutants formed appressoria that failed to elaborate extensive infection hyphae, while *abc4* mutants were defective in appressoria formation. However, it cannot be ruled out that both transporters have a partial overlap in function. All these findings clearly show that fungal ABC transporters can be involved in pathogenesis and it is possible that multiple members of this large family could be involved in host-fungal interaction in the same species.

Mycosphaerella fijiensis, a hemibiotrophic pathogen, causes the disease known as black Sigatoka, the most important threat for the banana and plantain industry worldwide (Fahleson et al., 2009; Vásquez et al., 2009; Abiala et al., 2010). The fungus affects leaf tissues causing a reduction of photosynthetic area, which leads to premature fruit ripening and loss of production. The methods being used to control *M. fijiensis* (chemical control and cultural practices) have failed or are ineffi-

cient (Romero and Suton, 1998; Amil et al., 2007; Orozco et al., 2008). Rapid acquisition of resistance to strobilurin (Qo respiration inhibitors) and benzimidazole (interfering with mitosis) fungicides has occurred (Sierotzki et al., 2000; Albertini et al., 1999; Cañaz-Gutiérrez et al., 2006). In both cases, the resistance is the result from a single change at the nucleotide level of target genes. Very little is still known about *M. fijiensis* pathogenicity or virulence factors. However, we hypothesized that ABC transporters are involved in the virulence of this fungus. Therefore, we set out an *in silico* strategy to identify putative virulence related ABC transporters in this important pathogen on the basis of homology (Igarashi et al., 2004; Piehler et al., 2008; Seret et al., 2009; Sturm et al., 2009). The closest related fungus with the same infection strategy as *M. fijiensis* in which a virulence-related ABC transporter has been identified is *M. graminicola,* a hemibiotrophic pathogen of wheat. Seven ABC transporters denominated *MgAtr1* to *MgAtr7* have been described in this fungus. Besides MgAtr7 which is involved in the maintenance of iron homeostasis (Zwiers et al., 2007), most of them play a role in providing protection against toxic compounds. A role in pathogenicity has only been attributed to *MgAtr4*. The expression of *MgAtr4* occurs concomitantly with the development of necrotic lesions in infected wheat leaves and MgAtr4 disruption mutants displayed reduced intercellular growth and an impaired capacity to colonize substomatal cavities (Stergiopoulos et al., 2003). Here, we reported the full cloning of the putative *MgAtr4* homolog in *M. fijiensis*, the sequence characterization of *MfAtr4* and the analysis of its expression in naturally infected banana leaves with different degrees of black Sigatoka disease. This study is a first step in improving our understanding of the pathogenicity of *M. fijiensis* on banana.

MATERIALS AND METHODS

Biological material

M. fijiensis strain C1233 was grown on modified solid V8 medium according to Mourichon et al. (1987). Briefly, 200 ml V8 juice were added to 2 g/L $CaCO_3$ and 2% agar-agar, autoclaved, and placed on Petri dishes. Individual plates were inoculated with 16 mm^2 mycelium, and left to grow at 26 ± 2°C, with a 12 h light/12 h dark photoperiod. Liquid V8 culture medium was prepared by the same procedure but without agar. The liquid medium was inoculated with 0.5 ml of *M. fijiensis* mortar and pestle disaggregated mycelium (1 g mycelium from an active culture disaggregated in 5 ml sterile water), using the same temperature and light conditions stated previously. For DNA extraction, mycelium was harvested after 15 days of culture, filtered through two pieces of fine cheesecloth, weighed, and distributed in portions of 0.3 g mycelium and immediately snap-frozen in liquid nitrogen and stored at -80°C until DNA extraction.

DNA extraction

Genomic DNA extraction was carried out according to Johanson (1997). DNA concentration in samples was determined using a spectrophotometer (Genesys 10 UV).

MfAtr4 cloning

To improve the chance to obtain an ortholog of *MgAtr4* from *M. fijiensis* a two- step strategy was followed. First, the MgAtr4 protein (AAK15314) was analyzed for the presence of particular specific motifs by comparison with the other known ABC-G transporters from *M. graminicola*; MgAtr1 (CAB46279), MgAtr2 (CAB46280), MgAtr3 (AAK62341), MgAtr5 (AAK62340) and MgAtr7 (EF062310); this strategy successfully identified amino acids characteristic for MgAtr4 (Supplementary 1). Furthermore, to prevent the selection of motifs unique for *M. graminicola*, MgAtr4 and the other MgAtrs were aligned with ABC transporter proteins from fungal plant and human pathogen species: CAC40023 (*G. pulicaris;* Sordariomycete), T30541 (*M. grisea;* Sordariomycete), CAD10327 (*Aspergillus fumigatus;* Eurotiomycete), CAF32148 (*A. fumigatus; Eurotiomycete*), CAC42218 (*Emericella nidulans;* Eurotiomycete), CAC41639 (*Botryotinia fuckeliana;* Leotiomycete), AAF05069 (*Candida glabrata;* Saccharomycotina), O74676 (*C. glabrata;* Saccharomycotina), P43071 (*Candida albicans;* Saccharomycotina), BAC67160 (*Botryotinia fuckeliana;* Leotiomycete), AAN28699 (*Trichophyton rubrum;* Eurotiomycete), AAK62810 (*Venturia inaequalis;* Dothideomycete) and CAA93140 (*E. nidulans;* Eurotiomycete); a phylogenetic tree was made using MEGA 4.0 (Figure 7). In a second step, a third alignment was developed with sequences of ABC transporters clustering in the same clade with MgAtr4 protein (AAN28699, AAK62810, BAC67160 and CAA93140).

Motifs identified in the first multi-alignment were manually searched in the last one. Degenerated primers were designed on motifs (mentioned from amino to carboxyl ends) EVDKHFP (forward 1; 320 degenerancies), and AFYHPATE (reverse 1; 2728 degeneracies), TFSTAEVLV (forward 2; 2180 degenerancies) and FAHMCIAA (reverse 2; 136 degenerancies). Nucleotide sequences of primers are given in Table 1. Amplification was performed by standard polymerase chain reaction (PCR) in 25 µL final volume containing 2 µM of each one of the degenerated primers, 0.2 µM of each dNTP, 0.2 mM $MgCl_2$, 25 ng of *M. fijiensis* genomic DNA and 1 µL (10 U) Taq DNA polymerase (Invitrogen). PCR cycle conditions were; 4 min of 95°C; followed by 30 cycles of 95°C for 30 s, 60°C for 40 s, and 72°C for 1.2 min; and a final elongation at 72°C for 10 min. The PCR products were analyzed on 1% agarose gel electrophoresis and photographs were taken in a UV-Gel DOC photodocumentation system (Bio Rad). The 1 Kb DNA ladder (Invitrogen) was used as reference for size. The amplicon was cloned in the pGEM-Teasy vector (Promega) according to the manufacturer instructions, transferred into *E. coli* and then sequenced.

During our research, the full genome sequence of *M. fijiensis* (JGI, http://genome.jgi-psf.org/cgi-bin/runAlignment?db= Mycfi1& advanced=1/) became publicly available and we benefitted from this by using the cloned sequence as query to retrieve the full genomic DNA sequence. Specific primers (ORF-MfAtr4-5´, ORF-MfAtr4-3´, Table 1) were designed on the basis of the downloaded genomic sequence. The complete ORF was amplified by long distance-PCR using similar PCR mixture as above, but using 5 U GoTaq DNA polymerase (Promega). PCR was performed as above, but extension step was for 5.2 min at 72°C each cycle. The PCR product was ligated into pGEM®-T Easy Vector (PROMEGA) and sequenced.

Determination of intron exon boundaries

RNA from *M. fijiensis* was obtained according to Islas-Flores (2006)

Table 1. List of primers used in this study.

Primer name	Type	Sequence (5´- 3´)	Observation
dAtr4-F1	Degenerate	CARGARGTIGAYAARCAYTTYCC	dAtr4-F1 + dAtr4-R1
dAtr4-R1	Degenerate	CIGTIGCIGGRTGRTARAAIGC	Expected the amplification of a *MfAtr4* fragment
dAtr4-F2	Degenerate	GTITTYMGIMGIGGICAYGTICC	dAtr4-F2 + dAtr4-R2
dAtr4-R2	Degenerate	ATIGCIGCIATRCACATRTGIGC	Expected the amplification of a *MfAtr4* fragment
ORF-MfAtr4-5´	Specific	GCCACCATGTCGTCAACGGACAAGGAC	ORF-MfAtr4-5´ + ORF-MfAtr4-3´ Amplification of complete *MfAtr4* ORF (from ATG to TGA)
ORF-MfAtr4-3´	Specific	CTAAATGATCTGGGCATTCCTCCTATTC	
IFAtr4	Specific	TACGGCTACACATACGATCATG	IFAtr4 + IRAtr4, primers flanking the putative intron
IRAtr4	Specific	AAGGAAAGCACAGATAGACCAAG	
MfAtr4267F	Specific	GGTCTTCTCTACGATCGTGCAG	Specific primers to amplify a 267 bp fragment of *M. fijiensis MfAtr 4* gene
MfAtr4267R	Specific	GAAGGTCGATGCATAGATCAAGAAG	
MfAct247F	Specific	CATCACCATTTGGCAACGAGC	Specific primers to amplify a 247 bp fragment of *M. fijiensis* actin gene
MfAct247R	Specific	GATCTTGACCTTCATGCTGG	
Mac267F	Specific	CTGCTGGTATCCATGAGACC	Specific primers to amplify a 267 bp fragment of *M. acuminate* actin gene
Mac267R	Specific	CCTTGGAGATCCACATCTGC	

and cDNA synthesis was conducted using SuperScript III (Invitrogen) according to supplier's instructions. Primers IFAtr4 and IRAtr4 (Table 1) flanking the putative intron were used to amplify a fragment of *MfAtr4*, using *M. fijiensis* gDNA and cDNA as templates. Resulting PCR amplified cDNA or DNA product was ligated into pGEM®–T Easy Vector (PROMEGA) and then sequenced.

Software and websites for bioinformatics analysis

Tools to analyze protein structure were used directly in the ExPASy Server (Expert Protein Analysis System), proteomics server of the Swiss Institute of Bioinformatics (SIB) (http://www.expasy.org). The Prosite (Bairoch 1991) was used to determine the Nucleotide Binding Domains (NBDs), TMHMM and SOSUI program (http:// www.expasy.org) were used to predict the Transmembrane Domains (TMD's). Topology prediction was carried out in the PredictProtein website (http://www.predictprotein.org). Fungal ABC PDRs were retrieved by multiple blastp searches against the National Center for Biotechnology Information website and using the *M. graminicola* ABC transporters (ATRs) as queries.

Phylogenetic analysis was performed with the program package MEGA4 (Tamura et al., 2007) using neighbor-joining algorithm and bootstrapping with 500 replicates. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. In the last phylogenetic analysis, all virulence-associated ABC proteins identified so far were included, independently of the ABC family to which they belong; accession numbers are indicated in the figures. The percent amino acid identity and amino acid similarity between MfAtr4 and MgAtr4 sequences were calculated by pair-wise analyses using the Matrix Global Alignment Tool (MatGAT) v.2.01 (Campanella et al., 2003), and comparing complete sequences or particular domains.

RT-PCR and *MfAtr4* expression analysis at different stages of the interaction *M. fijiensis-Musa acuminate* cv Grande Naine.

Banana plants (*Musa acuminate* cv. Grande Naine) naturally infected with *M. fijiensis* were collected in an experimental banana plantation located at Uxmal, Yucatan, Mexico.

The plant materials were cotton-cleaned using 70% ethanol, leaf areas showing stages I, II, III, IV and V of Sigatoka disease were excised with sterile knife and immediately stored in liquid nitrogen and then transported to the laboratory. Different stages of the disease were selected according to Fouré (1985).

Total RNA was obtained using the Concert^{TM} reagent (Invitrogen) according to the instructions of the manufacturer (0.25 g leave tissues/1.5 ml reagent).

Total RNA samples (5 µg/10 µL) were independently DNAse I (Sigma) treated for 30 min at room temperature. RNA samples were ethanol precipitated, air dried by 5 min and resuspended in distilled sterile RNAse-free water (10 µL). Of each RNA sample, 2 µg was used as template for cDNA synthesis using the SuperScript III RT-PCR kit (Invitrogen), according to instructions of the manufacturer. Subsequently, 500 ng of cDNA was used independently for RT-PCR, with primers to amplify fragments of the *M. fijiensis* genes, *MfAtr4* (amplicon 267 bp) and actin (247 bp), and the *M. acuminate* actin (267 bp) (sequences of primers in Table 1).

As negative control, uninfected banana were included. The result was a representative of at least three independent experiments.

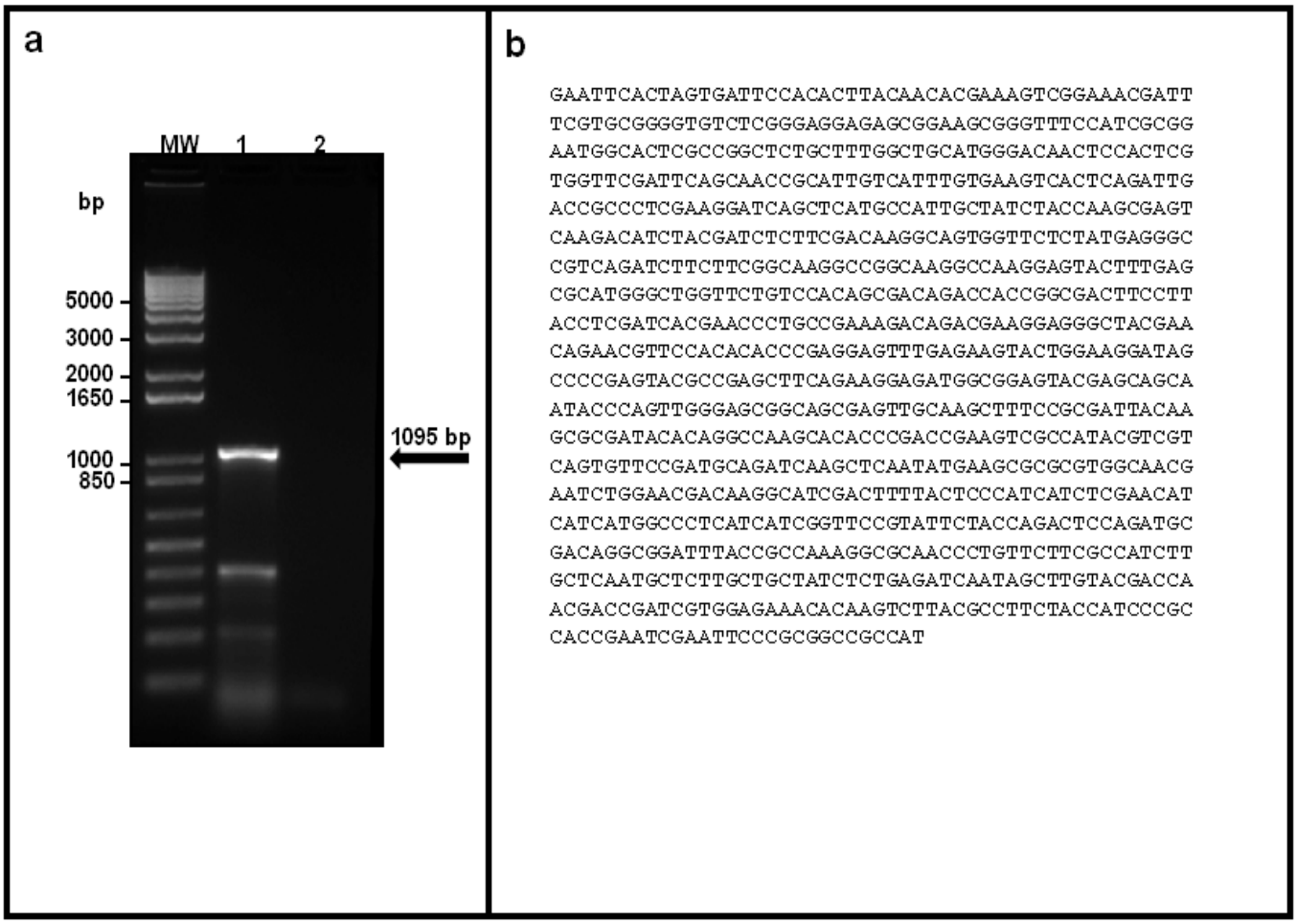


Figure 1. *MfAtr4* genomic fragment amplified by degenerated PCR. (A) PCR product separated on a 1% agarose gel. The arrow indicates the DNA band with the expected size, which was purified from gel and cloning for sequencing. (B) The nucleotide sequence obtained for two independent clones.

RESULTS

Cloning and *in silico* characterization of *MfAtr4*

The degenerated PCR amplification yielded few un-specific bands and also a ~1095 bp amplicon (Figure 1a) that was purified and cloned. Two clones were picked and 940 bp sequenced; both clones yielded an identical sequence (Figure 1b). The BlastX analysis using the sequence of the 940 bp DNA fragment as query against the NCBI database gave highest hit with MgAtr4 ($E = 2e^{-124}$, showing amino acid 73% identity and 82% amino acid similarity). Upon the availability of the whole genome sequence of *M. fijiensis*, the 940 bp nucleotide sequence was also used to query the whole genome sequence of *M. fijiensis* by BLASTN, which resulted in one hit with a gene with local 98% homology with the query. This gene was annotated as *MfAtr4*. Pair-wise comparison of the deduced full amino acid sequences of MfAtr4 and MgAtr4 results in 73% identity and 82% similarity. Furthermore, *in silico* PCR with these degenerate primers and annotated ABC-G genes in the *M. fijiensis* genome predicted only short amplicons (51-104 nt; data not shown), thus validating our approach.

MfAtr4 was amplified from the deduced translational start to translational stop (ATG to TAG), and this resulted in an amplicon of 4977 nucleotides that was fully sequenced twice in two independent clones to rule out possible PCR or sequencing errors. Comparison of the *MfAtr4* nucleotide sequence obtained in this study (*M. fijiensis* strain C1233) to the sequence from the *M. fijiensis* genome portal (isolate CIRAD86) indicated 99.1% identity. Most changes were silent.

A comparison of protein level between the MfAtr4 from isolate C1233 and from CIRAD86 indicated that the predicted proteins exhibited a 99.8% similarity and a 99.7% identity. In general, changes were conservative, that is- glutamine to histidine at the C-terminal end, alanine to valine in NBD1, isoleucine to alanine in TMS2 and lysine to arginine in NBD2-TMS7 linkage.

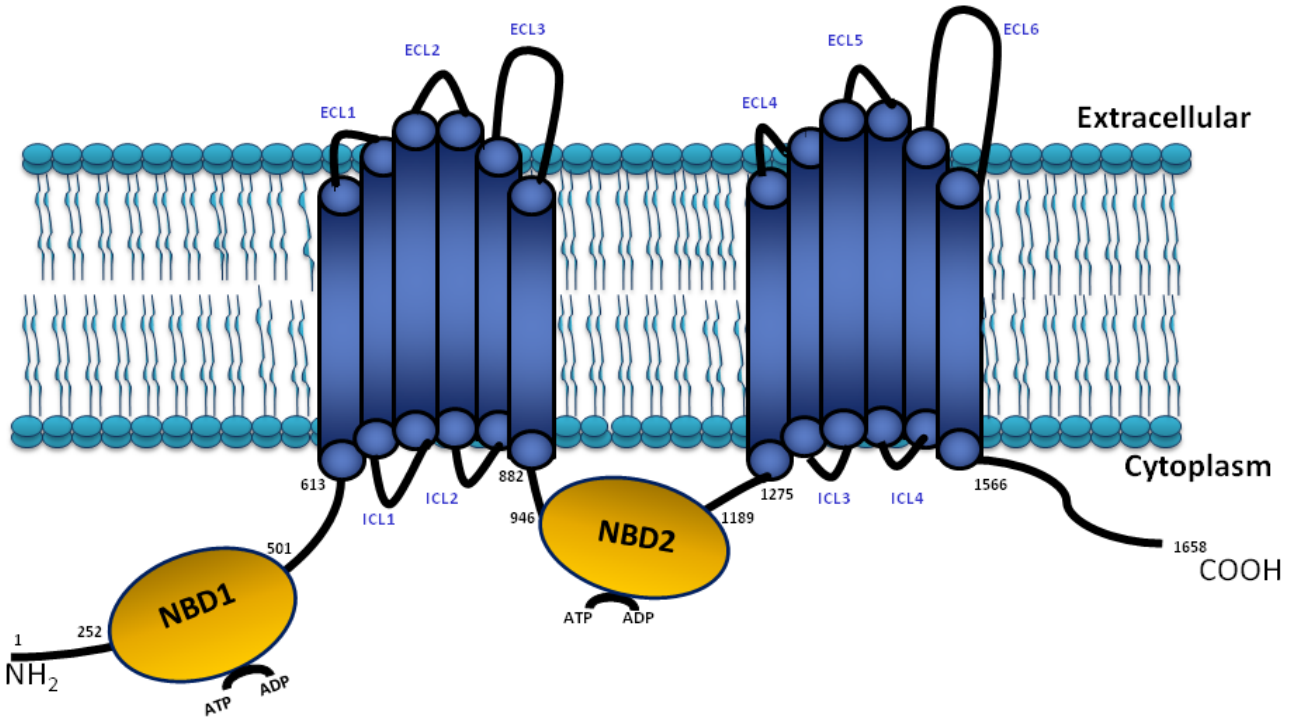


Figure 2. Two-dimensional topological model of MfAtr4. The model shows the 12 transmembrane helices, the two NBDs, the six extracellular loops (ECL 1–6) and the four intracellular loops (ICL 1–4), with amino and carboxyl terminal ends and NBD motifs oriented toward cytoplasm. SOSUI website was used to deduce structure and PredictProtein website for topology. Number of first and last amino acids in each NBD and TMD are indicated.

Features of *MfAtr4*

The predicted MfAtr4 structure consisted of two hydrophilic nucleotide binding domains (NBDs) located at the cytoplasmic surface, and two transmembrane domains (TMDs). Within each of the putative TMD (amino acid residues 613 to 880 and 1279 to 1566), six membrane-spanning segments (TMS) were predicted. The amino and carboxyl ends of the protein are oriented toward the cytoplasm (Figure 2). Four small intracellular loops were predicted (ICLs), ICL1 (25 amino acids), ICL2 (8 amino acids), ICL3 (32 amino acids) and ICL4 (13 amino acid) and all them inside of the cell. On the extracellular side, MfAtr4 has four small extracellular loops (ECLs), ECL1 (5 amino acids), ECL2 (11 amino acids), ECL4 (10 amino acids), ECL5 (5 amino acids), and two large ECLs (ECL3, between TMS5/6, and ECL6, between TMS11/12) of 77 and 91 amino acid residues, respectively.

The amino terminal Walker A and Walker B motifs of MfAtr4 (GRPGSG**C**ST and **LAAWD**NSTRGLD) are degenerated when compared to the canonical motifs (Walker A: GXXGXG**K**S/T, Walker B: ϕϕϕϕD, where ϕ is any hydrophobic amino acid), (Walker et al., 1982). The conserved lysine in the Walker A motif is replaced in MfAtr4 by a cysteine amino acid (Figure 3). Walker motifs are flanking the ABC signature motif of MfAtr4, sequence G**VSGGE**RKRVSIAEMA (canonical sequence is LSGGQ). The Walker A motif of the C-terminal NBD of MfAtr4 (GTSGAG**K**T) contains the canonical lysine; the Walker B sequence is LLFL**D**EPTSGLD and the second signature ABC sequence **LNVEQ**RKLLTIGVELAA (Figure 3).

MfAtr4 classification

MfAtr4 has the predicted NBD-TMS_6-NBD-TMS_6 topology (Figure 2; Table 3). This topology is characteristic for the ABC-G transporter sub-family, in contrast to the reverse $(TMS_6\text{-NBD})_2$ topology observed in the ABC-A, ABC-B (MDR), ABC-C (MRP) and ABC-D sub-families (Table 3; Kovalchuk and Driessen, 2010). The predicted topology of MfAtr4 corresponds to the topology of eukaryotic-type exporters (Igarashi et al., 2004; Cannon et al., 2009; Coleman and Mylonakis, 2009).

Comparative analysis with MgAtr4

Comparison of the deduced MfAtr4 protein with MgAtr4 showed 63.2% identity and 74.4% similarity on amino acid level. Major differences between MfAtr4 and MgAtr4

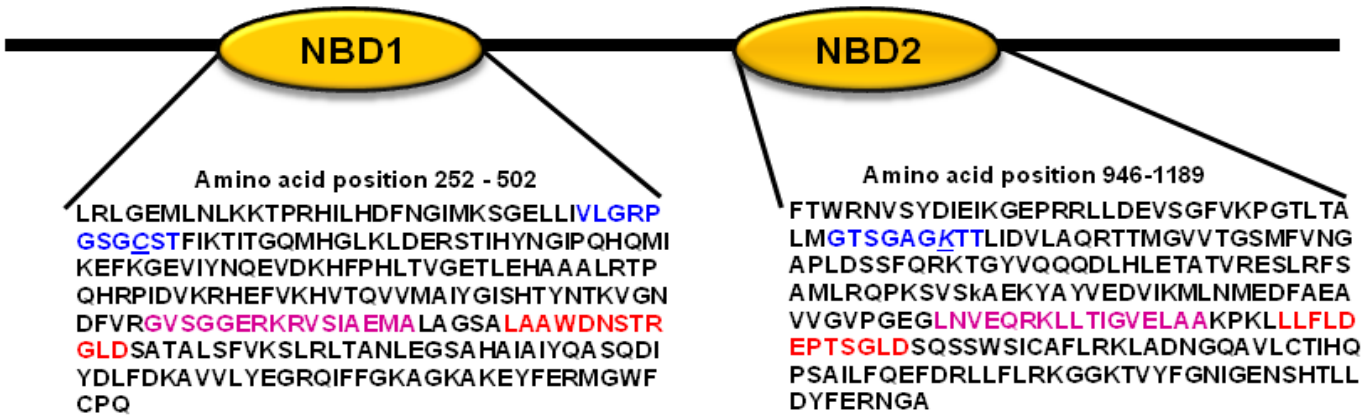


Figure 3. Prosite program identification of the two NBD domains in MfAtr4. NBDs were located 252 to 502 and 846-1189 residues downstream to the amino terminal end. Sequences of Walker A, Walker B and the signature sequence in each NBD domain are indicated with blue, red and pink letters respectively. The cysteine amino acid in the Walker A in NBD1 and the equivalent position of lysine in NBD2 are indicated with cursive underlined letters.

Table 2. Pair-wise comparison of MfAtr4 and MgAtr4.

Comparison of:	% Identity	% similarity
Complete amino acid sequences	63.2	74.4
From NBD1 to TMS12	73.1	84.5
NBD1	78.9	91.6
TMD1	75.7	88.4
NBD2	91.8	96.3
TMD2	74.6	86.1

proteins are at the N-(cytosolic stretch of amino acids before NBD1) and C-(cytosolic stretch of amino acids after TMS12) terminal ends. Both proteins were 73.1% identical and 84.5% similar when comparing from NBD1 to TMS12 (Table 2). In contrast to *MgAtr4* that lacks introns, *MfAtr4* is predicted to contain an intron of 52 nucleotides (Figure 4; Table 3), which splits the gene in two exons of 2979 and 1998 nucleotides. Amplification of a fragment of *MfAtr4* on gDNA and cDNA with primers flanking the putative intron resulted in amplicons with different sizes (Figure 4a). Sequencing of the CDS fragment corroborates the occurrence of the 52 nucleotides intron at the predicted position (Figure 4b). Comparison of the sizes of *MfAtr4* PCR products amplified with different combinations of primer pairs, and using *M. fijiensis* gDNA and cDNA, excluded the presence of other introns in this gene (data not shown).

Phylogenetic relationship between *MfAtr4* and other fungal ABCs

All ABC transporters which cluster with MfAtr4 belonged to fungi in the Pezizomycotina subphylum. This group is separated from the ABC-G proteins in the Saccharomycotina subphylum´s (*S. cerevisiae, C. albicans, K. lactis*) and the Basidiomycetes phylum (*Ustilago maydis, Criptococcus neoformans, Coprynopsis cinerea*). As expected, MfAtr4 clustered in the same clade as the ABC transporters, initially used in the design of the degenerated primers (Figure 5).

Phylogenetic relationship between *MfAtr4* and other ABCs involved in virulence

Phylogenetic analysis of multiple ABC-G proteins from several fungi indicated that MgAtr4 and MfAtr4 clustered together and in a different clade than the other ABC transporters with a proven function in pathogenicity. Virulence-associated ABC transporters fall in three different PDR-(ABC-G) subgroups and one non-ABC-G group. One ABC-G clade contains MgAtr4 and MfAtr4, the second ABC-G cluster consists of ABC1 from *M. grisea,* ABC1 *from G. pullicaris* and ABC1 from *F. culmorum* and the third cluster contain *B. fuckeliana* BcAtrB. The non ABC-G group includes *C. albicans* MLT1 (a MRP ABC transporter) and *M. grisea* ABC4 (a MDR ABC transporter) and cluster separate from all the other virulence-associated ABC-G members (Figure 5).

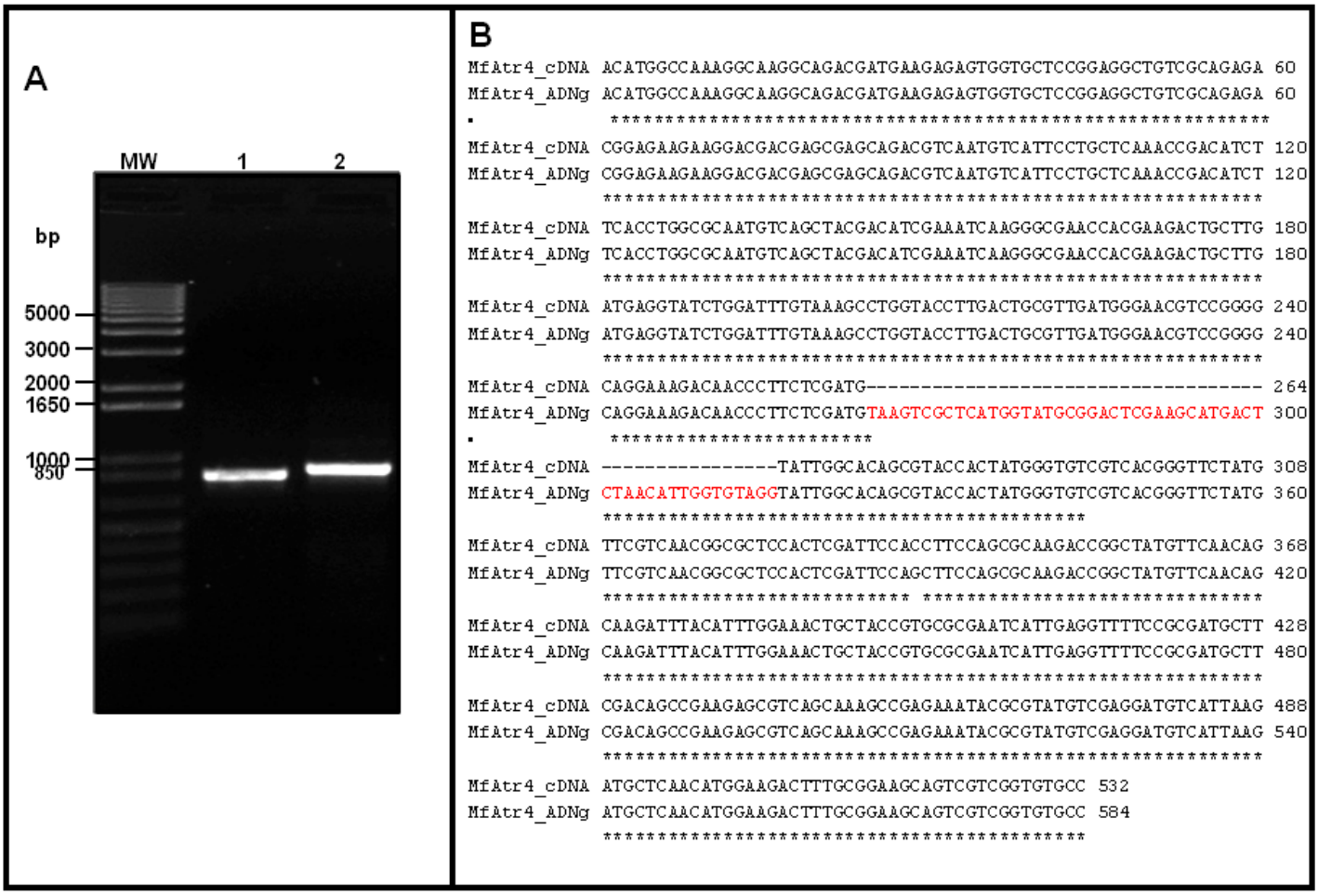


Figure 4. Presence of intronic sequence in *MfAtr4*. (A) Amplification of a fragment of *MfAtr4* with primers flanking the expected intron. Lane 1, amplicon obtained by using *M. fijiensis* cDNA as template; lane 2, using *M. fijiensis* gDNA as template; Mw, molecular markers. (B) Comparison of the nucleotide sequences obtained in each case. Red letters show nucleotides in gDNA which are absent in the cDNA.

Table 3. Comparative analysis of MfAtr4 and MgAtr4 features.

Parameter	MfAtr4	MgAtr4
Class	PDR (ABC-G family)	PDR (ABC-G family)
CDS size (ATG-TGA)	4977	4908
Peptide (number of amino acids)	1658	1635
Introns	One	None
Topology	$(NBD\text{-}TMS_6)_2$	$(NBD\text{-}TMS_6)_2$
Function	Exporter	Exporter
Role in virulence	Not determined	Yes
Walker A-1	VLGRPGSGCST	VLGRPGSGCST
Q-loop 1	VG**E**TL	VGQTL
Signature-1	VSGGERKRVSIAEMA	VSGGERKRVSIAEMA
Walker B-1	LAAWDNSTRGLD	LAAWDNSTRGLD
Walker A-2	GTSGAGKTT	GTSGAGKTT
Q-loop 2	VQQQD	VQQQD
Signature-2	LNVEQRKLLTIGVELAA	LNVEQRKLLTIGVELAA
Walker B-2	LLFLDEPTSGLD	LLFLDEPTSGLD
Symmetry	Asymmetric	Asymmetric

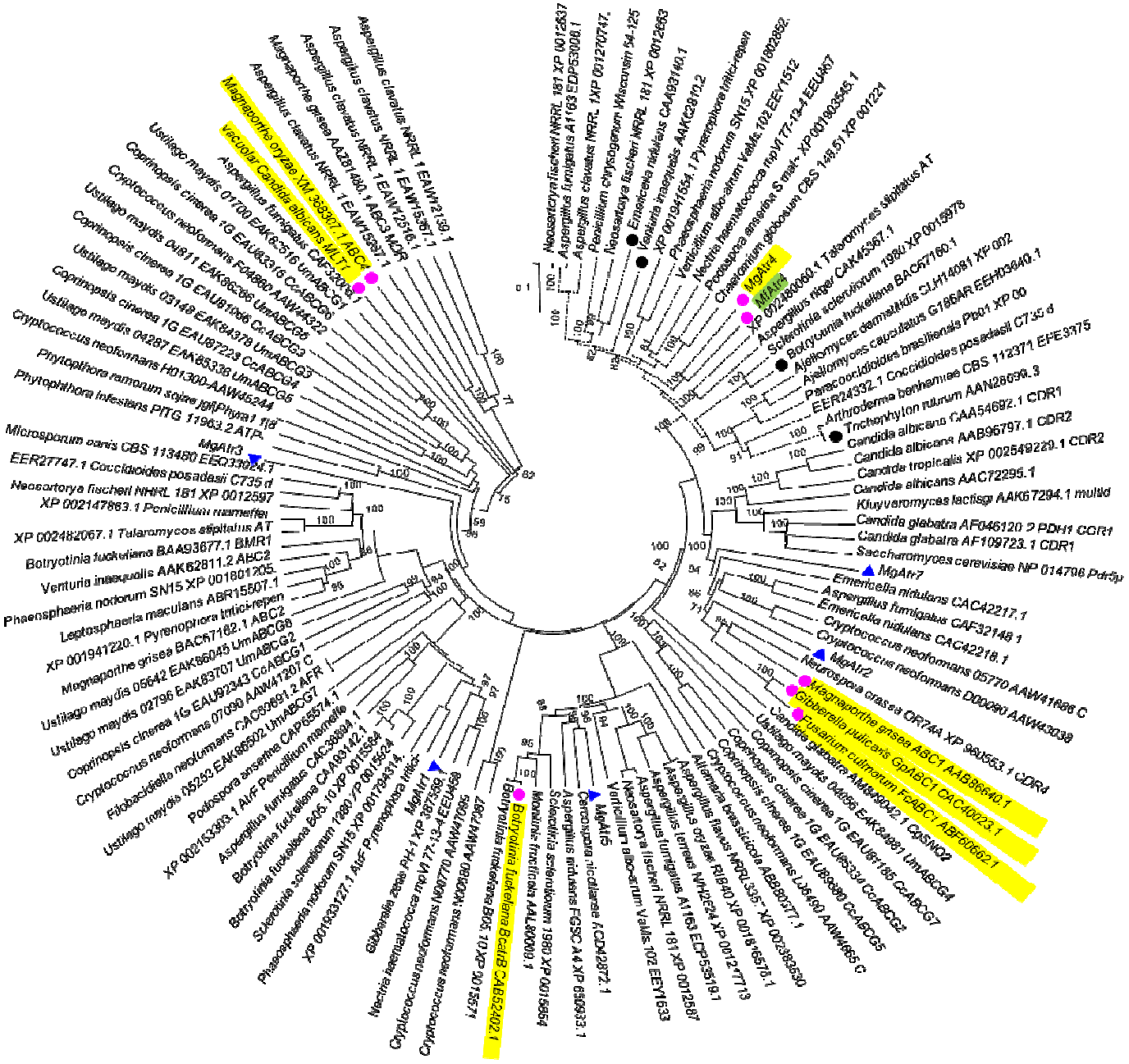


Figure 5. Phylogenetic tree of ABC transporters related to MfAtr4. The evolutionary history was inferred using the Neighbor-Joining method with the MEGA program version 4. Numbers on the branches indicate the percentage of 500 bootstrap replications (only >50% are shown). GenBank accession numbers are given for each sequence, except for *M. graminicola* Atrs (MgAtr1-7) and MfAtr4. MgAtrs are highlighted with blue triangles. Virulence-associated ABC transporters are highlighted with pink circles and yellow labeled (MfAtr4 is highlighted in green). The clade clustering the MfAtr4 is indicated with dotted branches. The ABC transporters clustering with MgAtr4 in Figure 7 (tree which helped to design the degenerated primers to amplify the first fragment of MfAtr4) are highlighted with black circles.

Analysis of expression of *MfAtr4* in black Sigatoka-infected banana leaves

Symptomatic plant material showing visual stages I, II, III, IV and V of the Sigatoka disease was selected in the field and then each stage individually harvested for the analysis (Figure 6, panel A). The actin genes from *M. fijiensis* and *M. acuminata* were used as reference genes (Figure 6, panels C, D). *Mf*-actin expression was lower at stages I and II than in later stages (Figure 6, panel C), which is congruent with the fungal biomass increment in the banana tissues with the disease progress (Arzanlou

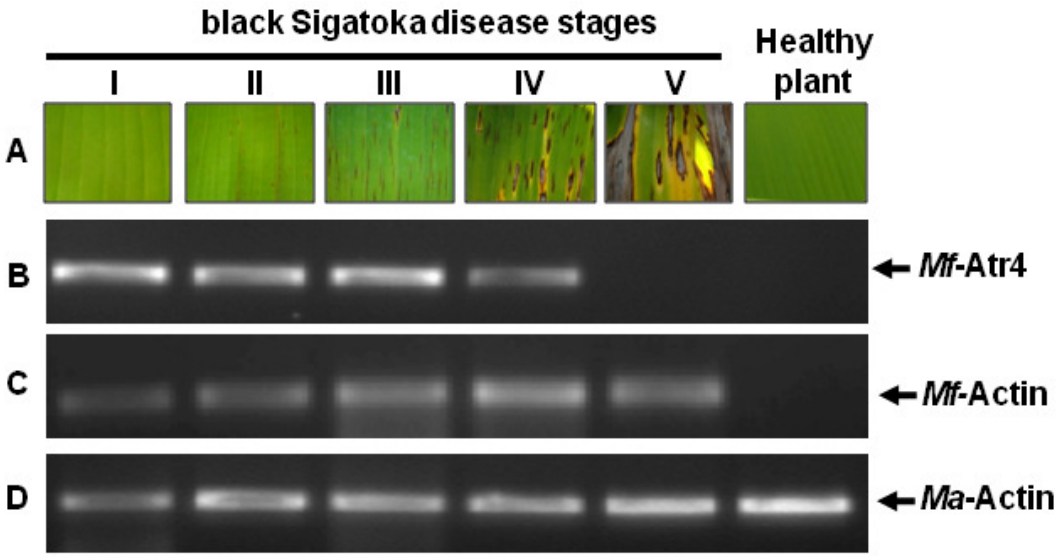


Figure 6. Analysis of expression of *MfAtr*4 in field samples of *Musa acuminate* cv. Grande Naine with black-Sigatoka disease, at different stages. The photographs show the material used for this analysis (A). Reverse transcription–polymerase chain reactions (RT-PCR) of MfAtr4 (B). RT-PCR of *M. fijiensis* actin, as reference fungal gene (C). RT-PCR of *M. acuminata* actin, as reference plant gene (D). cDNA prepared from healthy banana leaves was included as negative control.

et al., 2007). RT-PCR revealed the expression of *MfAtr4* in banana infected material and its probable temporal regulation during the infection process (Figure 6, panel B). Compared to the Mf-actin expression, the *MfAtr4* expression was highest in the initial infection stages and decreased with the progress of the necrotrophic phase (stages III and later). The seemingly complete absence of *MfAtr4* expression during the necrotrophic stage V was very striking and could definitely not be attributed to the absence of fungal biomass. Panel D shows the expression of the *M. acuminata* actin as reference gene.

DISCUSION

Cloning

The degenerated primers enabled us to get a fragment of *MfAtr4* in the first attempt. Therefore, these primers could be suitable to clone MgAtr4 homologs from closely related fungi with no available genomes and other Dothideomycetes, particularly in the order capnodiales to which *M. graminicola* and *M. fijiensis* belong. In addition, this strategy for designing primers could be extrapolated to clone other particular members or subfamilies in the ABC transporter family, or in other large gene families (kinases and permeases).

Comparison at nucleotide level of *MfAtr4* as cloned in this study and *MfAtr4* from the *M. fijiensis* genomic portal showed an identity of 99.7% and a similarity of 99.8%. This suggested a low degree of polymorphism in MfAtr4. Single nucleotide polymorphisms with similar degree occur in PDR5, an important ABC transporter implicated in pleiotropic drug resistance in *S. cerevisiae* (Guan et al., 2010), and also in *Candida glabratra* CDR1 (Haque et al., 2007), an ortholog of ScPDR5. In this ABC protein, the polymorphism, although low, is supposed to be significant for azole resistance. Some reports show that virulence-associated ABC transporters can contribute to resistance against fungicides and other cytotoxic xenobiotics (Gupta and Chattoo, 2008; Schoonbeek et al., 2001; Zwiers et al., 2003), but occurrence and contribution of polymorphism to tolerance to natural substrates or xenobiotics in these or other classes of fungal ABC transporters remains to be determined.

MfAtr4 classification

The predicted MfAtr4 topology (NBD-TMS_6- NBD-TMS_6), the presence of a cysteine residue in the N-terminal Walker A motif instead of a lysine residue, and the specific LNVEQ motif in the C-terminal ABC signature are all characteristics of a full-sized ABC-G (PDR) type transporter *sensu stricto* (Seret et al., 2009; Figure 3; Table 3). Many members of the ABC-G (PDR) family are involved in the prevention of the intracellular accumulation of toxicants (Cannon et al., 2009; Coleman and Mylonakis, 2009). Except for one, all the virulence-associated ABC transporters identified so far in fungal phytopathogens are members of the PDR family of ABC transporters. The only exception is ABC4 of *M. grisea* that belongs to the ABC-B (MDR) family (Gupta and Chattoo, 2008; Coleman and Mylonakis, 2009).

Features of *MfAtr4*

The ABC signature in the N-terminal NBD of MfAtr4 is canonical while the signature in the C-terminal NBD is degenerated; an asymmetric organization that is quite common in fungal ABC transporters (Rai et al., 2006; Preeti et al., 2006; Ernst et al., 2008; Cannon et al., 2009). The conserved lysine in the N-terminal Walker A motif is replaced in MfAtr4 by a cysteine amino acid (Figure 3).

This seems to be a feature characteristic for most of the fungal ABC-G transporters (Preeti et al., 2006), but the functional relevance of the change of the lysine by the cysteine amino acid is unknown.

Phylogenetic relationship between *MfAtr4* and other fungal ABCs

MfAtr4 clusters in a different clade than other PDR virulence associated ABC transporters. This suggests that fungal ABC transporters with roles in pathogenicity might have diversified in different times. Virulence-associated ABC1 transporter members are apparently ancient since they cluster with *Cryptococcus neoformans* (a Basidiomycete fungus) PDRs, suggesting these PDRs existed before the diversification of the major fungal lineages Ascomycetes and Basidiomycetes. MgAtr2 and MgAtr7 fall in this clade (Figure 5). Similar to other ABC families (ABC-B, ABC-C subfamilies) that are all present as multigene families in the genome of eukaryotic fungal species (Kovalchuc and Driessen, 2010), the fungal ABC-G (PDR) family might have become expanded by a series of gene duplications (Lupski, 2007; Seret et al., 2009). PDR transporters have taken a massive expansion in fungal genomes, especially in species belonging to the Pezizomycotina group, and several groups of these proteins are specific for this subphylum (Kovalchuk and Driessen, 2010). This seems to be the case of the clade containing the MfAtr4 and the MgAtr4. All ABC transporter proteins in this clade are PDRs from fungi belonging to the Pezizomycotina group, belonging to the classes Dothideomycetes, Leotiomycetes, Eurotiomycetes and Sordariomycetes, thus suggested that these PDRs evolved after the divergence of the main fungal lineages.

MfAtr4 and MgAtr4 fall in a different clade than MgAtr1, MgAtr2, MgAtr3, MgAtr5, and MgAtr7, the other ABC-G transporters identifie in *M. graminicola*. Each of these PDR members clustered separately from each other (Figure 5). They are paralogous among themselves, but according to the phylogenetic tree, with putative orthologues in other fungi. Because of the complexity of the PDR family, this is common in fungi (Cannon et al., 2009; Kovalchuk and Driessen, 2010).

Intron in MfAtr4

ABC transporters grouping in the same clade as MgAtr4 (Figure 7) have no introns. However, this is not a characteristic feature of genes present in the clade clustering with MfAtr4 (in Figure 5). Fifty percent of the PDRs in this clade contain 4 to 6 introns, but remarkably the Dothideomycetes PDRs in this clade (*Venturia inaequalis*, *Pyrenophora tritici*-repentis, *Phaeosphaeria nodorum*, *Alternaria brassicicola*) have no introns, thereby suggesting that the intron is a recent gain in *MfAtr4*. Occurrence of intron gain in fungal individual genes or gene families has been previously reported. Nielsen et al. (2004) analyzed *in silico* a set of orthologous 1-phos-phoribosyl-5-pyrophosphate (PRPP) synthetase genes and found a significant higher number of introns in *N. crassa* (six introns) and in *M. grisea* (fourteen introns) as compared to the PRPPs of other fungi. Nielsen et al. (2004) suggested that intron gain is a significant driving force that might be involved in the evolution of genes in fungi. Haugen et al. (2004) aligned Ascomycete and Basidiomycete S788 intron family and inferred that S788 gained access to Basidiomycete by lateral transferring and vertical inheritance. Puntual deletion events in S788 introns (example, by unequal crossing over, or by stepwise deletion) drive to genetic changes. In *Aspergillus*, intron gain is the outcome of the error-prone repair of DNA mediated by the capture of DNA fragments during non-homologous end joining of double strand breaks; intron gain or loss is the dynamics of evolution that cause changes in the rates of mutations, thus, introducing variants (mutation bias) or transmitting variants which may further be fixed or eliminated by selection (Zhang et al., 2010; Farlow et al., 2011).

As mentioned above, the PDR ABC transporter family is rapidly evolving in this kingdom, particularly by gene duplication (Coleman and Mylonakis, 2009). In addition to gene duplication, intron gain may be contributing to the evolution of individual genes; such seems to be the case of MfAtr4. Except *MgAtr4*, all PDR-ABC transporter encoding genes in *M. graminicola* contain introns, 19 introns in MgAtr7 (Zwiers et al., 2007), supporting a potential important role of introns in fungal PDR gene evolution.

Is *MfAtr4* an ortholog of *MgAtr4*?

ABC transporter orthologs can be identified by neighborhood and similarity searches (Seret et al., 2009). Eukaryotic ABC transporters have no substrate binding component as prokaryotes, but ligand recognition and specificity are mediated by the TMS (Igarashi et al., 2004). The active pocket has to allocate a variety of structurally different compounds because most ABC transporters can have multiple substrates. Congruent with their function, these structural components are the most divergent regions in ABC transporters. When TMDs are used as BLAST queries, generally this retrieves only proteins belonging to the same subfamily (Kovalchuk and

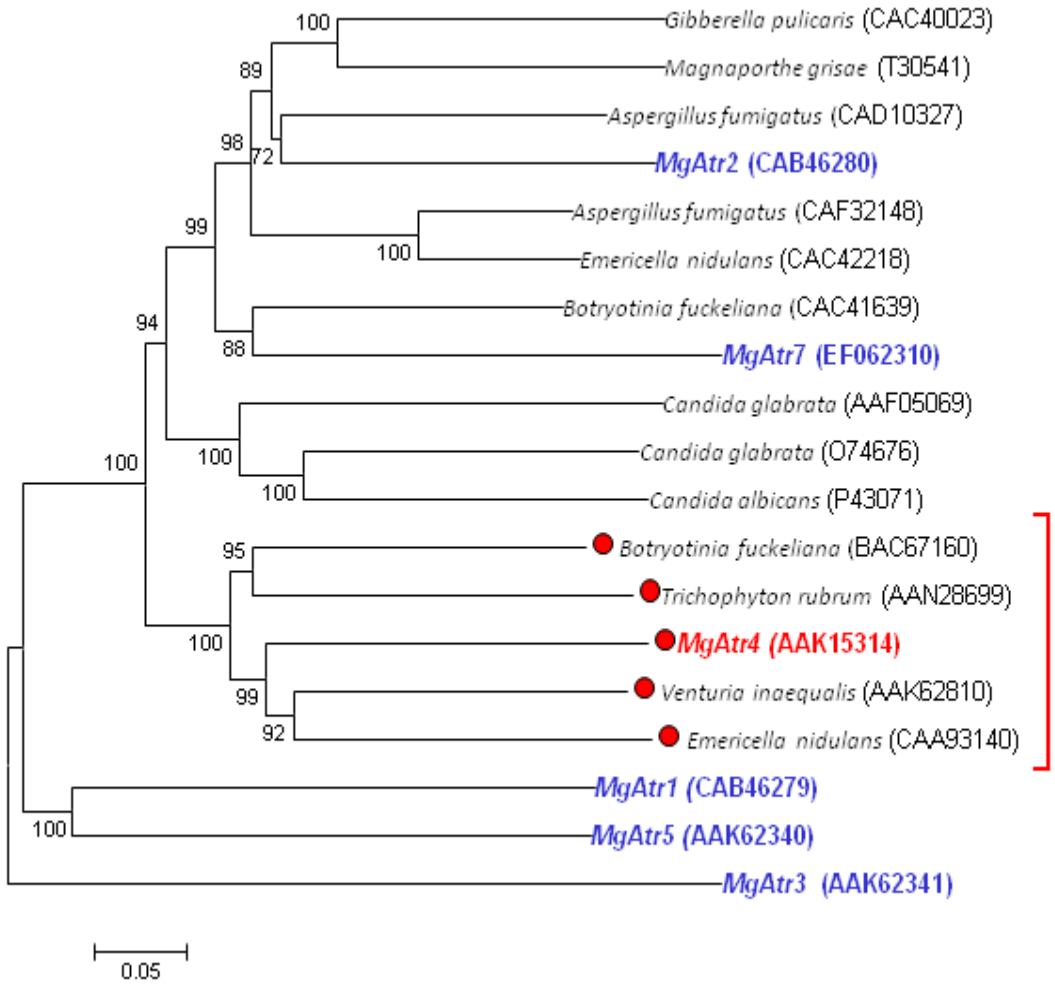


Figure 7. Neighbor-joining phylogenetic tree of MgAtrs and other fungal ABC (PDR) transporters. MgAtrs are highlighted with blue written names, except MgAtr4 which is in red. The sub-clade clustering MgAtr4 is indicated with a red bracket. Red circles indicate ABC transporters selected for a next alignment to search and exclude *M. graminicola* specific motifs in MgAtr4. GenBank accession numbers are given in parentheses after the name of each fungus species. The tree was constructed using the MEGA program version 4. Numbers on the branches indicate the percentage of 500 bootstrap replications.

Driessen, 2010). The first MfAtr4 fragment (obtained in this study) contained the MTS1 and MTS2; when this fragment was used as query to Blast the GenBank it retrieved as first hit the MgAtr4.

Considering that MgAtr4 and MfAtr4 cluster in the same clade in the phylogenetic tree (Figure 5) and the high overall similarity between them (Tables 2 and 3), we hypothesized that MfAtr4 is the ortholog of MgAtr4. Although, *M. graminicola* belongs to the same genus as *M. fijiensis*, it was phylogenetically more distant to *M. fijiensis* than other *Mycosphaerella* species. Closer phylogenetic relatives of *M. fijiensis* are *M. musicola* and *M. eumusae*, *M. africana*, *M. keniensis*, *M. marksii*, among many others (Carlier et al., 2000; Goodwin et al., 2001). Therefore, orthologs of MfAtr4 may exist in other *Mycosphaerella* species.

Analysis of expression of *MfAtr4* in black Sigatoka-infected banana leaves

It was previously demonstrated that ABC4 from *M. grisea* (Gupta and Chatoo, 2008) and *MgAtr4* from *M. graminicola* (Stergiopoulos et al., 2003), are involved in fungal virulence of these plant pathogens. Disruption or deletion of these genes reduced the ability of mutant strains to colonize the hosts. Molecular analysis of the full infection process using an artificial infection assay of wheat with *M. graminicola* showed no expression of *MgAtr4* during biotrophic phase. *MgAtr4* expression was observed from days 12 to 18 post-inoculation, while at day 22 no expression of *MgAtr4* was detectable; these times corresponded to the early/middle and late necrotrophic phase respectively (Stergiopoulos et al., 2003).

A similar pattern of expression was found in this study for *MfAtr4* gene in field samples of black Sigatoka infected-banana leaves (Figure 6, panel B). The expression of *MfAtr4* was higher at early necrotrophic stages in comparison with later stages of the fungal infection. As *MgAtr4* in *M. graminicola*-wheat pathosystem, *MfAtr4* expression was undetectable in the late necrotrophic phase of *M. fijiensis.* The positive expression of the fungal reference gene (Mf-actin) indicated that the absence of *MfAtr4* transcripts at this stage was not due to the absence of fungal biomass, but could be explained by assuming a regulation dependent on the disease progress. This suggests a role of *MfAtr4* during the early-middle stages of the disease progress, although a role of *MfAtr4* during the biotrophic phase of *M. fijiensis* cannot be ruled out. Further exploration of *MfAtr4* expression during the biotrophic stages of black Sigatoka disease is therefore necessary.

Taking together the analysis presented here, it is suggested that *MfAtr4* could play a role in *M. fijiensis* pathogenesis, similar to the role previously described for ABC4 and *MgAtr4* of *M. grisea* and *M. graminicola,* respectively. A number of reports have proposed that virulence-associated ABC transporters may be primarily involved in protection against exogenous compounds (Urban et al., 1999; Del Sorbo et al., 2000; Fleibner et al., 2002; Stefanato et al., 2009). Therefore, although its role in the efflux of fungal secondary metabolites or virulence factors cannot be discarded (Cruz-Cruz et al., 2009; Chuc-Uc et al., 2011), *MfAtr4* could be involved in the efflux of banana defense toxic compounds, example, preformed phytoprotectants as banana phytoanticipins (Cruz-Cruz et al., 2010) or inducible banana phytoalexins (Lazzaro et al., 2004). Research is currently being conducted to analyze the role of *MfAtr4* in *M. fijiensis* virulence and its probable role in detoxification of banana toxicants.

ACKNOWLEDGEMENTS

We are grateful to the Joint Genome Institute for the facilities provided to gain access to the sequence of the *M. fijiensis* genome, and to CONACyT for the economical support to project No. 45788Z and for the Ph. D., scholarship No. 204766 to Y. Couoh-Uicab.

REFERENCES

Abiala MA, Ogunjobi AA, Odebode AC, Ayodele MA (2010). Microbial control of *Mycosphaerella fijiensis* Morelet a notable pathogen of bananas and plantains. Nat. Sci. 8 (10): 299-305.

Albertini C, Grend M, Leroux P (1999). Mutations of the β-tubulin gene associated with different phenotypes of benzimidazole resistance in the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis*. Pestic. Biochem. Physiol. 64: 17-31.

Amil AF, Heaney SP, Stanger C, Shaw MW (2007). Dynamic of QoI sensivity in *Mycosphaerella fijiensis* in Costa Rica during 2000 to 2003. Phytopathology, 97(11): 1451-1457.

Andrade AC, Del Sorbo G, Van Nistelrooy JGM, Waard MAD (2000). The ABC transporter AtrB from *Aspergillus nidulans* mediates resistance to all major classes of fungicides and some natural toxic compounds. Microbiology, 146: 1987-1997.

Arzanlou M, Waalwijk C, Guzmán M, Crous PW, Carlier J (2007). Molecular diagnostics for the Sigatoka disease complex of banana. Phytopathology, 97(9): 1112-1118.

Bairoch A (1991). Prosite: a dictionary of sites and patterns in proteins. Nucleic Acids Res. 19: 2241-2245.

Campanella JJ, Bitincka L, Smalley J (2003). MatGAT: An application that generates similarity/identity matrices using protein or DNA sequences. BMC Bioinformatics, 4: 29-29.

Cannon RD, Lamping E, Holmes AR, Niimi K, Baret PV, Keniya MV, Tanabe K, Niimi M, Goffeau A, Monk BC (2009). Efflux-mediated antifungal drug resistance. Clin. Microbiol. Rev. 22(2): 291-321.

Cañaz-Gutiérrez GP, Patiño LF, Rodríguez-Arango E, Arango R (2006). Molecular characterization of benomyl resistant isolates of *Mycosphaerella fijiensis*, Collected in Colombia. J. Phytopathol.154: 403-409.

Carlier J, Zapater MF, Lapeyre F, Jones DR, Mourichon X (2000). Septoria leaf spot of banana: a newly discovered disease caused by *Mycosphaerella eumusae*. Phytopathology, 90: 884-890.

Coleman JJ, Mylonakis E (2009). Efflux in Fungi: La Pie`ce de Re´sistance. PLoS Pathog. 5 (6): e1000486. doi:10.1371/journal.ppat.1000486.

Chuc-Uc J, Brito-Argáez L, Canto-Canché B, Tzec-Simá M, Rodríguez-García C, Peraza-Echeverría L, Peraza-Echeverría S, James-Kay A, Cruz-Cruz CA, Peña-Rodróguez LM, **I**slas-Flores I. (2011). The in vitro secretome of *Mycosphaerella fijiensis* induces cell death in banana leaves. Plant Physiol. Biochem. 49 (6): 572-578.

Cruz-Cruz CA, Escalante-Erosa F, Peña-Rodríguez LM (2009). Production of hydrophilic phytotoxins by *Mycosphaerella fijiensis*. J. Gen. Plant Pathol. 75: 191-195.

Cruz-Cruz CA, Ramírez-Tec G, García-Sosa K, Escalante-Erosa F, Hill L, Osbourn AE and Peña-Rodríguez LM (2010). Phytoanticipins from banana (*Musa acuminate* cv. Grande Naine) plants, with antifungal activity against *Mycosphaerella fijiensis*, the causal agent of black Sigatoka. Eur. J. Plant Pathol. 126: 459-463.

Davidson AL, Maloney PC (2007). ABC transporters: how small machines do a big job. Trends Microbiol. 15 (10): 448-455.

Del Sorbo G, Schoonbeek H, De Waard MA (2000). Fungal transporters involved in efflux of natural toxic compounds and fungicides. Fungal Genet. Biol. 30: 1-15.

De Waard M, Andrade A, Hayashi K, Schoonbeek H, Stergiopoulus I, and Zwiers L-H (2006). Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. Pest. Manage. Sci. 62: 195-207.

Ernst R, Kueppers D, Klein C, Schwarzmueller T, Kuchler K, Schmitt L (2008). A mutation of the H-loop selectively affects rhodamine transport by the yeast multidrug ABC transporter Pdr5. Proc. Natl. Acad. Sci. USA, 105: 5026-5074.

Fahleson J, Nakyanzi M, Okori P, Seal S, Kenyon L, Dixelius C (2009). Genetic analysis of *Mycosphaerella fijiensis* in the Ugandan lake Victoria region. Plant Pathol. 58: 888-897.

Farlow A, Meduri E, Schlötterer C (2011). DNA double-strand break repair and the evolution of intron density. Trends Genet. 27(1): 1-5.

Fleibner A, Sopalla C, Weltring KM (2002). An ATP-binding cassette multidrug-resistance transporter is necessary for tolerance of *Gibberella pulicaris* to phytoalexins and virulence on potato tubers. Mol. Plant-Microbe Interact. 15(2): 102-108.

Fouré E (1985). Black leaf streak disease of bananas and plantains (*Mycosphaerella fijiensis* Morelet). Study of the symptoms and stages of the disease in Gabon. CIRAD-*IRFA*, París, p. 20.

Goodwin SB, Dunkle LD, Zismann VL (2001). Phylogenetic analysis of *Cercospora* and *Mycosphaerella* based on the internal transcribed spacer region of ribosomal DNA. Phytopathology, 91: 648–658.

Gottesman MM, Fojo T, Bates SE (2002). Multidrug resistance in cancer: role of ATP-dependent transporters. Nat. Rev. Cancer, 2: 48-58.

Guan W, Jiang H, Guo X, Mancera E, Xu L, et al. (2010). Antagonistic changes in sensitivity to antifungal drugs by mutations of an Important ABC transporter gene in a fungal pathogen. PLoS One 5(6): e11309. doi:10.1371/journal.pone.0011309.

Gupta A, Chattoo BB (2008). Functional analysis of a novel ABC transporter ABC4 from *Magnaporthe grisea*. FEMS Microbiol. Lett . 278: 22-28.

Haque A, Rai V, Bahal BS, Shukla S, Lattif AA, Mukhopadhyay G, and Prasad R (2007). Allelic variants of ABC drug transporter Cdr1p in clinical isolates of *Candida albicans*. Biochem. Biophys. Res. Commun. 352: 491-497.

Haugen P, Runge HJ, Bhattacharya D (2004). Long-term evolution of the fungal nuclear small subunit rRNA group I introns. RNA, 10: 1084-1096.

Higgins C (1992). ABC transporter: From microorganism to man. Annu. Rev. Cell Biol. 8: 67-113.

Igarashi Y, Aoki KF, Mamitsuka H, Kuma KI, Kanehisa M (2004). The evolutionary repertoires of the eukaryotic-type ABC transporters in terms of the phylogeny of ATP-binding domains in eukaryotes and prokaryotes. Mol. Biol. Evol. 21: 2149-2160.

Islas-Flores I, Peraza-Echeverría L, Canto-Canché B, Rodríguez-García C (2006). Extraction of high-quality melanin free RNA from *Mycosphaerella fijiensis* for cDNA preparation. Mol. Biotechnol. 34(1): 45-50.

Jie X, Lifang F, Dongxia Y, Chengjie F, Wei M (2010). Genome-wide identification and evolution of ATP-binding cassette transporters in the ciliate *Tetrahymena thermophila*: a case of functional divergence in a multigene family. BMC Evol. Biol. 10: 330, doi:10.1186/1471-2148-10-330.

Johanson A (1997). Detection of Sigatoka leaf spot pathogens of banana by the polymerase chain reaction. Natural Resources Institute, Chatham UK, p. 38.

Jones PM, George AM (2004). The ABC transporter structure and mechanisms: perspectives on recent research. Cell Mol. Life Sci. 61: 682-699.

Kenneth L, Higgins C (2007). Structure and function of ABC transporters: the ATP switch provides flexible control. Eur. J. Physiol. 453: 555-567.

Kovalchuk A, Driessen AJM (2010). Phylogenetic analysis of fungal ABC transporters. BMC Genomics, 11(177): 1-21.

Kuo D, Tan K, Zinman G, Ravasi T, Bar-Joseph Z, Ideker T (2010). Evolutionary divergence in the fungal response to fluconazole revealed by soft clustering. Genomics Biol. 11: R77.

Kretschner M, Leroch M, Mosbach A, Walker AS, Fillinger S, Mernke D, Schoonbeek HJ, Pradier JM, Leroux P, De Ward MA, Hahn M (2009). Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. PLoS Pathog. 5(12): e1000696; Doi:10.371/journal.ppar.1000696.

Laleh-Zereshki Nobar, Reza Azarbaijani, Mostafa Valizadeh, Mohammad Saeid Hejazi (2008). Cloning and sequencing of ABC transporter ATP binding protein encoding gene from *Streptomyces minoensis*. Biotechnology, 7(2): 182-187.

Lazzaro A, Corominas M, Martí C, Flors C, Izquierdo R, Grillo TA, Luis JG, Nonell S (2004). Light- and singlet oxygen-mediated antifungal activity of phenylphenalenone phytoalexins. Photochem. Photobiol. Sci. 3: 706-710.

Lupski JR (2007). An evolution revolution provides further revelation. BioEssays, 29: 1182-1184.

Mourichon X, Peter D, Zapater M (1987). Inoculation experimentale of *Mycosphaerella fijiensis* sur de juenes plantules de bananiers issues de culture *in vitro*. Fruits, 42(4): 195-198.

Nielsen CB, Friedman B, Birren B, Burge CB, Galagan JE (2004). Patterns of intron gain and loss in fungi. PLoS Biol. 2(12): e422.

Nikaido H (2009). Multidrug resistance in bacteria. Annu. Rev. Biochem. 78: 119-146.

Orozco-Santos M, Orozco-Romero J, Pérez-Zamora O, Manzo-Sánchez G, Farías-Larios J, da Silva Moraes W (2008). Prácticas culturales para el manejo de la Sigatoka negra en bananos y plátanos. Trop. Plant Pathol. 33(3): 189-196.

Piehler A, Hellum M, Wenzel J, Kaminski E, Foss KB, Kierulf P, Wolfgang K (2008). The human ABC transporter pseudogene family: evidence for transcription and gene pseudogene interference. BMC Genomics, 9: 165-178.

Preeti S, Akhtar N, Prasad R (2006). Chimeras of the ABC drug transporter Cdr1p reveal functional indispensability of transmembrane domains and nucleotide-binding domains, but transmembrane segment 12 is replaceable with the corresponding homologous region of the non-drug transporter Cdr3p. Microbiol. 152: 1559-1573.

Rai V, Gaur M, Shukla S, Shukla S, Ambudkar SV, Komath SS, Prasad R (2006). Conserved Asp327 of Walker B motif in the N-terminal nucleotide binding domain (NBD-1) of Cdr1p of *Candida albicans* has acquired a new role in ATP hydrolysis. Biochemistry, 45(49): 14726-14739.

Romero RA, Sutton T (1998). Characterization on Benomyl resistance *in Mycosphaerella fijiensis* cause of black Sigatoka of banana in Costa Rica. Plant Dis. 82 (8): 931-934.

Schoonbeek H, Del Sorbo G, De Waard MA (2001). The ABC transporter *BcatrB* affects the sensitivity of *Botrytis cinerea* to the phytoalexin resveratrol and the fungicide fenpiclonil. Mol. Plant-Microbe Interact. 14 (4): 562-571.

Seret M-L, Diffels JF, Goffeau A, Baret PV (2009). Combined phylogeny and neighborhood analysis of the evolution of the ABC transporters conferring multiple drug resistance in hemiascomycete yeasts. BMC Genomics 10(1): 459. Doi:10.1186/1471-2164-10-459.

Sierotzki H, Parisi S, Steinfeld U, Tenzer I, Poirey S, Gisi U (2000). Mode of resistance to respiration inhibitors at the cytochrome bc1 enzyme complex of *Mycosphaerella fijiensis* field isolates. Pest. Manage. Sci. 56: 833-841.

Skov J, Lemmens M, Giese H (2004). Role of a *Fusarium culmorum* ABC transporter (*FcABC1*) during infection of wheat and barley. Physiol. Mol. Plant Pathol. 64: 245-254.

Stefanato FL, Abou-Mansour E, Buchala A, Kretschmer M, Mosbach A, Hahn M, Bochet CG, Métraux JP, Schoonbeek HJ (2009). The ABC transporter BcatrB from *Botrytis cinerea* exports camalexin and is a virulence factor on *Arabidopsis thaliana*. Plant J. 58(3): 499-510.

Stergiopoulos I, Zwiers L-H, De Waard M (2002). Secretion of natural and synthetic toxic compounds from filamentous fungi by membrane transporter of the ATP-binding cassette and major facilitator superfamily. Eur. J. Plant Pathol. 108: 719-734.

Stergiopoulos I, Zwiers L-H, De Waard M (2003). The ABC Transporter *MgAtr4* is a virulence factor of *Mycosphaerella graminicola* that affects colonization of substomatal cavities in wheat leaves. Mol. Plant-Microbe Interact. 16(8): 689-698.

Sturm A, Cunningham P, Dean M (2009). The ABC transporter gene family of *Daphnia pulex*. BMC Genomics, 10: 170-188.

Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM (2006). Targeting multidrug resistance in cancer. Nat. Rev. Drug Discovery, 5: 219-234.

Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.

Theiss S, Kretschmar M, Nichterlein T, Hof H, Agabian N, Hacker J, Kohler GA (2002). Functional analysis of a vacuolar ABC transporter in wild-type *Candida albicans* reveals its involvement in virulence. Mol. Microbiol. 43: 571-584.

Urban M, Bhargava T, Hamer JE (1999). An ATP-driven efflux pump is a novel pathogenicity factor in rice blast disease. EMBO J. 18(3): 512-521.

Vásquez LE, Guzmán F, Patarroyo M, Arango R (2009). *In Vitro* evaluation of antimicrobial peptides against *Mycosphaerella fijiensis* Morelet and their interaction with some chemical fungicides. Rev. Fac. Natl. Agr. Medellín, 62(2): 5063-5069.

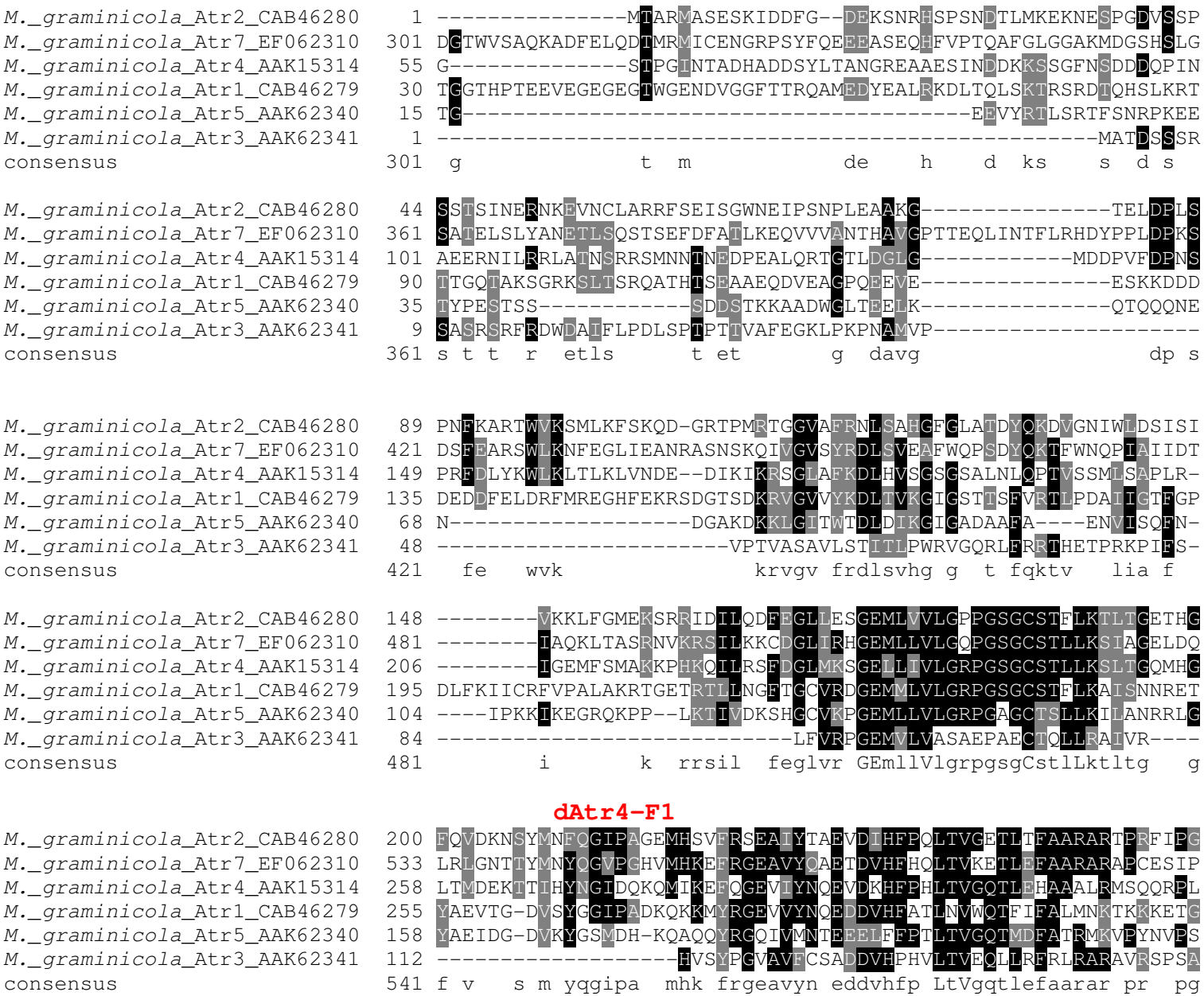
Walker JE, Sarasate M, Runswick MJ, Gay NJ (1982). Distantly related sequences in the alpha and betha subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and common nucleotide binding fold. EMBO J. 269: 32592-32597. Zhang L-Y, Yang Y-F, Niu D-K (2010). Evaluation of models of the mechanism underlying intron loss and gain in *Aspergillus fungi*. J. Mol. Evol. DOI: 10.1007/s00239-010-9391-6.

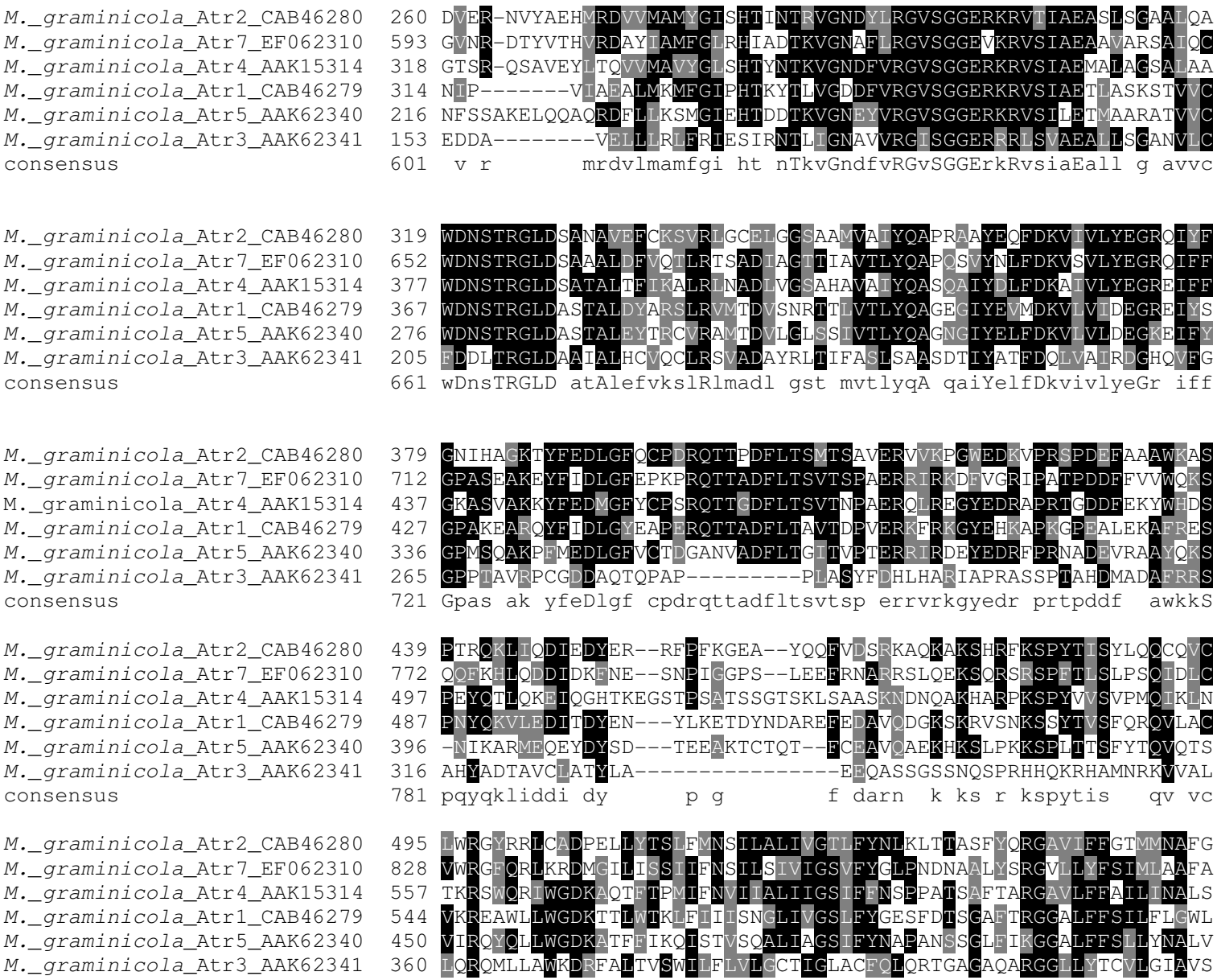
Zwiers L-H, Roohparvar R, De Waard MA (2007). MgAtr7, a new type of ABC transporter from *Mycosphaerella graminicola* involved in iron homeostasis. Fungal Genet. Biol. 44: 853-863.

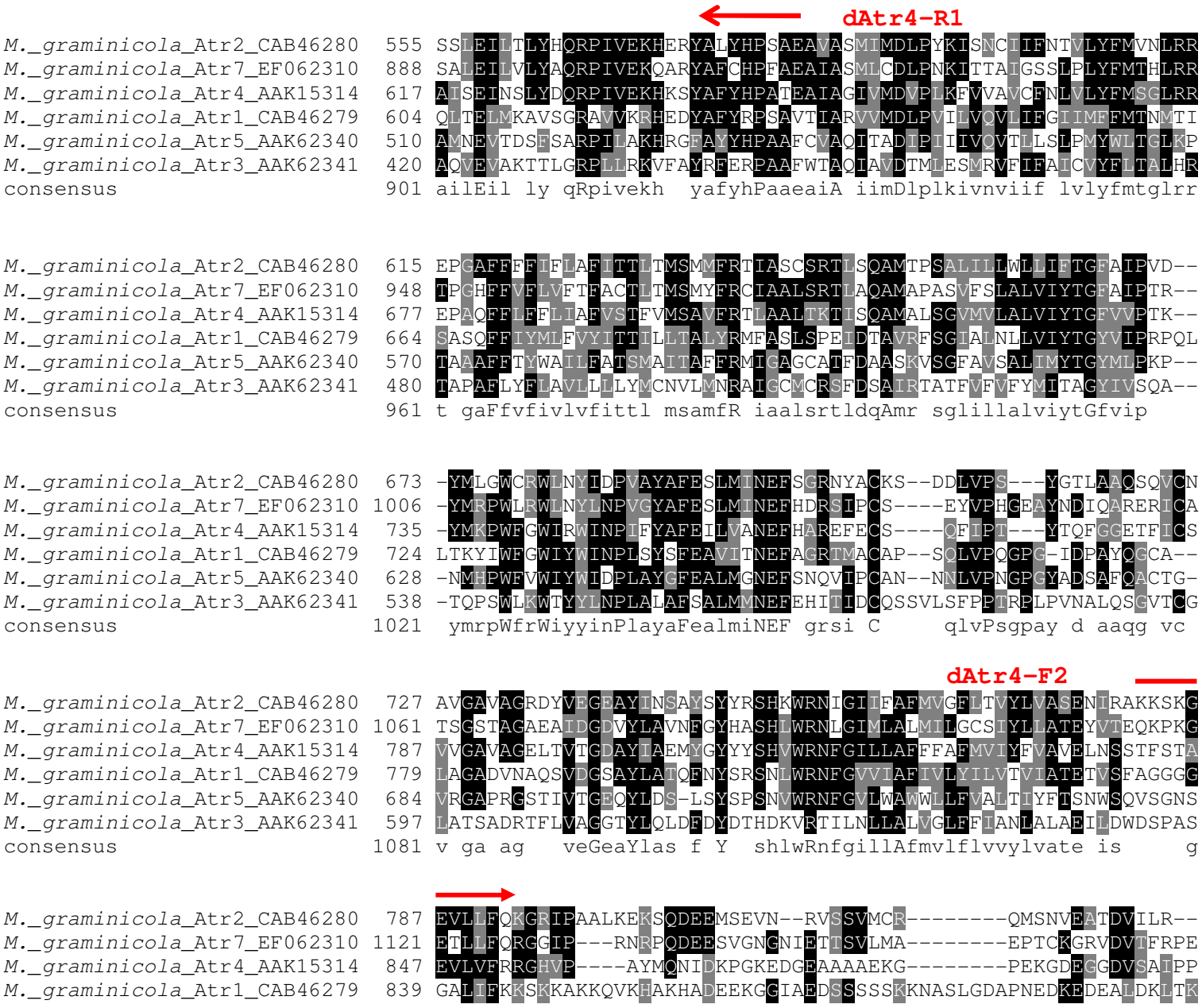
Zwiers L-H, Stergiopoulos I, Gielkens MM, Goodall SD, De Waard MA (2003). ABC transporters of the wheat pathogen *Mycosphaerella graminicola* function as protectants against biotic and xenobiotic toxic compounds. Mol. Genet. Genomics, 269: 499-507.

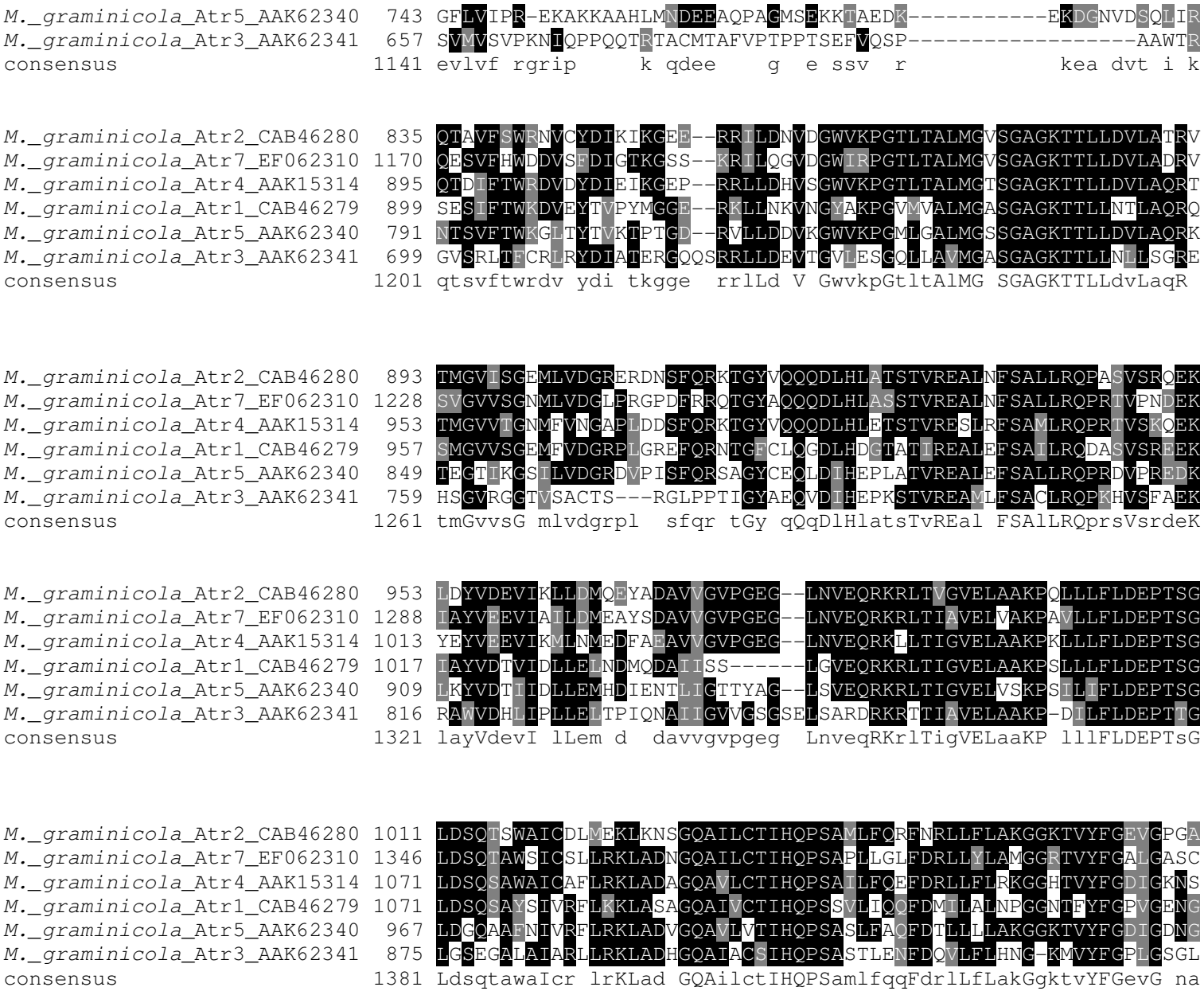
Supplementary material 1. ClustalX alignment of amino acid sequences of *Mycosphaerella graminicola* Atrs. Identical amino acids are shaded in black and conservative substitutions are shaded in grey.

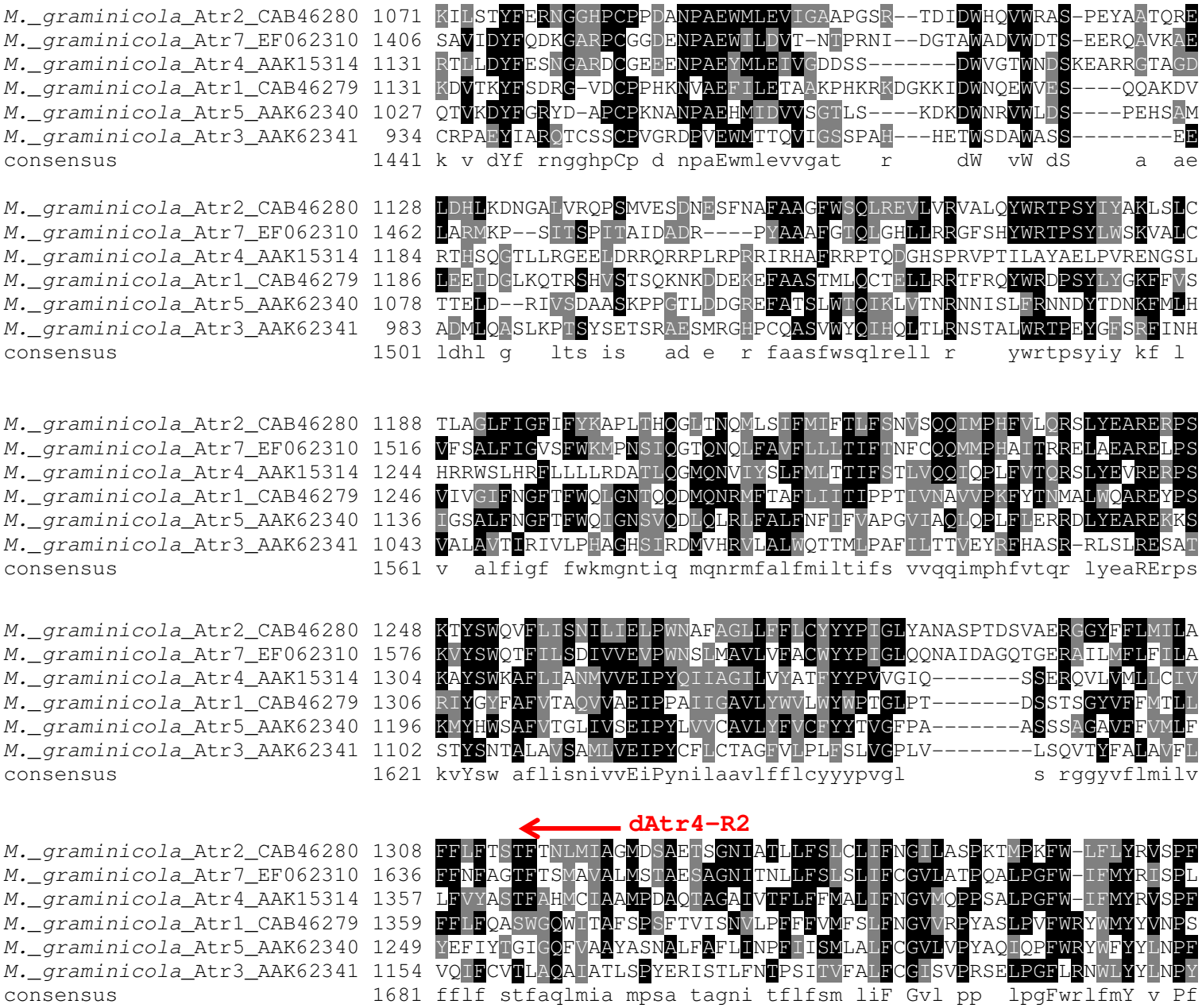
```
M._graminicola_Atr2_CAB46280     1 ------------------------------------------------------------
M._graminicola_Atr7_EF062310     1 MNASNSSLIASDPDGICEKILDVILEYALHKFGDTKQKLALGRPKFLETIAGFVVQGRCI
M._graminicola_Atr4_AAK15314     1 ------------------------------------------------------------
M._graminicola_Atr1_CAB46279     1 ------------------------------------------------------------
M._graminicola_Atr5_AAK62340     1 ------------------------------------------------------------
M._graminicola_Atr3_AAK62341     1 ------------------------------------------------------------
consensus                        1

M._graminicola_Atr2_CAB46280     1 ------------------------------------------------------------
M._graminicola_Atr7_EF062310    61 QMCLPAFPFKSSNKIDKVLGTLPDKAEELALGRLNTMCAKVQAIHAPGAALTIISDGLVY
M._graminicola_Atr4_AAK15314     1 ------------------------------------------------------------
M._graminicola_Atr1_CAB46279     1 ------------------------------------------------------------
M._graminicola_Atr5_AAK62340     1 ------------------------------------------------------------
M._graminicola_Atr3_AAK62341     1 ------------------------------------------------------------
consensus                       61

M._graminicola_Atr2_CAB46280     1 ------------------------------------------------------------
M._graminicola_Atr7_EF062310   121 NDLLSISDKDTWAYGEALRSMAIAHEFQHIRFARIRDLIKFPGSEVLNEITYVASATNFR
M._graminicola_Atr4_AAK15314     1 ------------------------------------------------------------
M._graminicola_Atr1_CAB46279     1 ------------------------------------------------------------
M._graminicola_Atr5_AAK62340     1 ------------------------------------------------------------
M._graminicola_Atr3_AAK62341     1 ------------------------------------------------------------
consensus                      121

M._graminicola_Atr2_CAB46280     1 ------------------------------------------------------------
M._graminicola_Atr7_EF062310   181 RSLLNEFGKDDIDIDHEIATSEDTKMTYLGYRRFLESDLKHIFPLGSDRSANSYKRNVKF
M._graminicola_Atr4_AAK15314     1 ------------------------------------------------------------
M._graminicola_Atr1_CAB46279     1 ------------------------------------------------------------
M._graminicola_Atr5_AAK62340     1 ------------------------------------------------------------
M._graminicola_Atr3_AAK62341     1 ------------------------------------------------------------
consensus                      181

M._graminicola_Atr2_CAB46280     1 ------------------------------------------------------------
M._graminicola_Atr7_EF062310   241 LAKEMIIRGYAFAGAVRAAFPEHLRLSIHQSTGEHKISISLLHTKTGFTTPWHCSVARLA
M._graminicola_Atr4_AAK15314     1 ----MATGGSASTPIVGDYNPERVEQLFDIPRPEQGN--IYVEAENAARTPSGAQESQLS
M._graminicola_Atr1_CAB46279     1 ------------------------------MWGYSVDERKLQREDTNGPPINQWHNAQR
M._graminicola_Atr5_AAK62340     1 ---------------------------------------MTLQR-------SSSWGVQTG
M._graminicola_Atr3_AAK62341     1 ------------------------------------------------------------
consensus                      241                                        l       ps
```

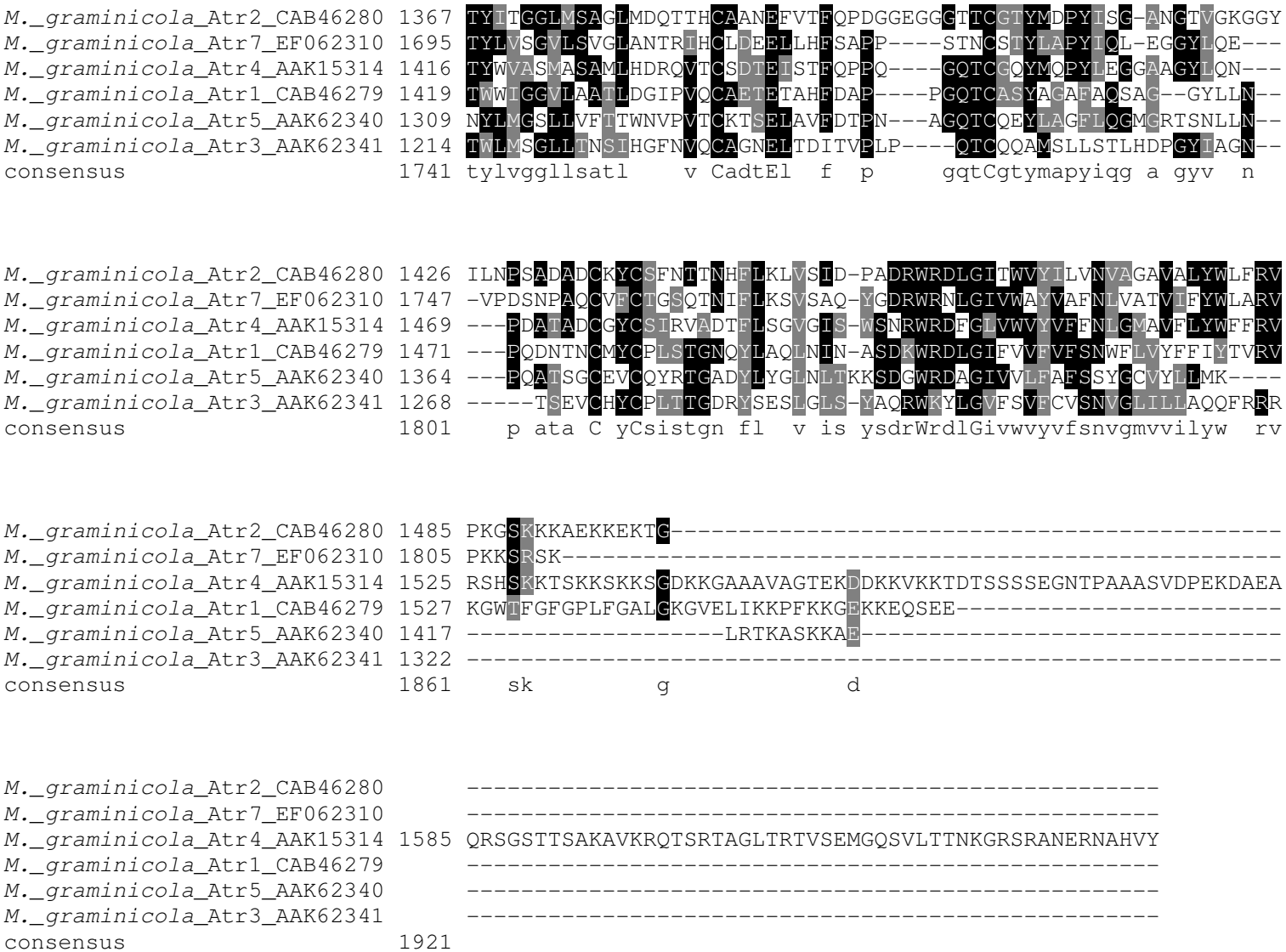

M._graminicola_Atr2_CAB46280
M._graminicola_Atr7_EF062310
M._graminicola_Atr4_AAK15314
M._graminicola_Atr1_CAB46279
M._graminicola_Atr5_AAK62340
M._graminicola_Atr3_AAK62341
consensus
dAtr4-F1


M._graminicola_Atr2_CAB46280 260 DVER-NVYAEHMRDVVMAMYGISHTINTRVGNDYLRGVSGGERKRVTIAEASLSGAALQA
M._graminicola_Atr7_EF062310 593 GVNR-DTYVTHVRDAYIAMFGLRHIADTKVGNAFLRGVSGGEVKRVSIAEAAVARSAIQC
M._graminicola_Atr4_AAK15314 318 GTSR-QSAVEYLTQVVMAVYGLSHTYNTKVGNDFVRGVSGGERKRVSIAEMALAGSALAA
M._graminicola_Atr1_CAB46279 314 NIP-------VIAFALMKMFGIPHTKYTLVGDDFVRGVSGGERKRVSIAETLASKSTVVC
M._graminicola_Atr5_AAK62340 216 NFSSAKELQQAQRDFLLKSMGIEHTDDTKVGNEYVRGVSGGERKRVSILETMAARATVVC
M._graminicola_Atr3_AAK62341 153 EDDA--------VELLLRLFRIESIRNTLIGNAVVRGISGGERRRLSVAEALLSGANVLC
consensus 601 v r mrdvlmamfgi ht nTkvGndfvRGvSGGErkRvsiaEall g avvc
M._graminicola_Atr2_CAB46280 319 WDNSTRGLDSANAVEFCKSVRLGCELGGSAAMVAIYQAPRAAYEQFDKVIVLYEGRQIYF
M._graminicola_Atr7_EF062310 652 WDNSTRGLDSAAALDFVQTLRTSADIAGTTIAVTLYQAPQSVYNLFDKVSVLYEGRQIFF
M._graminicola_Atr4_AAK15314 377 WDNSTRGLDSATALTFIKALRLNADLVGSAHAVAIYQASQAIYDLFDKAIVLYEGREIFF
M._graminicola_Atr1_CAB46279 367 WDNSTRGLDASTALDYARSLRVMTDVSNRTTLVTLYQAGEGIYEVMDKVLVIDEGREIYS
M._graminicola_Atr5_AAK62340 276 WDNSTRGLDASTALEYTRCVRAMTDVLGLSSIVTLYQAGNGIYELFDKVLVLDEGKEIFY
M._graminicola_Atr3_AAK62341 205 FDDLTRGLDAAIALHCVQCLRSVADAYRLTIFASLSAASDTIYATFDQLVAIRDGHQVFG
consensus 661 wDnsTRGLD atAlefvkslRlmadl gst mvtlyqA qaiYelfDkvivlyeGr iff
M._graminicola_Atr2_CAB46280 379 GNIHAGKTYFEDLGFQCPDRQTTPDFLTSMTSAVERVVKPGWEDKVPRSPDEFAAAWKAS
M._graminicola_Atr7_EF062310 712 GPASEAKEYFIDLGFEPKPRQTTADFLTSVTSPAERRIRKDFVGRIPATPDDFFVVWQKS
M._graminicola_Atr4_AAK15314 437 GKASVAKKYFEDMGFYCPSRQTTGDFLTSVTNPAERQLREGYEDRAPRTGDDFEKYWHDS
M._graminicola_Atr1_CAB46279 427 GPAKEARQYFIDLGYEAPERQTTADFLTAVTDPVERKFRKGYEHKAPKGPEALEKAFRES
M._graminicola_Atr5_AAK62340 336 GPMSQAKPFMEDLGFVCTDGANVADFLTGITVPTERRIRDEYEDRFPRNADEVRAAYQKS
M._graminicola_Atr3_AAK62341 265 GPPTAVRPCGDDAQTQPAP---------PLASYFDHLHARIAPRASSPTAHDMADAFRRS
consensus 721 Gpas ak yfeDlgf cpdrqttadfltsvtsp errvrkgyedr prtpddf awkkS
M._graminicola_Atr2_CAB46280 439 PTRQKLIQDIEDYER--RFPFKGEA--YQQFVDSFKAQKAKSHRFKSPYTISYLQQCQVC
M._graminicola_Atr7_EF062310 772 QQFKHLQDDIDKFNE--SNPIGGPS--LEEFRNARRSLQEKSQRSRSPFTLSLPSQIDLC
M._graminicola_Atr4_AAK15314 497 PEYQTLQKEIQGHTKEGSTPSATSSGTSKLSAASKNDNQAKHARPKSPYVVSVPMQIKLN
M._graminicola_Atr1_CAB46279 487 PNYQKVLEDITDYEN---YLKETDYNDAREFEDAVQDGKSKRVSNKSSYTVSFQRQVLAC
M._graminicola_Atr5_AAK62340 396 -NIKARMEQEYDYSD---TEEAKTCTQT--FCEAVQAEKHKSLPKKSPLTTSFYTQVQTS
M._graminicola_Atr3_AAK62341 316 AHYADTAVCLATYLA----------------EEQASSGSSNQSPRHHQKRHAMNRKVVAL
consensus 781 pqyqkliddi dy p g f darn k ks r kspytis qv vc
M._graminicola_Atr2_CAB46280 495 LWRGYRRLCADPELLYTSLFMNSILALIVGTLFYNLKLTTASFYQRGAVIFFGTMMNAFG
M._graminicola_Atr7_EF062310 828 VWRGFQRLKRDMGILISSIIFNSILSIVIGSVFYGLPNDNAALYSRGVLLYFSIMLAAFA
M._graminicola_Atr4_AAK15314 557 TKRSWQRIWGDKAQTFTPMIFNVIIALIIGSIFFNSPPATSAFTARGAVLFFAILINALS
M._graminicola_Atr1_CAB46279 544 VKREAWLLWGDKTTLWTKLFIIISNGLIVGSLFYGESFDTSGAFTRGGALFFSILFLGWL
M._graminicola_Atr5_AAK62340 450 VIRQYQLLWGDKATFFIKQISTVSQALIAGSIFYNAPANSSGLFIKGGALFFSLLYNALV
M._graminicola_Atr3_AAK62341 360 LQRQMLLAWKDRFALTVSWILFLVLGCTIGLACFQLQRTGAGAQARGGLLYTCVLGIAVS
consensus 841 v R yq lwgDkg lytslimnvilaliiGslfynlp t g y rGgvlffsilmna

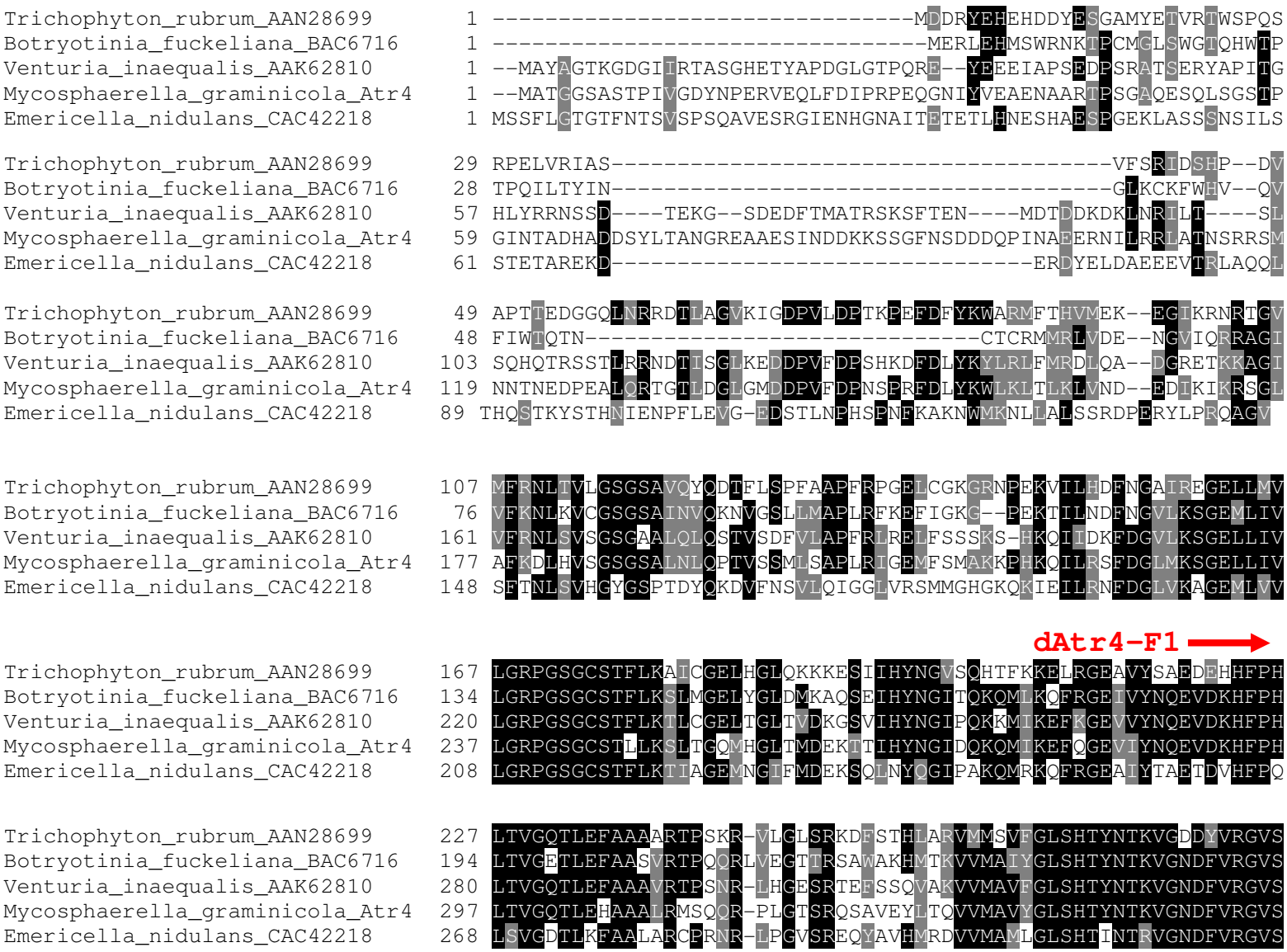

dAtr4-R1
dAtr4-F2
M._graminicola_Atr2_CAB46280 555 SSLEILTLYHQRPIVEKHERYALYHPSAEAVASMIMDLPYKISNCIIFNTVLYFMVNLRR
M._graminicola_Atr7_EF062310 888 SALEILVLYAQRPIVEKQARYAFCHPFAEAIASMLCDLPNKITTAIGSSLPLYFMTHLRR
M._graminicola_Atr4_AAK15314 617 AISEINSLYDQRPIVEKHKSYAFYHPATEAIAGIVMDVPLKFVVAVCFNLVLYFMSGLRR
M._graminicola_Atr1_CAB46279 604 QLTELMKAVSGRAVVKRHEDYAFYRPSAVTIARVVMDLPVILVQVLIFGIIMFFMTNMTI
M._graminicola_Atr5_AAK62340 510 AMNEVTDSFSARPILAKHRGFAYYHPAAFCVAQITADIPIIIVQVTLLSLPMYWLTGLKP
M._graminicola_Atr3_AAK62341 420 AQVEVAKTTLGRPLLRKVFAYRFERPAAFWTAQIAVDTMLESMRVFIFAICVYFLTALHR
consensus 901 ailEil ly qRpivekh yafyhPaaeaiA iimDlplkivnviif lvlyfmtglrr
M._graminicola_Atr2_CAB46280 615 EPGAFFFFIFLAFITTLTMSMMFRTIASCSRTLSQAMTPSALILLWLLIFTGFAIPVD--
M._graminicola_Atr7_EF062310 948 TPGHFFVFLVFTFACTLTMSMYFRCIAALSRTLAQAMAPASVFSLALVIYTGFAIPTR--
M._graminicola_Atr4_AAK15314 677 EPAQFFLFFLIAFVSTFVMSAVFRTLAALTKTISQAMALSGVMVLALVIYTGFVVPTK--
M._graminicola_Atr1_CAB46279 664 SASQFFIYMLFVYITTILLTALYRMFASLSPEIDTAVRFSGIALNLLVIYTGYVIPRPQL
M._graminicola_Atr5_AAK62340 570 TAAAFFTYWAILFATSMAITAFFRMIGAGCATFDAASKVSGFAVSALIMYTGYMLPKP--
M._graminicola_Atr3_AAK62341 480 TAPAFLYFLAVLLLLYMCNVLMNRAICCMCRSFDSAIRTATFVFVFYMITAGYIVSQA--
consensus 961 t gaFfvfivlvfittl msamfR iaalsrtldqAmr sglillalviytGfvip
M._graminicola_Atr2_CAB46280 673 -YMLGWCRWLNYIDPVAYAFESLMINEFSGRNYACKS--DDLVPS---YGTLAAQSQVCN
M._graminicola_Atr7_EF062310 1006 -YMRPWLRWLNYLNPVGYAFESLMINEFHDRSIPCS----EYVPHGEAYNDIQARERICA
M._graminicola_Atr4_AAK15314 735 -YMKPWFGWIRWINPIFYAFEILVANEFHARERFECS----QFIPT---YTQFGGETFICS
M._graminicola_Atr1_CAB46279 724 LTKYIWFGWIYWINPLSYSFEAVITNEFAGRTMACAP--SQLVPQGPG-IDPAYQCCA--
M._graminicola_Atr5_AAK62340 628 -NMHPWFVWIYWIDPLAYGFEALMGNEFSNQVIPCAN--NNLVPNGPGYADSAFQACTG-
M._graminicola_Atr3_AAK62341 538 -TQPSWLKWTYYLNPLALAFSALMMNEFEHITIDCQSSVLSFPPTRPLPVNALQSGVTCG
consensus 1021 ymrpWfrWiyyinPlayaFealmiNEF grsi C qlvPsgpay d aaqg vc
M._graminicola_Atr2_CAB46280 727 AVGAVAGRDYVEGEAYINSAYSYYRSHKWRNIGIIFAFMVGFLTVYLVASENIRAKKSKG
M._graminicola_Atr7_EF062310 1061 TSGSTAGAEAIDGDVYLAVNFGYHASHLWRNLGIMLALMILGCSIYLLATEYVTEQKPKG
M._graminicola_Atr4_AAK15314 787 VVGAVAGELTVTGDAYIAEMYGYYYSHVWRNFGILLAFFFAFMVIYFVAVELNSSTFSTA
M._graminicola_Atr1_CAB46279 779 LAGADVNAQSVDGSAYLATQFNYSRSNLWRNFGVVIAFIVLYILVTVIATETVSFAGGGG
M._graminicola_Atr5_AAK62340 684 VRGAPRGSTIVTGEQYLDS-LSYSPSNVWRNFGVLWAWWLLFVALTIYFTSNWSQVSGNS
M._graminicola_Atr3_AAK62341 597 LATSADRTFLVAGGTYLQLDFDYDTHDKVRTILNLLALVGLFFIANLALAEILDWDSPAS
consensus 1081 v ga ag veGeaYlas f Y shlwRnfgillAfmvlflvvylvate is g
M._graminicola_Atr2_CAB46280 787 EVLLFQKGRIPAALKEKSQDEEMSEVN--RVSSVMCR--------QMSNVEATDVILR--
M._graminicola_Atr7_EF062310 1121 EILLFQRGGIP---RNRPQDEESVGNGNIETTSVLMA--------EPTCKGRVDVTFRPE
M._graminicola_Atr4_AAK15314 847 EVLVFRRGHVP----AYMQNIDKPGKEDGEAAAAEKG--------PEKGDEGGDVSAIPP
M._graminicola_Atr1_CAB46279 839 GALIFKKSKKAKKQVKHAKHADEEKGGIAEDSSSSSKKNASLGDAPNEDKEDEALDKLTK

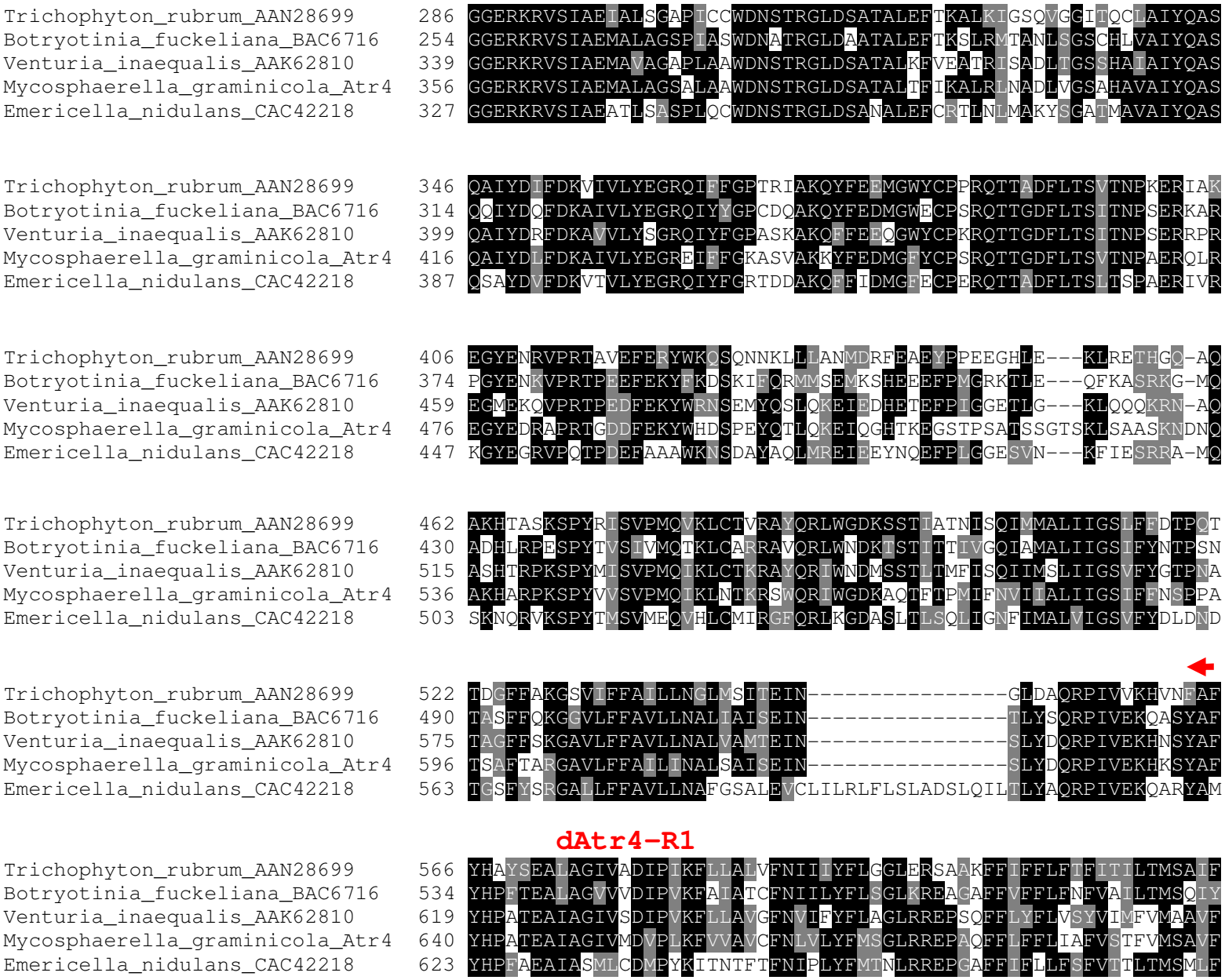

M._graminicola_Atr5_AAK62340 743 GFLVIPR-EKAKKAAHLMNDEEAQPAGMSEKKTAEDK----------EKDGNVDSQLIR
M._graminicola_Atr3_AAK62341 657 SVMVSVPKNIQPPQQTRTACMTAFVPTPPTSEFVQSP----------AAWTR
consensus 1141 evlvf rgrip k qdee g e ssv r kea dvt i k
M._graminicola_Atr2_CAB46280 835 QTAVFSWRNVCYDIKIKGEE--RRILDNVDGWVKPGTLTALMGVSGAGKTTLLDVLATRV
M._graminicola_Atr7_EF062310 1170 QESVFHWDDVSFDIGTKGSS--KRILQGVDGWIRPGTLTALMGVSGAGKTTLLDVLADRV
M._graminicola_Atr4_AAK15314 895 QTDIFTWRDVDYDIEIKGEP--RRLLDHVSGWVKPGTLTALMGTSGAGKTTLLDVLAQRT
M._graminicola_Atr1_CAB46279 899 SESIFTWKDVEYTVPYMGGE--RKLLNKVNGYAKPGVMVALMGASGAGKTTLLNTLAQRQ
M._graminicola_Atr5_AAK62340 791 NTSVFTWKGLTYTVKTPTGD--RVLLDDVKGWVKPGMLGALMGSSGAGKTTLLDVLAQRK
M._graminicola_Atr3_AAK62341 699 GVSRLTFCRLRYDIATERGQQSRRLLDEVTGVLESGQLLAVMGASGAGKTTLLNLLSGRE
consensus 1201 qtsvftwrdv ydi tkgge rrlLd V GwvkpGtltAlMG SGAGKTTLLdvLaqR
M._graminicola_Atr2_CAB46280 893 TMGVISGEMLVDGRERDNSFQRKTGYVQQQDLHLATSTVREALNFSALLRQPASVSRQEK
M._graminicola_Atr7_EF062310 1228 SVGVVSGNMLVDGLPRGPDFRRQTGYAQQQDLHLASSTVREALNFSALLRQPRTVPNDEK
M._graminicola_Atr4_AAK15314 953 TMGVVTGNMFVNGAPLDDSFQRKTGYVQQQDLHLETSTVRESLRFSAMLRQPRTVSKQEK
M._graminicola_Atr1_CAB46279 957 SMGVVSGEMFVDGRPLGREFQRNTGFCLQGDLHDGTATIREALEFSAILRQDASVSREEK
M._graminicola_Atr5_AAK62340 849 TEGTIKGSILVDGRDVPISFQRSAGYCEQLDIHEPLATVREALEFSALLRQPRDVPREDK
M._graminicola_Atr3_AAK62341 759 HSGVRGGTVSACTS---RGLPPTIGYAEQVDIHEPKSTVREAMLFSACLRQPKHVSFAEK
consensus 1261 tmGvvsG mlvdgrpl sfqr tGy qQqDlHlatsTvREal FSAlLRQprsVsrdeK
M._graminicola_Atr2_CAB46280 953 LDYVDEVIKLLDMQEYADAVVGVPGEG--LNVEQRKRLTVGVELAAKPQLLLFLDEPTSG
M._graminicola_Atr7_EF062310 1288 IAYVEEVIAILDMEAYSDAVVGVPGEG--LNVEQRKRLTIAVELVAKPAVLLFLDEPTSG
M._graminicola_Atr4_AAK15314 1013 YEYVEEVIKMLNMEDFAEAVVGVPGEG--LNVEQRKLLTIGVELAAKPKLLLFLDEPTSG
M._graminicola_Atr1_CAB46279 1017 IAYVDTVIDLLELNDMQDAIISS------LGVEQRKRLTIGVELAAKPSLLLFLDEPTSG
M._graminicola_Atr5_AAK62340 909 LKYVDTIIDLLEMHDIENTLIGTTYAG--LSVEQRKRLTIGVELVSKPSILIFLDEPTSG
M._graminicola_Atr3_AAK62341 816 RAWVDHLIPLLELTPIQNAIIGVVGSGSELSARDRKRTTIAVELAAKP-DILFLDEPTIG
consensus 1321 layVdevI lLem d davvgvpgeg LnveqRKrlTigVELaaKP lllFLDEPTsG
M._graminicola_Atr2_CAB46280 1011 LDSQTSWAICDLMEKLKNSGQAILCTIHQPSAMLFQRFNRLLFLAKGGKTVYFGEVGPGA
M._graminicola_Atr7_EF062310 1346 LDSQTAWSICSLLRKLADNGQAILCTIHQPSAPLLGLFDRLLYLAMGGRTVYFGALGASC
M._graminicola_Atr4_AAK15314 1071 LDSQSAWAICAFLRKLADAGQAVLCTIHQPSAILFQEFDRLLFLRKGGHTVYFGDIGKNS
M._graminicola_Atr1_CAB46279 1071 LDSQSAYSIVRFLKKLASAGQAIVCTIHQPSSVLIQQFDMILALNPGGNTFYFGPVGENG
M._graminicola_Atr5_AAK62340 967 LDGQAAFNIVRFLRKLADVGQAVLVTIHQPSASLFAQFDTLLLLAKGGKTVYFGDIGDNG
M._graminicola_Atr3_AAK62341 875 LGSEGALAIARLLRKLADHGQAIACSIHQPSASTLENFDQVLFLHNG-KMVYFGPLGSGL
consensus 1381 LdsqtawaIcr lrKLad GQAilctIHQPSamlfqqFdrlLfLakGgktvYFGevG na


M._graminicola_Atr2_CAB46280 1071 KILSTYFERNGGHPCPPDANPAEWMLEVIGAAPGSR--TDIDWHQVWRAS-PEYAATQRE
M._graminicola_Atr7_EF062310 1406 SAVIDYFQDKGARPCGGDENPAEWILDVT-NTPRNI--DGTAWADVWDTS-EERQAVKAE
M._graminicola_Atr4_AAK15314 1131 RTLLDYFESNGARDCGEEENPAEYMLEIVGDDSS-------DWVGTWNDSKEARRCTAGD
M._graminicola_Atr1_CAB46279 1131 KDVTKYFSDRG-VDCPPHKNVAEFILETAAKPHKRKDGKKIDWNQEWVES----QQAKDV
M._graminicola_Atr5_AAK62340 1027 QTVKDYFGRYD-APCPKNANPAEHMIDVVSGTLS----KDKDWNRVWLDS----PEHSAM
M._graminicola_Atr3_AAK62341 934 CRPAEYIARQTCSSCPVGRDPVEWMTTQVIGSSPAH---HETWSDAWASS--------EE
consensus 1441 k v dYf rngghpCp d npaEwmlevvgat r dW vW dS a ae
M._graminicola_Atr2_CAB46280 1128 LDHLKDNGALVRQPSMVESDNESFNAFAAGFWSQLREVLVRVALQYWRTPSYIYAKLSLC
M._graminicola_Atr7_EF062310 1462 LARMKP--SITSPITAIDADR----PYAAAFGTQLGHLLRRGFSHYWRTPSYLWSKVALC
M._graminicola_Atr4_AAK15314 1184 RTHSQGTLLRGEELDRRQRRPLRPRRIRHAFRRPTQDGHSPRVPTILAYAELPVRENGSL
M._graminicola_Atr1_CAB46279 1186 LEEIDGLKQTRSHVSTSQKNKDDEKEFAASTMLQCTELLRRTFRQYWRDPSYLYGKFFVS
M._graminicola_Atr5_AAK62340 1078 TTELD--RIVSDAASKPPGTLDDGREFATSLWTQIKLVTNRNNISLFRNNDYTDNKFMLH
M._graminicola_Atr3_AAK62341 983 ADMLQASLKPTSYSETSRAESMRGHPCQASVWYQIHQLTLRNSTALWRTPEYGFSRFINH
consensus 1501 ldhl g lts is ad e r faasfwsqlrell r ywrtpsyiy kf l
M._graminicola_Atr2_CAB46280 1188 TLAGLFIGFIFYKAPLTHQGLTNQMLSIFMIFTLFSNVSQQIMPHFVLQRSLYEARERPS
M._graminicola_Atr7_EF062310 1516 VFSALFIGVSFWKMPNSIQGTQNQLFAVFLLLTIFTNFCQQMMPHAITRRELAEARELPS
M._graminicola_Atr4_AAK15314 1244 HRRWSLHRFLLLLRDATLQGMQNVIYSLFMLTTIFSTLVQQIQPLFVTQRSLYEVRERPS
M._graminicola_Atr1_CAB46279 1246 VIVGIFNGFTFWQLGNTQQDMQNRMFTAFLIITIPPTIVNAVVPKFYTNMALWQAREYPS
M._graminicola_Atr5_AAK62340 1136 IGSALFNGFTFWQIGNSVQDLQLRLFALFNFIFVAPGVIAQLQPLFLERRDLYEARERKKS
M._graminicola_Atr3_AAK62341 1043 VALAVTIRIVLPHAGHSIRDMVHRVLALWQTTMLPAFILTTVEYRFHASR-RLSLRESAT
consensus 1561 v alfigf fwkmgntiq mqnrmfalfmiltifs vvqqimphfvtqr lyeaRErps
M._graminicola_Atr2_CAB46280 1248 KTYSWQVFLISNILIELPWNAFAGLLFFLCYYYPIGLYANASPTDSVAERGGYFFLMILA
M._graminicola_Atr7_EF062310 1576 KVYSWQTFILSDIVVEVPWNSLMAVLVFACWYYPIGLQQNAIDAGQTGERAILMFLFILA
M._graminicola_Atr4_AAK15314 1304 KAYSWKAFLIANMVVEIPYQIIAGILVYATFYYPVVGIQ-------SSERQVLVMLLCIV
M._graminicola_Atr1_CAB46279 1306 RIYGYFAFVTAQVVAEIPPAIIGAVLYWVLWYWPTGLPT-------DSSTSGYVFFMTLL
M._graminicola_Atr5_AAK62340 1196 KMYHWSAFVTGLIVSEIPYLVVCAVLYFVCFYYTVGFPA-------ASSSAGAVFFVMLF
M._graminicola_Atr3_AAK62341 1102 STYSNTALAVSAMLVEIPYCFLCTAGFVLPLFSLVGPLV--------LSQVTYFALAVFL
consensus 1621 kvYsw aflisnivvEiPynilaavlfflcyyypvgl s rggyvflmilv
dAtr4-R2
M._graminicola_Atr2_CAB46280 1308 FFLFTSTFTNLMIAGMDSAETSGNIATLLFSLCLIFNGILASPKTMPKFW-LFLYRVSPF
M._graminicola_Atr7_EF062310 1636 FFNFAGTFTSMAVALMSTAESAGNITNLLFSLSLIFCGVLATPQALPGFW-IFMYRISPL
M._graminicola_Atr4_AAK15314 1357 LFVYASTFAHMCIAAMPDAQIAGAIVTFLFFMALIFNGVMQPPSALPGFW-IFMYRVSPF
M._graminicola_Atr1_CAB46279 1359 FFLFQASWGQWITAFSPSFTVISNVLPFFFVMFSLFNGVVRPYASLPVFWRYWMYYVNPS
M._graminicola_Atr5_AAK62340 1249 YEFIYTGIGQFVAAYASNALFAFLINPFIISMLALFCGVLVPYAQIQPFWRYWFYYLNPF
M._graminicola_Atr3_AAK62341 1154 VQIFCVTLAQAIATLSPYERISTLFNTPSITVFALFCGISVPRSELPGFLRNWLYYLNPY
consensus 1681 fflf stfaqlmia mpsa tagni tflfsm liF Gvl pp lpgFwrlfmY v Pf


M._graminicola_Atr2_CAB46280 1367 TYITGGLMSAGLMDQTTHCAANEFVTFQPDGGEGGGTTCGTYMDPYISG-ANGTVGKGGY
M._graminicola_Atr7_EF062310 1695 TYLVSGVLSVGLANTRIHCLDEELLHFSAPP----STNCSTYLAPYIQL-EGGYLQE---
M._graminicola_Atr4_AAK15314 1416 TYWVASMASAMLHDRQVTCSDTEISTFQPPQ----GQTCGQYMQPYLEGGAAGYLQN---
M._graminicola_Atr1_CAB46279 1419 TWWIGGVLAATLDGIPVQCAETETAHFDAP----PGQTCASYAGAFAQSAG--GYLLN--
M._graminicola_Atr5_AAK62340 1309 NYLMGSLLVFTTWNVPVTCKTSELAVFDTPN---AGQTCQEYLAGFLQGMGRTSNLLN--
M._graminicola_Atr3_AAK62341 1214 TWLMSGLLINSIHGFNVQCAGNELTDITVPLP----QTCQQAMSLLSTLHDPGYIAGN--
consensus 1741 tylvggllsatl v CadtEl f p gqtCgtymapyiqg a gyv n
M._graminicola_Atr2_CAB46280 1426 ILNPSADADCKYCSFNTTNHFLKLVSID-PADRWRDLGITWVYILVNVAGAVALYWLFRV
M._graminicola_Atr7_EF062310 1747 -VPDSNPAQCVFCTGSQTNIFLKSVSAQ-YGDRWRNLGIVWAYVAFNLVATVIFYWLARV
M._graminicola_Atr4_AAK15314 1469 ---PDATADCGYCSIRVADTFLSGVGIS-WSNRWRDFGLVWVYVFFNLGMAVFLYWFFRV
M._graminicola_Atr1_CAB46279 1471 ---PQDNTNCMYCPLSTGNQYLAQLNIN-ASDKWRDLGIFVVFVFSNWFLVYFFIYTVRV
M._graminicola_Atr5_AAK62340 1364 ---PQATSGCEVCQYRTGADYLYGLNLTKKSDGWRDAGIVVLFAFSSYGCVYLLMK----
M._graminicola_Atr3_AAK62341 1268 -----TSEVCHYCPLTTGDRYSESLGLS-YAQRWKYLGVFSVFCVSNVGLILLAQQFRRR
consensus 1801 p ata C yCsistgn fl v is ysdrWrdlGivwvyvfsnvgmvvilyw rv
M._graminicola_Atr2_CAB46280 1485 PKGSKKKAEKKEKTG----------
M._graminicola_Atr7_EF062310 1805 PKKSRSK----------
M._graminicola_Atr4_AAK15314 1525 RSHSKKTSKKSKKSGDKKGAAAVAGTEKDDKKVKKTDTSSSSEGNTPAAASVDPEKDAEA
M._graminicola_Atr1_CAB46279 1527 KGWTFGFGPLFGALGKGVELIKKPFKKGEKKEQSEE----------
M._graminicola_Atr5_AAK62340 1417 ----------LRTKASKKAE----------
M._graminicola_Atr3_AAK62341 1322 ----------
consensus 1861 sk g d
M._graminicola_Atr2_CAB46280 ----------
M._graminicola_Atr7_EF062310 ----------
M._graminicola_Atr4_AAK15314 1585 QRSGSTTSAKAVKRQTSRTAGLTRTVSEMGQSVLTTNKGRSRANERNAHVY
M._graminicola_Atr1_CAB46279 ----------
M._graminicola_Atr5_AAK62340 ----------
M._graminicola_Atr3_AAK62341 ----------
consensus 1921

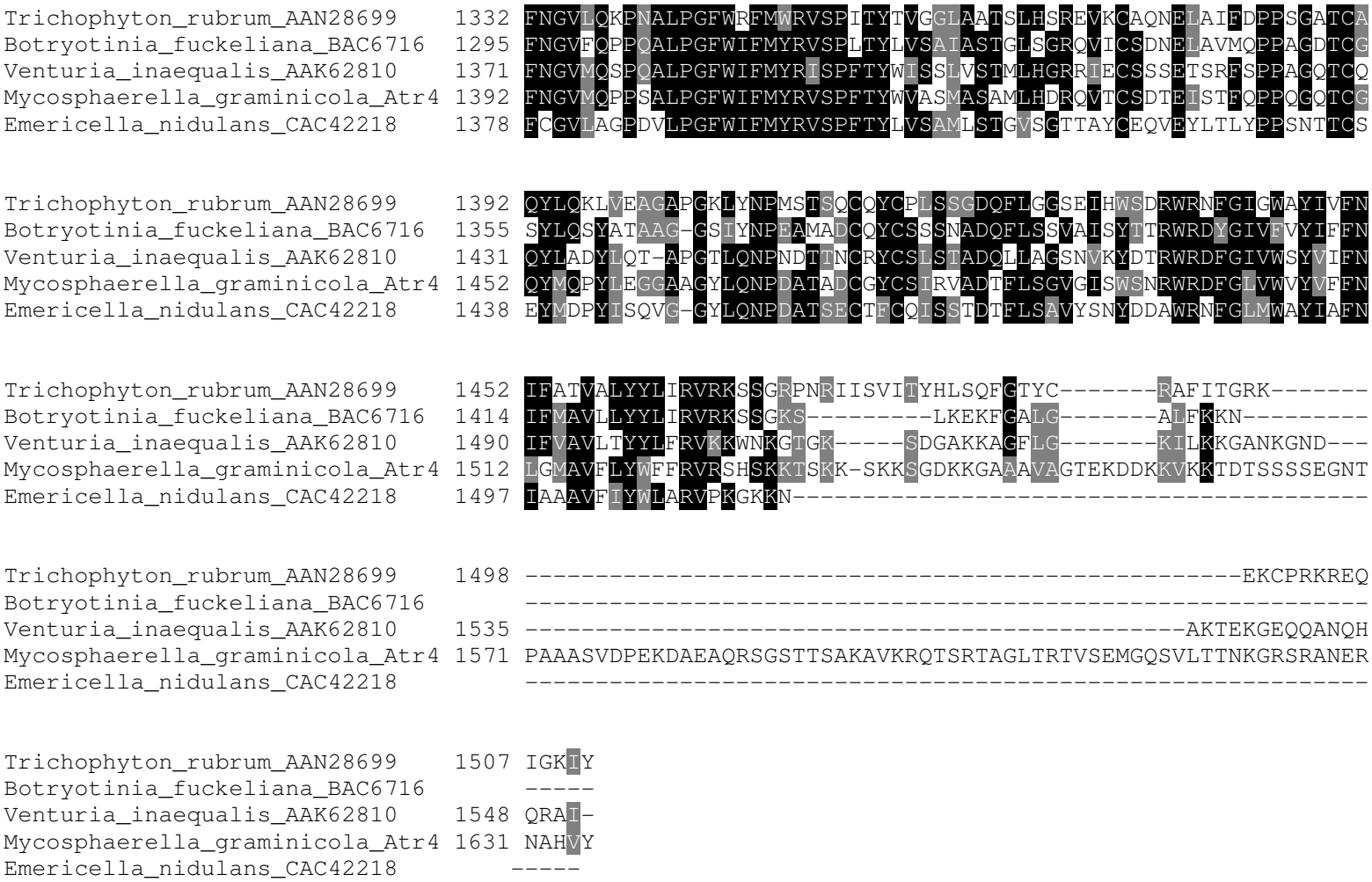
Supplementary material 2. ClustalX alignment of amino acid sequences of fungal ABC transporters from the subclade clustering the MgAtr4 in supplement 2. The arrows indicate the amino acids used to design the degenerated primers after manual reviewing of the alignment to discard *M. graminicola* specific motifs in MgAtr4. Beginning and end of the arrows indicate the first and last amino acid used for each primer.




Trichophyton_rubrum_AAN28699 286 GGERKRVSIAEIALSGAPICCWDNSTRGLDSATALEFTKALKIGSQVGGITQCLAIYQAS
Botryotinia_fuckeliana_BAC6716 254 GGERKRVSIAEMALAGSPIASWDNATRGLDAATALEFTKSLRMTANLSGSCHLVAIYQAS
Venturia_inaequalis_AAK62810 339 GGERKRVSIAEMAVAGAPLAAWDNSTRGLDSATALKFVEAIRISADLIGSSHAIAIYQAS
Mycosphaerella_graminicola_Atr4 356 GGERKRVSIAEMALAGSALAAWDNSTRGLDSATALTFIKALRLNADLVGSAHAVAIYQAS
Emericella_nidulans_CAC42218 327 GGERKRVSIAEATLSASPLQCWDNSTRGLDSANALEFCRTLNLMAKYSGATMAVAIYQAS
Trichophyton_rubrum_AAN28699 346 QAIYDIFDKVIVLYEGRQIFFGPTRIAKQYFEEMGWYCPPRQTTADFLTSVTNPKERIAK
Botryotinia_fuckeliana_BAC6716 314 QQIYDQFDKAIVLYEGRQIYYGPCDQAKQYFEDMGWECPSRQTTGDFLTSITNPSERKAR
Venturia_inaequalis_AAK62810 399 QAIYDRFDKAVVLYSGRQIYFGPASKAKQFFEEQGWYCPKRQTTGDFLTSITNPSERRPR
Mycosphaerella_graminicola_Atr4 416 QAIYDLFDKAIVLYEGREIFFGKASVAKKYFEDMGFYCPSRQTTGDFLTSVTNPAERQLR
Emericella_nidulans_CAC42218 387 QSAYDVFDKVTVLYEGRQIYFGRTDDAKQFFIDMGFECPERQTTADFLTSLTSPAERIVR
Trichophyton_rubrum_AAN28699 406 EGYENRVPRTAVEFERYWKQSQNNKLLLANMDRFEAEYPPEEGHLE---KLRETHGQ-AQ
Botryotinia_fuckeliana_BAC6716 374 PGYENKVPRTPEEFEKYFKDSKIFQRMMSEMKSHEEEFPMGRKTLE---QFKASRKG-MQ
Venturia_inaequalis_AAK62810 459 EGMEKQVPRTPEDFEKYWRNSEMYQSLQKEIEDHETEFPIGGETLG---KLQQQKRN-AQ
Mycosphaerella_graminicola_Atr4 476 EGYEDRAPRTGDDFEKYWHDSPEYQTLQKEIQGHTKEGSTPSATSSGTSKLSAASKNDNQ
Emericella_nidulans_CAC42218 447 KGYEGRVPQTPDEFAAAWKNSDAYAQLMREIEEYNQEFPLGGESVN---KFIESRRA-MQ
Trichophyton_rubrum_AAN28699 462 AKHTASKSPYRISVPMQVKLCTVRAYQRLWGDKSSTIATNISQIMMALIIGSLFFDTPQT
Botryotinia_fuckeliana_BAC6716 430 ADHLRPESPYTVSIVMQTKLCARRAVQRLWNDKISTITTIVGQIAMALIIGSIFYNTPSN
Venturia_inaequalis_AAK62810 515 ASHTRPKSPYMISVPMQIKLCTKRAYQRIWNDMSSTLTMFISQIIMSLIIGSVFYGTPNA
Mycosphaerella_graminicola_Atr4 536 AKHARPKSPYVVSVPMQIKLNTKRSWQRIWGDKAQTFTPMIFNVIIALIIGSIFFNSPPA
Emericella_nidulans_CAC42218 503 SKNQRVKSPYTMSVMEQVHLCMIRGFQRLKGDASLTLSQLIGNFIMALVIGSVFYDLDND
Trichophyton_rubrum_AAN28699 522 TDGFFAKGSVIFFAILLNGLMSITEIN----------GLDAQRPIVVKHVNFAF
Botryotinia_fuckeliana_BAC6716 490 TASFFQKGGVLFFAVLLNALIAISEIN----------TLYSQRPIVEKQASYAF
Venturia_inaequalis_AAK62810 575 TAGFFSKGAVLFFAVLLNALVAMTEIN----------SLYDQRPIVEKHNSYAF
Mycosphaerella_graminicola_Atr4 596 TSAFTARGAVLFFAILINALSAISEIN----------SLYDQRPIVEKHKSYAF
Emericella_nidulans_CAC42218 563 TGSFYSRGALLFFAVLLNAFGSALEVCLILRLFLSLADSLQILTLYAQRPIVEKQARYAM
dAtr4-R1
Trichophyton_rubrum_AAN28699 566 YHAYSEALAGIVADIPIKFLLALVFNIIIYFLGGLERSAAKFFIFFLFTFITILTMSAIF
Botryotinia_fuckeliana_BAC6716 534 YHPFTEALAGVVVDIPVKFAIATCFNIILYFLSGLKREAGAFFVFFLFNFVAILTMSQIY
Venturia_inaequalis_AAK62810 619 YHPATEAIAGIVSDIPVKFLLAVGFNVIFYFLAGLRREPSQFFLYFLVSYVIMFVMAAVF
Mycosphaerella_graminicola_Atr4 640 YHPATEAIAGIVMDVPLKFVVAVCFNLVLYFMSGLRREPAQFFLFFLIAFVSTFVMSAVF
Emericella_nidulans_CAC42218 623 YHPFAEAIASMLCDMPYKITNTFTFNIPLYFMTNLRREPGAFFIFLLFSFVTTLTMSMLF


Trichophyton_rubrum_AAN28699 626 RTLAAATKTIPQALALAGVMILALVIYTGFTLQPSYMHPWFKWILYINPIAYAYEALLVN
Botryotinia_fuckeliana_BAC6716 594 RSIAAATKTISQALAIAGVATLAIVIYTGFVIPRPLMHPWFKWISWINPVAYAFEALFVN
Venturia_inaequalis_AAK62810 679 RTMAAVTKTISQAMSLAGVLVLALVIYTGFVIPVSYMKPWFGWIHYINPIYYAFEILIAN
Mycosphaerella_graminicola_Atr4 700 RTLAALTKTISQAMALSGVMVLALVIYTGFVVPTKYMKPWFGWIRWINPIFYAFEILVAN
Emericella_nidulans_CAC42218 683 RTMAATSRTLSQALVPAAILILGLVIYTGFTIPTRNMLGWSRWMNYIDPIAYGFESLMVN
Trichophyton_rubrum_AAN28699 686 EVHGNRYRCA--TPIPP----YGSGKNFACAVAGAVPGEMSVSGDAWVESSYDYSYAHIW
Botryotinia_fuckeliana_BAC6716 654 ELHGKEFVCS--TLVPIGPGYVQAGNNFVCAVAGSVVGATTVSGDDYLQAQFQYSYSHIW
Venturia_inaequalis_AAK62810 739 EFHGRDFTCS--AIIPAYT--PLQGDSWICSIVGAVPGRRTVSGDDFIMQMYQYSYSHVW
Mycosphaerella_graminicola_Atr4 760 EFHAREFECS--QFIPTYT--QFGGETFICSVVGAVAGELTVTGDAYIAEMYGYYYSHVW
Emericella_nidulans_CAC42218 743 EFHGRLFPCSESELVPSYG----DTANRVCAVVGATPGELMVNGTTYLRESYQYTKSHEW
dAtr4-F2
Trichophyton_rubrum_AAN28699 740 RNLGILLGFLAFFYFVYLMVSELNLSSASSAEFLVFRRGHLPKNFQGSKDEEAAAGGVMH
Botryotinia_fuckeliana_BAC6716 712 RNLGFLFAFMIFFLAFYLLAIEFNASTDSKAEVLVFRRGHVPTNLLAA--EKAAK-----
Venturia_inaequalis_AAK62810 795 RNFGILLGFLCGFMCIYFVGVEVNSSTSSAAEFLIFRRGYVPAYMQDD----------
Mycosphaerella_graminicola_Atr4 816 RNFGILLAFFFAFMVIYFVAVELNSSTFSTAEVLVFRRGHVPAYMQNI----------
Emericella_nidulans_CAC42218 799 RNLGIMFAFMAFFLFTYLTATEYISEAKSKGEVLLFRRGQAPPSVNDV----------
Trichophyton_rubrum_AAN28699 800 PNDPARLPPTNTNGAAGETAPGGSTVAVIPPQKDIFTWRNVTYDITIKGEPRRLLDNISG
Botryotinia_fuckeliana_BAC6716 765 -NDEEAHAGNGSAVKEGNSDKQGDEVQALAPQTDIFTWKDVCYDIKIKNEPRRLLDNVSG
Venturia_inaequalis_AAK62810 843 PKHAGND-EEKMADGTTDAKEDGGDVSAIPPQKDIFTWRDIVYDIQIKGEDRRLLDHVIG
Mycosphaerella_graminicola_Atr4 864 DKPGKEDGEAAAAEKGPEKGDEGGDVSAIPPQTDIFTWRDVDYDIEIKGEPRRLLDHVSG
Emericella_nidulans_CAC42218 847 -------ETHSPATAGEKVDQSTQDVANIQRQTAIFHWKDVCYDIKIKNEPRRILDHVDG
Trichophyton_rubrum_AAN28699 860 WVRPGTLTALMGVSGAGKTTLLDALAQRTTMGVITGDMLVNGRPLDSSFQRKTGYVQQQD
Botryotinia_fuckeliana_BAC6716 824 WVKPGTLTALMGVSGAGKTTLLDVLAQRVSMGVITGDMLVSGKPLDASFQRKTGYVQQQD
Venturia_inaequalis_AAK62810 902 WVRPGTLTALMGVSGAGKTTLLDVLAQRTTMGVITGDMLVNGKPLDASFQRKTGYVQQQD
Mycosphaerella_graminicola_Atr4 924 WVKPGTLTALMGTSGAGKTTLLDVLAQRTTMGVVTGNMFVNGAPLDDSFQRKTGYVQQQD
Emericella_nidulans_CAC42218 900 WVKPGTCTALMGVSGAGKTTLLDVLATRVTMGVVTGEMLVDGRPRDQSFQRKTGYVQQQD
Trichophyton_rubrum_AAN28699 920 LHLETTTVREALRFSADLRQPKSVSRKEKYEYVEDVIKMLSMEDFSEAVVGNPGEGLNVE
Botryotinia_fuckeliana_BAC6716 884 LHLETTTVREALRFSAMLRQPKTVSKKEKYDFVEEVIKMLNMEEFSEAVVGVPGEGLNVE
Venturia_inaequalis_AAK62810 962 LHLETATVRESLRFSAELRQPKTVTLQEKFDYVEDVIKMLNMEDFAEAIVGSPGEGLNVE
Mycosphaerella_graminicola_Atr4 984 LHLETSTVRESLRFSAMLRQPRTVSKQEKYEYVEEVIKMLNMEDFAEAVVGVPGEGLNVE
Emericella_nidulans_CAC42218 960 LHLHTTTVREALRFSALLRQPAKTPRQEKLDYVEEVIKLLGMEAYADAVVGVPGEGLNVE


Trichophyton_rubrum_AAN28699 980 QRKLLTIGVELAAKPQLLLFLDEPTSGLDSQSSWSIVTFLRKLADNGQAVLSTIHQPSGI
Botryotinia_fuckeliana_BAC6716 944 QRKLLTIGVELAAKPALLLFLDEPTSGLDSQSSWAIVSFLRKLADNGQAVLATIHQPSAI
Venturia_inaequalis_AAK62810 1022 QRKLLTIGVELAAKPKLLLFLDEPTSGLDSQSAWAICAFLRKLADAGQAVLCTIHQPSAI
Mycosphaerella_graminicola_Atr4 1044 QRKLLTIGVELAAKPKLLLFLDEPTSGLDSQSAWAICAFLRKLADAGQAVLCTIHQPSAI
Emericella_nidulans_CAC42218 1020 QRKRLTIGVELAAKPQLLLFLDEPTSGLDSQTSWSILDLIDTLTQHGQAILCTIHQPSAM
Trichophyton_rubrum_AAN28699 1040 LFEQFDRLLFLAKGGRTVYFGDIGKNSETLLNYFETHGAEPCGPSENPAEYMLNIVGAGP
Botryotinia_fuckeliana_BAC6716 1004 LFQEFDRLLFLAKGGRTVYFGDIGHNSETLLNYFESHGAAKCGEDENPAEYMLTMVGAGA
Venturia_inaequalis_AAK62810 1082 LFQEFDRLLFLAKGGKTVYFGPVGKNSETLIDYYESNGARKCGEEENPAEYMLEIVNKGS
Mycosphaerella_graminicola_Atr4 1104 LFQEFDRLLFLRKGGHTVYFGDIGKNSRTLLDYFESNGARDCGEEENPAEYMLEIVGDDS
Emericella_nidulans_CAC42218 1080 LFQRFDRLLFLAKGGKTVYFGEIGEKSSTLASYFERNGAPKLPADANPAEWMLEVIGAAP
Trichophyton_rubrum_AAN28699 1100 SG--KSNIDWPVVWKESEESRHVQQELDRIQSETSKRNEGHGQSAEKEPGEFAMPFTSQL
Botryotinia_fuckeliana_BAC6716 1064 QG--KSTQDWHEVWKASDEAKGIQTEISRIQQEMGHQ---PSQDDSNSHGEFAMPFTVQL
Venturia_inaequalis_AAK62810 1142 S---GQGQDWHEVWKGSKEREAVNEELKQIHKEKEGEAI-AGANEEGAQDEFAMPFTAQV
Mycosphaerella_graminicola_Atr4 1164 SDWVGTWNDSKEARRGTAGDRTHSQGTLLRGEELDRRQR-RPLRPRRIRHAFRRP-TQDG
Emericella_nidulans_CAC42218 1140 GS--HSDIDWPAVWRESPERQAVHQHLAELKETLSQKPTETSASDPSEYNEFAAPFSVQL
Trichophyton_rubrum_AAN28699 1158 YCVTTRVFQQYWRIPSYIWGKLLLGLASALFIGFSFFLQNSSMAGLQNSLFSIFMLTTIF
Botryotinia_fuckeliana_BAC6716 1119 LEVMKRVFQQYWRIPGYVYSKLVLGVASALFIGFSFFHADASQQGLQDVIFSIFMITTIF
Venturia_inaequalis_AAK62810 1198 KAVTVRVFQQYWRMPSYVFAKWALGIASGLFIGFSFFQANTTQQGVQNVLFSAFMIATIF
Mycosphaerella_graminicola_Atr4 1222 HSPRVPTILAYAELP--VRENGSLHRRWSLHR-FLLLLRDATLQGMQNVIYSLFMLTTIF
Emericella_nidulans_CAC42218 1198 WECLVRVFSQYWRSPVYIYSKAALSTLTSLYIGFSFFQAQNTRQGLQNQMFSIFMLMTIF
Trichophyton_rubrum_AAN28699 1218 SSLVQQIMPRFVTQRDLFEVRERPSRAYSWKVFLLANIIVEIPYQILLGIIAWASLFYPT
Botryotinia_fuckeliana_BAC6716 1179 TTLVQQIMPRFILQRDLYEVRERPSKAYSWKAFIIANIAVEIPYQIILGIMVFASYFYPI
Venturia_inaequalis_AAK62810 1258 SSLVQQIMPLFVNQRSLYEVRERPSKAYSWKAFMIANIVVEIPYNIFLGVPVFACYLYAI
Mycosphaerella_graminicola_Atr4 1279 STLVQQIQPLFVTQRSLYEVRERPSKAYSWKAFLIANMVVEIPYQIIAGILVYATFYYPV
Emericella_nidulans_CAC42218 1258 GNLVQQIMPNFVTQRALYEVRERPSKAYSWKAFMTANILVELPWNTLMAVIMYFCWYYPV
dATr4-R2
Trichophyton_rubrum_AAN28699 1278 ----FGAH--LSSERQGILLLYCVQFFIFASTFAQMIIAGLPDAETAGGIATTMFGLMVT
Botryotinia_fuckeliana_BAC6716 1239 ----YTKNGIPPSGRQGLILLLLIQFFVFASTFAHMLISALPDAETAGNIATLMFSLTLT
Venturia_inaequalis_AAK62810 1318 ----AGII---SSVRQVLILLLMIQFFVYAGTFAAMCIAALPDAETAAAVVTLLFATSLT
Mycosphaerella_graminicola_Atr4 1339 ----VGIQ---SSERQVLVMLLCIVLFVYASTFAHMCIAAMPDAQTAGAIVTFLFFMALI
Emericella_nidulans_CAC42218 1318 GLYRNAEPTDSVHERGALMFLLILAFLLFTSTFAHMIIAGIETAETGGNIAQLLFSLCLI


Trichophyton_rubrum_AAN28699 1332 FNGVLQKPNALPGFWRFMWRVSPITYTVGGLAATSLHSREVKCAQNELAIFDPPSGATCA
Botryotinia_fuckeliana_BAC6716 1295 FNGVFQPPQALPGFWIFMYRVSPLTYLVSAIASTGLSGRQVICSDNELAVMQPPAGDTCG
Venturia_inaequalis_AAK62810 1371 FNGVMQSPQALPGFWIFMYRISPFTYWISSLVSTMLHGRRIECSSSETSRFSPPAGQTCQ
Mycosphaerella_graminicola_Atr4 1392 FNGVMQPPSALPGFWIFMYRVSPFTYWVASMASAMLHDRQVTCSDTEISTFQPPQGQTCG
Emericella_nidulans_CAC42218 1378 FCGVLAGPDVLPGFWIFMYRVSPFTYLVSAMLSTGVSGTTAYCEQVEYLTLYPPSNTTCS
Trichophyton_rubrum_AAN28699 1392 QYLQKLVEAGAPGKLYNPMSTSQCQYCPLSSGDQFLGGSEIHWSDRWRNFGIGWAYIVFN
Botryotinia_fuckeliana_BAC6716 1355 SYLQSYATAAG-GSIYNPEAMADCQYCSSSNADQFLSSVAISYTTRWRDYGIVFVYIFFN
Venturia_inaequalis_AAK62810 1431 QYLADYLQT-APGTLQNPNDTTNCRYCSLSTADQLLAGSNVKYDTRWRDFGIVWSYVIFN
Mycosphaerella_graminicola_Atr4 1452 QYMQPYLEGGAAGYLQNPDATADCGYCSIRVADTFLSGVGISWSNRWRDFGLVWVYVFFN
Emericella_nidulans_CAC42218 1438 EYMDPYISQVG-GYLQNPDATSECTFCQISSTDTFLSAVYSNYDDAWRNFGLMWAYIAFN
Trichophyton_rubrum_AAN28699 1452 IFATVALYYLIRVRKSSGRPNRIISVITYHLSQFGTYC-------RAFITGRK-------
Botryotinia_fuckeliana_BAC6716 1414 IFMAVLLYYLIRVRKSSGKS---------LKEKFGALG-------ALFKKN---------
Venturia_inaequalis_AAK62810 1490 IFVAVLTYYLFRVKKWNKGIGK------SDGAKKAGFLG-------KILKKGANKGND---
Mycosphaerella_graminicola_Atr4 1512 LGMAVFLYWFFRVRSHSKKTSKK-SKKSGDKKGAAAVAGTEKDDKKVKKTDTSSSSEGNT
Emericella_nidulans_CAC42218 1497 IAAAVFIYWLARVPKGKKN----------
Trichophyton_rubrum_AAN28699 1498 ----------EKCPRKREQ
Botryotinia_fuckeliana_BAC6716 ----------
Venturia_inaequalis_AAK62810 1535 ----------AKTEKGEQQANQH
Mycosphaerella_graminicola_Atr4 1571 PAAASVDPEKDAEAQRSGSTTSAKAVKRQTSRTAGLTRTVSEMGQSVLTTNKGRSRANER
Emericella_nidulans_CAC42218 ----------
Trichophyton_rubrum_AAN28699 1507 IGKIY
Botryotinia_fuckeliana_BAC6716 -----
Venturia_inaequalis_AAK62810 1548 QRAI-
Mycosphaerella_graminicola_Atr4 1631 NAHVY
Emericella_nidulans_CAC42218 -----