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ORIGINAL PAPER

Tagging target genes of the MAT1-2-1 transcription factor in *Fusarium verticillioides* (*Gibberella fujikuroi* MP-A)

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Abstract Mating type in filamentous ascomycetes is controlled by idiomorphic alleles, named *MAT1-1* and *MAT1-2*, which contain 1–3 genes. Of these genes *MAT1-1-1* and *MAT1-2-1* encode putative transcription factors and are thus considered to be the major regulators of sexual communication and mating. Fungi with no known sexual stage may also have fully functional mating type genes and therefore it was plausible to hypothesize that the MAT products may also regulate other types of genes not involved directly in the mating process. To identify putative target genes of these transcription factors in *Fusarium verticillioides*, Δ*MAT1-2-1* knock out mutants were produced and transcript profiles of mutant and wild type were compared by means of differential cDNA hybridization. Clones, either up- or down-regulated in the Δ*MAT1-2-1* mutant were sequenced and a total of 248 sequences were blasted against the NCBI database as well as the *Gibberella zeae* and *Gibberella moniliformis* genomes. Fifty-five percent of the clones were down-regulated in the mutant, indicating that the MAT1-2-1 product positively affected these tagged sequences. On the other hand, 45% were found to be up-regulated in the mutant, suggesting that the MAT1-2-1 product also exerted a negative regulatory function on this set of genes. Sequences involved in protein synthesis and metabolism occurred more frequently among the clones up-regulated in the mutant, whereas genes belonging to cell signalling and communication were especially frequently tagged among the sequences down-regulated in the mutant.

Keywords *Fusarium verticillioides* · Gene disruption · *MAT1-2-1* · Transcription profiles

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Introduction

Sexual development in filamentous fungi results in the formation of fruiting bodies, specialized structures providing space and protection for the meiosporangia that contain ascospores, the final products of meiotic recombination. This process needs molecular communication between mating

partners, followed by finely organized morphogenetic changes, regulated by a number of genes that are affected by either intrinsic and/or environmental stimuli, like age of the culture, temperature, nutrient status or light. Early studies (Dyer et al. 1992) suggested that sexual morphogenesis in fungi is under polygenic control and several genes involved in sexual communication have already been characterized in detail (Shen et al. 1999; Vallim et al. 2000; Pöggeler and Kück 2001; Kim et al. 2002; Turina et al. 2003; Kim and Borkovich 2004). Nevertheless the molecular mechanisms of this series of events are still not fully understood (Coppin et al. 1997; Kronstadt and Staben 1997; Shiu and Glass 2000; Trail et al. 2003).

Filamentous ascomycetes can be either heterothallic or homothallic. In heterothallic fungi, mating occurs only between strains belonging to opposite mating type, whereas the homothallic species are self-fertile. Mating type in heterothallic fungi is controlled by idiomorphic alleles, named *MAT1-1* and *MAT1-2* (Yoder et al. 1986; Yun et al. 2000) that may contain one to three *MAT* genes (Staben and Yanofsky 1990; Glass et al. 1990). On the other hand, homothallic fungi, such as *Gibberella zeae (Fusarium graminearum)* are equipped with all these four *MAT* genes, linked tightly together at the same locus (Yun et al. 2000). Of the genes located within the two mating type idiomorphs, *MAT1-1-1* and *MAT1-2-1* are the major regulators of sexual communication (Turgeon 1998). The translation products of these genes have the conserved regions of α1-domain and HMG domain transcription factors, respectively (Nelson 1996). Similarly to yeast species, the proven targets of these translation products are pheromone precursor and pheromone receptor genes (Debuchy 1999; Pöggeler 2000; Pöggeler and Kück 2001; Kim and Borkovich 2004). The role of pheromone signalling in communication between female and male partners has earlier been demonstrated (Bistis 1983), but apart from this early recognition of partners with opposite mating type, important post-fertilization events, like internuclear recognition were also found to be influenced by pheromones (Arnaise et al. 1997; Debuchy 1999).

Fungi with no known sexual stage have been reported to contain fully functional, constitutively transcribed *MAT* genes (Sharon et al. 1996; Wirsel et al. 1998; Arie et al. 2000; Yun et al. 2000; Kerényi et al. 2004) indicating that the absence of sexuality in these fungi is not due to adverse mutations of the mating type genes. The reasons of the presence of functional *MAT* genes in fungi, in which sexual structures have never been observed, are unknown. One possible explanation is that these fungi may have a cryptic sexual cycle (Turgeon 1998), and consequently their teleomorphs have not been observed due to the extreme rarity of mating events. However, one may also hypothesize, that the *MAT* derived transcription factors could have an influence on other events of the life cycle and may regulate additional genes not directly involved in mating.

The aims of this study were to (i) find additional targets of the *MAT* genes in filamentous ascomycetes and (ii) identify biological processes that are potentially governed by the *MAT1-2-1* gene in both sexually and asexually reproducing fungi.

Transcription factors are transcribed at low rates and are therefore difficult to isolate, so it is problematic to study their effects directly by means of biochemical techniques. Therefore, we followed another experimental strategy where we produced a Δ*MAT1-2-1* mutant of *Fusarium verticillioides* and compared the expression profile of this mutant with that of the wild type parental strain. *F. verticillioides*, a heterothallic fungus (teleomorph: *Gibberella moniliformis*; synonymous names *Fusarium moniliforme, Gibberella fujikuroi* mating population A) was selected for this study due to its economic importance. This fungus that occurs globally and is one of the most commonly reported fungal species infecting maize (Oren et al. 2003), producing fumonisins, a well known group of mycotoxins that cause human and animal diseases (Nelson et al. 1993; Moretti et al. 1997; Marasas 2001). Moreover, *F. verticillioides* is member of a species complex that contains both sexual and asexual species of agronomic importance (O'Donnell et al. 1998). A better insight into the role of the MAT transcription factors in regulating mating and/or other important events of the life cycle of this and related fungal species helps to understand the mechanisms responsible for the successful

adaptation of these organisms to ecologically diverse conditions and contributes to the development of strategies that can be applied to control these detrimental fungi.

Materials and methods

Fungal strains and growth conditions

F. verticillioides strains FGSC 7600 and FGSC 7603 were received from the Fungal Genetic Stock Center, University of Kansas, Medical Center, Kansas City, Kansas, USA. The genotypes of these strains are MATA-1 (*MAT1-1)* and MATA-2 (*MAT1-2)*, respectively according to the nomenclature proposed by Kerényi et al. (1999). Complete medium, CM (Correll et al. 1987) and carrot agar, CA (Klittich and Leslie 1988) were used to compare growth and morphology of the wild type strains, FGSC 7600 and 7603 as well as the Δ*FvMAT1-2-1* mutants. For transformation, *F. verticillioides* FGSC 7603 was grown on YEPD-2G medium (Proctor et al. 1997) containing yeast extract (3.0 g), peptone (10.0 g), glucose (20.0 g) per litre of distilled water as shaken culture (180 rpm) for 9–10 h at 28°C. All the other incubations occurred at 23/24°C under a 12 h of light–12 h of darkness diurnal cycle. Strains used in this study were maintained as conidial suspensions in 15% glycerol at –70°C.

Isolation and manipulation of nucleic acids

Fungal genomic DNA was isolated from four-day-old cultures grown on liquid CM and purified as described previously (Kerényi et al. 1999) using a modified version of the CTAB (cetyltrimethylammonium bromide) procedure developed by Murray and Thompson (1980). Total RNA used in Northern analyses was isolated from five-day-old cultures grown on either CM or CA by using TRI REAGENT™ (Sigma, St. Louis, Missouri, USA). DNA and RNA electrophoreses, blotting, Southern and Northern hybridizations, and general recombinant DNA techniques were performed according to Sambrook and Russell (2001).

Disruption of FvMAT1-2-1

A 4002 bp fragment of the *MAT1-2-1*gene was amplified by PCR from *F. verticillioides* FGSC 7603 using the primers FMATp1 and FMATp2 (Table 1). This fragment was ligated into pBluescript II KS+ (Stratagene, La Jolla, California, USA). Subsequently, a 1973 bp *Nde*I fragment of the *FvMAT1-2-1* sequence was replaced with a 3805 bp hygromycin expression cassette

Table 1 PCR primers used for amplification of *FvMAT1-2-1* and *hph* sequences

Name of primers	Nucleotide sequence (5′-3′)	Description
FMATp1	ACCTAGTGCAACAAGAAACAAAGCGAGTG	*F. verticillioides MAT1-2-1*(AF100926.1) forward
FMATp2	AACCCTGTGTCCTTTAACC(GC)TGAGCAT	*F. verticillioides MAT1-2-1*(AF100926.1) reverse
GfMAT2-f	ACCGTAAGGAACGTCACCATT	*F. verticillioides MAT1-2-1*(AF100926.1) reverse
GfMAT2-r	GGGGTACTGTCGGCGATGTT	*F. verticillioides MAT1-2-1*(AF100926.1) forward
GfMAT2-r	GGGGTACTGTCGGCGATGTT	*F. verticillioides MAT1-2-1*(AF100926.1) forward
FMATp2	AACCCTGTGTCCTTTAACC(GC)TGAGCAT	*F. verticillioides MAT1-2-1*(AF100926.1) reverse
TMAT_for	CTTCCAGCCCCATCGTCTTC	*F. verticillioides MAT1-2-1*(AF100926.1) reverse
TMAT_rev	TAGGCGGTCATCTGCTGTGTAAC	*F. verticillioides MAT1-2-1*(AF100926.1) forward
FMATp1	ACCTAGTGCAACAAGAAACAAAGCGAGTG	*F. verticillioides MAT1-2-1*(AF100926.1) forward
hp_check2	CACGGCGGGAGATGCAATAGGTC	*E. coli hph* (Z32698.1) reverse
FMATp2	AACCCTGTGTCCTTTAACC(GC)TGAGCAT	*F. verticillioides MAT1-2-1*(AF100926.1) reverse
hph_check1	GGCGCAGACCGGGAACACA	*E. coli hph* (Z32698.1) forward
hph_check1	GGCGCAGACCGGGAACACA	*E. coli hph* (Z32698.1) forward
hph_EcoRV_rev*	(ATC)TATTGGGTGTTACGGAGCATTCA	*E. coli hph* (Z32698.1) reverse

* The 'TATTGGGTGTTACGGAGCATTCA' sequence is a reverse complementary sequence of a portion of the *hph* fragment of plasmid pAN7-1. The (ATC) sequence is an addition to this sequence and corresponds to a partial *Eco*RV recognition site. This construct allowed ligation of the *hph* sequence into an *Eco*RV site of plasmid pKS

containing the *hygB* (hygromycin B phosphotransferase) gene from *Escherichia coli* by using blunt-end ligation, yielding the plasmid pMAT7603/hph. Ten μg plasmid DNA was linearized by digestion with *Not*I and used to transform *F. verticillioides* FGSC 7603 protoplasts, isolated from exponentially growing mycelial cultures of the fungus by digestion with a mixture of Lyzing Enzymes from *Trichoderma harzianum*, Driselase and Chitinase (all from Sigma). PEG-mediated transformation was performed according to Proctor et al. (1997). Transformants were selected on regeneration medium (0.1% yeast extract, 0.1% casein hydrolysate, 1.6% bacto agar and 0.8 M sucrose) containing 200 μg ml^{-1} hygromycin B (HygB, Duchefa, Haarlem, The Netherlands). Resistant transformants detected after 4–5 days incubation were transferred to CM plates amended with HygB (200 μg ml^{-1}); they were then single-spored and tested for stability by repeated sub-culturing on antibiotic-free CM. The site specific integration of the *hph* cassette was confirmed by PCR, using the primer pairs GfMAT2-f—GfMAT-2-r, FMATp1—hph_check2 and FMATp2—hph_check1 (Table 1) and Southern hybridization. Probes used in Southern hybridization were PCR generated fragments of the *MAT1-2-1* gene from *F. verticillioides* and the *hygB* gene, respectively (Fig. 1A, B).

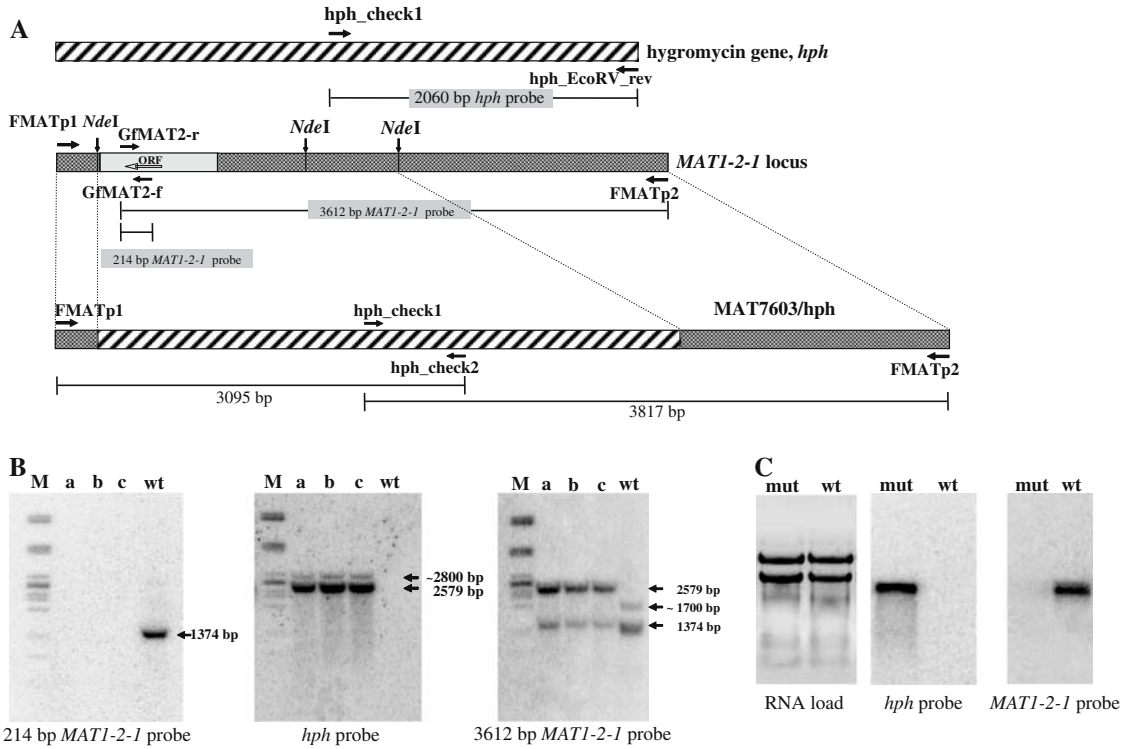

Fig. 1 Disruption of the *FvMAT1-2-1* gene. (**A**) Schematic illustration of the gene replacement strategy. (**B**) Southern analysis of *NdeI* and *Hind*III digested DNA samples of three Δ*FvMAT1-2-1* transformants and the wild type strain of *F. verticillioides* FGSC 7603 probed with a 214 bp *FvMAT1-2-1* fragment (panel I), a 2060 bp *hph* fragment (panel II), and a 3612 bp *FvMAT1-2-1* fragment (panel III). Lane 1, λ *Pst*I ladder; lanes 2–4, Δ*FvMAT1-2-1* transformants (Δ*FvMAT1-2-1/6, 7, 15*); lane 5, wild type strain. Expected numbers and sizes of fragments were: (I)—no fragment in the mutants and one fragment (1374 bp) in the wild type, (II)—two fragments (a 2579 bp long and an unpredictable one) in the mutants and no fragment in the wild type, (III)—two fragments in the Δ*FvMAT1-2-1* mutants (a 2579 bp long and an unpredictable one), and three fragments in the wild type, a 1349 and a 1374 bp long, as well as an unpredictable one. (NB. The 1349 and the 1374 bp co-migrating fragments could not be separated, they gave a thick band around 1350 bp.) (**C**) Northern analysis of RNA samples of the Δ*FvMAT1-2-1/15* mutant and the wild type strain, FGSC 7603. Northern blots were probed with a 2060 bp *hph* fragment and a 740 bp *MAT1-2-1* fragment. wt, wild type; mut, mutant

Sexual crossing protocol

Strain FGSC 7603 (*MAT1-2*) or its Δ*FvMAT1-2-1* derivatives (Δ*FvMAT1-2-1*/6, Δ*FvMAT1-2-1*/7, Δ*FvMAT1-2-1*/15) were crossed on carrot agar with strain FGSC 7600 (*MAT1-1*) in separate crosses where mating partners acted either as males or as females. When used as female partners, strains were grown on carrot agar and sprinkled with a conidial suspension (obtained from water agar plates) of the opposite partner and fertility was evaluated according to Klittich and Leslie (1988). Carrot agar plates were incubated for 4 weeks at 23/24°C under a diurnal cycle of 12/12 h light/darkness and regularly monitored using a stereo-microscope until the growth of perithecia were observed.

Nucleic acid hybridization—High density filter technique

Total RNA was extracted from 50 mg mycelium of both the wild type (wt), *F. verticillioides* FGSC 7603 and the Δ*FvMAT1-2-1/15* mutant grown on carrot agar using the Qiagen RNA isolation kit (Qiagen Inc., Chatsworth, California, USA). Subsequently poly (A^+) RNA was purified with the Oligotex kit (Qiagen) and cDNA was synthesized with the superscript kit from Invitrogen (Groningen, The Netherlands). After second strand synthesis and adaptor ligation, part of this cDNA was used for directional cloning into the vector pSPORT-1, digested with *Sal*I and *Not*I and the ligation mix was used to transform electrocompetent *E. coli* DH10B cells. EST libraries were constructed from both the wt and the Δ*FvMAT1-2-1* mutant. From each library 7680 clones were picked into twenty 384-well plates and with the aid of a 384-pin tool these clones were spotted onto Hybond N+ filters. Each high density filter contained colonies of four 384-well plates, spotted in duplicate on a single 8×12 cm filter, amounting $2 \times 4 \times 384 = 3072$ colonies per filter.

Another part of the cDNA preparations from the wt and from the Δ*FvMAT1-2-1* mutant was labelled using the random prime labelling kit from Boehringer (Mannheim, Germany) with α–^{32}P-dATP and random hexamer primers. These probes were used for hybridization to filters carrying clones from the wt library as well as filters with clones from the mutant library. Because duplicate filters were hybridized with the same cDNA from the wt as well as with that of the mutant, comparisons between hybridization signals could be achieved. Filters were scanned using a phospho-imager and the images were imported into the image analysis software program AIS 4.0 (Imaging Research Inc., 500 Glenridge Avenue, St. Catharines, Ontario Canada, L2S 3A1, www.imagingresearch.com). A grid, with 3×3 spots per quadrant of a 384 formatted filter, was superimposed onto the image and local intensities were recorded. Gray values were imported into an Excel table where local variations in background density were compensated by subtracting of the signal of the central (empty) spot. Subsequently, averages of the duplicate spots were calculated.

Sequence comparisons

The hybridization signals obtained with the wt probe and the mutant probe were compared to identify clones that were differentially expressed between the wt strain, FGSC 7603 and its Δ*MAT1-2-1* mutant. Colonies that showed at least a 4-fold difference in hybridization intensity were selected for sequencing. The 5′end of the cDNA was sequenced using the T7 primer and DYEnamic ET terminator kit (Amersham, UK) on an ABI 3700 sequencer. Sequence data were analyzed with the Lasergene (DNAStar Inc., Madison, Wisconsin, USA) software package and the FGENESH program (www.softberry.com). Subsequently, three different BLAST searches (Altschul et al. 1997) were performed: BlastN to the GenBank EST-database; BlastX to the GenBank nr-database and BlastN to the *F. graminearum/G. zeae* (http://www.mips.gsf.de/genre/proj/fusarium/), as well as the *F. verticillioides/G. moniliformis* (http://www.broad.mit.edu/annotation/fungi/fusarium_verticillioides/) genome sequences.

Results

Molecular and phenotypic characterization of the Δ*FvMAT1-2-1* mutants

Fifty stable, single-spored hygromycin resistant transformants were assayed to detect sequence replacement events by PCR as described in Materials and Methods. Four transformants gave PCR patterns indicating disruption of the *FvMAT1-2-1* gene through double recombination. PCRs generated the expected 3095 bp and 3817 bp fragments, respectively in these strains, which were therefore regarded as potential Δ*FvMAT1-2-1* mutants. The sequence replacement in these mutants was confirmed by Southern hybridization. Figure 1B demonstrates that a double cross-over occurred in these mutants between the 1973 bp *Nde*I fragment of the *MAT1-2-1* gene and the 3.8 kb fragment of the *hph* cassette. No *MAT1-2-1* transcripts were observed in the Δ*FvMAT1-2-1* mutants by Northern analyses using a PCR fragment of the *MAT1-2-1* gene (generated by primer pairs TMAT_for—TMAT_rev) as probe, indicating that the *MAT1-2-1* gene was indeed inactivated in these strains (Fig. 1C). The same Northern hybridization with the *hph* probe is also shown in Fig. 1C.

All Δ*FvMAT1-2-1* mutants grew normally on CM and carrot agar. No signs of a reduction in sporulation or spore germination were observed. No morphological aberrations occurred either and by cultural characters the mutants were indistinguishable from the wild type: both macro- and microconidia showed a normal appearance and the cultures formed a typical white floccose mycelium on potato dextrose agar. However Δ*FvMAT1-2-1* mutants were unable to mate with the tester strain, *F. verticillioides* FGSC 7600 irrespective of whether they were used as male or as female partners. One of these mutants, Δ*FvMAT1-2-1/15* was used in subsequent experiments.

Gene expression profiling

Two cDNA libraries were generated using mRNAs isolated from the wild type and the Δ*FvMAT1-2-1/15* mutant grown on carrot agar for 5 days at 20/22°C with a photoperiod of 12 h dark/light. Northern experiments showed that these culture conditions favour the expression of the *MAT1-2-1* gene as compared to solid or liquid CM (Fig. 2). *E. coli* Electromax DH10B colonies, harbouring the cDNAs were grown overnight on LB in 384-well microtiter plates, and then transferred to Hybond N+ filters, using a 384-pin tool. Altogether 7680 cDNA clones of each strain were arrayed and hybridized to labelled random cDNA fragments prepared from both the mutant and the wild type. An automatic image analysis allowed the identification of cDNA clones of genes, differentially expressed in the wild type and the Δ*FvMAT1-2-1/15* mutant. An example of a typical hybridization array is shown in Fig. 3.

Selecting and sequencing cDNA clones originated from genes differentially expressed in the wild type and the Δ*FvMAT1-2-1/15* mutant

Clones which showed different signal intensities between cDNA probes from the wild type and those of the Δ*FvMAT1-2-1/15* mutant were selected for further analysis. A four-fold threshold of differences in hybridization intensities was chosen to assure that the selected clones

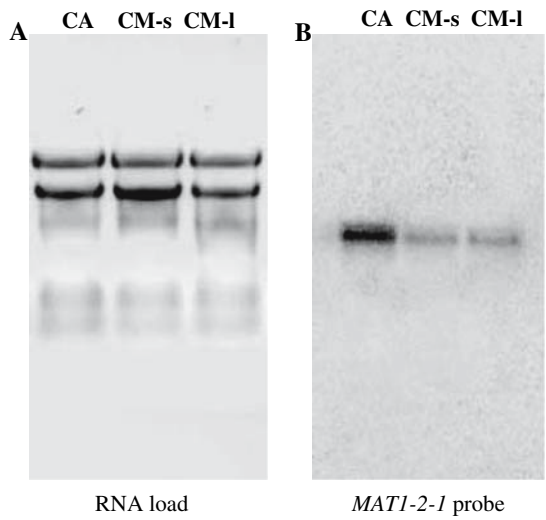

Fig. 2 Comparison of the expression levels of *FvMAT1-2-1* transcripts on different media. *F. verticillioides*, FGSC 7603 was grown on carrot agar (CA), solid complete medium (CM-s) and liquid CM (CM-l)

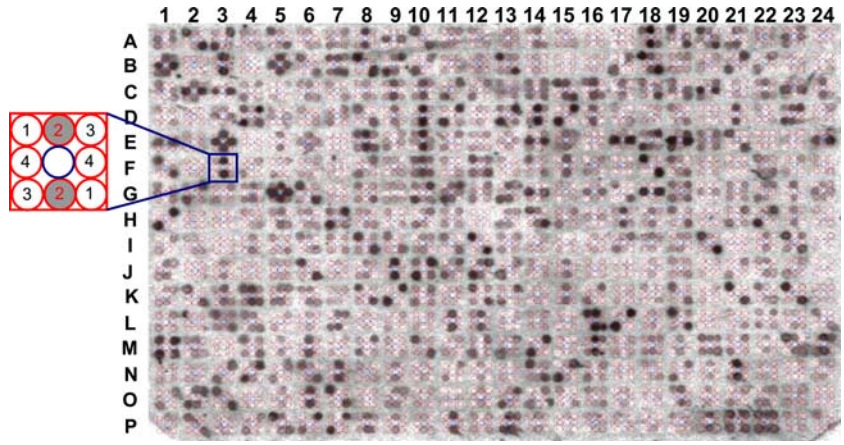

Fig. 3 High density filter used for comparing transcription patterns. A magnified 3 × 3 quadrant at the upper left corner shows the arrangement of the colonies. Spots denoted by the same number are duplicates of the same colony. The central spot is an empty colony (without fungal insert) used as absolute control

contained indeed tagged sequences of genes that had been differentially regulated in the mutant as a result of targeted disruption of the *FvMAT1-2-1* gene. Using this threshold, a total of 272 individual cDNA clones were selected for sequencing. Individual sequences were analyzed by using the SeqMan II module of the Lasergene v. 5.0 software (DNASTAR, Madison, WI, USA) software. 248 sequences (91.2%) were of good quality, with a read-length ranging between 82 bp and 1476 bp, with an average length of ~ 494 bp. The remaining 24 (248 + 24 = 272) gave no hits to any sequences of the three different BLAST searches we done.

BLAST search, performed at individual cDNA sequence level identified 171 sequences that gave best hits to known fungal genes. Forty sequences only gave matches to proteins from distantly related organisms, 23 matched with hypothetical proteins, whereas no match was found with 13 sequences (Table 2). Not surprisingly, one of the sequences (clone 97), up-regulated in the Δ*FvMAT1-2-1/15* mutant was the *hph* sequence, originating from the transforming construct. This clone was omitted from statistical analyses. [Contrary to our expectations, no ESTs of the *MAT1-2-1* gene were found among the sequences down-regulated in the Δ*FvMAT1-2-1* mutant, although this gene was clearly expressed in five-day-old carrot agar cultures of the wild type strain (Fig. 2) and no expression occurred in the mutant in Northern experiments (Fig. 1C). A possible explanation for this discrepancy is that the libraries we used for cDNA/DNA hybridizations were not wholly representative: 7680 clones of each library were tested, and the *MAT1-2-1* gene happened to escape from this dragnet.]

Two-hundred-eleven cDNA clones, which gave hits to known proteins, were assembled by the SeqMan II program into 150 singletons and 17 multiple-sequence contigs. (Sequences belonging to a given contig are denoted by the same superscript letter in Table 2.) Of the 211 singletons and contigs, 117 (55.5%) were down-regulated in the mutant, indicating that the MAT1-2-1 product positively affected these tagged sequences. On the other hand, this gene product also exerted a negative regulatory function on a set of other genes: 94 sequences (44.5%) were found to be up-regulated in the mutant, as a result of the targeted disruption of the *FvMAT1-2-1*.

Contigs and singletons were assigned to functional categories following the classification system developed by White and Kerlavage (1996) with modifications proposed by Sacadura and Saville (2003). Figure 4 shows the results of functional annotation. Percentage distribution of ESTs into functional categories were calculated from the total numbers of clones rather than contigs in order to demonstrate the relative levels of the expressed genes in each category. Great differences were observed in expression patterns between the wild type and the Δ*FvMAT1-2-1* mutant. ESTs involved in protein synthesis and metabolism occurred much more frequently among the sequences, up-regulated in the mutant (21.3 and 51%, respectively as compared to 2.5 and 34.2% in the down-regulated category). On the other hand, among the sequences, down-regulated in the mutant, genes belonging to cell

Table 2 List of sequences differentially expressed in *F. verticillioides*, strain FGSC 7603 and its Δ*FvMAT1-2-1* mutant

Clone	Regulation in the mutant		Similarity	Species	Accession	*E* value
	up	down				
001[a]		+	No hits found	–	–	–
002	+		Oxysterol binding protein (Osh5), putative	*A. fumigatus*	EAL92408	2E–40
003		+	Elongation factor 3-like protein	*M. grisea*	AAX07692	2E–88
004		+	N-acetylglucosaminyltransferase	*N. crassa*	EAA32165	1E–103
005		+	Hypothetical protein	*G. zeae*	EAA67654	2E–81
006		+	No hits found	–	–	–
007	+		Hydrolase, alpha/beta fold family	*P. putida*	AAN69537	6E–83
008[b]		+	No hits found	–	–	–
009		+	Hypothetical protein	*G. zeae*	EAA73884	3E–43
010		+	No hits found	–	–	–
011	+		Carbonic anhydrase family protein	*A. fumigatus*	EAL85418	2E–89
012		+	Hypothetical protein	*G. zeae*	EAA73871	1E–23
013		+	No hits found	–	–	–
014	+		UV excision repair protein RadW	*A. fumigatus*	XP_754028	8E–31
015		+	Hypothetical protein	*G. zeae*	EAA71288	9E–27
016		+	No hits found	–	–	–
017		+	ATP-dependent RNA helicase (Drs1), putative	*A. fumigatus*	EAL90830	1E–32
018	+		40S ribosomal protein S13	*G. zeae*	EAA76607	8E–71
019		+	Histidine biosynthesis trifunctional protein (his-3)	*N. crassa*	CAE76555	3E–120
020		+	Hypothetical protein	*G. zeae*	EAA77227	5E–48
021		+	No hits found	–	–	–
022	+		5-aminolevulinate synthase	*G. fujikuroi*	BAB68405	1E–122
023		+	Vacuolar ATP synthase subunit B	*G. zeae*	EAA67943	3E–151
024	+		Hypothetical protein	*G. zeae*	EAA67972	3E–55
025		+	Related to tRNA-splicing endonuclease beta chain	*N. crassa*	CAB88602	2E–46
026		+	Phosphoglycerate mutase family domain protein	*A. fumigatus*	EAL92577	1E–114
027		+	Alcohol dehydrogenase, zinc-containing	*A. fumigatus*	EAL89964	4E–60
028		+	T-complex protein 1, epsilon subunit, putative	*A. fumigatus*	EAL88607	1E–93
029[b]		+	No hits found	–	–	–
030	+		No hits found	–	–	–
031	+		40S ribosomal protein S12	*G. zeae*	EAA77525	2E–64
032		+	Mitochondrial import inner membrane translocase subunit TIM17	*G. zeae*	EAA74619	3E–79
033		+	Arginase	*A. fumigatus*	EAL92438	1E–91
034[c]	+		40S ribosomal protein S23	*G. zeae*	EAA74990	1E–72
035	+		Mitochondrial phosphate carrier protein (Mir1), putative	*A. fumigatus*	EAL90845	2E–68
036	+		Related to acetate kinase	*N. crassa*	CAE76499	2E–106
037		+	Probable pyruvate dehydrogenase beta chain precursor (PDB1)	*N. crassa*	CAB97287	6E–81
038	+		Hypothetical protein	*G. fujikuroi*	CAG28683	5E–38
039[d]	+		Heat shock protein SSC1-like protein	*M. grisea*	AAX07628	2E–56
040	+		Putative vacuolar targeting protein	*C. albicans*	XP_711465	1E–20
041		+	No hits found	–	–	–
042		+	No hits found	–	–	–
043		+	No hits found	–	–	–
044		+	No hits found	–	–	–
045[e]		+	No hits found	–	–	–
046[f]		+	No hits found	–	–	–
047		+	Protein kinase, putative	*A. fumigatus*	EAL88773	9E–05

Table 2 continued

Clone	Regulation in the mutant		Similarity	Species	Accession	*E* value
	up	down				
048		+	No hits found	–	–	–
049[g]		+	D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase	*A. fumigatus*	EAL86321	1E–26
050		+	SNARE protein (Ufe1), putative	*A. fumigatus*	EAL93262	8E–41
051[g]		+	No hits found	–	–	–
052		+	Related to fructosyl amino acid oxidase	*N. crassa*	CAE85581	2E–18
053		+	U1 small nuclear ribonucleoprotein 70 kDa	*A. fumigatus*	EAL91268	2E–46
054[a]		+	HAD-superfamily hydrolase, putative	*P. syringae*	AAZ34446	2E–34
055	+		Hypothetical protein	*G. fujikuroi*	CAG28683	5E–38
056		+	Hypothetical protein	*M. grisea*	XP_367120	2E–20
057	+		Ketol-acid reductoisomerase	*G. zeae*	EAA67345	2E–147
058	+		40S ribosomal protein S7	*G. zeae*	EAA76320	3E–107
059	+		No hits found	–	–	–
060		+	No hits found	–	–	–
061	+		Probable phosphoglyceromutase	*N. crassa*	CAF05897	1E–68
062[h]		+	Hypothetical protein AdehDRAFT_0700	*A. dehalogenans*	ZP_00401899	4E–02
063	+		Fibrinogen A-alpha-chain	*S. scrofa*	BAA07817	1E–03
064	+		Probable cytochrome P450 monooxygenase	*Z. mays*	T02955	2E–11
065		+	Glutamine synthetase	*G. fujikuroi*	CAC27836	2E–146
066[d]	+		Heat shock protein SSC1-like protein	*M. grisea*	AAX07628	2E–56
067	+		NADP-dependent glutamate dehydrogenase	*G. fujikuroi*	CAC45043	9E–140
068	+		67 kDa myosin-cross-reactive antigen family protein	*A. fumigatus*	EAL91605	7E–06
069	+		Protein kinase (Lkh1), putative	*A. fumigatus*	EAL91008	1E–23
070	+		DNA polymerase epsilon, catalytic subunit A	*S. cerevisiae*	P21951	4E00
071[e]		+	No hits found	–	–	–
072		+	Phosphatidate cytidylyltransferase	*A. fumigatus*	XP_751919	5E–39
073		+	Hypothetical protein	*G. zeae*	EAA68557	4E–34
074	+		Argininosuccinate synthase	*S. cerevisiae*	CAA62528	2E–43
075[a]		+	Elicitin	*P. infestans*	AAV92919	6E–52
076	+		Aminotransferase, class V, putative	*A. fumigatus*	EAL90276	1E–92
077	+		Mannitol-1-phosphate 5-dehydrogenase-like protein	*M. grisea*	AAX07705	1E–115
078		+	Endoglycoceramidase	*A. fumigatus*	EAL86246	7E–05
079	+		Ketol-acid reductoisomerase, mitochondrial precursor	*G. zeae*	EAA67345	8E–134
080[h]		+	Similar to Anaphase promoting complex subunit 2(APC2)	*B. taurus*	XP_887169	1E–25
081[h]		+	Phosphatidylinositol 3-kinase, putative	*L. major*	CAJ04126	3E00
082	+		Mitochondrial ribosomal protein L36	*S. pombe*	NP_595638	6E–07
083	+		60S ribosomal protein L27a (L29)	*G. zeae*	EAA78050	2E–82
084	+		Gamma-glutamyltranspeptidase	*P. fluorescens*	ABA75303	1E–99
085	+		No hits found	–	–	–
086		+	No hits found	–	–	–
087		+	Y55B1BR.3	*C. elegans*	NP_497198	3E–02
088	+		Eukaryotic translation initiation factor 3 subunit 11-likeprotein	*M. grisea*	AAW69356	1E–86
089	+		No hits found	–		
090	+		Probable 40S ribosomal protein S24	*N. crassa*	CAD71100	5E–49
091	+		MSF membrane transporter	*A. fumigatus*	EAL90365	2E–59

Table 2 continued

Clone	Regulation in the mutant		Similarity	Species	Accession	*E* value
	up	down				
092		+	Probable NADPH quinone oxidoreductase homolog PIG3	*N. crassa*	CAD70876	2E–85
093		+	Manganese resistance 1 protein	*S. cerevisiae*	CAA04646	6E–25
094	+		Pyruvate decarboxylase PdcA, putative	*A. fumigatus*	EAL92474	3E–70
095		+	No hits found	–	–	
096	+		GLP_14_41229_37921	*G. lamblia*	EAA39748	5E00
097	+		Hygromycin B phosphotransferase	*synthetic construct*	CAA83647	3E–167
098		+	alpha,alpha-trehalose-phosphate synthase subunit TPS2,putative	*A. fumigatus*	EAL92998	6E–67
099	+		Probable phosphoglyceromutase	*N. crassa*	CAF05897	2E–120
100		+	Putative exported protein	*B. parapertussis*	CAE40185	8E–26
101	+		PH domain protein	*A. fumigatus*	EAL89069	7E–30
102	+		Unknown protein	*O. sativa*	XP_475204	6E–34
103	+		Probable glucosamine-6-phosphate deaminase	*N. crassa*	CAE85549	1E–06
104	+		Retrotransposon protein, putative, unclassified	*O. sativa*	ABA97830	6E–01
105	+		Heat shock protein 60, mitochondrial precursor	*G. zeae*	EAA73737	4E–94
106	+		Mitochondrial ribosomal protein of the small subunit; Mrps35p	*S. cerevisiae*	NP_011681	2E–06
107	+		No hits found	–	–	–
108		+	No hits found	–	–	–
109		+	Ca/CaM-dependent kinase-1	*N. crassa*	AAL14118	5E–118
110	+		Formate dehydrogenase (NAD-dependent formate dehydrogenase)	*G. zeae*	EAA75069	7E–127
111		+	pH regulatory protein	*G. moniliformis*	AAO64251	3E–114
112		+	Annexin ANXC3.1	*A. fumigatus*	EAL85432	2E–28
113[f]		+	S222	*T. aestivum*	AAD10252	2E–93
114[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
115[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
116[j]		+	Allergen, putative	*C. neoformans*	AAW44097	7E–12
117	+		Esterase, putative	*A. fumigatus*	EAL91616	1E–85
118		+	RIKEN cDNA 8430410A17	*M. musculus*	AAH24401	7E–108
119[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
120[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
121[k]		+	Putative alpha glucosidase	*P. minioluteum*	CAC09327	5E–69
122		+	GTP-binding protein ypt1	*G. zeae*	EAA74326	4E–103
123[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
124[l]		+	Similar to Olf-1/EBF-like-3 transcription factor	*P. troglodytes*	XP_519669	6E–01
125[m]		+	MGC53673 protein	*X. laevis*	AAH43850	4E–72
126	+		40S ribosomal protein S27	*N. crassa*	XP_324798	5E–41
127	+		Malate synthase, glyoxysomal	*G. zeae*	EAA70869	8E–50
128		+	Putative receptor kinase	*S. bicolor*	AAM94320	1E00
129		+	Hypothetical protein	*G. zeae*	EAA73978	3E–31
130[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
131	+		Phosphoribosyl diphosphate synthase isoform 4	*A. fumigatus*	EAL89710	6E–132
132	+		Extracellular matrix protein precursor	*F. oxysporum*	AAL47843	1E–89
133	+		Delta-6-elongase	*P. tricornutum*	AAW70157	2E–22
134	+		Related to cell division protein CDC50	*N. crassa*	CAD21366	3E–52
135[j]		+	Allergen, putative	*C. neoformans*	AAW44097	1E–25

Table 2 continued

Clone	Regulation in the mutant		Similarity	Species	Accession	*E* value
	up	down				
136		+	60S ribosomal protein L10-A-like protein	*M. grisea*	AAW69346	5E–107
137		+	Beta-1,3-glucan binding protein	*N. crassa*	CAC28724	4E–28
138[l]		+	Similar to Olf-1/EBF-like-3 transcription factor	*P. troglodytes*	XP_519669	6E–01
139	+		Vacuolar ATP synthase subunit d	*G. zeae*	EAA74514	2E–137
140	+		ADP-ribosylation factor	*G. zeae*	EAA67817	2E–101
141		+	Putative protein serine/threonine kinase	*D. discoideum*	XP_646132	1E00
142[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
143		+	Phosphoglycerate kinase	*G. zeae*	EAA73460	7E–146
144		+	Nitroalkane oxidase	*F. oxysporum*	AAL57485	1E–155
145[m]		+	Transcription-repair coupling factor - superfamily II helicase	*C. glutamicum*	BAB98356	3E00
146		+	Serine/threonine kinase receptor associated protein, putative	*C. neoformans*	AAW41455	8E–15
147		+	Hypothetical protein	*G. zeae*	EAA70655	4E–53
148	+		Delta-6-elongase	*P. tricornutum*	AAW70157	2E–22
149		+	Putative alpha glucosidase	*P. minioluteum*	CAC09327	9E–93
150[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
151[j]		+	Allergen, putative	*C. neoformans*	AAW44097	2E–25
152[k]		+	Putative alpha glucosidase	*P. minioluteum*	CAC09327	9E–93
153[n]	+		Carbon catabolite repression regulator	*G. fujikuroi*	CAA76330	2E–89
154[c]	+		40S ribosomal protein S23	*G. zeae*	EAA74990	7E–78
155	+		60S ribosomal protein L11	*S. pombe*	Q10157	3E–67
156[n]	+		Carbon catabolite repression regulator	*G. fujikuroi*	CAA76330	2E–89
157		+	No hits found	–	–	–
158		+	Preprotrypsin	*F. oxysporum*	AAB27568	4E–114
159	+		MIPC synthase subunit SurA	*A. fumigatus*	XP_747610	1E–42
160[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
161[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
162	+		Putative OPT family transporter	*C. albicans*	XP_718105	1E–10
163	+		Polyubiquitin	*S. pombe*	AAC64787	3E–152
164		+	esdC homologue	*S. macrospora*	CAH03680	6E–41
165[n]	+		Carbon catabolite repression regulator	*G. fujikuroi*	CAA76330	2E–89
166°		+	Nitroalkane oxidase	*F. oxysporum*	AAL57485	1E–155
167		+	ABC transporter, ATP-binding protein	*B. pseudomallei*	ABA49992	3E00
168		+	Catalase-peroxidase	*N. crassa*	AAL66352	4E–82
169		+	40S ribosomal protein S15a (PPCB8)	*B. napus*	Q00332	2E–37
170		+	No hits found	–	–	–
171	+		Potential zinc RING finger protein	*C. albicans*	EAK96719	5E–08
172		+	Siderochrome-iron transporter (Sit1), putative	*A. fumigatus*	EAL86866	8E–86
173		+	Ctr copper transporter family	*A. fumigatus*	EAL85758	4E–34
174[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
175	+		Glycine dehydrogenase	*A. fumigatus*	EAL84525	1E–104
176	+		Saccharopine dehydrogenase	*S. pombe*	Q09694	2E–34
177	+		Tubulin alpha chain	*G. zeae*	EAA67945	4E–127
178	+		Similar to Olf-1/EBF-like-3 transcription factor	*P. troglodytes*	XP_519669	6E–01
179	+		Cell wall integrity and stress response component 4 precursor	*S. cerevisiae*	P38739	7E00
180[m]		+	GCN5-related N-acetyltransferase	*A. variabilis*	ABA21858	2E00
181[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
182[m]		+	No hits found	–	–	–
183	+		Olfactory receptor Olfr973	*M. musculus*	AAS99804	5E00
184	+		Nitrate reductase	*S. oleracea*	BAA13047	6E–16

Table 2 continued

Clone	Regulation in the mutant		Similarity	Species	Accession	*E* value
	up	down				
185	+		Probable porphobilinogen synthase	*N. crassa*	CAD70821	3E–81
186	+		Probable transcription factor BTF3a	*N. crassa*	CAE76548	1E–58
187	+		No hits found	–	–	–
188	+		Outer membrane usher protein	*S. enterica*	YP_149528	4E00
189		+	alpha-ketoglutarate dependent xanthine dioxygenase	*E. nidulans*	CAI47587	3E–119
190	+		Related to hexokinase	*N. crassa*	CAE85550	4E–61
191		+	Related to cell cycle regulation and aging protein	*N. crassa*	CAE76477	6E–71
192[p]		+	ThiJ/PfpI family protein	*A. fumigatus*	EAL86037	1E–16
193	+		Carbon catabolite repression protein CreD, putative	*A. fumigatus*	EAL88643	1E–47
194		+	Stomatin-like protein	*G. fujikuroi*	BAB68403	4E–135
195		+	Similar to CG8713-PA	*A. mellifrea*	XP_393264	7E–01
196[p]		+	ThiJ/PfpI family protein	*A. fumigatus*	EAL86037	2E–16
197	+		Phospholipase/Carboxylesterase	*Nocardioides* sp.	ZP_00660025	3E–11
198	+		Probable methionyl aminopeptidase	*N. crassa*	CAD71035	1E–101
199		+	Cell cycle arrest in response to pheromone-related protein	*C. neoformans*	AAW40619	2E00
200		+	No hits found	–	–	–
201		+	Related to neutral amino acid permease	*N. crassa*	CAE76088	2E–67
202[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
203		+	Ethylene-responsive proteinase inhibitor I precursor	*L. esculentum*	P20076	5E–47
204		+	No hits found	–	–	–
205		+	Similar to odd-skipped-related 2 protein	*G. gallus*	XP_418353	3E–01
206	+		Probable 1, 4-Benzoquinone reductase	*N. crassa*	CAE76242	2E–79
207		+	Related to B56-delta regulatory subunit of protein phosphatase 2A	*N. crassa*	CAC28812	1E–113
208	+		Probable ammonium transporter MEPa	*N. crassa*	CAD21326	1E–113
209		+	Woronin body major protein	*N. crassa*	AAB61278	4E–65
210		+	esdC homologue	*S. macrospora*	CAH03680	5E–35
211	+		Surface-exposed outer membrane protein	*X. fastidiosa*	AAF84325	1E–02
212	+		60S ribosomal protein L36 (TRP36)	*G. zeae*	EAA68099	2E–41
213[j]		+	Allergen, putative	*C. neoformans*	AAW44097	1E–25
214[l]		+	Similar to Olf-1/EBF-like-3transcription factor	*P. troglodytes*	XP_519669	6E–01
215		+	Membrane bound C2 domain protein (vp115), putative	*A. fumigatus*	EAL84710	2E–60
216		+	Hypothetical protein	*G. zeae*	EAA75931	3E–32
217		+	Cation exchanger, putative	*A. fumigatus*	EAL84481	3E–38
218		+	Related to dnase1 protein	*N. crassa*	CAD21357	5E–17
219		+	Putative cell wall adhesin	*C. albicans*	XP_714666	2E–04
220	+		Dihydrodipicolinate synthetase family protein	*A. fumigatus*	EAL85271	1E–47
221	+		Protein binding / ubiquitin-protein ligase/ zinc ion binding	*A. thaliana*	NP_850478	3E00
222[q]		+	Related to rasp f 7 allergen	*N. crassa*	CAD70798	5E–15
223[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77

Table 2 continued

Clone	Regulation in the mutant		Similarity	Species	Accession	*E* value
	up	down				
224	+		a-agglutinin core protein AGA1 homolog - fission yeast	*S. pombe*	T42367	8E–02
225		+	Plasma membrane zinc ion transporter	*A. fumigatus*	XP_731508	4E–59
226	+		60s ribosomal protein l21	*A. fumigatus*	EAL92872	2E–52
227	+		Auxin Efflux Carrier superfamily	*A. fumigatus*	EAL90922	5E–41
228	+		Hypothetical protein	*G. zeae*	EAA68333	2E–10
229°		+	Nitroalkane oxidase	*F. oxysporum*	AAL57485	1E–152
230	+		No hits found	–	–	–
231		+	similar to Importin alpha-2 subunit	*M. musculus*	XP_895972	5E–01
232		+	bZIP transcription factor LziP	*A. fumigatus*	XP_753012	8E–05
233		+	P-type calcium ATPase, putative	*A. fumigatus*	EAL90415	1E–93
234[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
235		+	D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase	*A. fumigatus*	EAL92505	4E–51
236		+	Putative alpha glucosidase	*P. minioluteum*	CAC09327	3E–61
237	+		Tyrosinase precursor	*N. crassa*	CAE81941	7E–34
238		+	Clock-controlled protein 6 (CCG-6)	*N. crassa*	CAD70877	2E–17
239		+	Methyltransferase FkbM	*T. erythraeum*	ZP_00675486	4E00
240[l]		+	Hypothetical protein	*G. zeae*	EAA68216	2E–105
241		+	White collar 2 protein-like protein	*M. grisea*	AAX07710	2E–07
242	+		Serine/threonine kinase with two-component sensor domain	*Nostoc sp.*	BAB72843	6E00
243		+	Golgi reassembly stacking protein	*A. fumigatus*	XP_750751	3E–64
244[l]		+	Hypothetical protein	*G. zeae*	EAA68216	2E–105
245[l]		+	Hypothetical protein	*G. zeae*	EAA68216	2E–105
246		+	Cation exchanger, putative	*A. fumigatus*	EAL84481	7E–04
247		+	Hypothetical protein	*G. zeae*	EAA77344	1E–61
248		+	Predicted protein	*N. crassa*	XP_322154	5E–33
249		+	Probable homeoprotein	*N. crassa*	CAE76096	3E–43
250	+		No hits found	–	–	–
251		+	No hits found	–	–	–
252	+		A-agglutinin attachment subunit precursor	*S. cerevisiae*	P32323	3E–02
253		+	Mannose-1-phosphate guanylyltransferase	*H. jecorina*	AAC39498	2E–149
254	+		Pyruvate decarboxylase PdcA, putative	*A. fumigatus*	EAL92474	3E–84
255		+	No hits found	–	–	–
256[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
257	+		Putative MFS transmembrane transport protein	*P. chrysogenum*	AAY23170	9E–99
258	+		Probable translation elongation factor EF-Tu precursor	*N. crassa*	CAC28833	1E–88
259	+		Related to 60s ribosomal protein L2 (mitochondrial)	*N. crassa*	CAD21256	7E–46
260[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
261		+	Hypothetical protein	*G. zeae*	EAA72018	3E–52
262	+		Amino acid permease (Dip5), putative	*A. fumigatus*	EAL93176	6E–65
263		+	Mitogen-activated prtein kinase STY1	*N. crassa*	XP_327310	2E–146
264	+		Related to hexokinase	*N. crassa*	CAE85550	5E–66
265		+	Opsin-like protein	*G. fujikuroi*	CAD97459	9E–143
266[q]		+	Related to rasp f 7 allergen	*N. crassa*	CAD70798	7E–31
267		+	No hits found	–	–	–
268	+		Amino acid permease family protein, putative	*A. fumigatus*	EAL84500	1E–85

Table 2 continued

Clone	Regulation in the mutant		Similarity	Species	Accession	*E* value
	up	down				
269	+		Mitochondrial F1 ATP synthase beta subunit	*A. thaliana*	CAC81058	1E–04
270[i]		+	Nitrilase, putative	*A. fumigatus*	EAL84990	8E–77
271		+	Signal transduction histidine kinase	*M. magneticum*	BAE49077	6E00
272		+	Hypothetical protein	*G. zeae*	EAA70773	5E–74

[a–q] Sequences denoted by identical superscript letters belong to the same contig.

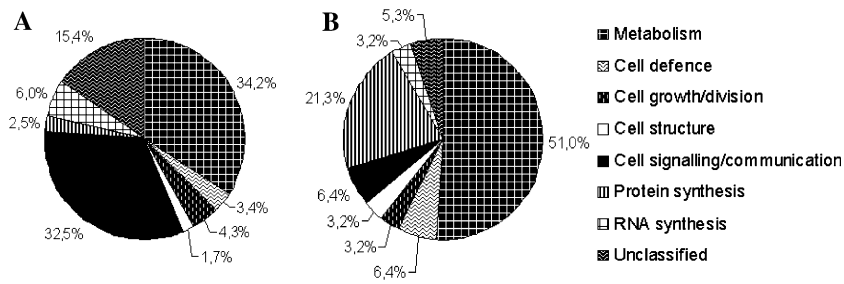

Fig. 4 Functional classification of clones, down-regulated (**A**) and up-regulated (**B**) in the Δ*FvMAT1-2-1/15* mutant

signalling and communication were especially frequently tagged. Furthermore, a significant portion (15.4%) of unclassified ESTs was found in this set of clones, indicating that the MAT1-2-1 product positively affects a number of genes, which are completely unknown at the present stage of knowledge. This unclassified category amounted only 5.3% among the down-regulated sequences.

Discussion and conclusions

In this study, transcript profiles of a wild type strain of *F. verticillioides* and its Δ*MAT1-2-1* mutant were compared by using the high-density filter technique. The results clearly demonstrated that targeted disruption of the *MAT1-2-1* sequence affected the transcription of a number of genes with various roles in cell biology. Approximately 55% of the tagged sequences were found to be down-regulated in the mutant and 45% of the sequences were up-regulated indicating that the putative transcription factor encoded by the *MAT1-2-1* gene may have both positive and negative regulatory function depending on the target gene. Similar expression profiles were seen in microarray experiments using different cultural conditions for *F. graminearum* (Güldener et al. 2006).

Several precautionary measures were done to minimize the risks of misinterpretation of signal intensity differences when filters hybridized with cDNAs from the wt and the mutant, respectively were compared: (i) all the experiments were done in parallel with each probe in one tube, (ii) the same DNA preparations were used throughout, and (iii) a strict, four-fold threshold was used instead of the widely accepted two-fold one to compensate differences that might derive from the technical makeup of nucleic acid manipulation, hybridization or filter quality. Furthermore, all individual DH10B colonies (containing inserts of *F. verticillioides* origin) were placed on the filter in duplicate, at opposite positions (see Fig. 3, upper left corner). The image analysis software calculated average values of signal intensities of these duplicates: if variation between the duplicates was high, due to background unevenness of the X-ray or the proximity of a highly intense spot, that particular clone was automatically excluded by the program from the analysis. The use of such strict measures may have caused losses, i.e. we probably missed quite a few genes regulated differentially by the MAT1-2-1 transcription factor. On the other hand, the

differences we obtained under these conditions can be trustworthily interpreted as real transcriptional differences.

The regulation patterns of clones grouped into the same contig by the SeqMan program were further analyzed to test the robustness of the selection procedure. All members of contig *i* (putative nitrilase), contig *k*(alpha glucosidase), contig *o* (nitroalkane oxidase), contig *p* (Thij/PfpI family protein), and contig *q* (rasp f7 allergen) showed down-regulation in the Δ*FvMAT1-2-1* mutant, whereas members of contigs *c* (40 S ribosomal protein), *d* (putative heat shock protein), and *n* (carbon catabolite repression regulator protein) were all up-regulated in the mutant. Thus all genes tagged in this study (and probably driven by the same promoter) were unequivocally assigned into the up- or down-regulated category illustrating the validity of the methodology we used here.

It should be emphasized, that functional assignments of the tagged sequences were only based on BLAST comparisons following the classification system developed by White and Kerlavage (1996) with modifications proposed by Sacadura and Saville (2003). Although gene functions have not been tested yet, some of the ESTs negatively affected by the inactivation of the *MAT1-2-1* gene deserve a more detailed discussion as they are likely to be involved in sexual development. The appearance of such sequences in a study like this could be foreseen. Their occurrence indicates further that the majority of other sequences whose roles can not be postulated at this stage of the research are probably not artefacts either.

Nitrilase encoding fragments (contiguous sequences denoted by the superscript 'i') were especially frequently identified among the sequences down-regulated in the Δ*FvMAT1-2-1* mutant. This frequent occurrence was further accompanied with significant transcriptional differences between the mutant and the wild type, indicating that a putative nitrilase gene of *F. verticillioides* is definitely affected by the MAT1-2-1 transcription factor. Nitrilases constitute a large superfamily of enzymes comprising 12 families of amidases, N-acyltransferases, carbamylases, nitrilases and various presumptive amidases. One subfamily of this highly heterogeneous superfamily, named branch 9, contains enzymes capable of reverse amidase activity that catalyzes a condensation reaction resulting in palmitylation of cystein residues. Some enzymes in this family are known to participate in biosynthesis of signalling molecules (Brenner 2002), but no information is available on the role of this type of nitrilases in fungi. Owing to its potential N-acetyltransferase activity, the putative nitrilase, tagged so frequently in the present experiment, may contribute to lipophilic additions needed to produce the mature pheromone in fungi, like *F. verticillioides*.

Clone 265 sheds light on another reasonable target of the MAT1-2-1 product. This clone represents a fragment of the *carO* gene, described recently from *Fusarium fujikuroi*, a close relative of *F. verticillioides* (Prado et al. 2004). The CarO protein belongs to the opsin family and was found to show significant degrees of identity with *ops* and *nop-1* from *Leptospaeria maculans* and *Neurospora crassa*, respectively. Opsins are membrane photoreceptors which play an important role in light detection in algae (Ridge 2002) and animals (Menon et al. 2001). However, understanding the biological function of these proteins in fungi needs further investigations. Disruption of *carO* caused no phenotypic alteration and photoinduction of the carotenoid biosynthesis was not affected either (Prado et al. 2004). Filamentous ascomycetes require light for mating and sexual morphogenesis, furthermore, asexual spore production is light-dependent in most fungi. The MAT1-2-1 transcription factor, produced constitutively both in sexually and asexually reproducing *Fusarium* species (Kerényi et al. 2004) may enhance photoreception in these organisms by up-regulating such an opsin-like protein encoding gene. This stimulating effect could be important under insufficient illumination that frequently occurs in natural habitats of fungi.

Other genes encoding predicted proteins involved in light regulated events were also found to be down-regulated in the Δ*FvMAT1-2-1* mutant, indicating indirectly that the MAT1-2-1 product enhances their transcription. Clone 241 showed homology to the WC-2 protein from *Magnaporthe grisea*. WC-2 forms the so-called white collar complex (WCC) together with another protein,

WC-1. WCC acts then as a transcription factor and mediates many blue-light-dependent responses in *Neurospora* including carotenogenesis, conidiation and phototropism of sexual structures (Linden et al. 1997). Not surprisingly, another clone (no. 109) showing similarity to a calcium/calmodulin-dependent protein kinase (CAMK-1) also occurred among the sequences down-regulated in the Δ*FvMAT1-2-1* mutant as there is a multiple interaction between the WCC complex and CAMK-1. WCC activates the transcription of *frq*, a gene encoding the circadian clock protein, FREQUENCY (FRQ), and FRQ – meanwhile repressing *frq* transcription – exerts a positive feedback on levels of the WC proteins (Lee et al. 2000). Posttranslational mechanisms also regulate the circadian clock functions through phosphorylation of these proteins maintaining thus the proper operation of the clock. Phosphorylation of FRQ is mainly done by CAMK-1, which in turn seems to be positively regulated by the MAT1-2-1 transcription factor according to our investigations.

Further down-regulated sequences (clones 199 and 238) showed similarity to a cell cycle arrest protein and a clock controlled protein, respectively. The cell cycle arrest protein is known to be influenced by a pheromone-related protein in *Cryptococcus neoformans*, whereas the clock-controlled protein 6 (CCG-6) known from *N. crassa* is involved in the circadian expression of the developmental rhythm. Two ESDC (essential for sexual development C) homologues known to be essential for sexual development in *Aspergillus nidulans* (Nowrousian et al. 2005) were also found among the ESTs down-regulated in the Δ*FvMAT1-2-1* mutant and a MAP kinase sequence, involved in regulation of mating (Jenczmionka et al. 2003) has also been tagged.

Previous studies (Verma and Ballester 1999) demonstrated that mating type genes play a role in maintaining cell wall integrity in *Saccharomyces cerevisiae*. Of the clones, down-regulated in the Δ*FvMAT1-2-1* mutant clone 137 showed similarity to a putative β-1,3-glucan binding protein. Furthermore, a putative cell wall adhesin protein encoding gene (clone 219) has also been tagged among the sequences down-regulated in the mutant. This protein has been reported to play a role in maintaining cell wall integrity and is required for efficient mating in *Candida albicans*.

Two papers closely related to the present work have recently been published. Noworousian et al. (2005) compared gene expression profiles of developmental mutants of *Sordaria macrospora* using *N. crassa* cDNA microarrays hybridized with RNA probes from a wild type strain of *S. macrospora* and three 'pro' mutants of the fungus. These 'pro' mutants, although they carried mutations in different genes were all blocked at the protoperithecium formation stage. Their analysis could thus lead to the identification of genes involved in sexual development. The other study (Lee et al. 2006) aimed to identify down-regulated genes in a *MAT1-2*-deleted transgenic mutant of *Gibberella zeae* (*Fusarium graminearum*) in a growth stage, when abundant perithecium development occurs in the wild type. The three works, including ours gave only partially overlapping results, which could be expected, when the differences in the experimental designs and methodologies are considered. The two previous works studied a physiological stage when sexual development is switched on, whereas we wanted to examine the effect of the *MAT1-2-1* gene in an early stage of growth with the aim to trace the role of the MAT product during vegetative growth. Our philosophy was that such an approach might help to understand the function of the constitutively transcribed mating type genes in the absence of mating, i.e. during asexual growth of fungi with a sexual lifestyle or in fungi with no known sexual stage.

In spite of these differences, a number of ESTs are worthy to be mentioned here, as they occurred both in the present research as well as in one or the other previous studies, cited above. The opsin-like protein and the putative MAP kinase sequence were both found to be down-regulated by Lee et al. (2006) in the *MAT1-2*-deleted strain of *G. zeae*, similarly to our findings. The ESDC homologous sequence was similarly down-regulated in the 'pro' mutants of *S. macrospora* (Nowroussian et al. 2005) and the Δ*FvMAT1-2-1* mutant (this study). Similar coincidences were observed with a number of other ESTs, including an alcohol dehydrogenase (clone 27), a methyltransferase (clone 239), an

EFB-like transcription factor (contig *l*), a putative elongation factor (clone 3), a GTP-binding protein (clone 122), a putative alpha glucosidase (clone 149) and a rasp f7 allergen (contig *q*) encoding sequence. Some of these ESTs are likely to be involved in sexual development (e.g. opsin, MAP kinase, ESDC), whereas such a role for the other sequences cannot be postulated at this moment. On the other hand, some of the ESTs, like a mitochondrial ATP synthase protein, the WC-2 protein, the methyltransferase and several ribosomal protein sequences, were found to be differentially regulated in one or the other of the three studies, indicating that many genes are influenced by the MAT1-2-1 transcription factor in a growth stage dependent manner.

As pheromone precursor and pheromone receptor genes are well documented targets of the *MAT1-2-1* derived transcription factor, we postulated at the beginning of these experiments that fragments of such genes should have been found among the clones, down-regulated in the Δ*FvMAT1-2-1* mutant. However, no traces of such genes were tagged by us and such sequences did not appear either in the work done by Lee et al. (2006) with the *MAT1-2*-deleted mutant of *G. zeae*. On the other hand, the pheromone precursor genes, *ppg1* and *ppg2* were differentially expressed in the wild type and the 'pro' mutants of *S. macrospora*, but – somewhat unexpectedly – these genes were up-regulated in mutants with deficiencies in fruiting body formation (Nowrousian et al. 2005). The lack of pheromone precursor and pheromone receptor sequences among the ESTs which showed significantly stronger intensities in the wild type strain in comparison to the Δ*FvMAT1-2-1* mutant suggests, that these genes are not so abundantly expressed in the early stage of growth (*nota bene* five-day-old cultures were used for RNA extraction) and/or in the absence of the other mating partner.

In the present study we identified a number of genes influenced either positively or negatively by the MAT1-2-1 product. Based on sequence homologies, some of the tagged genes are likely to be involved in sexual development, whereas the role of others remained unclear. However, we would not claim that all targets of the putative MAT1-2-1 protein were found in these experiments, moreover it may also be that some of the sequence tags were not true targets of this transcription factor. Our experiments, although carefully designed, were by necessity not fully satisfactory: the fungal material we used for RNA extraction was a mixture of cells, comprised by differentiated mycelial cells, conidiophores, phialides, conidia, germ tubes and this might cause some discrepancies. The internal genetic program of these different cells is necessarily varied and this variation may contribute to the transcriptional differences we found when compared the wild type and its Δ*FvMAT1-2-1* mutant.

In conclusion, we have identified 248 ESTs affected by the *MAT1-2-1* gene of *F. verticillioides*. Due to their significant similarities to other known genes, many of these sequences are promising candidates for future investigations. With the recent development of suitable transformation and gene inactivation systems for a number of *Fusarium* species (Proctor et al. 1997; Fernández-Martín et al. 2000; Trail et al. 2003; this study), the specific roles of the tagged sequences in mating and/or other aspects of the fungal life cycle can be uncovered in the near future.

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