The effect of natamycin on the transcriptome of conidia of *Aspergillus niger*

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Abstract: The impact of natamycin on *Aspergillus niger* was analysed during the first 8 h of germination of conidia. Polarisation, germ tube formation, and mitosis were inhibited in the presence of 3 and 10 µM of the anti-fungal compound, while at 10 µM also isotropic growth was affected. Natamycin did not have an effect on the decrease of microviscosity during germination and the concomitant reduction in mannitol and trehalose levels. However, it did abolish the increase of intracellular levels of glycerol and glucose during the 8 h period of germination.

Natamycin hardly affected the changes that occur in the RNA profile during the first 2 h of germination. During this time period, genes related to transcription, protein synthesis, energy and cell cycle and DNA processing were particularly up-regulated. Differential expression of 280 and 2586 genes was observed when 8 h old germlings were compared with conidia that had been exposed to 3 µM and 10 µM natamycin, respectively. For instance, genes involved in ergosterol biosynthesis were down-regulated. On the other hand, genes involved in endocytosis and the metabolism of compatible solutes, and genes encoding protective proteins were up-regulated in natamycin treated conidia.

Key words: antibiotics, *Aspergillus niger*, conidia, germination, natamycin, transcriptome.

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INTRODUCTION

Conidia are stress-resistant dispersal vehicles that are produced by many fungal species. Fungi belonging to the genera *Aspergillus* and *Penicillium* produce large numbers of airborne conidia. These conidia easily contaminate and colonise food, which explains why *Aspergillus* and *Penicillium* are among the most important food-spoiling organisms. Preservatives as sorbic acid and natamycin (Plumridge *et al.* 2004, Stark 2007) prevent fungal growth in or on a food source. There are clear indications that dormant conidia are more resistant to antifungal compounds than growing hyphae. Dormant conidia of *Aspergillus fumigatus* survive concentrations of 50 µg/mL of the polyene antibiotic amphotericin B, but become sensitive to 20 and 1–2 µg/mL of the antifungal after 2 and 4 h of germination, respectively (Russel *et al.* 1975, 1977). Similarly, conidia of *A. niger* and *Penicillium discolor* survive a treatment with 45 µM of the polyene antibiotic natamycin, which equals ten times the minimal inhibitory concentration for germinating conidia. Notably, conidia start to germinate upon removal of the antibiotic (van Leeuwen *et al.* 2010).

It is the aim of this study to evaluate the cellular mechanisms that explain these variations in antifungal sensitivity. Novel insights may lead to new prevention strategies of fungal contamination in agriculture and the food industry. As a model system the antifungal compound natamycin that is used in the food industry (Stark 2007) is used. In contrast to other polyene antifungals, natamycin does not induce membrane permeability (Te Welscher *et al.* 2008, van Leeuwen *et al.* 2009). It does inhibit endocytosis in germinating conidia of *P. discolor* in a time and dose dependent manner (van Leeuwen *et al.* 2009). Moreover, natamycin interferes with vacuole fusion in yeast cells as well as filamentous fungi (Te Welscher *et al.* 2010). Very recent work has shown that natamycin also reversibly inhibits transport of different nutrient molecules into the cell (Te Welscher *et al.* 2012).

In order to study the changes that occur in conidia that are challenged with antifungal compounds, the transcriptome of conidia of *Aspergillus niger* was studied in the presence of natamycin and compared with data of untreated germinating conidia. Recently, RNA profiles of dormant and germinating conidia of *A. niger* were reported (van Leeuwen *et al.* 2013). It was shown that the RNA composition of dormant conidia was most distinct when compared to conidia that had been germinating for 2, 4, 6, and 8 h. Dormant conidia contain high numbers of transcripts of genes involved in formation of protecting components such as trehalose, mannitol, heat shock proteins and catalase. Transcripts of the functional gene classes protein synthesis, cell cycle and DNA processing and respiration were over-represented in the up-regulated genes after 2 h of germination, whereas metabolism and cell cycle and DNA processing were over-represented in the up-regulated genes after 4 h of germination. No functional gene classes were over- or under-represented in the differentially expressed genes after 6 and 8 h of germination. From these data it was concluded that the RNA profile of conidia changes especially during the first 2 h of germination and that this coincides with protein synthesis and respiration.

We here show that 3 and 10 µM natamycin hardly affect the transcriptome during the first 2 h of germination, but it inhibits further stages of germination as judged by several cellular parameters. The transcriptome after 8 h was less affected when spores were kept in 3 µM natamycin compared to those treated in a concentration of 10 µM. For instance, genes involved in endocytosis, and genes involved in protection of conidia were up-regulated. On the other hand, genes involved in ergosterol biosynthesis were down-regulated.

MATERIALS AND METHODS

Organism and growth conditions

The *A. niger* strain N402 (Bos *et al.* 1988) and its derivative RB#9.5 were used in this study. The latter strain expresses a gene encoding a fusion of sGFP and the histone protein H2B under regulation of the *mpdA* promoter (R. Bleichrodt, unpubl. results). For spore isolation, strains were grown for 12 d at 25 °C on complete medium (CM) containing per liter: 1.5 % agar, 6.0 g $NaNO_3$, 1.5 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4$, 4.5 g D-glucose, 0.5 % casamino acids, 1 % yeast extract and 200 µl trace elements (containing per liter: 10 g EDTA, 4.4 g $ZnSO_4 \cdot 7H_2O$, 1.0 g $MnCl_2 \cdot 4H_2O$, 0.32 g $CoCl_2 \cdot 6H_2O$, 0.32 g $CuSO_4 \cdot 5H_2O$, 0.22 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.5 g $CaCl_2 \cdot 2H_2O$, and 1.0 g $FeSO_4 \cdot 7H_2O$). Conidia were harvested in ice-cold ACES-buffer (10 mM ACES, 0.02 % Tween-80, pH 6.8), filtered through sterile glass wool, washed in ice-cold ACES-buffer and resuspended in CM (van Leeuwen *et al.* 2013). The conidia were kept on melting ice until further processing on the same day. An aliquot of 3.10^9 conidia were added to 300 ml CM in 500 ml Erlenmeyers. Cultures were shaken at 125 rpm in the absence or presence of 3 or 10 µM natamycin. Stock solutions of natamycin (10 mM) were freshly made in 85 % DMSO (Brik 1981).

Transcriptome analysis

Data analysis was performed on biological triplicates, each based on three cultures. At each time point, 15 ml of each of the three cultures was pooled. The (germinating) conidia were pelleted at 1100 g at 5 °C for 5 min and immediately frozen in liquid nitrogen. RNA extraction, cDNA labeling, microarray hybridisation and data analysis were done as described (van Leeuwen *et al.* 2013). The array data has been deposited in NCBI's Gene Expression Omnibus (Edgar *et al*. 2002) and is accessible through GEO Series accession number GSE36440 (www.ncbi.nlm.nih.gov/geo/).

HPLC analysis

Dormant, germinating or treated conidia ($5 \cdot 10^7$ - $1 \cdot 10^8$) were frozen at -80 ºC and homogenised with a Qiagen Tissuelyser® (2 min at 30 strokes /sec: Qiagen, Venlo, The Netherlands) using a stainless steel grinding jar cooled with liquid nitrogen. After an additional round of grinding with 1 ml milliQ, the samples were thawed and quickly transferred to a 2 ml Eppendorf tube. Samples were centrifuged at 4 °C for 30 min at 20.817 g. The supernatant was stored at -80 °C until analysis. Prior to HPLC analysis samples were filtered through an Acrodisc® 0.2 µm PTFE syringe filter (Sigma-Aldrich, Zwijndrecht, The Netherlands). A volume of 10 µl was subjected to HPLC analysis, using a Waters 717 plus autosampler equipped with a 515 HPLC pump with control module II (Waters Corporation, Etten-Leur, The Netherlands). The mobile phase consisting of 0.1 mM Ca EDTA in water was maintained at a flow rate of 0.5 ml/min. The Sugar Pak I Ca^+ cation-exchange column was kept at 65 °C with a Waters WAT380040 column heater module (Laborgerätebörse GmbH, Burladingen, Germany). Sugars and polyols were detected with an IR 2414 refractive index detector (Waters Corporation, Etten-Leur, The Netherlands). As standards, trehalose, mannitol, D-(+)-glucose, glycerol, erythritol and arabitol were used (Sigma-Aldrich, Zwijndrecht, The Netherlands). Peak integrations and quantitative calculations were performed with the Waters Empower software (Waters Corporation, Etten-Leur, The Netherlands).

ESR spectroscopy

Germinating conidia were centrifuged at 8000 rpm for 2 min. The supernatant was discarded and the conidia were resuspended in 25 µl perdeuterated TEMPONE-potassium ferricyanide solution (1 mM and 120 mM, respectively). Micro-viscosity was determined and calculated as described in (van Leeuwen *et al.* 2010).

Fluorescence microscopy

Samples of liquid cultures were placed on poly-l-lysine (Sigma) coated cover slips (van Leeuwen *et al.* 2008). The medium was removed and the cover slips with the immobilised conidia were placed upside-down onto an object glass with a < 0.5 mm layer of 2 % water agar. Images were taken with a Zeiss Axioskop 2 plus microscope (Zeiss, Oberkochen, Germany) equipped with a HBO 100 W mercury lamp and a AxioCam MRc (Zeiss, Germany) camera using standard FITC (λ = 450–490 nm, FT510, LP520) filters.

RESULTS

Morphological responses to natamycin during conidial germination

Light microscopy showed that germination of *A. niger* conidia is inhibited in natamycin-treated conidia compared to untreated cells (Fig. 1A). Untreated conidia swell slightly during the first 2 h of germination. The surface area of the cells on the micrographs increased from 17 to 22.6 µm² (Fig. 1B). The conidia enlarged gradually to 46 µm² between 2- and 6 h and their volume further increased up to 8 h. At this stage, the variability in size of the cells was largely due to differences in germ tube emergence and growth. After 6 and 8 h, 10 % and 80 % of the conidia had started to form germ tubes, respectively (Fig. 1C).

Conidia that had been exposed to 3 µM natamycin showed a similar swelling as control cells during the first 2 h. The surface area of the cells on the micrographs increased from 17.7 to 22.1 µm². Between 2- and 6 h, the surface area of the conidia enlarged to 35.6 µm², which had further increased to 40.1 µm² after 8 h of germination. Notably, polarisation and germ tube formation were not observed during the 8 h incubation time (Fig. 1C). The surface area of conidia that had been exposed to 10 µM natamycin for 2 h increased from 17.3 to 21.8 µm² (Fig. 1B). After 3 h the conidia had reached a surface area of 23.4 µm², which remained unchanged up to 8 h of incubation. Polarised cells and germ tubes were not formed throughout culturing (Fig. 1C). All considering, these results show that polarisation and germ tube formation are inhibited at

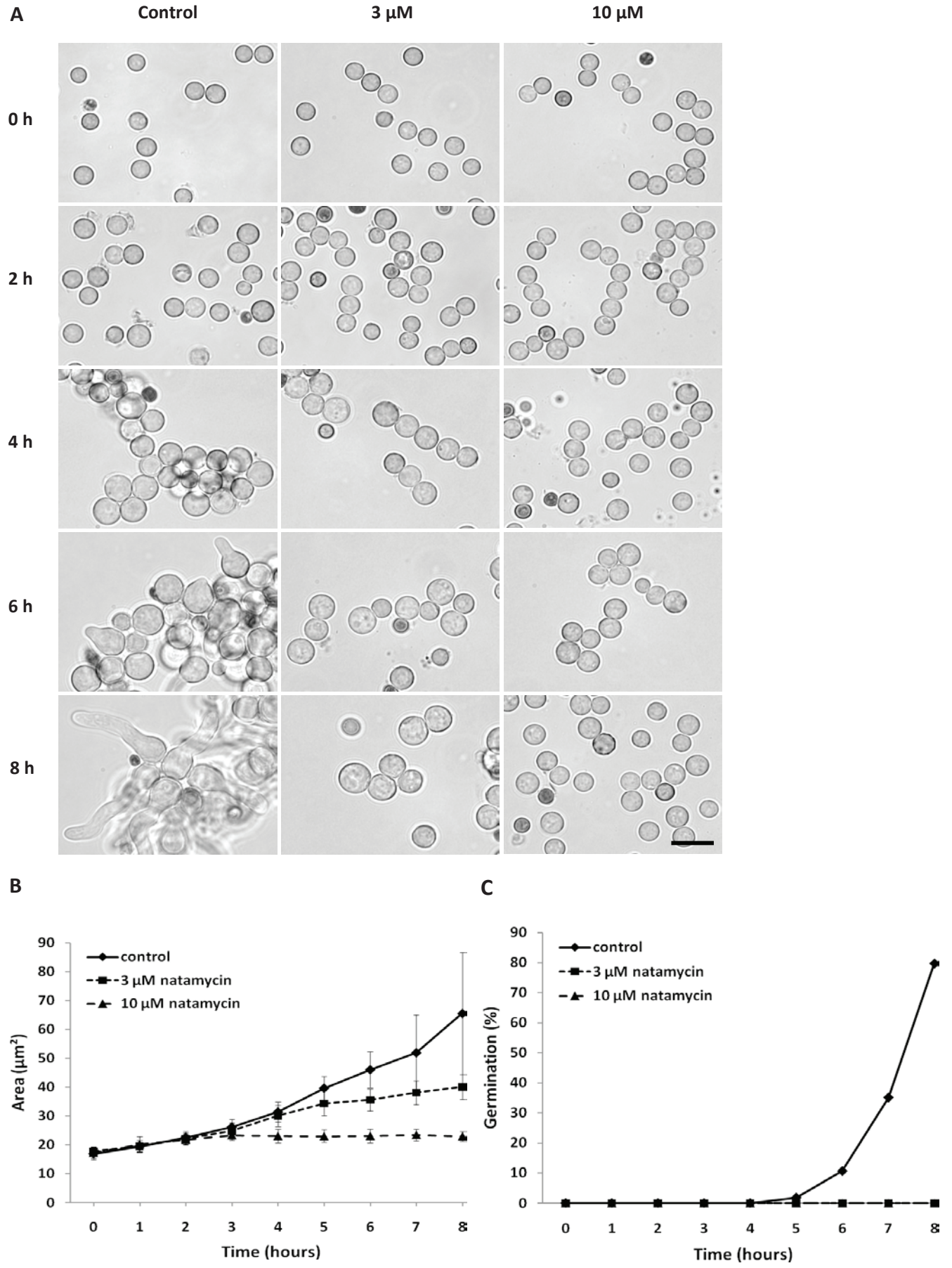

Fig. 1. Effect of natamycin on germination of *A. niger* conidia. Morphology (A), increase in surface area (B) and percentage of conidia forming germ tubes (C) in the absence or presence of 3 µM and 10 µM natamycin. Bar represents 10 µm.

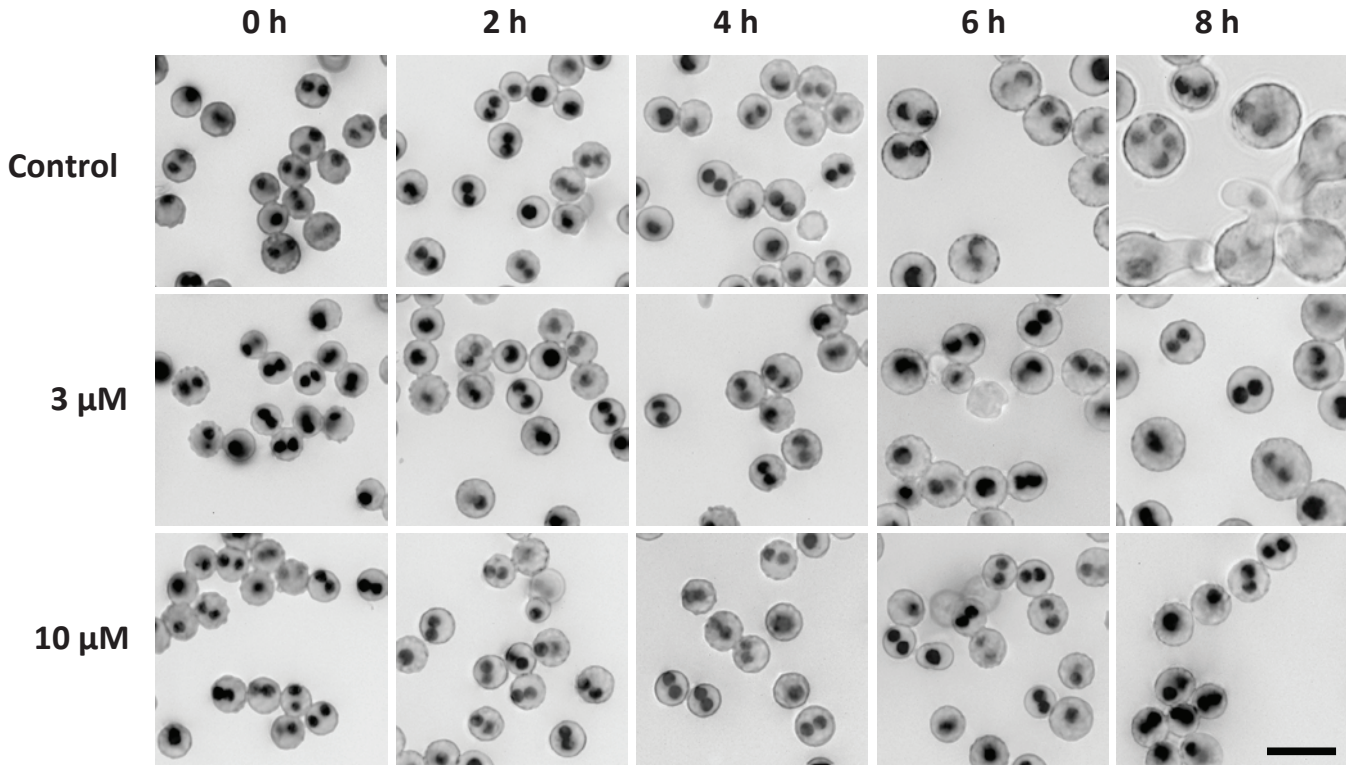

Fig. 2. Number of nuclei in conidia of the *A. niger* strain RB#9.5 in the absence or presence of 3 µM and 10 µM natamycin as visualised with fluorescence microscopy. Bar represents 10 µm.

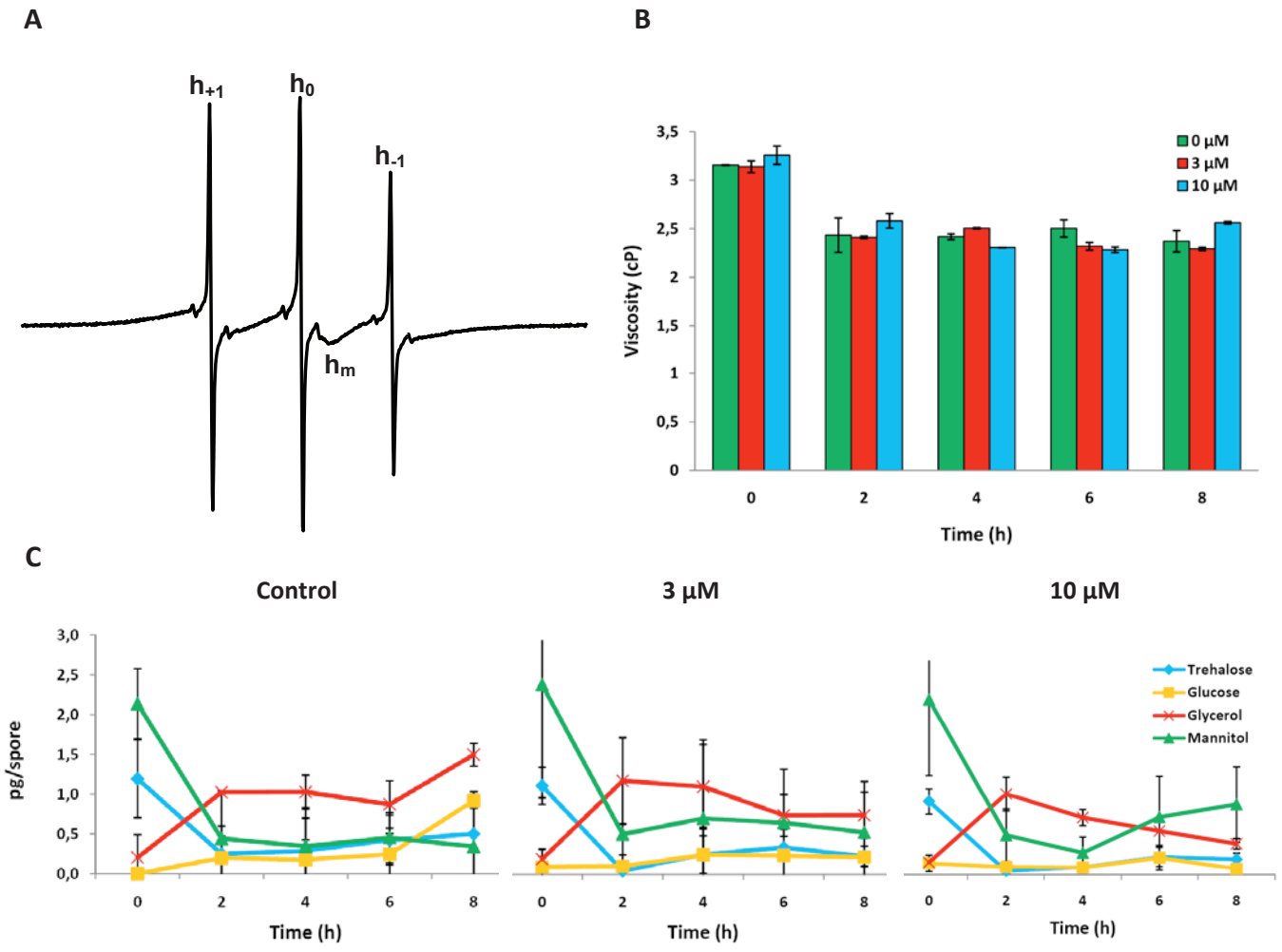

Fig. 3. Viscosity (A, B) and glucose, trehalose, glycerol and mannitol levels (C) in germinating *A. niger* conidia and in conidia treated with 3 µM and 10 µM natamycin. In (A), h_0 and h_{-1} represent the low-field and high-field lines of the electron spin resonance (ESR) signals, respectively, which are used to calculate micro-viscosity (B). h_m is the ESR signal from melanin which is present in the conidial cell wall.

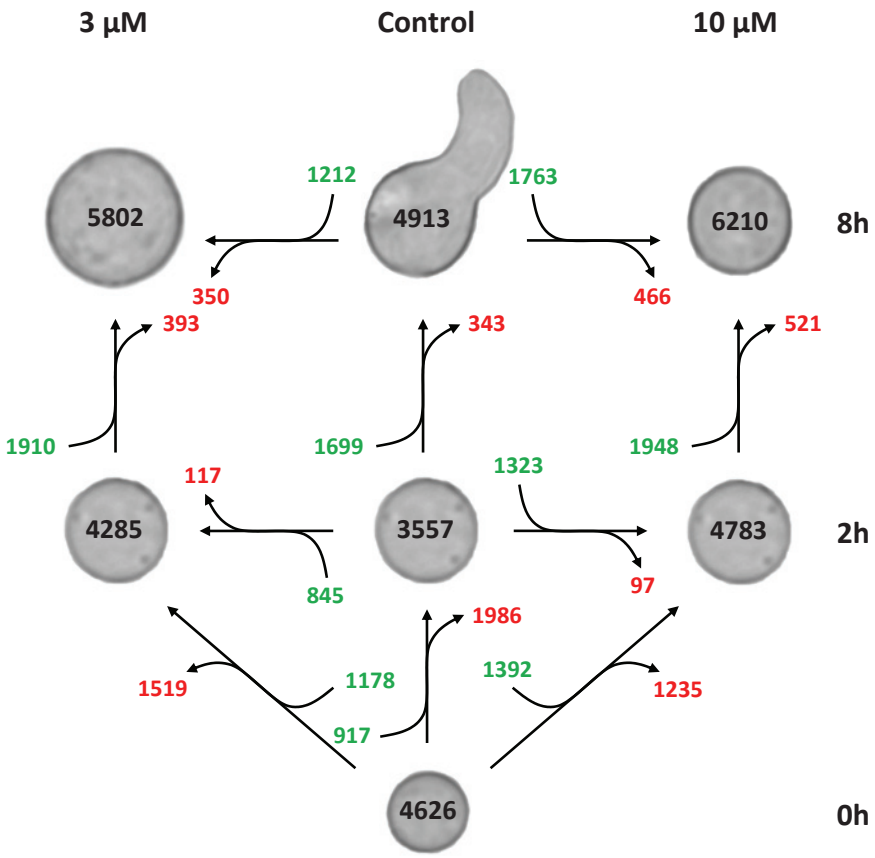

Fig. 4. Overview of the global changes in the transcriptome of conidia that had either or not been treated with 3 µM or 10 µM natamycin. Inside the conidia the number of expressed transcripts is given. Green and red numbers represent genes with an absent to present call and a present to absent call between two stages, respectively.

3 and 10 µM natamycin, while at 10 µM also isotropic growth is inhibited.

An *A. niger* reporter strain expressing a fusion of the H2B histone protein and the sGFP protein under control of the *mpdA* promoter was used to monitor nuclear division (Fig. 2). Dormant conidia of this RB#9.5 strain were, with 85 % (n = 86), binucleate (van Leeuwen *et al.* 2013). Nuclear division was shown to occur prior to the formation of germ tubes namely between 6 and 8 h of germination. Conidia did not show mitosis after 8 h of incubation when exposed to 3 or 10 µM natamycin.

Intracellular viscosity and compatible solutes during germination

The dormancy of fungal spores has been correlated to the viscosity of the interior of the cell (Dijksterhuis *et al.* 2007). Viscosity within *A. niger* conidia was analysed by electron spin resonance (ESR) using the spin probe perdeuterated TEMPONE (PDT). The spectra included a narrow and a broad component. The narrow component represents the cytoplasmic signal and was detectable after subtraction of the broad component originating from cell wall located PDT (Dijksterhuis *et al.* 2007, van Leeuwen *et al.* 2010). The narrow spectrum showed a central line (h_0), flanked by a low-field line (h_{-1}), high-field line (h_{+1}) and an m-line (h_m). The latter represents melanin, which has its own paramagnetic properties (Fig. 3A, Dijksterhuis *et al.* 2007). Central and high-field components were used to calculate rotational correlation time and the viscosity (η) based on the Stokes-Einstein equation. The effective viscosity of dormant conidia ranged between 3.14 and 3.26 cP. After 2 h of germination, viscosity decreased with 20–30% irrespective of the presence of natamycin (Fig. 3B). As germination proceeded, no further change in viscosity was observed in all cases. This was irrespective of the presence of natamycin. These results show that natamycin does not affect the decrease in cytoplasmic viscosity as observed during germination of conidia *of A. niger*.

A dormant conidium of *A. niger* contains on average 2.1 ± 0.4 and 1.2 ± 0.5 pg of the compatible solutes mannitol and trehalose, respectively (Fig. 3C). These values drop to ≤ 0.5 pg within the first 2 h of germination. In contrast, the level of glycerol increased in this time span from almost undetectable to 1.0 ± 0.0 pg per cell. Levels of glycerol remained unchanged until 6 h of germination, but had increased to 1.5 ± 0.1 pg after 8 h. The level of glucose slowly increased to approximately 0.3 ± 0.3 pg during the first 6 h of germination, after which it increased to 0.9 ± 0.1 pg per cell. Natamycin did not affect the degradation of compatible solutes as well as the appearance of glycerol in the cytoplasm during the first 2 h. After 8 h, however, no further increase in the glycerol level had occurred in natamycin-treated cells. Instead, glycerol levels decreased between 2 and 8 h in conidia treated with 10 µM natamycin. Furthermore, no increase in glucose levels was observed in the presence of natamycin after 8 h. Between 4 and 8 h mannitol increased to 1.0 ± 0.5 pg per cell when conidia were exposed to 10 µM natamycin. Taken together, natamycin decreases glycerol and glucose levels in conidia after degradation of mannitol and trehalose, In addition, mannitol levels stay higher and in 10 µM natamycin increase after 8 h.

Transcriptional profiling and comparison of gene expression

RNA from conidia that had been treated for 2 or 8 h with 3 or 10 µM natamycin was hybridised to whole genome microarrays. MAS5.0 detection calls showed that the number of genes with a present call was invariably higher upon treatment with natamycin as compared with the controls (Fig. 4). Untreated conidia showed a marked decrease in the number of expressed genes after 2 h

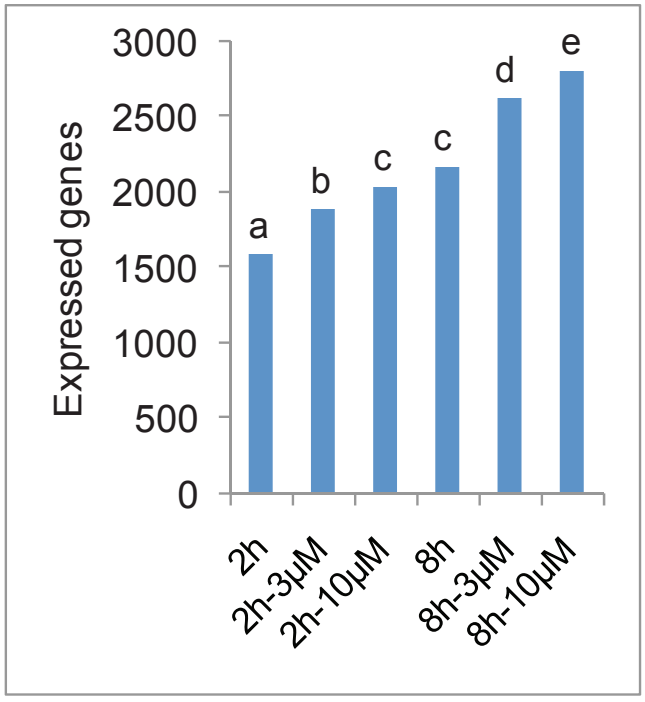

Fig. 5. The number of expressed genes with hybridisation values between 100 and 1000 in treated conidia and in controls. The triplicates were tested by means of ANOVA and different lettering indicates a significant difference ($p < 0.05$).

of germination (*i.e.* from 4626 to 3557; see also van Leeuwen *et al.* 2013). The decrease was much less in the presence of 3 µM natamycin (*i.e.* from 4626 to 4285), whereas at 10 µM natamycin the number of expressed genes had even increased (*i.e.* from 4626 to 4783) (Fig. 4). The number of genes that lost their transcripts during the first 2 h of germination dropped from 1986 (control) to 1519 (3 µM) and 1235 (10 µM), while the number of genes whose expression was activated increased (917, 1178 and 1392 genes, respectively). The conidia treated with 10 µM natamycin had 34 % more expressed genes than the controls after 2h of germination. A similar difference was also observed after 8 h. The number of transcripts reached 6210 (this is 43 % of all ORFs identified in *A. niger*) in the case of treatment with 10 µM natamycin and 5802 and 4913 in the case of conidia treated with 3 µM or untreated cells, respectively. The increase in expressed genes was significant in the case of moderately to highly expressed genes (Fig. 5).

Correlation of the RNA profiles showed that dormant conidia were most different compared to the other samples. Conidia treated for 8 h with 10 µM natamycin showed the strongest resemblance to dormant conidia (Fig. 6). The correlation between the profiles at t = 2 h and t = 8 h was 0.76 for the control, 0.61 for the samples treated with 3 µM natamycin and 0.57 for 10 µM natamycin. All considering, these data indicate that RNA profiles of natamycin-treated conidia change to a higher extent when compared to non-treated cells.

Differential gene expression in natamycin-treated cells.

The number of genes that was more than 2-fold up-regulated within the first 2 h ranged between 947 and 1152 in the absence or presence of natamycin (Fig. 7). The number of down-regulated genes ranged between 1343 and 1968. When the profiles at t = 2 h were compared, 1 and 9 genes were ≥ 2-fold down- and up-regulated, respectively. Apparently, the changes that occur in the RNA profile during the first 2 h of germination are hardly affected by natamycin. Indeed, the correlation of the profiles at t = 2 h was between 0.87 and 0.9 (Fig. 6). In all cases, transcripts belonging to the functional categories protein synthesis, energy and cell cycle and DNA processing were over-represented in the up-regulated genes at t = 2 h (Table 1). The functional gene class cell cycle and DNA processing was over-represented in the up-regulated genes and the functional gene class transcription was over-represented in the down-regulated genes when the profiles of t = 2 and t = 8 h were compared (Table 2). This was irrespective of natamycin treatment. In conidia treated with 10 µM natamycin, the categories C-compound and carbohydrate utilisation and lipid and fatty acid breakdown were overrepresented in the up-regulated genes. At 8 h, 280 genes were ≥ 2-fold up- or down-regulated (*i.e.* 173 and 103, respectively) when germinating controls were compared to the non-germinating conidia that had been exposed to 3 µM natamycin for 8 h (Fig. 7). Changes were clearly more pronounced between the controls and conidia treated with 10 µM natamycin. In this case, 1713 and 873 genes up- and down-regulated, respectively. Indeed, the correlation in the RNA profile was higher between the control and 3 µM natamycin at t = 8 h than between the control and 10 µM natamycin (*i.e.* 0.8 and 0.71, respectively; Fig. 6). The fact that the 10 µM natamycin sample at t = 8 h is more different from the control than the 3 µM sample is also reflected in a Fisher exact test (Table 3).

Specific transcriptional changes associated to conidial germination

In the following paragraphs expression of selected groups of genes in conidia that had been incubated in medium with or without 10 µM natamycin will be discussed. The tables also show the values for dormant conidia and conidia treated for 8 h with 3 µM natamycin.

Ergosterol and desaturated fatty acids

Natamycin specifically binds to ergosterol. Ergosterol is formed from acetyl CoA, which involves 22 enzymes in *S. cerevisae* (Onyewu *et al.* 2003, Da Silva Ferreira *et al.* 2005, Mysyakina & Funtikova, 2007). Fourteen out of 24 genes with homology to ergosterol biosynthesis genes showed ≥ 2-fold lower expression in conidia that had been incubated in the presence of 10 µM natamycin when compared to the control (Table 4). The most severe down-regulation was observed for genes with similarity to HMG-CoA synthase (*erg13, HMGS*, An02g06320), *erg1* (An01g03350), *erg3* (An16g02930, An15g00150), *erg5* (An01g02810), a gene similar to squalene monooxygenase (*erg1*, An03g03770), *erg25* (An03g06410), and a lanosterol 14 alpha-demethylase like gene (*erg11,* An11g02230). These genes were 6.5-fold to 40 times down-regulated. In contrast, the HMG-CoA reductase (*hmg1*, An04g00610, Basson *et al.* 1986) was 10-fold up-regulated in the presence of natamycin.

Δ9-stearic acid desaturases (Wilson *et al*. 2004) and Δ12-oleic acid desaturases (Calvo *et al.* 2001, Chang *et al.* 2004) are important for the generation of desaturated fatty acids. As such, they influence the amount of (poly)unsaturated fatty acids in membranes. Transcripts of four desaturases (An07g01960, An12g09940, An08g05160 and An14g06980) are strongly down regulated in conidia exposed to natamycin. For instance, transcripts of the enzyme *odeA* (An08g05160) were down-regulated 25.8-fold.

Vesicle trafficking

Ergosterol is involved in fusion and fission events in fungal cells (Jin *et al.* 2008) including endocytosis (Heese-Peck *et al.* 2002,

	0h	2h	8h	2h-3µM	8h-3µM	2h-10µM	8h-10µM
0h	0.92	0.42	0.47	0.43	0.49	0.45	0.52
2h		0.96	0.76	0.90	0.64	0.87	0.56
8h			0.97	0.70	0.80	0.72	0.71
2h-3µM				0.88	0.61	0.89	0.53
8h-3µM					0.87	0.64	0.89
2h-10µM						0.85	0.57
8h-10µM							0.96

Fig. 6. Correlation of the RNA profiles of dormant or germinating conidia and conidia which are kept in natamycin for 2 and 8 h.

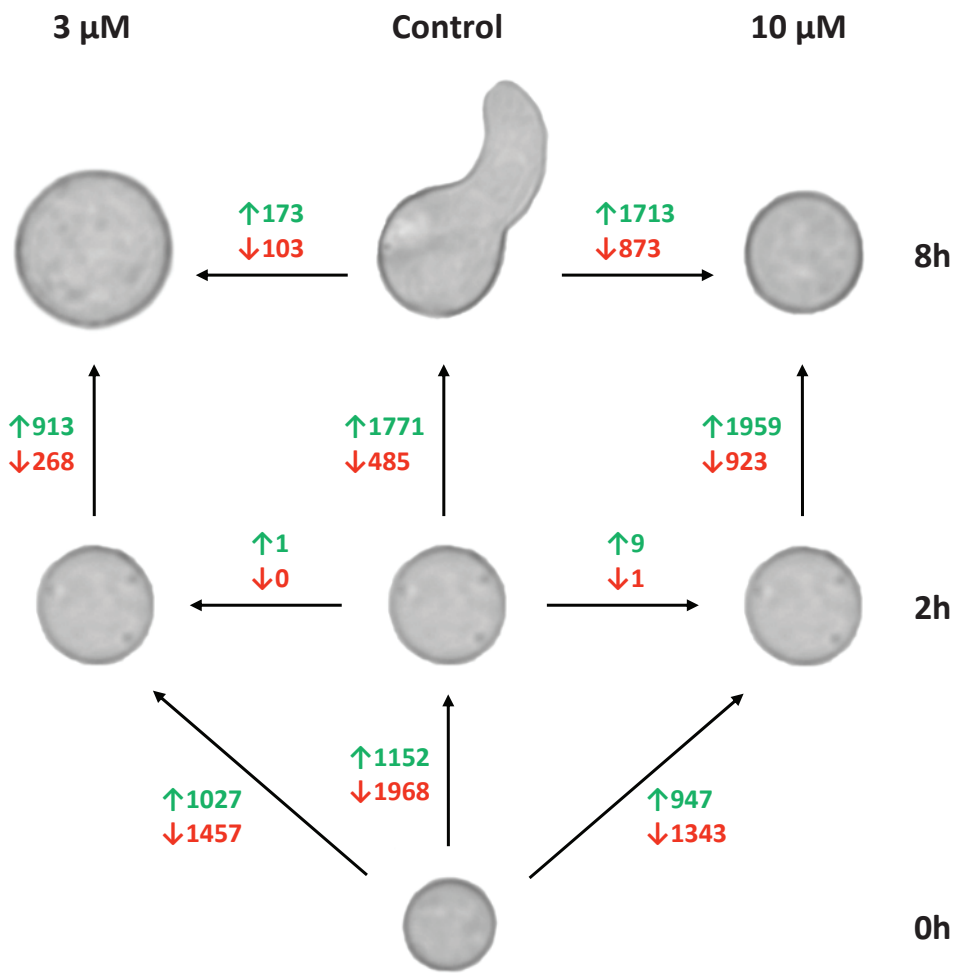

Fig. 7. Overview of the global changes in the transcriptome of conidia that had either or not been treated with 3 µM or 10 µM natamycin. The number of differentially expressed genes is indicated.

van Leeuwen *et al.* 2009) and vacuole fusion (te Welscher *et al.* 2010). Earlier work (van Leeuwen *et al.* 2009, te Welscher *et al.* 2010) has provided evidence that natamycin interferes with vesicle trafficking and fusion events in fungal cells. Twenty six out of 77 genes involved in vesicle recognition and fusion, endocytosis and vesicle secretion were ≥ 2-fold up-regulated in conidia that had been incubated for 8 h in 10 µM natamycin (Table 5). For instance, a gene (An12g07570) similar to synaptobrevin SNC2, a protein involved in vesicle recognition, is over 3.7-fold higher expressed in natamycin. Up-regulation was also observed for genes encoding a FTI1-like protein (An01g00170) and the endosomal protein SNF7 (An18g05430 and An04g05670, Weiß *et al.* 2008) that showed 2.1-, 4-, and 3.7-fold higher transcript levels. The gene encoding a VPS33-like protein (An02g05380, Subramanian *et al.* 2004) that is active during both endosome and vacuole fusion, is 6.6-fold higher expressed in the presence of the anti-fungal compound. Genes involved in vesicle secretion and endocytosis were also up-regulated in natamycin exposed conidia including members of the actin-organising arp2/3 complex that is involved in vesicle uptake. Moreover, the Sec15p homologue An15g00010 that is predicted to be involved in exocytosis (Köhli *et al.* 2008) was upregulated 4.0-fold.

Membrane rafts (Martin & Konopka 2004, Malinska *et al.* 2004) are small stabilised domains of the plasma membrane that contain ergosterol and capture specific landmark or transport proteins like Pma1. The *A. niger* gene An09g05950 has similarity to this

Table 1. Over- (E) and under- (S) representation of functional gene classes in the pool of genes that were up- and down-regulated in conidia of *A. niger* that had been incubated for 2 h in medium with or without 3 or 10 µM natamycin.

	0h vs 2h		3µM [0h vs 2h]		10µM [0h vs 2h]	
	UP	DOWN	UP	DOWN	UP	DOWN
01 METABOLISM		E	S		S	
01.01.10 amino acid degradation (catabolism)	S		S			
01.02.01 nitrogen and sulfur utilisation	S		S			
01.03 nucleotide metabolism	E					
01.03.01 purine nucleotide metabolism	E		E		E	
01.03.04 pyrimidine nucleotide metabolism	E		E			
01.05.01 C-compound and carbohydrate utilisation	S		S		S	E
01.05.07 C-compound, carbohydrate transport	S		S		S	
01.06.04 breakdown of lipids, fatty acids and isoprenoids			S		S	E
01.20.05 biosynthesis of acetic acid derivatives	S	S	S		S	
01.20.35 biosynthesis of secondary products derived from L-phe and L-tyr	S					
01.20.37 biosynthesis of peptide derived compounds	S					
02 ENERGY	E		E		E	
02.11.05 accessory proteins of electron transport and energy conservation	E		E		E	
02.13 respiration	E		E			
02.13.03 aerobic respiration	E		E		E	
03 CELL CYCLE AND DNA PROCESSING	E		E		E	
04 TRANSCRIPTION	E		E	S	E	S
04.01.01 rRNA synthesis	E		E		E	
04.01.04 rRNA processing	E		E		E	
04.03.03 tRNA processing			E		E	
04.03.06 tRNA modification			E		E	
04.05.05 mRNA processing (splicing, 5'-, 3'-end processing)		S	E		E	
04.05.01 mRNA synthesis	S		S		S	
05 PROTEIN SYNTHESIS	E	S	E	S	E	S
05.04 translation	E		E		E	
05.04.01 initiation	E		E		E	
06 PROTEIN FATE (folding, modification, destination)	E	E	E	E	E	
06.07.05 modification by ubiquitination, deubiquitination	S					
06.13.01 cytoplasmic and nuclear degradation			S		S	
08 CELLULAR TRANSPORT AND TRANSPORT MECHANISMS	E		E			
11 CELL RESCUE, DEFENSE AND VIRULENCE	S		S		S	
29 TRANSPOSABLE ELEMENTS, VIRAL AND PLASMID PROTEINS	S		S			
40 SUBCELLULAR LOCALISATION	E	S	E		E	
67 TRANSPORT FACILITATION	S		S			
99 UNCLASSIFIED PROTEINS	S	S	S		S	

protein and is 10-fold upregulated in the presence of natamycin. The gene encoding a Sur7-like protein (An07g6530) is 27 times up-regulated. Sur7 can be found in membrane rafts, but also in eisosomes. Eisosomes are large protein complexes underlying the plasma membrane that co-localise with sites of endocytosis (Walther *et al.* 2006, Fröhlich *et al.* 2009, Loibl *et al.* 2010). A central protein in this complex is PilA, which is also observed in the plasma membrane of *A. nidulans* conidia (Vangelatos *et al.* 2010). Dormant conidia of *A. niger* contain transcripts of genes with similarity to PilA (*i.e.* An11g0175 and An07g08890). In the presence of natamycin these genes are 8.9-fold and 5.4-fold up-regulated, respectively. Walther *et al.* (2006) reported a network of interactions of eisosome components with known endocytic effectors. Five genes of *A. niger* with similarity to proteins of this network, *i.e.* RVS 161 (An17g01970), RVS 167 (An09g04300), Sla2 (An11g10320), Pan1 (An13g00290) and ABP1 (An03g06960) were 2.1- to 3.7-fold up-regulated in the presence of natamycin.

Table 2. Over- (E) and under- (S) representation of functional gene classes in the pool of genes that were up- and down-regulated in conidia of *A. niger* that had been incubated for 2 or 8 h in medium with or without 3 or 10 µM natamycin.

	2h vs 8h		3µM [2h vs 8h]		10µM [2h vs 8h]	
	UP	DOWN	UP	DOWN	UP	DOWN
01 METABOLISM	E			S	E	
01.01.01 amino acid biosynthesis						E
01.01.10 amino acid degradation (catabolism)						S
01.03.16 polynucleotide degradation		E				
01.05.01 C-compound and carbohydrate utilisation		S		S	E	S
01.05.07 C-compound, carbohydrate transport	S					S
01.06.04 breakdown of lipids, fatty acids and isoprenoids					E	S
01.20.05 biosynthesis of acetic acid derivatives	S				S	S
02 ENERGY	E					E
02.11.05 accessory proteins of electron transport and energy conservation						E
03 CELL CYCLE AND DNA PROCESSING	E		E		E	
03.01.03 DNA synthesis and replication	E		E		E	
03.03.01 mitotic cell cycle and cell cycle control	E					
04 TRANSCRIPTION	S	E	S	E	S	E
04.01.01 rRNA synthesis		E		E		E
04.01.04 rRNA processing	S	E		E	E	E
04.03.03 tRNA processing						E
04.03.06 tRNA modification		E		E		E
04.05.01 mRNA synthesis	S		S			S
04.05.05 mRNA processing (splicing, 5'-, 3'-end processing)	S					E
05 PROTEIN SYNTHESIS	S	E	S		S	E
05.04.01 initiation				E		E
05.04.02 elongation						E
05.99 other protein-synthesis activities						E
06 PROTEIN FATE (folding, modification, destination)	E	S	E		E	E
06.07.03 modification by phosphorylation, dephosphorylation					E	S
06.07.99 other protein modifications					E	
06.13.01 cytoplasmic and nuclear degradation	E		E			
06.13.04 lysosomal and vacuolar degradation			E			
08 CELLULAR TRANSPORT AND TRANSPORT MECHANISMS	E		E			
11 CELL RESCUE, DEFENSE AND VIRULENCE	S					S
13 REGULATION OF / INTERACTION WITH CELLULAR ENVIRONMENT	E				E	
14 CELL FATE	E					
29 TRANSPOSABLE ELEMENTS, VIRAL AND PLASMID PROTEINS	S					
30 CONTROL OF CELLULAR ORGANIZATION	E					
40 SUBCELLULAR LOCALISATION	E					E
99 UNCLASSIFIED PROTEINS	S	S	S	S	S	S

Compatible solutes

Compatible solutes accumulate in conidia to protect proteins and membranes during drought and other stressors. Most of the trehalose-synthesising and degrading enzymes are expressed in natamycin-treated conidia. Gene An07g08720, which has strong similarity to trehalose-phosphate synthase and the acid trehalase encoding gene (An01g01540) were 4.7- and 3-fold up-regulated respectively, in the treated conidia (Table 6). Mannitol-synthesising and degrading enzymes (see also Ruijter *et al.* 2003, Aguilar-Osorio *et al.* 2010) were also up-regulated in the presence of the anti-fungal (5.4-fold for *mpdA*, An02g05830 and 4.8-fold for *mtdA*, An15g05450). Moreover, a gene with similarity to a mannitol transporter (An02g06710) was 42 times up-regulated in the presence of natamycin.

Table 3. Over- (E) and under- (S) representation of functional gene classes in the pool of genes that were up- and down-regulated in conidia of *A. niger* that had been incubated for 8 h in medium with or without 3 or 10 µM natamycin.

	8h [0 vs 3µM]		8h [0 vs 10µM]	
	UP	DOWN	UP	DOWN
01 METABOLISM		E	E	E
01.01.01 amino acid biosynthesis			E	E
01.05.01 C-compound and carbohydrate utilisation				S
01.05.07 C-compound, carbohydrate transport				S
01.20.05 biosynthesis of acetic acid derivatives			S	S
02 ENERGY			E	E
02.11.05 accessory proteins of electron transport and energy conservation				E
03 CELL CYCLE AND DNA PROCESSING				E
04 TRANSCRIPTION			E	E
04.01.01 rRNA synthesis				E
04.01.04 rRNA processing			E	E
04.05.01 mRNA synthesis				S
04.05.05 mRNA processing (splicing, 5'-, 3'-end processing)			S	
05 PROTEIN SYNTHESIS			E	E
05.04.01 initiation			E	E
05.99 other protein synthesis activities				E
06 PROTEIN FATE (folding, modification, destination)			E	
08 CELLULAR TRANSPORT AND TRANSPORT MECHANISMS		E		
11 CELL RESCUE, DEFENSE AND VIRULENCE		E		
13 REGULATION OF / INTERACTION WITH CELLULAR ENVIRONMENT		E	E	
29 TRANSPOSABLE ELEMENTS, VIRAL AND PLASMID PROTEINS			S	
40 SUBCELLULAR LOCALISATION				E
99 UNCLASSIFIED PROTEINS		S	S	S

Glyoxylate cycle

Several genes that encode proteins predicted to be involved in fermentation, gluconeogenesis and glyoxylate cycle show strong up-regulation in the presence of natamycin (Table 7). This included an alcohol dehydrogenase (An13g00950, 39-fold), D-lactate dehydrogenase (An11g09520, 12.4-fold); pyruvate decarboxylate (An 09g01030, 11.3-fold); isocitrate lyase (An01g09270, 81-fold) and a malate synthase gene (An15g01860, 52-fold). A gene with similarity to 2-methylisocitrate lyase (An12g07630) that could have a role in fatty acid oxidation (Upton & McKinney 2007) was 2.6-fold upregulated.

Heat shock proteins

The expression of a number of genes involved in cell protection are shown in Table 8. Some of the genes show strong up-regulation in the presence of natamycin. For instance, a gene with similarity to the protective LEA proteins (An02g07350, Browne *et al.* 2002, Chakrabortee *et al.* 2007) was 16.1-fold up-regulated. Similarly, genes encoding dehydrin-like proteins (An13g01110 and An14g05070, Wong Sak Hoi *et al.* 2011) and a small heat shock protein (hsp9p; An06p01610) were 22-, 101- and 14.6-fold up-regulated, respectively. Other up-regulated genes included putative catalases (An08g08920 and An01g01830, 14.4- and 19.5-fold), a gene predicted to be involved in glutathione synthesis (An09g06270, 7.2-fold) and a gene similar to a glutathione transferase (An16g06100, 47-fold). A number of genes predicted to encode chaperonins were significantly down-regulated. For example, An16g09260 predicted to encode a Dnak-type chaperonine was 8-fold down-regulated. The other down-regulated genes are similar to *hsp10, hsp60*, *hsp70* and *hsp78*.

DISCUSSION

In this study the impact of natamycin on germination of conidia of *A niger* was analysed. In the absence of natamycin, conidia swell, initiate polarised growth and undergo one round of mitosis when they are incubated in medium for an 8 h period. Conidia were unable to initiate polarised growth in the presence of 3 µM natamycin, whereas 10 µM natamycin even blocked isotropic swelling. In addition, mitosis did not occur at both concentrations of the anti-fungal. Earlier studies have shown that conidia of *Penicillium* and *Aspergillus* are not killed by natamycin. They survived a period of 20 h in 45 µM natamycin (van Leeuwen *et al.* 2010) and initiated germination upon removal of the compound. A similar response is observed in conidia of *Penicillium paneum* that are exposed to the self-inhibitor 1-octen-3-ol (Chitarra *et al.* 2004). This component prevents germination of conidia at high densities, the so-called crowding phenomenon. It was shown that 1-octen-3-ol has a clear effect on the proteome of conidia (Chitarra *et al.* 2004, 2005).

Table 4. Transcript levels of genes involved in synthesis of ergosterol and desaturated fatty acids in dormant conidia and conidia that were incubated for 8 h in medium in the absence or presence of natamycin. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 2500 expression units. The value of gene expression is significantly differentially expressed (≥ 2-fold) compared to the 8 h old germling if the outline of the box is dashed. SS = strong similarity; S = similarity. Calb = *Candida albicans*; Gfuj = *Gibberella Fujikuroi*; Ncra = *Neurospora crassa*; Pita = *Penicillium albican*s; Scer = *Saccharomyces cerevisiae*; Spom = *Schizosaccharomyces pombe*.

Name	Description	Dormant	8h	8h-3µM	8h-10µM
ergosterol					
An16g09190	SS to cytosolic acetyl-CoA C-acetyltransferase Erg10 - Scer	325	2615	1304	871
An04g00610	SS to the hmg-CoA reductase Hmg1 - Spom[truncated ORF]	12	92	799	923
An07g08280	SS to hmg-CoA reductase HmgR - Gfuj	109	416	299	260
An02g06320	SS to hydroxymethylglutaryl-CoA synthase HmgS - Scer	124	942	305	70
An04g02190	SS to mevalonate kinase Erg12 - Scer	81	68	52	41
An14g04010	SS to phosphomevalonate kinase Erg8 - Scer	63	204	169	147
An04g01540	SS to diphosphomevalonate decarboxylase Erg19 - Scer	12	197	117	81
An08g07570	SS to isopentenyl-diphosphate Delta-isomerase Idi1 - Scer	102	438	407	365
An02g10350	SS to farnesyl-pyrophosphate synthetase Erg20 - Gfuj	27	699	830	961
An12g01890	SS to squalene synthase Erg9 - Candida utilis	549	544	264	158
An01g03350	SS to C-8 sterol isomerase Erg1 - Ncra	15	642	168	64
An03g03770	SS to squalene monooxygenase Erg1 - Rattus norvegicus	50	1072	95	52
An13g00090	SS to eburicol 14 α-demethylase cyp51 Erg11 - Uncinula necator	376	1273	895	671
An11g02230	SS to lanosterol 14 α-demethylase Cyp51 Erg11 - Pita	46	1780	468	242
An01g07000	SS to C-14 sterol reductase Erg24 - Scer	12	1341	2809	2442
An03g06410	SS to methyl sterol oxidase Erg25 - Scer	29	2378	259	60
An15g03090	SS to C-3 sterol dehydrogenase/C-4 decarboxylase Erg26 - Calb	169	453	368	347
An02g03580	SS to lipid metabolism protein YER044c patent WO200058521-A2 (Erg28) - Scer	14	284	152	102
An02g05150	SS to C-8,7 sterol isomerase (Erg2) - Arabidopsis thaliana	31	93	105	105
An16g02930	SS to C-5 sterol desaturase Erg3 - Scer	333	1680	261	64
An15g00150	SS to C-5 sterol desaturase Erg3 - Scer	65	394	110	61
An18g03480	SS to the sterol delta14,15-reductase Erg3 - Ncra	12	116	76	42
An01g02810	SS to the cytochrome P-450 sterol delta22-desaturase Erg5 - Scer	81	1330	392	112
An07g09690	SS to sterol C-24(28) reductase STS1 Erg4 - Spom	14	303	116	65
desaturases					
An04g01320	SS fatty acid desaturase from patent WO9846764-A1 - Homo sapiens	143	125	72	63
An12g09940	SS to stearoyl-CoA desaturase Ole1 - Ajellomyces capsulata	19	195	12	12
An07g01960	SS to stearoyl-CoA desaturase P-Ole1 - Pichia angusta	2441	2486	642	334
An08g05160	SS to oleate delta-12 desaturase OdeA - Aspergillus nidulans	139	1464	367	57
An14g06980	SS to delta-12 fatty acid desaturase - Mortierella alpina	56	705	554	84

Natamycin did not affect germination of conidia during the first 2 h of the process. Degradation of compatible solutes, the decrease in viscosity and swelling were similar to control conidia. Moreover, natamycin hardly affected the transcriptome during the first 2 h of incubation. The functional gene classes energy, protein synthesis and transcription were overrepresented in the up-regulated genes irrespective of the presence of the polyene antibiotic. It has been shown that ergosterol cannot be observed in the plasma membrane of *P. discolor* (van Leeuwen *et al.* 2008) during early stages of germination. Absence of ergosterol would explain why we could not find an effect of natamycin during the first stages of germination of *A. niger* conidia.

Natamycin did affect the transcriptome of conidia after an 8 h exposure. This was most notable at 10 µM natamycin of the anti-fungal. Several genes involved in biosynthesis of ergosterol were down-regulated upon exposure to 10 µM natamycin. In fungi, sterols are asymmetrically distributed and can be found in membranes at sites of cytokinesis and polarised growth (Wachtler 2003, Martin & Konopka 2004). The decrease in expression of ergosterol biosynthesis genes after polyene treatment is also observed in the case of *Saccharomyces cerevisiea* (Zhang *et al.* 2002) and *Candida albicans* (Liu *et al.* 2005). This suggests that natamycin and other polyene antibiotic not only exert their effect by binding to ergosterol but also by reducing the concentration of the sterol in the cell. These effects would impact the formation of an ergosterol cap at the site of polarised growth, as observed in the fungal species *P. discolor, A. niger, Fusarium oxysporum* and *Verticillium fungicola* (van Leeuwen *et al.* 2008, 2010). This would explain why formation of germ tubes is abolished upon natamycin exposure.

Recently, it has been shown that natamycin also blocks growth of yeast and fungi via inhibition of amino acid and glucose transport

Table 5. Transcript levels of genes involved in trafficking, fission and fusion of vesicles in dormant conidia and conidia that had been incubated for 8 h in medium in the absence or presence of natamycin. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 2200 expression units. The value of gene expression is differentially expressed (≥ 2-fold) compared to the 8 h old germling if the outline of the box is dashed. SS = strong similarity; S = similarity; Hsap = *Homo sapiens*.

Name	Description	Dormant	8h	8h-3µM	8h-10µM
An02g05390	SS to t-SNARE Sec9p - Scer	246	175	240	226
An12g07570	SS to synaptobrevin Snc2 - Scer	1083	340	1268	1265
An02g05380	SS to vacuolar protein-sorting protein Vps33 - Scer	102	61	221	405
An04g05670	S to vacuolar sorting protein Snf7 - Scer	348	128	387	480
An01g00170	S to Fti1 protein - Scer	531	230	478	479
An18g05430	SS to endosomal protein Snf7 - Scer	355	115	343	458
An07g08290	S to actin cytoskeleton organiser Spa2 - Scer	38	89	203	215
An02g06360	S to Arp2/3 complex 16kD subunit Arc16 - Hsap	65	110	308	355
An16g01570	SS to Arp2/3 complex 21kDa subunit Arc21 - Hsap	52	173	381	368
An15g00010	SS to exocyst complex vesicular traffic control protein Sec15p - Scer [truncated ORF]	320	90	233	364
An17g01970	SS to Rvs161 - Scer	170	253	754	905
An09g04300	SS to protein Rvs167 - Scer	279	259	767	970
An11g10320	SS to cytoskeleton assembly control protein homolog Sla2 - Scer	97	256	480	661
An13g00290	SS to poly(A)-specific ribonuclease Pan1 - Scer	42	98	185	213
An03g06960	SS to actin-binding protein Abp1 - Scer	329	771	1447	1654
An07g06530	SS to multicopy suppressor Sur7 - Scer	378	79	983	2177
An09g05950	SS to plasma membrane ATPase Pma1 - Kluyveromyces lactis	1073	64	343	659
An11g01750	S to hypothetical protein YGR086c - Scer	2205	12	67	106
An07g08890	SS to hypothetical protein YGR086c - Scer	1370	409	1179	2218

Table 6. Expression of genes involved in the synthesis of trehalose and mannitol in dormant conidia and conidia that had been incubated for 8 h in medium in the absence or presence of natamycin. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 4100 expression units. The value of gene expression is differentially expressed (≥ 2-fold) compared to the 8 h old germling if the outline of the box is dashed. SS = strong similarity. Anid = *Aspergillus nidulans*; Anig = *Aspergillus niger*; Smut = *Streptococcus mutans*.

Name	Description	Dormant	8h	8h-3µM	8h-10µM
An08g10510	trehalose-6-phosphate synthase subunit 1 TpsA - Anig	871	270	278	376
An14g02180	SS to trehalose-6-phosphate synthase TpsB - Anig	456	134	168	196
An07g08710	α, α-trehalose-phosphate synthase 2 tpsB - Anig	121	99	128	176
An02g07770	trehalose-6-phosphate synthase subunit 1 TpsA - Anig	139	494	307	573
An13g00400	SS to reg. sub. treh-6-P synthase/phosphatase complex Tps3 - Scer	392	45	60	65
An07g08720	SS to 123K chain α,α-trehalose-phosphate synthase Tsl1 - Scer	286	27	71	125
An11g10990	SS to TPP of patent WO200116357-A2 - Scer	96	198	205	230
An01g09290	SS to neutral trehalase (TreB) - Anid	1203	178	193	255
An01g01540	SS to α,α-trehalase TreA - Anid	22	329	675	1000
An02g05830	SS to mannitol-1-phosphate 5-dehydrogenase MtlD - Smut	140	153	536	822
An15g05450	SS to NADPH-dependent carbonyl reductase S1 - Candida magnoliae	425	862	2129	4145
An03g02430	SS to mannitol dehydrogenase MtlD - Pseudomonas fluorescens	645	200	183	109
An02g07610	SS to mannitol transporter Mat1 - Apium graveolens	467	12	278	498

across the plasma membrane (te Welscher *et al.* 2012). In agreement, an up-regulation of transport proteins is observed when conidia are exposed to natamycin. This may be a strategy to try to counteract this effect of natamycin. Some of the most extremely up-regulated genes are An06g02270 (similar to an arabinose transport protein, 168-fold); An03g02190 (similarity to the sugar transporter Sut 1, 136-fold) and An13g00840 (similarity to amino acid protein GAP1, 132-fold).

Genes encoding proteins related to eisosomes were also over-expressed in natamycin-exposed conidia. Eisosomes are structures that are present in *Aspergillus* conidia (Vangelatos *et al.* 2010) and that are associated with endocytosis and

Table 7. Expression of genes involved in glycolysis, fermentation and gluconeogenesis in dormant conidia and conidia that had been incubated for 8 h in medium in the absence or presence of natamycin. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 1700 expression units. The value of gene expression is differentially expressed (≥ 2-fold) compared to the 8 h old germling if the outline of the box is dashed. SS = strong similarity. Klac = *Kluyveromyces lactus*.

Name	Description	Dormant	8h	8h-3µM	8h-10µM
An01g09270	SS to isocitrate lyase AcuD - Anid	2774	21	812	1723
An15g01860	SS to malate synthase AcuE - Anid	765	17	668	866
An09g01030	SS to pyruvate decarboxylase DcpY - Aspergillus parasiticus	91	74	573	835
An11g09520	SS to D-lactate dehydrogenase KlDld - Klac	27	29	154	358
An12g07630	SS to 2-methylisocitrate lyase Icl2 - Scer	101	259	469	680
An13g00950	SS to alcohol dehydrogenase B AlcB -b - Anid	18	12	194	471

Table 8. Transcript levels of genes involved in cell protection in dormant conidia and conidia that had been incubated for 8 h in medium in the absence or presence of natamycin. The normalised average values of three independent experiments are given. White to black shading indicate expression levels from absent (12 units of expression) to > 6700 expression units. The value of gene expression is differentially expressed (≥ 2-fold) compared to the 8 h old germling if the outline of the box is dashed. SS = strong similarity; S = similarity; WS = weak similarity. Ncra = *Neurospora crassa*; Zmay = *Zea mays*.

Name	Description	Dormant	8h	8h-3µM	8h-10µM
An02g07350	WS to group 3 Lea protein Mgl3 - Zmay	4559	74	663	1196
An13g01110	S to hypothetical protein An14g05070 (dehydrin) - Anig	550	18	256	398
An14g05070	WS to heterokaryon incompatibility protein Het-C (dehydrin) - Ncra	785	13	808	1313
An06g01610	SS to the heat shock protein Hsp9p - Spom	4577	460	3264	6715
An07g09990	SS to heat shock protein 70 Hsp70 - Ajellomyces capsulata	4139	1895	912	386
An11g00550	SS to chaperonin Hsp10 - Scer	303	685	364	130
An08g05300	SS to heat shock protein Hsp70 pss1+ - Spom	251	558	278	142
An12g04940	SS to mitochondrial heat shock protein Hsp60 - Scer	522	1036	449	167
An16g09260	SS to dnaK-type molecular chaperone Ssb2 - yeast Scer	3585	6103	2266	765
An08g03480	SS to the mitochondrial heat shock protein Hsp78p - Scer	618	361	163	146
An08g08920	SS to catalase C CatC - Anid	78	29	180	412
An01g01830	SS to catalase/peroxidase CpeB - Streptomyces reticuli	555	61	496	1194
An09g06270	SS put.glutath.-depend. formald. dehydrogen. SPBC1198.01 - Spom	6489	323	1248	2320
An16g06100	S to glutathione S-transferase Gst1 - Ascaris suum	64	12	221	564

putative membrane rafts. Up-regulation of the genes related to eisosomes may be a way of the conidium to counteract the inhibition of endocytosis by natamycin (van Leeuwen *et al.* 2009). Genes involved in the biosynthesis of protecting compounds and genes encoding protective proteins were also up-regulated in natamycin exposed conidia. For instance, one gene involved in trehalose biosynthesis and two genes in mannitol biosynthesis and degradation were up-regulated in conidia that had been treated for 8 h with 10 µM natamycin. Trehalose levels did not increase in natamycin-treated spores when compared to the control. This indicates that the compatible solute is used for energy generation (D'Enfert & Fontaine 1997) as a result of the activity of the acid trehalase, which showed up-regulation in 8 h-treated cells. In contrast, the level of mannitol inside treated cells had increased after 8 h, which correlates to a more marked upregulation of genes involved in mannitol metabolism compared to trehalose biosynthesis. Furthermore, genes encoding LEA-like proteins, dehydrins (Wong Sak Hoi *et al.* 2011), Hsp9 (Sales *et al.* 2000), catalase and a glutathion synthesising enzyme were up-regulated. These data indicate that a stress response is activated in natamycin-exposed conidia. This may also explain the up-regulation of genes of the glyoxylate cycle in natamycin-treated cells. The glyoxylate cycle is an important shunt of the citric acid cycle. It is involved in fatty acid and acetate metabolism and has a role in gluconeogenesis (Eastmond & Graham 2001). Conidia of *Aspergillus fumigatus* that are stressed due to exposure to neutrophils also show up-regulation of catalase, glutathione and glyoxylate cycle enzymes (Sugui *et al.* 2008).

All considering, this study shows that natamycin does not have an impact on conidia during the first stages of germination. However, longer exposure to natamycin shifts the transcriptome to a state of survival with some similarities to the dormant conidium. The conidia respond to the presence of the anti-fungal compound by activating genes that are involved in stress resistance.

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