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

M.R. van Leeuwen1, P. Krijgsfeld2, T.T. Wyatt1, E.A. Golovina3,4, H. Menke3, A. Dekker5, J. Stark3, H. Stam6, R. Bleichrodt6, H.A.B. Wösten2, and J. Dijksterhuis7

1Applied and Industrial Mycology, CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands; 2Microbiology and Kluver Centre for Genomics of Industrial Fermentation, Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands; 3Laboratory of Biophysics, Wageningen University, and Wageningen NMR Centre, Dreijenlaan 3, 6703 HA, Wageningen, The Netherlands; 4Laboratory of Plant Physiology, Wageningen University, Arboretumlaan 4, 6703 BD, Wageningen, The Netherlands; 5DSM Food Specialties, PO Box 1, 2600 MA, Delft, The Netherlands

Correspondence: Jan Dijksterhuis, j.dijksterhuis@CBS.knaw.nl

Abstract: The impact of natamycin on Aspergillus niger was analysed during the first 8 h of germination of conidia. Polariation, 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: antiobiotics, Aspergillus niger, conidia, germination, natamycin, transcriptome.

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 amphoterocin 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 mppA 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_4·7H_2 O, 1.0 g MnCl_2·4H_2 O, 0.32 g CoCl_2·6H_2 O, 0.32 g CuSO_4·5H_2 O, 0.22 g (NH_4)_2MoO_4·4H_2 O, 1.5 g CaCl_2·2H_2 O, and 1.0 g FeSO_4·7H_2 O). 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 4 °C for 30 min at 20.817 g. The supernatant was centrifuged at 4 °C for 30 min at 20.817 g. The supernatant was discarded and the conidia were resuspended in CM containing per liter: 10 g EDTA, 4.4 g ZnSO_4·7H_2 O, 1 % yeast extract and 200 µl trace elements (containing per liter: 10 g EDTA, 4.4 g ZnSO_4·7H_2 O, 1.0 g MnCl_2·4H_2 O, 0.32 g CoCl_2·6H_2 O, 0.32 g CuSO_4·5H_2 O, 0.22 g (NH_4)_2MoO_4·4H_2 O, 1.5 g CaCl_2·2H_2 O, and 1.0 g FeSO_4·7H_2 O). 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).

HPLC analysis

Dormant, germinating or treated conidia (5-10^7 - 1·10^8) were frozen at -80 °C 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^2+ 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 TEMPO-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 an 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.
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 (h₀), flanked by a low-field line (h₋₁), high-field line (h₊₁) and an m-line (hₘ). 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
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. cerevisiae (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 (An01g33530), erg3 (An16g02930, An15g00150), erg5 (An01g02810), a gene similar to squalene monoxygenase (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,
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 similarly to this
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. RVS161 (An17g01970), RVS167 (An09g04300), Sla2 (An11g10320), Pan1 (An13g00290) and ABP1 (An03g06960) were 2.1- to 3.7-fold up-regulated in the presence of natamycin.

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.

| 01 METABOLISM | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|---------------|----------|----------|----------|
| 01.01.10 amino acid degradation (catabolism) | E | S | S |
| 01.02.01 nitrogen and sulfur utilisation | S | S | S |
| 01.03 nucleotide metabolism | E | S | S |
| 01.03.01 purine nucleotide metabolism | E | E | E |
| 01.03.04 pyrimidine nucleotide metabolism | E | S | S |
| 01.05.01 C-compound and carbohydrate utilisation | S | S | S |
| 01.05.07 C-compound, carbohydrate transport | S | S | S |
| 01.06.04 breakdown of lipids, fatty acids and isoprenoids | S | E | E |
| 01.20.05 biosynthesis of acetic acid derivatives | S | S | S |
| 01.20.35 biosynthesis of secondary products derived from L-phe and L-tyr | S | S | S |
| 01.20.37 biosynthesis of peptide derived compounds | S | S | S |

| 02 ENERGY | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|------------|----------|----------|----------|
| 02.11.05 accessory proteins of electron transport and energy conservation | E | E | E |
| 02.13 respiration | E | E | E |
| 02.13.03 aerobic respiration | E | E | E |

| 03 CELL CYCLE AND DNA PROCESSING | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|----------------------------------|----------|----------|----------|

| 04 TRANSCRIPTION | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|------------------|----------|----------|----------|
| 04.01.01 rRNA synthesis | E | E | E |
| 04.01.04 rRNA processing | E | E | E |
| 04.03.03 RNA processing | E | E | E |
| 04.03.06 RNA modification | E | E | E |
| 04.05.05 mRNA processing (splicing, 5’-, 3’-end processing) | S | E | E |
| 04.05.01 mRNA synthesis | S | E | E |

| 05 PROTEIN SYNTHESIS | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|----------------------|----------|----------|----------|
| 05.04 translation | E | E | E |
| 05.04.01 initiation | E | E | E |

| 06 PROTEIN FATE (folding, modification, destination) | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|----------------------------------------------------|----------|----------|----------|
| 06.07.05 modification by ubiquitination, deubiquitination | S | E | E |
| 06.13.01 cytoplasmic and nuclear degradation | S | S | S |

| 08 CELLULAR TRANSPORT AND TRANSPORT MECHANISMS | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|-----------------------------------------------|----------|----------|----------|

| 11 CELL RESCUE, DEFENSE AND VIRULENCE | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|--------------------------------------|----------|----------|----------|
| 29 TRANPOSABLE ELEMENTS, VIRAL AND PLASMID PROTEINS | S | S | S |

| 40 SUBCELLULAR LOCALISATION | 0h vs 2h | 0h vs 2h | 0h vs 2h |
|-----------------------------|----------|----------|----------|
| 67 TRANSPORT FACILITATION | S | S | S |
| 99 UNCLASSIFIED PROTEINS | S | S | S |

78
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.
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 (An09g01030, 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) that could have a role in fatty acid oxidation (Upton & McKinney 2007) was 2.6-fold upregulated.

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 (An09g01030, 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.

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 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.

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

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.
Disruption of germination by natamycin

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 = similarly. Cab = Candida albicans; Gfu = Gibberella Fujikuroi; Ncra = Neurospora crassa; Pita = Penicillium albicans; 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 | 2815 | 1304 | 871 |
| An04g00610 | SS to the hmg-CoA reductase Hmg1 - Spom[truncated ORF] | 12 | 92 | 728 | 469 |
| An07g08280 | SS to hmg-CoA reductase Hmg-R - Gfu | 109 | 416 | 236 | 206 |
| 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 |
| An06g07570 | SS to isopentenyl-diphosphate Delta-isomerase Idi1 - Scer | 102 | 438 | 407 | 365 |
| An02g10350 | SS to farnesyl-pyrophosphate synthase Erg20 - Gfu | 27 | 699 | 830 | 161 |
| 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 monoxygenase Erg1 - Rattus norvegicus | 50 | 1072 | 52 | 52 |
| An13g00090 | SS to eburicol 14 α-demethylase cyp51 Erg11 - Uncinula necator | 376 | 1273 | 895 | 277 |
| An11g02230 | SS to lanosterol 14 α-demethylase Cyp51 Erg11 - Pita | 46 | 1780 | 468 | 292 |
| An01g07000 | SS to C-14 sterol reductase Erg24 - Scer | 12 | 1341 | 2809 | 2442 |
| An03g06410 | SS to methyl sterol oxidase Erg25 - Scer | 29 | 2378 | 259 | 69 |
| 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 |
| An15g01150 | SS to C-5 sterol desaturase Erg3 - Scer | 65 | 394 | 110 | |
| An18g03480 | SS to the sterol delta14,15-reductase Erg3 - Ncra | 12 | 116 | 76 | 42 |
| An01g02610 | 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 cerevisiae* (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    | 229     |
| 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   | 297    | 480     |
| An01g00170       | S to F1/1 protein - Scer                          | 531      | 230   | 478    | 475     |
| An16g05430       | SS to endosomal protein Snf7 - Scer               | 355      | 115   | 343    | 450     |
| An07g08290       | S to actin cytoskeleton organising 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   |        |         |
| An15g00010       | SS to exocyst complex vesicular traffic control protein Sec15p - Scer [truncated ORF] | 320 | 90 | 233     |
| An17g01970       | SS to Rvs161 - Scer                               | 170      | 253   | 754    | 905     |
| An09g04300       | SS to protein Rvs167 - Scer                        | 279      | 259   | 767    | 970     |
| An11g01320       | SS to cytoskeleton assembly control protein homolog Sla2 - Scer | 97       | 256   | 480    | 681     |
| An13g00290       | SS to poly(A)-specific ribonuclease Pan1 - Scer   | 42       | 98    | 185    | 211     |
| An03g06960       | SS to actin-binding protein Abp1 - Scer            | 329      | 771   | 1447   | 1654    |
| An07g06530       | SS to multicopy suppressor Sur7 - Scer             | 376      | 79    | 983    | 2177    |
| An09g05950       | SS to plasma membrane ATPase Pma1 - Klyuyveromyces 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   | 276    | 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    | 82      |
| An11g01990       | SS to TTP of patent WC0200116357-A2 - Scer        | 96       | 198   | 205    | 230     |
| An01g09290       | SS to neutral trehalase (Treh) - Anid              | 1203     | 178   | 193    | 255     |
| An01g01540       | SS to α,α-trehalase TrehA - Anid                   | 22       | 329   | 675    | 1000    |
| An02g05830       | SS to mannitol-1-phosphate 5-dehydrogenase MttM - Smut | 140 | 153 | 536    | 872     |
| An15g05450       | SS to NADPH-dependent carbonyl reductase S1 - Candida magnoliae | 425 | 862 | 2129   | 4145    |
| An03g02430       | SS to mannitol dehydrogenase MttD - Pseudomonas fluorescens | 645 | 200 | 183    | 109     |
| An02g07610       | SS to mannitol transporter Mat1 - Apium graveolens | 467 | 12  | 276    | 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 arabinoarabinose transport protein, 168-fold); An03g02190 (similarly 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...
upative 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 manitol inside treated cells had increased after 8 h, which correlates to a more marked upregulation of genes involved in manitol 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.

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  | 2774    | 21   | 812    | 1722   |
| An15g01860   | SS to malate synthase AcuE   | 785     | 17   | 668    | 866    |
| An09g10130   | SS to pyruvate decarboxylase DcpY | 91     | 74   | 573    | 835    |
| An11g09520   | SS to D-lactate dehydrogenase KIDd | 27     | 29   | 154    | 558    |
| An12g07630   | SS to 2-methylisocitrate lyase Icl2 | 101   | 259  | 469    |        |
| An13g00950   | SS to alcohol dehydrogenase B AlkB | 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    | 199    |
| An13g01110   | S to hypothetical protein An14g0570 (dehydrin) - Anig | 550   | 18   | 256    | 398    |
| An14g0570    | WS to heterokaryon incompatibility protein Het-C (dehydrin) - Ncra | 785  | 13   | 808    | 1813   |
| An06g0970    | SS to the heat shock protein Hsp9p - Spom | 4577  | 460  | 3264   | 6715   |
| An07g09990   | SS to heat shock protein 70 Hsp70 - Ajellomyces capsulata | 4139  | 1859 | 912    |        |
| An11g05550   | SS to chaperonin Hsp10 - Scer | 303  | 685  | 364    | 130    |
| An08g05300   | SS to heat shock protein Hsp70 ps1+ - Spom | 251  | 558  | 278    | 142    |
| An12g04940   | SS to mitochondrial heat shock protein Hsp60 - Scer | 522  | 1036 | 449    |        |
| An16g09260   | SS to dnaK-type molecular chaperone Ssb2 - yeast Scer | 3585  | 6103 | 2266   | 1255   |
| An08g03480   | SS to the mitochondrial heat shock protein Hsp78p - Scer | 616  | 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   | 5320   |
| An16g06100   | S to glutathione S-transferase Gst1 - Ascaris suum | 64   | 12   | 221    | 504    |
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