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Transcriptional profiling of *Scedosporium apiospermum* enzymatic antioxidant gene battery unravels the involvement of thioredoxin reductases against chemical and phagocytic cells oxidative stress

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Abstract

Scedosporium species rank the second, after *Aspergillus fumigatus*, among the filamentous fungi colonizing the airways of patients with cystic fibrosis (CF). Development of microorganisms in the respiratory tract depends on their capacity to evade killing by the host immune system, particularly through the oxidative response of macrophages and neutrophils, with the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS). This is particularly true in the airways of CF patients which display an exacerbated inflammatory reaction. To protect themselves, pathogens have developed various enzymatic antioxidant systems implicated in ROS degradation, including superoxide dismutases, catalases, cytochrome C peroxidases, chloroperoxidases and enzymes of the glutathione and thioredoxin systems, or in RNS degradation, that is, flavohemoglobins, nitrate reductases, and nitrite reductases. Here we investigated the transcriptional regulation of the enzymatic antioxidant gene battery in 24-h-old hyphae of *Scedosporium apiospermum* in response to oxidative stress induced chemically or by exposure to activated phagocytic cells. We showed that 21 out of the 33 genes potentially implicated in the oxidative or nitrosative stress response were overexpressed upon exposure of the fungus to various chemical oxidants, while they were only 13 in co-cultures with macrophages or neutrophils. Among them, genes encoding two thioredoxin reductases and to a lesser extent, a peroxiredoxin and one catalase were found to be overexpressed after chemical oxidative stress as well as in co-cultures. These results suggest that thioredoxin reductases, which are known to be virulence factors in other pathogenic fungi, play a key role in pathogenesis of scedosporiosis, and may be new drug targets.

Key words: *Scedosporium apiospermum*, ROS, RNS, oxidative stress, enzymatic antioxidant, thioredoxin reductase.

Introduction

Although saprophytes usually live in highly polluted soils and contaminated water, *Scedosporium* species are opportunistic pathogens that cause in receptive hosts various diseases ranging from localized infections such as subcutaneous mycetomas to severe and often fatal disseminated infections in

immunocompromised individuals.[1,2] About 10 species are recognized today in the *Scedosporium* genus,[2] but the prominent species remain the closely related *S. boydii* and *S. apiospermum* in Europe,[3,4] and *S. aurantiacum* in Australia.[5]

With a prevalence ranging from 2 to 17.4%, *Scedosporium* species rank the second, after *Aspergillus fumigatus*, among the filamentous fungi colonizing the respiratory tract of patients with cystic fibrosis (CF).[6–11] These fungi, now considered as prominent pathogens in the CF context, are usually responsible for a chronic colonization of the airways.[3,12] However, they may also cause true respiratory infections, similar to those due to *A. fumigatus* like bronchitis and allergic bronchopulmonary mycoses,[7,13] as well as severe and often fatal disseminated infections following lung transplantation.[14–18]

To colonize the respiratory tract, fungi must adhere to the host tissues and acquire the extracellular iron necessary for numerous essential physiological processes like respiration and ergosterol synthesis, but they also need to cope the host immune response. Macrophages and neutrophils are major effector cells of the innate immune system, releasing various antimicrobial compounds during the inflammatory reaction triggered by the pathogens, including reactive oxygen species (ROS) as superoxide anions and hydrogen peroxide, and reactive nitrogen species (RNS) as nitric oxide.[19–23] This is particularly true in the CF lung, which is characterized by an exacerbated inflammatory reaction.[24,25]

To prevent macromolecules degradation during oxidative and nitrosative stresses, pathogens have developed a variety of enzymatic antioxidants, including superoxide dismutases (SODs), catalases, cytochrome c peroxidases, chloroperoxidases, enzymes of the glutathione or thioredoxin systems, flavohemoglobins, nitrate and nitrite reductases.[23,26,27]

Prior to the sequencing of *S. apiospermum*,[28] few pathogenic factors have been identified in *Scedosporium* species. Studies in this field focused on proteolytic enzymes thought to be involved in the host tissue invasion[29–31] or in protection of the fungus against the host immune defenses. In *S. boydii*, a cytosolic Cu/Zn-SOD called SODC[32] and three distinct catalases have been identified, and one of them, that is, catalase A1 (CatA1), was purified and characterized.[33] Genes encoding SODC and CatA1 were sequenced, and it was demonstrated that *CatA1* gene was highly expressed in response to the oxidative stress.[34] Recently, a genome-wide analysis in *S. apiospermum* permitted to identify 31 other genes encoding proteins putatively implicated in ROS or RNS detoxification.[35] In order to get a better knowledge of the pathogenic mechanisms of *Scedosporium* species, we investigated here the transcriptional response of these genes to chemical oxidative stresses and in co-culture with activated phagocytic cells.

Methods

Fungal strain and growth conditions

Study was conducted on the reference strain *Scedosporium apiospermum* IHEM 14462 originally isolated from a sputum sample from a CF patient and previously used for whole genome sequencing.[28] The fungus was maintained by weekly passages on potatoe-dextrose-agar (PDA, containing in g/L: dextrose, 20; infusion from potatoes, 200; and bacteriological agar, 15; CONDALAB, Madrid, Spain) supplemented with chloramphenicol 0.5%, with incubation at 37°C. Conidia from 9-day-old cultures collected by scraping as previously described[34] were inoculated in yeast extract-peptone-dextrose (YPD; containing in g/L: yeast extract, 10; peptone, 20; glucose, 20; and chloramphenicol 0.5%, pH 7) broth in Petri dishes (30 mL per dish; 2×10^6 conidia per mL), which were incubated for 24 h at 37°C to obtain hyphae.

For some experiments, the fungus was also cultivated under environmental conditions encountered in the CF airways. In this aim, the fungus was cultivated in YPD broth supplemented with 90 mM NaCl or buffered at pH 5.3 by the addition of 0.5 M 2-(N-morpholino)ethanesulfonic acid with incubation under normal air conditions, or in YPD broth with incubation under 5% CO_2. After 24 h of incubation, hyphae were recovered by centrifugation (4000 *g*, 15 min) and stored at −80°C before total RNA extraction. All experiments were performed in triplicate and hyphae grown in YPD broth under normal air conditions were used as control.

Chemical induction of an oxidative or nitrosative stress

For these experiments, 24-h-old hyphae were incubated for 3 h at 37°C in the presence of various chemicals (from Sigma Aldrich, Saint-Quentin Fallavier, France) at a concentration ranging from 31.25 μM to 4 mM: (i) menadione (single addition at T0) or hydrogen peroxide (addition at T0 and then every hour), which induce a general stress; (ii) diamide or cumene hydroperoxide (single addition at T0), which generate an oxidative stress triggering specifically the glutathione and thioredoxin systems; and (iii) DETA NO (single addition at T0), which releases NO and thus generates a nitrosative stress. In sum, 24 h-old hyphae grown in YPD broth under normal air conditions and incubated for 3 h without any oxidant were used as control. After 3 h of incubation, hyphae were recovered and stored at −80°C before total RNA extraction.

Co-cultivation with phagocytic cells

Co-culture experiments were realized as previously described[34] with slight modifications. The human myelomonocytic leukemia

cell line THP-1 (ATCC) was cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium, and the human promyelocytic cell line HL60 (ATCC) in IMDM. Both culture media were supplemented by the addition of fetal calf serum (10%; PAA Laboratories, Pashing, Austria), L-glutamine (2 mM), sodium pyruvate (1 mM), nonessential amino acids (0.1 mM), HEPES (10 mM), and antibiotics (penicillin 100 U/ml and streptomycin 100 mg/mL, both from Lonza, Verviers, Belgium). Cell cultures were realized in T175 vented flasks at 37°C under 5% CO_2 with a density of 2×10^5 to 1×10^6 cells/ml, according to the recommendation of ATCC. Phorbol 12-myristate 13-acetate 50 ng/ml (Sigma Aldrich) was added to induce differentiation of THP-1 cells into macrophages (also referred as THP-1 macrophages). Dimethylsulfoxide (DMSO; Sigma Aldrich) 1.3% was used to induce differentiation of HL60 cells into neutrophils (also referred to as HL60 neutrophils). After differentiation, the cells were washed and incubated overnight in complete medium at a concentration of 1×10^5 cells per cm^2. Before co-cultures, the cells were activated by the addition of 2 mg/ml lipopolysaccharides from *Escherichia coli* O111:B4 (LPS; Sigma Aldrich). Co-cultures were performed at a ratio of one cell for three 24-h-old fungal hyphae in T175 flasks for 1 to 24 h of incubation for LPS-activated TPH-1 macrophages, and 15 to 100 min for LPS-activated HL60 neutrophils. Controls were realized with 24-h-old hyphae in RPMI medium with LPS but without phagocytic cells for each incubation time. The absence of changes in gene expression level in co-cultures with nonactivated phagocytic cells was also verified. After co-culture, hyphae were recovered and stored at −80°C. Biological triplicates were realized for each experiment.

RNA extraction and reverse transcription

For RNA extraction, hyphae were ground in liquid nitrogen, and extraction was performed on 100 mg of the homogenate with Nucleospin RNA plant kit (Macherey Nagel, Düren, Germany). Genomic DNA was then digested with DNase. Total RNA was quantified by fluorimetry on a qubit fluorimeter 2.0 (Invitrogen, Cergy Pontoise, France) and integrity was verified by 1% agarose gel electrophoresis.

Three micrograms of RNA were then reverse transcribed with the high capacity complementary DNA (cDNA) reverse transcription kit (Applied Biosystems, Waltham, MA, USA) according to the manufacturer's instructions. Reverse transcription was performed on thermal cycler Bioer Genepro, for 5 min at 25°C, followed by 2 h at 37°C and 5 min at 85°C. The cDNA were then 1/15th diluted in water and stored at −20°C before analysis.

Gene expression analysis

The expression of the 33 target genes was analyzed by real time (RT)- quantitative polymerase chain reaction (qPCR) on a Step One Plus thermalcycler (Applied Biosystems Foster City, CA, USA). RT-qPCR was performed in a 12.5-μl reaction volume containing 1 × Fast SYBR® Green Master Mix (Applied Biosystem), 0.4 μM of each primer (Supplementary Table 1), and 20 ng of cDNA. The PCR thermal profile consisted in an initial incubation at 95°C for 20 s, followed by 40 cycles of 30 s at 95°C, 30 s at 60°C, and 15 s of denaturation at 95°C with a final extension of 1 min at 60°C. Melting curves were obtained at the end of PCR, by increasing temperature from 60°C to 95°C at 0.3°C/s, to identify possible primer dimerization or off-target amplification. For each gene, RT-qPCR was done in duplicate for each of the three biological replicates. Genes encoding actin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used for normalization. Relative quantification (RQ) of gene expression was determined from the threshold cycle (Ct) values, according to the following formula[36] (Livak and Schmittgen 2001):

$$\Delta Ct_{experiment} = Ct_{target} - Ct_{reference}, \text{ corresponding to experimental conditions}$$

$$\Delta Ct_{control} = Ct_{target} - Ct_{reference}, \text{ corresponding to control conditions}$$

$$RQ = 2^{-(\Delta Ctexperiment - \Delta Ctcontrol)}$$

Statistical analysis

RT-qPCR data were compared using the Two-ANOVA test, and results were considered significant when $P < .05$.

Results

Cultivation in CF conditions has little effect on the expression of target genes

Gene expression was evaluated in three CF conditions (90 mM NaCl, acid pH, or 5% CO_2). Only a few genes were overexpressed in these conditions. Two genes encoding a thioredoxin and a flavohemoglobin (*SAPIO_CDS8608* and *SAPIO_CDS1359*, respectively) were overexpressed in the presence of 90 mM NaCl, with 3.2- and 3.1-fold changes in expression levels, respectively. Five genes were slightly overexpressed at pH 5.3, encoding respectively a catalase peroxidase, a peroxiredoxin, a thioredoxin, a thioredoxin reductase, and a nitrite reductase (*SAPIO_CDS10583, SaPrx2, SAPIO_CDS8608, SAPIO_CDS10274,* and *SAPIO_CDS10292*, respectively) with 2.8-, 4.7-, 4.2-, 6.8- and 4.3-fold changes in expression levels, respectively. Likewise, five genes were overexpressed under 5% CO_2, three of them being overexpressed also at pH 5.3 (*SAPIO_CDS10583*, RQ = 3.4; *SAPIO_CDS8608*,

RQ = 2.9; *SAPIO_CDS10292*, RQ = 5.5). The last two genes encoding a Cu/Zn-SOD (*SAPIO_CDS7433*) and a Mn-SOD (*SAPIO_CDS3426*) were overexpressed only under 5% CO_2 with a 5.1- and 5.0-fold increase in the expression level, respectively. A single gene was overexpressed in these three conditions, *SAPIO_CDS8608*, encoding thioredoxin (Supplemental Fig. 1).

Chemical oxidants strongly influence the expression of some enzymatic antioxidant genes

Gene expression was evaluated in hyphae stressed by menadione, a superoxide anion inductor, at concentrations ranging from 0.03125 to 2 mM to induce a global oxidative stress. And 13 out of the 33 genes studied were overexpressed (Fig. 1, Table 1). *SAPIO_CDS1830* encoding a thioredoxin reductase was the most overexpressed, followed by *SAPIO_CDS0416, SAPIO_CDS6039, SAPIO_CDS4327,* and *SAPIO_CDS8864* encoding a glutaredoxin, a glutathione reductase, a Cu/Zn-SOD, and a glutathione peroxidase, respectively. Although the maximum expression level was reached for low concentrations of menadione (0.25 or 0.5 mM) for some genes, in most cases the gene expression levels progressively increased with the concentration of menadione up to the highest concentration tested.

Exposure to hydrogen peroxide, that also induces a global oxidative stress, resulted in the overexpression of 12 out of the 33 target genes (Supplemental Fig. 2, Table 1). Eight of these genes were also overexpressed in response to menadione, including the abovementioned genes. *SAPIO_CDS4931* encoding a glutathione reductase was the most overexpressed, followed two other genes encoding proteins of the glutathione system (*SAPIO_CDS6039* and *SAPIO_CDS0416*) and four of the genes encoding proteins of the thioredoxin system (*SAPIO_CDS9937*, *SAPIO_CDS10274*, *SaPrx2* and *SAPIO_CDS1830*).

Diamide and cumene hydroperoxide, which specifically target proteins of the glutathione and thioredoxin systems, induced the overexpression of five of the 10 genes of the glutathione system and three of the six genes of the thioredoxin system for diamide (Supplemental Fig. 3A, Table 1), and of three and four genes of the glutathione and thioredoxin systems for cumene hydroperoxide (Supplemental Fig. 3B, Table 1). The highest fold changes were obtained for *SAPIO_CDS10274* with diamide and *SAPIO_CDS6039* with hydroperoxide cumene.

Finally, the use of DETA NO, that leads specifically to a nitrosative stress, induced the overexpression of two of the target genes, a flavohemoglobin (*SAPIO_CDS8682*) and a nitrite reductase (*SAPIO_CDS10292*) (Fig. 2, Table 1).

Genes of the thioredoxin system are strongly induced by co-cultivation with phagocytic cells

To better understand the potential role of enzymatical antioxidant gene battery in an innate immune context, the gene expression profile was also analyzed in 24-h-old hyphae co-cultivated with LPS-activated TPH-1 macrophages or HL60 neutrophils. No changes were seen in the gene expression levels after co-culture with nonactivated THP-1 macrophages or HL60 neutrophils classically used as control in such experiments since they produce ROS and RNS at a very low basal level.

Only four genes showed an overexpression in co-culture with activated TPH-1 macrophages. After 3 hours of co-culture, a maximum expression level was observed for *SAPIO_CDS1830* gene, encoding one of the thioredoxin reductases, (3.6-fold change compared to the control). The three others genes, *SAPIO_CDS10274* (encoding another thioredoxin reductase), *SaPrx2* (encoding a peroxiredoxin), and *SAPIO_CDS4185* (encoding one of the catalases) displayed a maximum level expression at 12 h of co-culture, with a 14.6-, 13.0-, and 9.4-fold increase, respectively (Fig. 3, Table 1). Expression of the other target genes remained unchanged.

Shorter times of incubation were used with LPS-activated HL60 neutrophils due to the quick release of ROS after their activation. In co-culture with these cells, 13 genes were overexpressed (Fig. 4, Table 1). For most of them (11), the maximum expression level was reached very quickly, after 30 min of co-culture whereas a 60 or 75 min of incubation was needed for the two other genes, that is, *SAPIO_CDS8682* and *SAPIO_CDS1359* genes, which encode flavohemoglobins. The highest relative expression levels were obtained for two genes of the thioredoxin system, that is, *SAPIO_CDS10274* and *SaPrx2*, which encode a thioredoxin reductase and a peroxiredoxin (11- and 10-fold changes, respectively), followed by a *SAPIO_CDS4185* and *SAPIO_CDS4327,* which encode a catalase and a Cu/Zn-SOD (8.4- and 8.3-fold changes, respectively; Fig. 4, Table 1).

To sum up, Figure 5 illustrates the genes significantly overexpressed in response to a general stress induced chemically (menadione or hydrogen peroxide) or generated by phagocytic cells. And 19 genes were found to be overexpressed in at least one condition, 10 of them being other expressed in two conditions (exposure to menadione and to hydrogen peroxide or to LPS-activated HL60 neutrophils) and four of them in three conditions (*SAPIO-CDS4327, SAPIO_CDS6474, SAPIO-CDS4185,* and *SAPIO-CDS10274* encoding a Cu/Zn-SOD, a Mn-SOD, a catalase and a thioredoxin reductase, respectively). Interestingly, only two genes encoding proteins of the thioredoxin system (*SAPIO_CDS1830* and *SaPrx2*) were overexpressed in the four conditions.

Discussion

Few data are available today regarding the pathogenesis of *Scedosporium* infections. To establish an infection, pathogens must evade the host immune defenses. One prominent mechanism in the host defense against pathogens is the oxidative burst

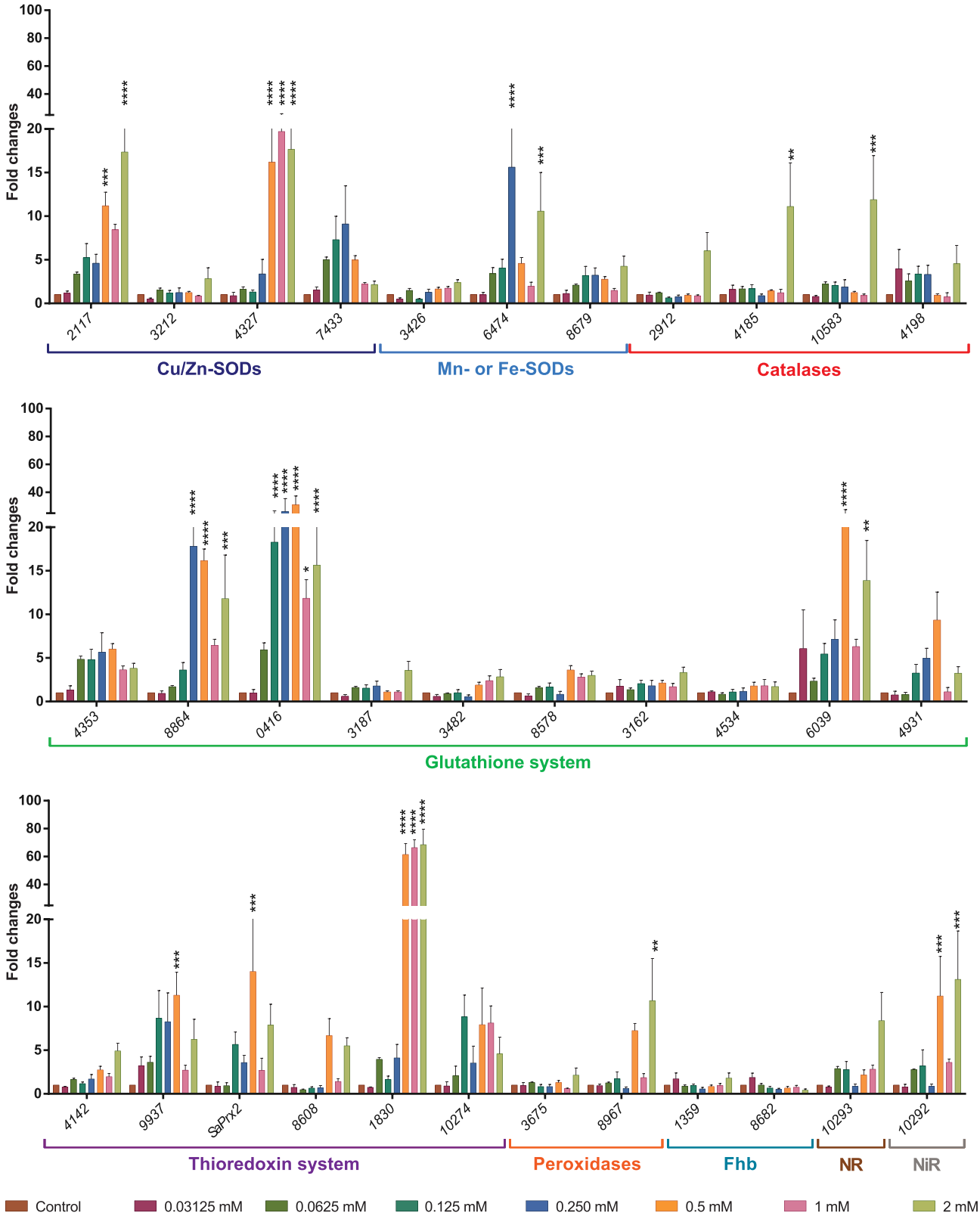

Figure 1. Relative gene expression levels in 24-h-old *S. apiospermum* hyphae after induction of oxidative stress by menadione. Coding sequences (CDS) are indicated in Genbank database with the prefix SAPIO_ for genome of *S. apiospermum* strain IHEM 14462 except for *SaPrx2*, which not considered as a coding sequence in the draft genome sequence[28] and was identified by a blastn analysis of the whole genome. Data were compared using the Two-ANOVA test (**** $P < .0001$; *** $.01 < P < .001$; ** $.05 < P < .01$; * $P < .05$). This Figure is reproduced in color in the online version of *Medical Mycology*.

Table 1. Gene expression levels in 24-h-old *S. apiospermum* hyphae exposed to a chemical oxidative stress or co-cultivated with LPS-activated phagocytes.

	Exposure to					Co-culture with LPS-activated	
Gene annotation and accession number	Menadione	H_2O_2	Diamide	Cumene	DETA NO	THP-1 macrophages	HL60 neutrophils
Cu/Zn-SODs							
CDS2117	**17.4 (2)**	5.3 (2)	/	/	/	2.2 (3 h)	2.7 (30 min)
CDS3212	2.8 (2)	4.2 (1)	/	/	/	1.5 (12 h)	1.9 (30 min)
CDS4327	**19.7 (1)**	**10.5 (2)**	/	/	/	3.5 (12 h)	**8.3 (30 min)**
CDS7433	9.1 (0.25)	5.1 (2)	/	/	/	1.3 (7 h)	1.4 (30 min)
Mn-SODs							
CDS3426	2.4 (2)	1.1 (2)	/	/	/	1.7 (3 h)	1.9 (30 min)
CDS6474	**15.6 (0.25)**	**8.6 (2)**	/	/	/	2.0 (7 h)	**4.4 (30 min)**
CDS8679	4.2 (2)	3.8 (0.25)	/	/	/	1.8 (3 h)	2.2 (30 min)
Catalases							
CDS2912	6.0 (2)	**9.4 (2)**	/	/	/	2.0 (24 h)	2.2 (30 min)
CDS4185	**11 (2)**	5.6 (2)	/	/	/	**9.4 (12 h)**	**8.4 (30 min)**
Catalases-peroxidases							
CDS4198	4.6 (2)	2.7 (1)	/	/	/	1.2 (7 h)	2.6 (30 min)
CDS10583	**11.9 (2)**	3.5 (2)	/	/	/	2.4 (3 h)	**3.8 (30 min)**
Glutathione peroxidases							
CDS4353	6.0 (0.5)	6.5 (2)	**7.5 (2)**	14.1 (4)	/	1.4 (12 h)	1.6 (60 min)
CDS8864	**17.8 (0.25)**	**9.6 (2)**	4.9 (1)	**72.1 (2)**	/	1.5 (12 h)	1.5 (30 min)
Glutaredoxins							
CDS0416	**31.1 (0.5)**	**28.6 (0.25)**	**15.3 (1)**	10.2 (1)	/	1.2 (12 h)	1.9 (30 min)
CDS3187	3.6 (2)	1.8 (2)	2.6 (1)	5.2 (1)	/	1.8 (3 h)	1.7 (30 min)
CDS3482	2.8 (2)	1.7 (0.25)	4.0 (4)	6.4 (2)	/	2.2 (1 h)	2.5 (30 min)
CDS8578	3.6 (0.5)	2.8 (2)	6.2 (4)	27.0 (4)	/	1.9 (3 h)	2.6 (30 min)
CDS3162	3.3 (2)	4.7 (0.25)	**7.3 (1)**	6.2 (1)	/	2.0 (1 h)	1.4 (30 min)
Glutathione reductases							
CDS4534	1.8 (1)	6.4 (0.25)	4.4 (1)	2.4 (2)	/	1.7 (1 h)	1.6 (60 min)
CDS4931	9.4 (0.5)	**79.4 (0.5)**	**22.8 (1)**	**26.5 (2)**	/	2.1 (12 h)	**7.7 (30 min)**
CDS6039	**21.6 (0.5)**	**34 (2)**	**13.0 (1)**	**85.0 (2)**	/	1.9 (24 h)	2.0 (30 min)
Peroxiredoxins							
CDS4142	4.9 (2)	5.9 (2)	3.2 (2)	12.0 (2)	/	1.7 (12 h)	1.7 (60 min)
CDS9937	**11.3 (0.5)**	**28.7 (2)**	**13.8 (1)**	**33.6 (2)**	/	1.3 (12 h)	1.2 (75 min)
SaPrx2	**14.0 (0.5)**	**20 (0.5)**	**22.9 (1)**	**25.6 (2)**	/	**13.0 (12 h)**	**10.0 (30 min)**
Thioredoxin							
CDS8608	6.7 (0.5)	4.5 (2)	7.7 (1)	10.1 (4)	/	2.1 (3 h)	2.7 (30 min)
Thioredoxin reductases							
CDS1830	**68.5 (2)**	**14.9 (0.25)**	4.1 (4)	**66.3 (4)**	/	**3.6 (3 h)**	**3.9 (30 min)**
CDS10274	8.1 (1)	**20.7 (0.5)**	**77.2 (1)**	**40.0 (2)**	/	**14.6 (12 h)**	**11 (30 min)**
Cytochrome C peroxidase							
CDS3675	2.1 (2)	1.3 (0.125)	/	/	/	1.6 (3 h)	1.9 (30 min)
Chloride peroxidase							
CDS8967	**10.7 (2)**	5.2 (2)	/	/	/	2.8 (12 h)	**5.4 (30 min)**
Flavohemoglobins							
CDS1359	1.8 (2)	**14.1 (0.25)**	/	/	6.1 (4)	2.6 (3 h)	**3.7 (75 min)**
CDS8682	1.9 (0.03125)	1.4 (0.25)	/	/	**18.4 (4)**	2.5 (12 h)	**3.5 (60 min)**
Nitrate reductase							
CDS10293	8.4 (2)	1.7 (0.25)	/	/	6.2 (4)	2.7 (3 h)	**4.5 (30 min)**
Nitrite reductase							
CDS10292	**13.1 (2)**	6.8 (2)	/	/	**54.4 (2)**	2.6 (24 h)	**7.4 (30 min)**

Coding sequences (CDS) are indicated in Genbank database with the prefix *SAPIO_* for genome of *S. apiospermum* strain IHEM 14462 except for SaPrx2, which was not considered as a coding sequence in the draft genome sequence[28], and was identified by a blastn analysis of the whole genome. Data correspond to the maximum expression levels (expressed in fold changes) for each gene and in parenthesis is indicated the concentration (in mM) of the chemical oxidant or the duration of co-culture needed to reach this value. Significant increases in the gene expression levels are highlighted in bold font. /: not determined.

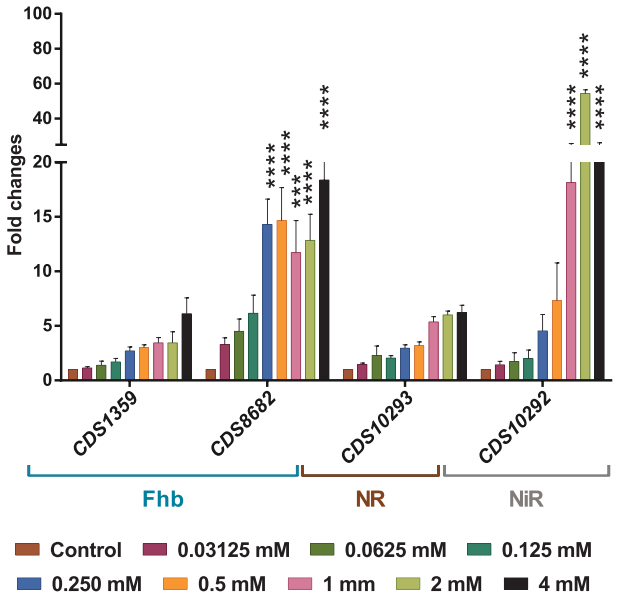

Figure 2. Relative gene expression levels in 24-h-old *S. apiospermum* hyphae after induction of nitrosative stress by DETA NO. Coding sequences (CDS) are indicated in Genbank database with the prefix SAPIO_ for genome of *S. apiospermum* strain IHEM 14462.[28] Data were compared using the Two-ANOVA test (**** $P < .0001$; *** $.01 < P < .001$). This Figure is reproduced in color in the online version of *Medical Mycology*.

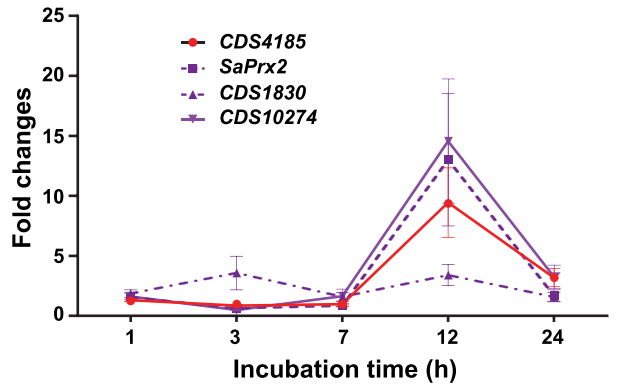

Figure 3. Relative gene expression levels in 24-h-old *S. apiospermum* hyphae co-cultivated with LPS-activated THP-1 macrophages. Coding sequences (CDS) are indicated in Genbank database with the prefix SAPIO_ for genome of *S. apiospermum* strain IHEM 14462 except for *SaPrx2,* which was not considered as a coding sequence in the draft genome sequence of *S. apiospermum,* and was identified by a blastn analysis of the whole genome.[28] This Figure is reproduced in color in the online version of *Medical Mycology*.

response of phagocytic cells and the subsequent release of ROS and RNS. The role of ROS in the host defense against pathogens is particularly well illustrated by the occurrence of infections in patients with chronic granulomatous disease (CGD). Indeed, mutations of *Nox* gene encoding the NADPH oxidase, which characterize this genetic disease, result in a defective production of superoxide anions by phagocytic cells, correlated with an increased susceptibility to respiratory pathogens.[37] Interestingly, most of the pathogens causing respiratory infections in CGD patients are also common CF pathogens, including *Staphylococcus aureus* and *Burkholderia cepacia* for bacteria, and *A. fumigatus, Scedosporium,* and *Lomentospora* species, the *Rasamsonia argillacea* species complex or *Exophiala dermatitidis* for filamentous fungi.[1,37–41]

Evasion of pathogens to the oxidative or nitrosative stress is essential to survive in the environment and to cause an infection. Regarding *S. apiospermum*, recent analysis of the genome revealed a set of 33 genes encoding SODs (7), catalases (4), proteins of the glutathione system (10), and of the thioredoxin system (6), chloroperoxidase (1), cytochrome c peroxidase (1), flavohemoglobins (2), nitrate reductase (1), and nitrite reductase (1), which are potentially implicated in protection of the fungus against the oxidative or nitrosative stress.[35] Here we showed overexpression of 21 of these genes in response to an oxidative or nitrosative stress induced chemically or by co-cultivation of the fungus with phagocytic cells, suggesting for these genes a role in the protection of the fungus against the host immune defenses.

Twelve of these genes were not significantly overexpressed in none of our experimental conditions, including genes encoding two Cu/Zn-SODs. Nevertheless, one cannot disregard the involvement of these genes in protection of the fungus against the host defenses. First, they may be constitutively expressed as the Cu/Zn-SOD of *Candida glabrata*.[42] The functional redundancy in the same protein family, as well as between some protein families also should be considered. For instance, expression of one of the four catalases identified in *S. apiospermum* was unchanged. This catalase (encoded by *SAPIO_CDS4198*) is a bifunctional catalase also degrading peroxide as glutathione peroxidases and peroxiredoxins, and several genes encoding these last enzymes were found to be overexpressed. Likewise, for each protein family, at least one of the encoding genes was found to be overexpressed, perhaps compensating the lack of response of the others. Moreover, the study was conducted on 24-h-old hyphae, and one may speculate the expression of some genes specifically in other morphological stages of the fungus. For example, expression of *SAPIO_CDS3212* encoding a Cu/Zn-SOD was unchanged in all of our experimental conditions. However, we showed previously that this enzyme is a glycosyl phosphatidyl inositol-anchored cell wall protein expressed exclusively at the surface of the conidia which are the infecting form of the fungus.[43] Likewise, four SOD genes were identified in *A. fumigatus* genome, two of them were highly expressed in conidia while *AfSOD3* was expressed only in hyphae.[44] Finally, since macrophages are less involved in the immune response against hyphae than neutrophils, our model could explain that a higher number of genes were modulated when 24-h-old hyphae of *S. apiospermum* were exposed to neutrophils (13 genes overexpressed vs. only four with macrophages).

Twenty-one genes were significantly overexpressed in at least one of our experimental conditions, and 12 upon exposure to both a chemically induced oxidative stress and an oxidative stress induced either by THP-1 or HL60 cells. Interestingly,

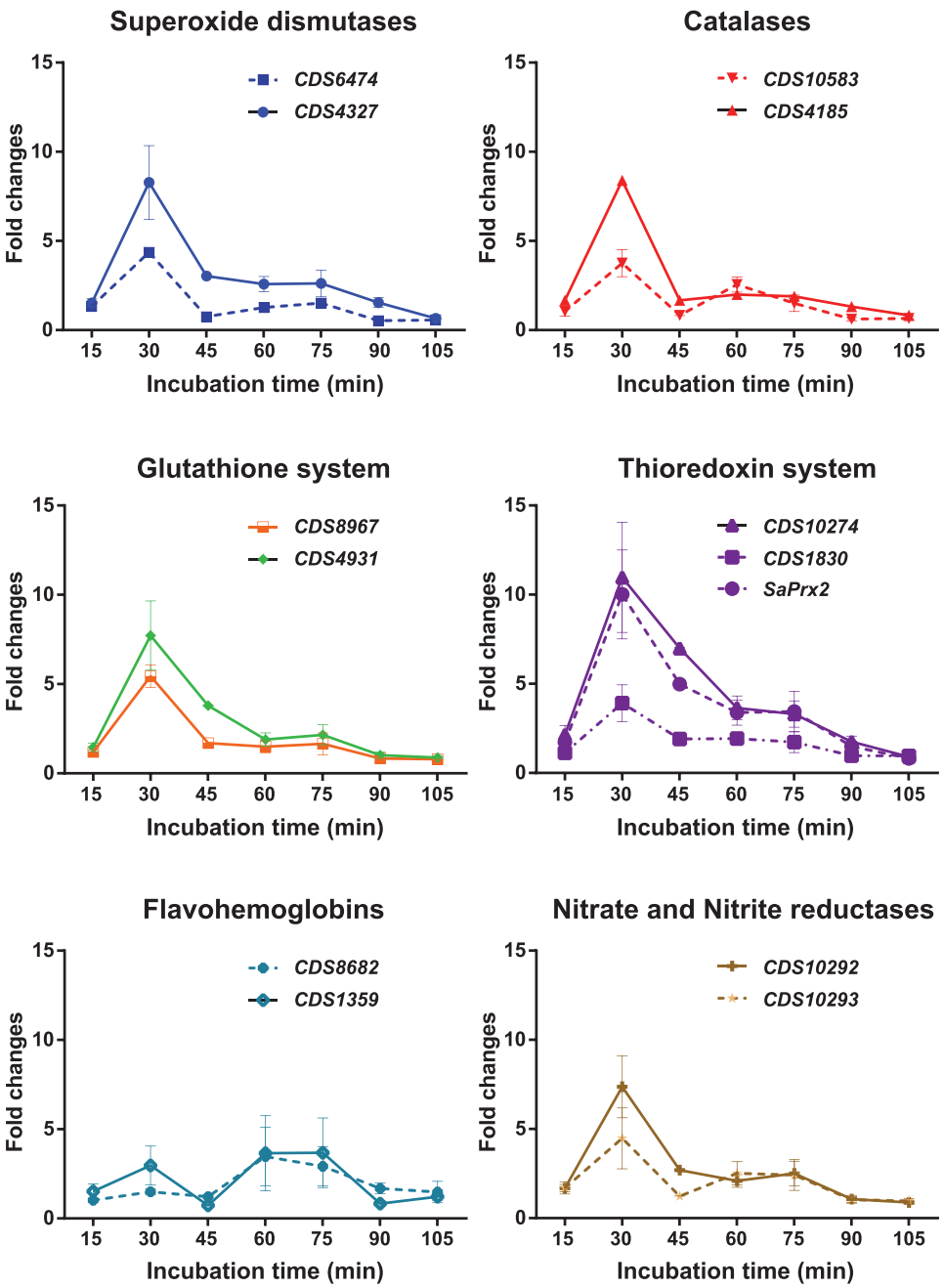

Figure 4. Relative gene expression levels in 24-h-old *S. apiospermum* hyphae co-cultivated with LPS-activated HL60 neutrophils Coding sequences (CDS) are indicated in Genbank database with the prefix SAPIO_ for genome of *S. apiospermum* strain IHEM 14462 except for *SaPrx2,* which not considered as a coding sequence in the draft genome sequence of *S. apiospermum,* and was identified by a blastn analysis of the whole genome.[28] This Figure is reproduced in color in the online version of *Medical Mycology*.

while some genes encoding proteins of the glutathione system were highly overexpressed in response to a chemically induced oxidative stress (the two genes encoding glutathione peroxidases, one of the five genes encoding a glutaredoxin, and two of the three genes encoding glutathione reductases), only one of them (*SAPIO_CDS4931* encoding a glutathione reductase) was found to be overexpressed in co-culture with LPS-activated HL60 neutrophils. In *C. albicans*, the glutathione reductase GLR1 is also induced upon exposure to hydrogen peroxide[45] as well as in co-culture with human neutrophils[46] and was shown to be involved in virulence of the yeast in the *Galleria mellonella* model.[47] Likewise, this enzyme is essential to virulence of *C. neoformans* in mice.[48] Further studies are needed to define the role of this glutathione reductase in the pathogenesis of scedosporiosis. As for the other genes of the glutathione system, their lack of response upon exposure to phagocytic cells suggests a prominent role for

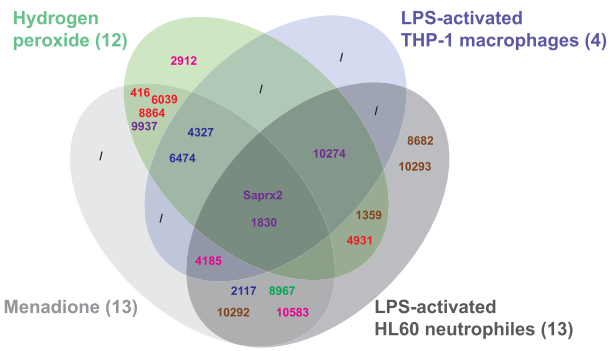

Figure 5. Venn representation of the genes significantly overexpressed in response to a general stress induced chemically (menadione or hydrogen peroxide) or generated by phagocytic cells. Accession numbers of the coding sequences (preceded in the Genbank database by the prefix SAPIO_CDS) that were significantly overexpressed in the different experimental conditions studied, are indicated. Only two CDS (in red and bold font) were overexpressed in each of the four conditions illustrated here. *SaPrx2*, which was not considered as a coding sequence in the draft genome sequence of *S. apiospermum* strain IHEM 14462,[28] was identified by a blastn analysis of the whole genome. In dark blue are indicated the Genbank accession number of *S. apiospermum* CDS encoding superoxide dismutases, in pink CDS encoding catalases, in red CDS encoding proteins of the glutathione system, in purple CDS encoding proteins of the thioredoxin system, in green CDS encoding peroxidases, and in brown CDS encoding proteins involved in NO degradation (the color figure is available in the online version of the manuscript). This Figure is reproduced in color in the online version of *Medical Mycology*.

other antioxidant genes, such as components of the thioredoxin system.

Only two genes encoding a thioredoxin reductase (*SAPIO_CDS1830*) and a peroxiredoxin (*SaPrx2*) were overexpressed upon exposure to both menadione and hydrogen peroxide as well as in co-culture with THP1 and HL60 cells, whereas two other genes were upregulated in these co-cultures as well as upon exposure to either menadione or hydrogen peroxide, that is, *SAPIO_CDS4185* and *SAPIO_CDS10274*, which encodes one catalase and the other thioredoxin reductase. These genes were also highly expressed in response to diamide and cumene hydroperoxide, particularly the thioredoxin reductase gene *SAPIO_CDS10274*, which showed the highest changes in expression level.

Catalases are known to detoxify hydrogen peroxide and to participate to evasion of the pathogen to the oxidative stress. Nevertheless, in most cases, they did not seem to be essential for virulence. For example, three catalases are described in *A. fumigatus*, including the spore-specific CatA and two mycelial catalases, the monofunctional catalase Cat1 and the catalase-peroxidase Cat2, but the delta CatA mutant was as virulent as the wild-type strain in a rat model of aspergillosis and the double mutant delta cat1 delta cat2 exhibited only delayed infection in this experimental model.[49] Likewise, four catalase genes have been described in *C. neoformans*, and no differences were seen in virulence in a mouse model between a wild-type strain and single or even quadruple mutants.[50] Conversely, a single catalase gene *CAT1* was identified in *C. albicans*, and a markedly decreased virulence was reported for the homozygous null mutant in a mouse model of disseminated candidiasis.[51]

The role of fungal peroxiredoxins in virulence is poorly documented. In *A. fumigatus*, the Asp f3 allergen was identified as a member of the peroxiredoxin protein family, and disruption of the encoding gene resulted in a hypersensitivity to external superoxide and in a total lack of virulence in mice, suggesting that these enzymes could be therapeutic targets.[52]

Our results also suggest that genes encoding the two thioredoxin reductases in *S. apiospermum* could play an important role in protecting the fungal cells against ROS and RNS. There is now an increasing body of evidence that these enzymes display a high potential for drug design and vaccine development.[53,54] For example, the *C. albicans* thioredoxin reductase (*Ca*TrxR or TRR1) is highly overexpressed under oxidative stress and is implicated in ROS and RNS degradation, especially in response to neutrophils,[46,55,56] as well as in virulence in a mouse model of candidiasis.[57] Likewise, recombinant *Ca*TrxR protein induced high levels of serum specific antibodies and reduced the fungal burden in experimental disseminated candidiasis in mice.[58] In addition, recent studies on the thioredoxin reductase inhibitor auranofin[59] highlighted its promising antimicrobial potential against various bacteria,[60–63] as well as some pathogenic yeasts[61,64] and filamentous fungi.[64]

Finally, our experiments also showed overexpression of the flavohemoglobin gene *SAPIO_CDS8682* and the nitrite reductase gene *SAPIO_CDS10292* upon exposure to DETA-NO. Although further investigations on these genes are needed, recent studies performed on *A. fumigatus* which revealed a prominent role of the flavohemoglobin FhpA in protection of the conidia against RNS, also showed no differences in killing of the conidia by macrophages and in virulence for mice between the wild-type strain and the *FhpA* mutant.[65]

In conclusion, this study provides new insights into the response of *S. apiospermum* to the oxidative or nitrosative stress. As observed in other human pathogenic fungi, several genes were deregulated in *S. apiospermum* hyphae upon exposure to ROS and RNS, suggesting a role in protecting the fungus. Further studies are needed to confirm their involvement in protection of the fungus against the host immune defenses. Considering literature data, a particular attention should be paid to genes encoding some proteins of the thioredoxin system, particularly *SaPrx2* and the thioredoxin reductase genes. These genes which play an essential role in virulence in other fungal pathogens, were overexpressed in most of our experimental conditions, and therefore could be considered as potential targets for drug development.

Supplementary material

Supplementary data are available at *MMYCOL* online.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Giraud S, Bouchara JP. *Scedosporium apiospermum* complex: diagnosis and species identification. *Curr Fungal Infect Rep*. 2014; 8: 211–219.
2. Ramirez-Garcia A, Pellon A, Rementeria A et al. *Scedosporium* and *Lomentospora*: an updated overview of underrated opportunists. *Med Mycol*. 2018; 56: 102–125.
3. Zouhair R, Rougeron A, Razafimandimby B, Kobi A, Bouchara JP, Giraud S. Distribution of the different species of the *Pseudallescheria boydii/Scedosporium apiospermum* complex in French patients with cystic fibrosis. *Med Mycol*. 2013; 51: 603–613.
4. Sedlacek L, Graf B, Schwarz C et al. Prevalence of *Scedosporium* species and *Lomentospora prolificans* in patients with cystic fibrosis in a multicenter trial by use of a selective medium. *J Cyst Fibros*. 2015; 14: 237–241.
5. Blyth C, Harun A, Middleton PG et al. Detection of occult *Scedosporium* species in respiratory tract specimens from patients with cystic fibrosis by use of selective media. *J Clin Microbiol*. 2010; 48: 314–316.
6. Cimon B, Carrère J, Chazalette J et al. Fungal colonization and immune response to fungi in cystic fibrosis. *J Mycol Méd*. 1995; 5: 211–216.
7. Cimon B, Carrère J, Vinatier JF, Chazalette JP, Chabasse D, Bouchara JP. Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis*. 2000; 9: 53–56.
8. Pihet M, Carrère J, Cimon B et al. Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis: a review. *Med Mycol*. 2008; 47: 387–397.
9. Paugam A, Baixench MT, Demazes-Dufeu N et al. Characteristics and consequences of airway colonization by filamentous fungi in 201 adult patients with cystic fibrosis in France. *Med Mycol*. 2010; 48: S32–36.
10. Borman AM, Palmer MD, Delhaes L et al. Lack of standardization in the procedures for mycological examination of sputum samples from CF patients: a possible cause for variations in the prevalence of filamentous fungi. *Med Mycol*. 2010; 48: S88–97.
11. Schwarz C, Vandeputte P, Rougeron A et al. Developing collaborative works for faster progress on fungal respiratory infections in cystic fibrosis. *Med Mycol*. 2018; 56: 42–59.
12. Defontaine A, Zouhair R, Cimon B et al. Genotyping study of *Scedosporium apiospermum* isolates from patients with cystic fibrosis. *J Clin Microbiol*. 2002; 40: 2108–2114.
13. Vázquez-Tsuji O, Campos Rivera T, Rondán Zárate A, García Mirabal, M. Endobronchitis by *Scedosporium apiospermum* in a child with cystic fibrosis. *Rev Iberoam Micol*. 2006; 23: 245–248.
14. Castiglioni B, Sutton DA, Rinaldi MG, Fung J, Kusne S. *Pseudallescheria boydii* (anamorph *Scedosporium apiospermum*). Infection in solid organ transplant recipients in a tertiary medical center and review of the literature. *Medicine*. 2002; 81: 333–348.
15. Husain S, Muñoz P, Forrest G et al. Infections due to *Scedosporium apiospermum* and *Scedosporium prolificans* in transplant recipients: clinical characteristics and impact of antifungal agent therapy on outcome. *Clin Infect Dis*. 2005; 40: 89–99.
16. Symoens F, Knoop C, Schrooyen M et al. Disseminated *Scedosporium apiospermum* infection in a cystic fibrosis patient after double-lung transplantation. *J Heart Lung Transplant*. 2006; 25: 603–607.
17. Sahi H, Avery RK, Minai OA et al. *Scedosporium apiospermum* (*Pseudallescheria boydii*) infection in lung transplant recipients. *J Heart Lung Transplant*. 2007; 26: 350–356.
18. Morio F, Horeau-Langlard D, Gay-Andrieu F et al. Disseminated *Scedosporium/Pseudallescheria* infection after double-lung transplantation in patients with cystic fibrosis. *J Clin Microbiol*. 2010; 48: 1978–1982.
19. Babior BM. The respiratory burst of phagocytes. *J Clin Invest*. 1984; 73: 599–601.
20. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat Rev Microbiol*. 2004; 2: 820–832.
21. Iovine NM, Pursnani S, Voldman A, Wasserman G, Blaser MJ, Weinrauch Y. Reactive nitrogen species contribute to innate host defense against *Campylobacter jejuni*. *Infect Immun*. 2008; 76: 986–993.
22. Campbell EL, Colgan SP. Neutrophils and inflammatory metabolism in antimicrobial functions of the mucosa. *J Leukoc Biol*. 2015; 98: 517–522.
23. Staerck C, Gastebois A, Vandeputte P et al. Microbial antioxidant defense enzymes. *Microb Pathog*. 2017; 110: 56–65.
24. Reid DW, Misso N, Aggarwal S, Thompson PJ, Walters EH. Oxidative stress and lipid-derived inflammatory mediators during acute exacerbations of cystic fibrosis. *Respirology*. 2007; 12: 63–69.
25. Galli F, Battistoni A, Gambari R et al. Oxidative stress and antioxidant therapy in cystic fibrosis. *Biochim Biophys Acta*. 2012; 1822: 690–713.
26. Belozerskaya TA, Gessler NN. Reactive oxygen species and the strategy of antioxidant defense in fungi: a review. *Appl Biochem Microbiol*. 2007; 43: 506–515.
27. Forrester MT, Foster MW. Protection from nitrosative stress: a central role for microbial flavohemoglobin. *Free Radic Biol Med*. 2012; 52: 1620–1633.
28. Vandeputte P, Ghamrawi S, Rechenmann M et al. Draft genome sequence of the pathogenic fungus *Scedosporium apiospermum*. *Genome Announc*. 2014; 2: pii: e00988-14.
29. Larcher G, Cimon B, Symoens F, Tronchin G, Chabasse D, Bouchara JP. A 33 kDa serine proteinase from *Scedosporium apiospermum*. *Biochem J*. 1996; 315: 119–126.
30. Pereira MM, Silva BA, Pinto MR, Barreto-Bergter E, dos Santos ALS. Proteins and peptidases from conidia and mycelia of *Scedosporium apiospermum* strain HLPB. *Mycopathologia*. 2009; 167: 25–30.
31. Silva BA, Souza-Gonçalves AL, Pinto MR, Barreto-Bergter E, Santos ALS. Metallopeptidase inhibitors arrest vital biological processes in the fungal pathogen *Scedosporium apiospermum*. *Mycoses*. 2011; 54: 105–112.
32. Lima OC, Larcher G, Vandeputte P et al. Molecular cloning and biochemical characterization of a Cu,Zn-superoxide dismutase from *Scedosporium apiospermum*. *Microbes Infect*. 2007; 9: 558–565.
33. Mina S, Marot-Leblond A, Cimon B et al. Purification and characterization of a mycelial catalase from *Scedosporium boydii*, a useful tool for specific antibody detection in patients with cystic fibrosis. *Clin Vaccine Immunol*. 2015; 22: 37–45.
34. Mina S, Staerck C, d'Almeida SM et al. Identification of *Scedosporium boydii* catalase A1 gene, a reactive oxygen species detoxification factor highly expressed in response to oxidative stress and phagocytic cells. *Fungal Biol*. 2015; 119: 1322–1333.
35. Staerck C, Vandeputte P, Gastebois A et al. Enzymatic mechanisms involved in evasion of fungi to the oxidative stress: focus on *Scedosporium apiospermum*. *Mycopathologia*. 2018; 183: 227–239.
36. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. *Methods*. 2001; 25: 402–408.
37. Roos D. Chronic granulomatous disease. *Br Med Bull*. 2016; 118: 50–63.
38. Kenney RT, Kwon-Chung KJ, Waytes AT et al. Successful treatment of systemic *Exophiala dermatitidis* infection in a patient with chronic granulomatous disease. *Clin Infect Dis*.1992; 14: 235–242.
39. Santos PE, Oleastro M, Galicchio M, Zelazko M. Fungal infections in paediatric patients with chronic granulomatous disease. *Rev Iberoam Micol*. 2000; 17: 6–9.
40. Bhat SV, Paterson DL, Rinaldi MG, Veldkamp PJ. *Scedosporium prolificans* brain abscess in a patient with chronic granulomatous disease: successful combination therapy with voriconazole and terbinafine. *Scand J Infect Dis*. 2007; 39: 87–90.
41. Giraud S, Pihet M, Razafimandimby B et al. *Geosmithia argillacea*: an emerging pathogen in patients with cystic fibrosis. *J Clin Microbiol*. 2010; 48: 2381–2386.
42. Briones-Martin-Del-Campo M, Orta-Zavalza E, Juarez-Cepeda J et al. The oxidative stress response of the opportunistic fungal pathogen *Candida glabrata*. *Rev Iberoam Micol*. 2014; 31: 67–71.
43. Ghamrawi S, Gastebois A, Zykwinska A et al. A multifaceted study of *Scedosporium boydii* cell wall changes during germination and identification of GPI-anchored proteins. *PLoS One*. 2015; 10: e0128680.

44. Lambou K, Lamarre C, Beau R, Dufour N, Latgé JP. Functional analysis of the superoxide dismutase family in *Aspergillus fumigatus*. *Mol Microbiol*. 2010; 75: 910–923.
45. Alarco AM, Raymond M. The bZip transcription factor Cap1p is involved in multidrug resistance and oxidative stress response in *Candida albicans*. *J Bacteriol*. 1999; 181: 700–708.
46. Fradin C, De Groot P, MacCallum D et al. Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Mol Microbiol*. 2005; 56: 397–415.
47. Tillmann AT, Strijbis K, Cameron G et al. Contribution of Fdh3 and Glr1 to glutathione redox state, stress adaptation and virulence in *Candida albicans*. *PloS One*. 2015; 10: e0126940.
48. Missall TA, Pusateri ME, Donlin MJ, Chambers KT, Corbett JA, Lodge JK. Post-translational, translational, and transcriptional responses to nitric oxide stress in *Cryptococcus neoformans*: implications for virulence. *Eukaryot Cell*. 2006; 5: 518–529.
49. Paris S, Wysong D, Debeaupuis JP et al. Catalases of *Aspergillus fumigatus*. *Infect Immun*. 2003; 71: 3551–3562.
50. Giles SS, Stajich JE, Nichols C et al. The *Cryptococcus neoformans* catalase gene family and its role in antioxidant defense. *Eukaryot Cell*. 2006; 5: 1447–1459.
51. Wysong DR, Christin L, Sugar AM, Robbins PW, Diamond RD. Cloning and sequencing of a *Candida albicans* catalase gene and effects of disruption of this gene. *Infect Immun*. 1998; 66: 1953–1961.
52. Hillmann F, Bagramyan K, Straßburger M et al. The crystal structure of peroxiredoxin Asp f3 provides mechanistic insight into oxidative stress resistance and virulence of *Aspergillus fumigatus*. *Sci Rep*. 2016; 6: 33396.
53. Abadio AKR, Kioshima ES, Teixeira MM, Martins NF, Maigret B, Felipe MSS. Comparative genomics allowed the identification of drug targets against human fungal pathogens. *BMC Genomics*. 2011; 12: 75.
54. Ianiri G, Idnurm A. Essential gene discovery in the basidiomycete *Cryptococcus neoformans* for antifungal drug target prioritization. *MBio*. 2015; 6: e02334–14.
55. Enjalbert B, Nantel A, Whiteway M. Stress-induced gene expression in *Candida albicans*: absence of a general stress response. *Mol Biol Cell*. 2003; 14: 1460–1467.
56. Kusch H, Engelmann S, Albrecht D, Morschhäuser J, Hecker M. Proteomic analysis of the oxidative stress response in *Candida albicans*. *Proteomics*. 2007; 7: 686–697.
57. Zaki N, Bakar F, Mahadi N, Murad A. *Candida albicans* TRR1 heterozygotes show increased sensitivity to oxidative stress and decreased pathogenicity. *Afr J Microbiol Res*. 2012; 6: 1796–1805.
58. Godoy JSR, Kioshima ÉS, Abadio AKR, Felipe MSS, de Freitas SM, Svidzinski TIE. Structural and functional characterization of the recombinant thioredoxin reductase from *Candida albicans* as a potential target for vaccine and drug design. *Appl Microbiol Biotechnol*. 2015; 100: 4015–4025.
59. Roder C, Thomson MJ. Auranofin: repurposing an old drug for a golden new age. *Drugs R D*. 2015; 15: 13–20.
60. Harbut MB, Vilchèze C, Luo X et al. Auranofin exerts broad-spectrum bactericidal activities by targeting thiol-redox homeostasis. *Proc Natl Acad Sci U S A*. 2015; 112: 4453–4458.
61. Fuchs BB, RajaMuthiah R, Souza ACR et al. Inhibition of bacterial and fungal pathogens by the orphaned drug auranofin. *Future Med Chem*. 2016; 8: 117–132.
62. Thangamani S, Mohammad H, Abushahba MFN et al. Antibacterial activity and mechanism of action of auranofin against multi-drug resistant bacterial pathogens. *Sci Rep*. 2016; 6: 22571.
63. Thangamani S, Mohammad H, Abushahba MFN, Sobreira TJP, Seleem MN. Repurposing auranofin for the treatment of cutaneous staphylococcal infections. *Int J Antimicrob Agents*. 2016; 47: 195–201.
64. Wiederhold NP, Patterson TF, Srinivasan A et al. Repurposing auranofin as an antifungal: *in vitro* activity against a variety of medically important fungi. *Virulence*. 2016; 8: 138–142.
65. Lapp K, Vödisch M, Kroll K et al. Characterization of the *Aspergillus fumigatus* detoxification systems for reactive nitrogen intermediates and their impact on virulence. *Front Microbiol*. 2014; 5: 469.