SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Comparative functional analysis of mitochondrial manganese SOD genes in *Aspergillus* species

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The PhD Defense takes place in the Lecture Hall of the Department of Internal Medicine, Building "A", Faculty of Medicine, University of Debrecen at 1:00 p.m., 17th of February, 2026.

Introduction

Aspergillus nidulans is used in basic and microbiological research around the world. Phylogenetically, it is one of the best-known species in *Aspergillus* genus and is essential for the study of the growth and development mechanisms, cell cycle and secondary metabolism of filamentous fungi. *Aspergillus fumigatus* is a saprophytic filamentous fungus, which occurs primarily on degradable organic matter, soil, compost and other plant residues. Although *A. fumigatus* is primarily saprophytic, it can be an opportunistic pathogen under certain conditions, especially in immunocompromised people. From food safety perspective this species can cause risk, but it is less significant than species like *A. flavus* or *A. niger*, which are well-known mycotoxin producers and cause major problems to the food industry. According to some studies, *A. fumigatus* can be present cereals, barley, rice, wheat, as well as nuts like hazel nuts, walnuts, and peanuts.

In our study we focused on investigating the effects of oxidative stress in these two species, with a focus on the role of the mitochondrial manganese-containing superoxide dismutase (MnSOD) enzyme. Oxidative stress management is quite important for fungi, as oxidative stress occurs in the natural environment and, in addition, this type of stress is growing in pathogenic fungi during host colonization. Understanding the survival strategies, the mechanism of cellular defense in fungi is important for finding potential targets for antifungal therapies and industrial strain improvement. For identifying gene function in this experiment, we used deletion mutants of both *Aspergillus* species lacking the gene encoding the mitochondrial MnSOD enzyme.

There is a close linkage between oxidative stress response and secondary metabolite production. The production of mycotoxins, such as aflatoxins, ochratoxins and trichothecenes, can be induced by oxidative stress, while the presence of antioxidants can suppress these processes. According to some hypotheses, secondary metabolites production helps to maintain the redox-balance or give a competitive advantage against other microorganisms in the case of fungi and in addition, these compounds provide a greater advantage over the host immune system.

In conclusion, understanding the oxidative stress responses of filamentous fungi is relevant from the perspective of industrial and food safety. Their adaptation to oxidative stress basically affected their survival and the production of some secondary metabolites – including confirmed or potentially toxic compounds. Since oxidative stress can induce or enhance the production of mycotoxins, the investigation of these processes may lead to preventing food

contamination. Moreover, understanding the fungal responses to stress may help us develop new industrial strains and identify new targets against fungi.

Objectives

The aim of my PhD work was the comparative functional analysis of the mitochondrial MnSOD enzymes in *A. nidulans* and *A. fumigatus*, especially their roles in oxidative stress defense. *A. nidulans* is an excellent model organism, which provides a trusted base to the transcriptomic works with its well-characterized genome and identified genes. Through the comparison with the transcriptome of *A. fumigatus,* the differences between the two species were better identifiable. Our investigation leads us to better understanding of the oxidative stress response of these two filamentous, conidia- and secondary metabolites producing fungi, which may be important from medical and industrial points of view. Like an opportunist, *A. fumigatus* has a particular importance from a clinical perspective. Comparing its transcriptomic data with the expression pattern of orthologs in *A. nidulans* may reveal important evolutionary and functional differences in oxidative stress defense.

In our work, we set out to implement the following steps:

1. Culture in liquid culture medium under normal and oxidative stress conditions, including RNA isolation, superoxide levels and SOD activity measurements. Comparison of oxidative stress sensitivity in solid media.
2. Evaluation of data from RNA sequencing, identification of differentially expressed genes, gene set enrichment analyses of gene clusters.
3. Validate the results by qPCR with genes selected from the transcriptomic data.
4. To carry out further work in the light of the results obtained (e.g. macrophage susceptibility testing).

Our work has addressed the following questions:

Physiological role of MnSOD enzymes:

1. What is the role of mitochondrial MnSOD enzymes in *A. nidulans* and *A. fumigatus* in defence against oxidative stress?

Effects of deletion of the sodB and sod2 genes:

2. To what extent does deletion of the *sodB* and *sod2* genes in *A. nidulans* and *A. fumigatus* increase the susceptibility of fungi to oxidative stress?

Alternative defence mechanisms:

3. Which antioxidant enzymes are activated to compensate for MnSOD deficiency?

Different stress responses of *A. nidulans* and *A. fumigatus*:

4. What differences in the stress response of the two strains are revealed by comparative functional analysis?

5. How can *A. fumigatus*-specific differences be exploited in future drug development?

Materials and methods

Comparison of the manganese SOD genes of the two Aspergillus species

A. nidulans sodB and *A. fumigatus sod2* genes were compared using nucleotide and amino acid sequences from the FungiDB database. Intron and UTR regions were also identified using FungiDB. Sequence alignments were performed using BioEdit, while homology between amino acid sequences was determined using the NCBI BLAST interface. The predicted 3D structural models of the two proteins were generated using AlphaFold 2 and aligned on the RCSB Protein Data Bank interface for similarity analysis. In addition, functional domains were determined using the online program SMART, which searches for homology in the given protein sequences based on known domain structures.

Strains and their breeding

The strains we used were *Aspergillus nidulans* THS30.3 and *ΔsodB*; *Aspergillus fumigatus akuB*^{ku80} and *Δsod2*/IP345. The strains were maintained on Barratt's minimal nitrate medium (AMM). Before the experiments, we inoculated from glycerol stock to produce fresh spores. Cultivation was carried out for 6 days in each case at 37 °C.

Mycelium cultivation for RNA isolation

We measured 5 x 10^7 db *A. nidulans* conidia into 100 ml Barratt's media, in the case of *A. fumigatus* into 100 ml modified media with diammonium-tartrate, in 500 ml Erlenmeyer flasks. The flasks were then incubated at 37 °C for 16 h for *A. nidulans* strains and 20 h for *A. fumigatus* strains at 3.7 Hz. After 16 h 0,16 mM MSB was added to the *A. nidulans* cultures. In

the case of *A. fumigatus* cultures 6 mM MSB was added in the flask after 20 h. Three to three biological replicates were generated from treated and untreated cultures. After 30 min of treatment, the mycelium was filtered and stored at -20 °C until further use.

Conditions of stress experiments on solid media

For *A. nidulans* strains we used Barratt's agar, for *A. fumigatus* we used the modified one with diammoium-tartrate. 25 µM MSB was mixed with the agar for the experiments with *A. nidulans* strains, and 0.5 µM, 1 µM and 15 µM for *A. fumigatus* strain. 5 µl conidia suspension was inoculated (from 2×10^7 conidia/ml stock solution) in the middle of agar plates. After that the Petri dishes were incubated at 37 °C for 5 days and the degree of inhibition was determined by measuring the diameter of the colonies.

In experiments for iron limitation with *A. fumigatus* strains we supplemented the medium with 0.8 mM deferiprone (DFP) and prepared the medium without iron. A 100 µl suspension of conidia (from 10^7 conidia/ml stock solution) was spread on the surface of the media. After that a hole was made in the center of each plate using a pipette tip. One part of the dishes we added 50 µl of 12 mM MSB solution into the holes, the rest of the dishes got this MSB treatment after 1 day incubation. The cultures were incubated at 37 °C for 4 days. The diameter of the inhibition zone was measured when the experiment was evaluated.

Samples for measurement of superoxide formation and SOD activity

Cultivation was performed under the same conditions to determine superoxide formation as for mycelium prepared for RNA isolation. The formation of superoxide was measured from the medium after the addition of MSB, and mycelium was filtered 5 hours after the measurement of SOD activity. In this case, we worked with three biological replicates too.

Measurement of superoxide formation

Dihydroethidium treatment was used to detect superoxide according to the protocol described by Carter et al (1994). A 100 µl of 2 mM dihydroethidium solution was added to a 20 ml culture and the samples were incubated for 1 hour on a shaker at 37 °C ~ 3.6 Hz (220 rpm). After this 6 ml culture was filtered and washed by distilled water from each sample. The mycelium was placed in an Eppendorf tube and 1 ml of 4 C°, 5 w / v% sulfosalicylic acid solution was added to each tube. Incubated on ice for 10-20 minutes, and after 10 minutes of centrifugation (4 °C, 10000 g) 500 µl 2 M NaOH solution was added to 500 µl supernatant. The fluorescence of the samples was determined by a spectrophotometer (λ_{ext} = 488 nm, λ_{em} = 610

nm) and the amount of ethidium formed was calculated after taking an ethidium bromide calibration line.

Measurement of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was determined by the Oberley and Spitz (1984) methods. 1 ml final volume reaction mixture containing a 10% v / v sample was used for the determination. The components of the reaction mixture were: 50 mM sodium phosphate buffer (pH 7.8), 70 µM nitro blue tetrazolium chloride (NBT), 1.4 mM diethylenetriaminepentacetic acid (DETAPAC), 0.2 mM xanthine, 10 U / l xanthine oxidase, and 1000 U/l catalase. The determination of enzyme activity was based on the monitoring of NBT oxidation, measured at a wavelength of 560 nm over 2 minutes. A sample-free reaction mixture was used as a control. Enzyme activity was calculated from the quotient of control and sample absorbance values, and 1 was subtracted from this.

Determination of protein content

The protein content of the samples was determined by the addition of a Bradford reagent. For supernatant samples, a Bradford reagent was added and after an incubation period of 10 minutes at room temperature, the samples were measured with a spectrophotometer at a wavelength of $\lambda = 595$ nm. From the absorbance data, the protein content was determined using a bovine serum albumin (BSA) calibration line, and the values thus obtained were given in mg / l relative to the volume of the fermentation broth.

Survival of *A. fumigatus* conidia against macrophages

Two types of macrophages were used for the experiment. There were M1 macrophage phenotypes created with granulocyte-macrophage colony stimulating factor (GM-CSF; Gentaur Molecular Products, London, UK, 80 ng / ml) and macrophage colony stimulating factor (M-CSF; PeproTech, Brussels, Belgium, 50 ng / ml) induced M2 phenotype. Macrophages were inoculated into 96-well cell culture plates at a cell density of 1×10^5 cells / 100 µl. A 100 µl 1×10^5 *A. fumigatus* conidia / RPMI 1640 medium suspension was added to each well. The control was 100 µl of conidia suspension and 100 µl of RPMI medium without macrophages. After 4 hours of incubation and centrifugation, the supernatant was removed, and cell lysis was induced with distilled water. The samples were inoculated on Barratt's minimum nitrate agar after a series of dilutions, and the number of viable cells was assessed at 37 °C after 36 hours of incubation by determination of colony forming units (CFU).

The RNA isolation procedure

RNA used for RNA sequencing and gene expression assays was isolated from lyophilized mycelia according to the Chomczynksi (1993) protocol with a TRIzol reagent (Invitrogen, Waltham, MA, USA) and then the RNA content of the samples was previously measured with Nanodrop UV / Vis (Nabi, μ2 Microdigital). Samples were stored at -70 °C until use. Furthermore, the purity of the RNA samples was checked by the staff of the DE ÁOK Genomi Medicine and Bioinformatics Service Laboratory with a microfluid electrophoresis system (Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA, USA).

Quantitative real-time polymerase chain reaction (RT-qPCR)

Gene expression results from RNA sequencing were validated by RT-qPCR. In the preparation of the RNA samples, DNA removal was the first step and then the qPCR reaction was measured according to the little modified protocol of the Xceed qPCR SG 1-step 2x Mix Lo-ROX Kit (Applied Biotechnologies, Praha, Czech Republic). The reference gene was AN9168 (a gene encoding a suspected glycerol transporter) for *A. nidulans* and AFUB_078400 (presumably encoding the catalytic subunit of 1,3-β-D-glucan synthase) for *A. fumigatus*. The Cq values obtained in RT-qPCR were compared to the corresponding reference gene, and then the ΔΔCP values were calculated based on four different comparisons. These were compared with $\log_2$FC values from sequencing data, which were also determined in the same comparisons.

High-throughput RNA sequencing procedure

The following samples were prepared by three biological replicates: *A. nidulans* THS30.3 untreated, THS30.3 MSB (0.16 mM) treated, *ΔsodB* untreated, *ΔsodB* MSB (0.16 mM) treated, and *A. fumigatus akuB*^{ku80} untreated, *akuB*^{ku80} MSB (6mM) treated, *Δsod2* untreated, *Δsod2* MSB (6 mM) treated. The RNA library required for single-read 75 bp Illumina RNA sequencing was created with the TruSeq RNS Library Prep Kit (Illumina, San Diego, CA, USA) as described by the manufacturer. Sequencing was performed on the Illumina NextSeq 500 instrument.

Evaluation of sequenced data (RNAseq)

Reads from sequencing were matched to the genome *A. nidulans* FGSC A4 and *A. fumigatus* A1163 by running the HISAT2 software (version 2.1.0). Exactly how many reads

belonged to a gene was determined by the featureCounts software (version 2.0.0). Differentially expressed genes (DEG) were obtained with DESeq2 (version 1.34.0) software. RPKM values were determined by the "rpkm" function of the edgeR packet, and PCA (main component analysis) was performed with the RStudio (https://rstudio-education.github.io/hopr/starting.html) "pcromp" function.

Evaluation of transcriptomic data

When comparing two transcriptomes, the upregulated and downregulated genes were defined as DEGs (corrected *p*-value <0.05), where the abundances of $|\log_2 FC|$ were greater than a defined threshold (unless otherwise indicated as 1). Log_2FC values were calculated using DESeq2 software (version 1.34.0), where we used the untreated cultures or wild-type cultures as reference. To characterize the composition of selected gene sets, we performed gene set enrichment analysis ("gene set enrichment analysis") on ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/) platform. Terms containing fewer than three genes or results containing only one gene were skipped from the analysis and only results with corrected values-*p* <0.05 were considered. Analyses were also performed on several subsets of DEGs obtained by setting different $\log_2 FC$ thresholds. We used $|\log_2 FC| > 0$, $|\log_2 FC| > 1$, $|\log_2 FC| > 2$ threshold values in the case of *A. nidulans*, while for the *A. fumigatus* data $|\log_2 FC| > 0$, $|\log_2 FC| > 0{,}5$ and $|\log_2 FC| > 1$ threshold values were used.

Analysis of transcriptomic data from *A. nidulans*

The results of gene set enrichment analysis of the "Fe-S cluster assembly", "Antioxidant enzyme", "Respiration" and "Sterigmatocystin cluster" were also examined using Fisher's exact test ("fisher.test" function in Project R; ww.R-projekt.org/). The "Fe-s cluster assembly" and "Respiration" gene groups were determined according to the FungiDB database. Antioxidant enzyme genes are also present in FungiDB (https://fungidb.org/fungidb/app) identified according to available data. The gene cluster responsible for sterigmatocystin production was collected from a study by Inglis et al. (2013). Finally stress genes were identified from the stress genes found in *A. nidulans* from the Fungal Stress Response Database (FSRD).

Analysis of transcriptomic data from *A. fumigatus*

The enrichment of genes belonging to the gene clusters of "Glycolysis", "Antioxidant enzyme", "Iron uptake", "Siderophore cluster", "Fe-S cluster protein", "Fe-S cluster assembly",

"Heme binding protein", "Heme biosynthesis", and "Ribosome protein" were also tested using the Fisher test ("fisher. test" function in the R project; www.R-project.org/). *A. fumigatus* Af293 were collected from the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database (https://www.kegg.jp/pathway/afm03010). The other gene sets were obtained from the following sites: "Glycolysis" genes from Flipphi et al. (2009), "Antioxidant enzyme", "Iron uptake", "Fe-S cluster protein", "Fe-S cluster assembly", "Heme binding protein", "Heme biosynthesis" by Emri et al (2022), and "Siderophore cluster" genes by Inglis et al (2013), as described in *A. fumigatus* Af293 gene list. Orthologs of *A. fumigatus* Af293 were determined using OrthoMCL v2.0 (https://orthomcl.org/orthomcl/app/) in *A. fumigatus* A1163. During the analysis process, we used the blast algorithm for homology search and the MCL (Markov Cluster Algorithm) algorithm (www.micans.org/mcl/index.html) for clustering the found orthologs.

New scientific results

Our data suggests that the mechanisms of the oxidative stress response in the two strains are quite different. Whereas the nature of stress response and the number of affected genes were similar in *A. nidulans* THS30.3 and in *ΔsodB* mutant strains, the changes in genes expression responses in A. fumifatus $akuB^{ku80}$ and *Δsod2* mutant strains were very different.

Sporulation and oxidative stress

Conidia of *A. nidulans* and *A. fumigatus* were more sensitive to the oxidative stress, because the activity of mitochondria encreases under germination, leading to elevetad ROS levels. According to our experiments, both the cultivation conditions and the timing of oxidative stress exposure (before or after germination) significantly influenced the sensitivity of the fungi.

The mutant strains *A. nidulans ΔsodB* and *A. fumigatus Δsod2* were especially vulnerable to the stress before germination. However, the differences decreased between wild type and mutant strains when the cultures were exposed to oxidative stress treatment after germination (e.g. in submerged culture). This leads us to conclude that targeting conidia may be a more effective antifungal strategy than targeting growing mycelia.

Compensation for the lack of MnSOD

Several antioxidant genes (such as *sodA*, *catB*, *ccp1*, *AN5440*, *trxR*, *tpxB*, and *trxA*) were upregulated in the *ΔsodB* mutant compared to the reference strain by MSB treatment. Despite

this the increased production of ethidium observed in the mutant under stress indicated a redox imbalance. Although SOD activity during treatment was not significantly different compared to wild type, alternative mechanisms were not sufficient to fully compensate. Similarly, lower SOD activity and higher superoxide levels were measured in response to MSB in the *A. fumigatus Δsod2* mutant strain, which indicates reduced stress tolerance due to lack of the Sod2 enzyme. Much more antioxidative genes were upregulated in the *Δsod2* mutant strain than in the wild type in the presence of MSB, which partly helped in the protection against oxidative stress. Examining other *sod* genes, we found that none of them compensated significantly for the gene deficiency, except AFUB-073150 gene (ortholog of *A. fumigatus* AF293 *sod4*), which showed a moderate transcriptional increase in the *Δsod2* mutant.

Effect of oxidative stress on the transcriptome of gene deletion and reference strains

Both *A. nidulans* strains showed similar transcriptomic changes both in their nature and the number of genes involved. Genes related to cell growth and division were downregulated, while those involved in autophagy, oxidative stress response, and Fe–S cluster assembly were upregulated in both strains. Based on our research MSB decreased the expression of the mitochondrial function genes (e.g. aerobic respiration, mitochondrial translation), while the genes responsible for the removal of damaged mitochondria were activated. Under these cultivation conditions it seemed that the removal of not repairable mitochondria was just as important as maintaining the integrity and function of mitochondria. In contrast, changes in the *A. fumigatus Δsod2* mutant transcriptome were more significantly different from those observed in the control strain. The number of genes responding to oxidative stress was significantly higher than those in the control strain. The cells enhanced the expression of mitochondrial function and antioxidant genes, also genes related in iron metabolism and Fe-S cluster assembly. The strong up-regulation of ribosomal protein genes indicates that the mutant was trying to compensate for oxidative stress-induced cell damage by increased protein synthesis. Although we identified several MSB-responsive genes in the *Δsod2* mutant, a major part of these were related to similar biological processes as in the wild type. This observation suggests that the absence of Sod2 did not activate completely new stress defense pathways but rather increased the intensity of the oxidative stress response. According to our results Sod2 plays a key role in reducing mitochondrial damage caused by MSB.

Differential transcriptomic effects of deletion of *sodB* and *sod2* genes

There was already a significant difference in the transcriptomes of the *A. nidulans ΔsodB* mutant and the reference strains without MSB treatment (808 genes), which was further increased by MSB stress (1741 genes). The genes of cell cycle, DNS replication, mitochondrial organization and stress response were upregulated, while the genes of ribosome biogenesis, conidial development and lipid biosynthesis were downregulated by *sodB* deletion. The genes of the sterigmatocystin biosynthetic cluster were downregulated, indicating a decrease in secondary metabolite production. The genes of aerobic respiration in both strains were downregulated under oxidative stress, but this effect was smaller in the mutant strain. The alternative oxidase gene (*aodA*) was activated in both strains. In contrast, the lack of *sod2* elicited more significant changes in the *A. fumigatus* strains. Before MSB treatment transcription of 446 genes were altered due to gene deletion, after treatment this number increased to 1940 genes. The mutant strain gave much stronger transcriptional response to MSB treatment than the wild type, but completely new stress defense mechanisms were not activated in *Δsod2*. The difference between the stress responses of the two strains of *A. fumigatus* was more in the intensity of the response. Although more genes responded in the mutant to the presence of MSB, these genes were mostly related to the same biological processes for the most part as in reference strain. In the mutant, more genes responded, especially in the areas of ribosomal, mitochondrial and heme-binding functions. The expression of genes associated with glycolysis and antioxidant enzymes increased in both strains, but to a greater extent than in the mutant.

Sensitivity to macrophages

The *A. fumigatus Δsod2* mutant strain showed increased sensitivity in interaction experiments against M-CSF- and GM-CSF-induced macrophages. According to our results the MnSOD enzyme plays an important role in defending against oxidative stress created by the host's immune system and may contribute to the survival of fungi against macrophage attack.

The effect of iron limitation and the possibility of combined therapy

The mutant strain *A. fumigatus Δsod2* showed increased sensitivity to both MSB-induced oxidative stress and deferiprone (DFP) induced iron-restriction. The combination of the two stressors caused strong growth inhibition in the mutant strain, suggesting that tolerance to oxidative stress is closely related to iron availability and effective iron metabolism

contributes to stress tolerance of the fungi. Since the virulence of the *Δsod2* strain has not been significantly reduced, the inhibition of MnSOD alone does not appear to be an effective therapeutic target. However, when combined with simultaneous inhibition of iron uptake, more significant oxidative damage can be induced, which increases the iron requirement of the fungus and thus makes the treatment more effective. This combined strategy may also be promising in the clinic, as it cannot only reduce virulence, but also help resolve the resistance that is spreading today.

To summarise the new scientific findings, I would like to list the answers to the questions posed in the objectives of my thesis and my more recent scientific results:

1. *A. nidulans* and *A. fumigatus* spores were more sensitive to oxidative stress than mycelia due to increased mitochondrial activity during germination, which naturally causes increased ROS production.

2. Deletion of the *A. fumigatus sod2* gene induced a more significant change in the stress response to MSB than the absence of the *A. nidulans sodB* gene.

3. In *A. nidulans*, the activity of some antioxidant genes (e.g. *sodA*, *catB*-catalase, peroxidases) is increased in the *sodB* deletion mutant, compensating for the lack of MnSOD.

4. In the A. fumigatus *Δsod2* strain, MSB also increased the expression of other antioxidant genes, but no significant changes were observed for the other *sod* genes.

5. Stress induced *A. nidulans* strains to increase the removal of damaged mitochondria as shown by transcriptomic data.

6. The efficient iron metabolism of *A. fumigatus* helps it to survive oxidative stress, and therefore, by inhibiting iron uptake, we observed a higher oxidative stress sensitivity.

7. In *A. fumigatus*, we observed a higher sensitivity to M-CSF- and GM-CSF-induced macrophages due to the deletion of *sod2* in the mutant strain.

8. Strategies to kill conidia may be more effective than methods developed for fungal lines already in growth.

9. Inhibition of MnSOD has been shown in the literature not to be an effective therapeutic target by itself, but in combination with inhibition of iron metabolism may induce more severe oxidative stress in fungal cells.

Summary

Protection of filamentous fungi against oxidative stress is very important for their survival and adaptation. This form of stress is ubiquitous, but pathogenic species are subjected to even higher levels of oxidative stress by the host immune system. Oxidative stress is mainly caused by ROS, including $O_2^{\bullet-}$, H_2O_2 and $^{\bullet}OH$, which damage cellular proteins, lipids and DNA after accumulation. Effective defence mechanisms against this form of stress are needed to protect cell integrity.

Manganese-containing superoxide dismutase (MnSOD), localized in the mitochondria, is an antioxidant enzyme in fungi that protects against oxidative stress. Because of its intracellular location, it plays a role in maintaining proper mitochondrial function. It converts superoxide anions generated during the electron transport chain into hydrogen peroxide, which is then neutralised by other members of the defence system. MnSOD helps maintain cellular homeostasis and prevent cell damage.

The *sodB* and *sod2* genes encode the MnSOD enzyme, which our transcriptomic analyses show to be crucial in protecting mitochondria and ensuring their proper function. We observed that the transcriptional responses of wild-type and *ΔsodB* deletion mutant strains of *A. nidulans* to oxidative stress were similar. While in the *Δsod2* mutant strain of *A. fumigatus*, the stress responses were very different from those of the control strain. Our results suggest that fungi may employ multiple strategies to protect against oxidative stress, which may be based on upregulation of alternative antioxidant enzyme genes or more efficient removal of damaged mitochondria.

In our experiments with the *Δsod2* mutant strain of *A. fumigatus*, we found an important link between iron metabolism and oxidative stress. The Sod2 enzyme is crucial for the protection of Fe-S cluster proteins in mitochondria. After deletion, the *Δsod2* mutant became highly sensitive to oxidative stress on iron-limited media. Our transcriptomic results suggest that MSB induces changes in the regulation of ribosomal and iron metabolism genes that are likely to promote cell survival.

In human pathogenic fungi, such as *A. fumigatus*, a very effective defence system against oxidative stress has been developed. So, if we consider that inhibition of MnSOD alone is not a sufficient strategy, but when combined with inhibition of iron metabolism, we can induce a stronger oxidative stress. Furthermore, we observed in our experiments that strategies targeting conidia before germination can be much more effective than attacks after germination.

Consideration of these combinations may be useful for the development of antifungal strategies in the future.

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Registry number: DEENK/127/2025.PL
Subject: PhD Publication List

Candidate: Klaudia Pákozdi
Doctoral School: Doctoral School of Nutrition and Food Sciences
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List of publications related to the dissertation

1. **Pákozdi, K.**, Antal, K., Pázmándi, K. L., Miskei, M., Szabó, Z., Pócsi, I., Emri, T.: Resynthesis of Damaged Fe-S Cluster Proteins Protects Aspergillus fumigatus Against Oxidative Stress in the Absence of Mn-Superoxide Dismutase.
   J. Fungi. 10, 1-16, 2024.
   DOI: https://doi.org/10.3390/jof10120823
   IF: 4.2 (2023)
2. **Pákozdi, K.**, Emri, T., Antal, K., Pócsi, I.: Global Transcriptomic Changes Elicited by sodB Deletion and Menadione Exposure in Aspergillus nidulans.
   J. Fungi. 9 (11), 1-15, 2023.
   DOI: http://dx.doi.org/10.3390/jof9111060
   IF: 4.2

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List of other publications

3. Leiter, É., Emri, T., **Pákozdi, K.**, Hornok, L., Pócsi, I.: The impact of bZIP Atf1ortholog global regulators in fungi.
   Appl. Microbiol. Biotechnol. 105, 5769-5783, 2021.
   DOI: http://dx.doi.org/10.1007/s00253-021-11431-7
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4. Szabó, Z., **Pákozdi, K.**, Murvai, K., Pusztahelyi, T., Kecskeméti, Á., Gáspár, A., Logrieco, A. F., Emri, T., Ádám, A. L., Leiter, É., Hornok, L., Pócsi, I.: FvatfA regulates growth, stress tolerance as well as mycotoxin and pigment productions in Fusarium verticillioides.
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5. Szabó, Z., **Pákozdi, K.**, Murvai, K., Kecskeméti, Á., Oláh, V., Logrieco, A. F., Madar, A., Dienes, B., Csernoch, L., Emri, T., Hornok, L., Pócsi, I., Leiter, É.: FvmnSOD is involved in oxidative stress defence, mitochondrial stability and apoptosis prevention in Fusarium verticillioides.
   J. Basic Microbiol. 60 (11-12), 994-1003, 2020.
   DOI: http://dx.doi.org/10.1002/jobm.202000560
   IF: 2.281

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The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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