New insights into catalases in the pathogenesis of *Sporothrix schenckii* research (#77047)

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- Article content is within the Aims and Scope of the journal.
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- Methods described with sufficient detail & information to replicate.
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- Are sources adequately cited? Quoted or paraphrased as appropriate?
- Is the review organized logically into coherent paragraphs/subsections?

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3

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New insights into catalases in the pathogenesis of *Sporothrix schenckii* research

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Pathogenic fungal infection success depends on the ability to bypass the immune response. Most strategies for fungal infection control are focused on the inhibition of virulence factors and increasing the effectiveness of antifungal drugs. Nevertheless, little attention has been focused on their physiological resistance to the immune system. Hints may be found in pathogenic fungi that also inhabit the soil. In nature, the saprophyte lifestyle of fungi is also associated with predators that can induce oxidative stress upon cell damage. The natural sources of nutrients for fungi are linked to cellulose degradation, which in turn generates reactive oxygen species (ROS). Overall, the antioxidant arsenal needed to thrive both in free-living and pathogenic lifestyles in fungi is fundamental for success. In this review, recent findings regarding catalases and oxidative stress in fungi and how these can be in close relationship with pathogenesis ~~are presented~~. Additionally, special focus is placed on catalases of *Sporothrix schenckii* as a pathogenic model with a dual lifestyle. It is assumed that catalase expression is activated upon exposure to H_2O_2, but there are reports where this is not always the case. Additionally, it may be relevant to consider the role of catalases in *S. schenckii* and why their study can assess their involvement in the virulence phenotype of different species of *Sporothrix* and when each of the three catalases are ~~most needed~~. Additionally, studying antioxidant mechanisms in other isolates of pathogenic and free-living fungi may be linked to the virulence phenotype and be potential therapeutic and diagnostic targets. Thus, the rationale for this review to place focus on fungal catalases and their role in pathogenesis in addition to counteracting the effect of immune system reactive oxygen species. ~~Compelling evidence indicates they are potential targets for treatment and diagnosis~~. Fungi that thrive in soil and have mammal hosts could shed light on the importance of these enzymes in the two types of lifestyles. We look forward to encouraging more research in a myriad of ~~research~~ areas on catalase biology with a focus on basic and applied objectives and to place these enzymes

as ~~potential~~ virulence determinants.

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Keywords: fungal catalase, virulence determinant, *Sporothrix schenckii*, reactive oxygen species, innate immunity.

Abstract

Pathogenic fungal infection success depends on the ability to ~~bypass~~ the immune response. Most strategies for fungal infection control are focused on the inhibition of virulence factors and increasing the effectiveness of antifungal drugs. Nevertheless, little attention has been focused on their physiological resistance to the immune system. Hints may be found in pathogenic fungi that also inhabit the soil. In nature, the saprophyte lifestyle of fungi is also associated with predators that can induce oxidative stress upon cell damage. The natural sources of nutrients for fungi are linked to cellulose degradation, which in turn generates reactive oxygen species (ROS). Overall, the antioxidant arsenal needed to thrive both in free-living and pathogenic lifestyles in fungi is fundamental for success. In this review, recent findings regarding catalases and oxidative stress in fungi and how these can be in close relationship with pathogenesis ~~are presented.~~ Additionally, special focus is placed on catalases of *Sporothrix schenckii* as a pathogenic model with a dual lifestyle. It is assumed that catalase expression is activated upon exposure to H_2O_2, but there are reports where this is not always the case. Additionally, it may be relevant to consider the role of catalases in *S. schenckii* and why their study can assess their involvement in the virulence phenotype of different species of *Sporothrix* and when each of the three catalases are ~~most needed~~. Additionally, studying antioxidant mechanisms in other isolates of pathogenic and free-living fungi may be linked to the virulence phenotype and be potential therapeutic and diagnostic targets. Thus, the rationale for this review to place focus on fungal catalases and their role in pathogenesis in addition to counteracting the effect of immune system reactive oxygen species. ~~Compelling evidence indicates they are potential targets for treatment and diagnosis~~. Fungi that thrive in soil and have mammal hosts could shed light on the importance of these enzymes in the two types of lifestyles. We look forward to encouraging more research ~~in a myriad of research areas~~ on catalase biology with a focus on basic and applied objectives and to place these enzymes as ~~potential~~ virulence determinants.

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42

43 1 Introduction

44 When cells are exposed to oxidative stress, specifically H_2O_2, it is assumed that
45 antioxidant enzymes are induced and perform their task to detoxify the cell milieu.
46 However, this is not always the case; sometimes, antioxidant enzymes are damaged by
47 the same molecules they should eliminate (Karakus, 2020; Nicholls, 2012).

48 Vertebrates use hydrogen peroxide as a biological weapon in combination with other
49 molecules to potentiate its effect. This is particularly efficient for damaging the
50 pathogen's DNA (Mahaseth and Kuzminov, 2017), resulting in a more complicated task
51 to survive the immune response.

52 Pathogens encode various antioxidant molecules, including catalases. Catalases (EC
53 1.11.1.6) are heme-containing enzymes that catalyze the dismutation of hydrogen
54 peroxide ($2H_2O_2$) into $2H_2O$ and oxygen (O_2). These molecules are widespread in
55 aerobic organisms and have been linked to survival during oxidative stress (Karakus,
56 2020; Nicholls, 2012). Catalases are homotetrameric proteins containing a heme group
57 buried deep in the protein. The access to the catalytic domain is through a 45 Å channel
58 where H_2O_2 residence is enhanced, rendering a selectivity for this substrate
59 (Dominguez et al., 2014) and having evolved to exclude water molecules. This allows a
60 high kinetic activity (which the km is in the range of 20 to 200 mM) (Dominguez et al.,
61 2010; Hansberg et al., 2012).

62 The sequence and structure of catalase domains are more divergent than previously
63 thought. This feature has rendered the classifications of these enzymes in three clades
64 (Dominguez et al., 2010; Horvath and Grishin, 2001). Clade I refers to catalases from
65 plants, green algae, and Clade III to archaea, bacteria, fungi, and animals (Dominguez
66 et al., 2010). These clades are proteins with subunits of 55 to 69 kDa. Clade II belongs
67 to bacteria, archaea, and fungi and is formed by larger subunits of 75 to 86 kDa; the
68 additional residues are located in the C-terminal domain and belong to type 1 glutamine
69 amidotransferase (Horvath and Grishin, 2001).

70 Catalases have complex reaction mechanisms for a simple dismutation reaction, which
71 has been a hot research topic. Although much information is available, it mostly focuses
72 on bacteria and some examples of fungal catalases. Nevertheless, catalases are still
73 being studied due to their diversity among prokaryotic and eukaryotic organisms. One
74 example is a catalase found with phenol oxidase activities and the interchange of
75 activities between catalase and phenol oxidase in *Scytalidium thermophilum* (Sutay
76 Kocabas et al., 2008). This has been observed to be relevant in polyphenol oxidation,
77 where H_2O_2 is released (Akagawa et al., 2003), thus affecting the free-living lifestyle of
78 bacteria and fungi. These enzymes have been demonstrated to have a bacterial origin
79 (Bacteroidetes) and have been found in another Ascomycota (Kamlárová et al., 2018).

In the case of some parasites that do have catalases, these enzymes have been demonstrated to play a key role against host defense mechanisms and survival. In some cases, only one catalase gene is present, but an important arsenal of other Reactive Oxygen Species (ROS) detoxifying enzymes ~~is~~ needed for survival (Kwok et al., 2004; Staerck et al., 2017), adding to our current understanding of the pathogenesis of protists.

In the literature, there are experimental conditions where fungal catalases are induced and needed for survival, with a focus on the signal transduction pathways that regulate their expression (Aguirre et al., 2006). Nevertheless, in fungal pathogens, this is not fully addressed because the best-studied Ascomycete catalases are encoded in the genome of *Neurospora crassa*, which have a link ~~in~~ morphogenesis and cell differentiation as well as for contending with environmental stressors (Aguirre et al., 2005; Fountain et al., 2016). Additionally, extensive structural studies have been carried out on *N. crassa* catalases, showing unique features for H_2O_2 binding and recognition in a water milieu (Dominguez et al., 2010) and complex inhibitory mechanisms by singlet oxygen, reducing its stability and resistance to degradation (Días et al., 2005). In the case of bovines, catalase possesses resistance to singlet oxygen. In turn, this ~~is~~ not known in pathogenic fungi and may become a potential target for treatment using other inhibitors (Kim et al., 2001).

In *Candida albicans*, the high expression of these enzymes may result in reduced fitness. High expression levels in clinical isolates result in a double-edged sword; on the one hand, it protects cells from stress conditions, but on the other hand, in the absence of stress, it reduces cell fitness that is alleviated by iron supplementation (Pradhan et al., 2017). ~~Additionally,~~ low expression levels impair the colonization of certain tissues in a mouse model (Pradhan et al., 2017).

ROS production in fungal organisms varies and asexual development is closely related to ROS present in the environment. When catalases are absent, the asexual cycle of the cell differentiation program is enhanced (Navarro-Sichán et al., 2003; Zamocky et al., 2009). Catalase expression, for instance, is related to redox balance control in plant pathogens, such as *Sclerotinia sclerotiorum*, where this enzyme is needed for cell integrity, oxidative stress resistance, pathogenicity, and antifungal resistance (Huang et al., 2021). What is truly striking in *S. sclerotiorum* is that the genome encodes seven catalases. Nevertheless, only one contributes to oxidative stress resistance (Huang et al., 2021). The role of the other catalases remains to be explored.

Determining the importance of catalases may impede the discovery of novel potential uses in diagnosing and treating pathogenic fungi. One such example is the presence of circulating antibodies in patients infected with *Histoplasma capsulatum* that recognize catalases B and P, serving as potential targets for diagnosis kits (Almeida et al., 2020), and these enzymes have been demonstrated to be required for virulence (Holbrook et al., 2013; Johnson et al., 2002).

For all the above, this review addresses the following question: why have catalases been neglected in pathogenic fungi research as both potential targets for treatment and

diagnosis? One important aspect that partially explains this is that these enzymes are assumed to be highly conserved and functionally defined in all kingdoms of life. However, oxidative stress has different outcomes in distinct organisms. Likewise, this review proposes a closer look on *Sporothrix schenkii* as an example of an emerging fungal pathogen with an evolutionary well-adapted saprophytic lifestyle.

2 Methodology

The literature was consulted through Pubmed and Google Scholar. Key words used were 'Catalase', 'Pathogenic fungi', '*Sporothrix schenckii*', and the Boolean 'and' for the combination of these keywords. Authors conducted independent review of the literature to prevent any bias.

The sequence analysis was conducted using BLASTp (Altschul et al., 1990). Protein structure prediction was conducted using AlphaFold2 (Jumper et al., 2021) with the default options, using the API hosted at Söding lab based on MMseqs2 server (Mirdita et al., 2019). Phylogenetic analysis was conducted with MEGA version 11.0.13 (Tamura et al., 2021).

3 The case of pathogenic fungi: *Sporothrix schenckii*

In the genome sequence of *S. schenckii*, three catalase coding genes were identified based on homology to *Aspergillus* and *Neurospora* genes. In RedoxiBase (http://peroxibase.toulouse.inra.fr/) (Savelli et al., 2019), only one catalase is annotated for *S. schenckii* (as KatE, accession number XP_016592737.1 or SPSK1099_11725-RA in the *S. schenckii* genome database). However, at least three were identified by BLAST analysis and expressed in response to oxidative stress (Román-Casiano et al., 2021). The work by Román-Casiano (2021) described the response of these three catalases in the presence of H_2O_2 and the relative expression levels, showing that Cat1, one of the small catalases, is highly expressed upon H_2O_2 exposure. The second catalase that is highly expressed is the large subunit catalase (81.4 kDa). When analyzing several fungi in RedoxiBase, the repertoire found for antioxidant enzymes is vast and varied in all species; this imposes a challenge when assessing their role, specifically in cases where two contrasting lifestyles are found in the same organism. In Ascomycota alone, catalases and catalase/peroxidases are the fourth most abundant antioxidant enzymes. The three front runners ahead of catalases are cytochrome C peroxidase, fungi-bacteria glutathione peroxidase, and hybrid ascorbate-cytochrome C peroxidase.

In the work by Román-Casiano and colleagues (2021), two isoforms (CAT1 and CAT 3, accession numbers: ERS99939.1 and ERT00986.1, respectively) were shown to be highly expressed upon exposure to oxidative stress. However, in a recent paper, Saucedo-Campa and collaborators showed that this organism's landscape is more complex than previously thought. Several moonlight proteins are induced by oxidative stress by H_2O_2 (Saucedo-Campa et al., 2022), suggesting that the arsenal for H_2O_2 detoxification in this organism is complex and involves proteins previously thought to be related to protein folding, lipid metabolism, or even metabolic enzymes that in the cell

wall may represent the first line of defense. Additionally, in the case of menadione-induced oxidative stress, other moonlight proteins are present in the cell wall as protection against oxidative stress (Félix-Contreras et al., 2020).

In the case of the catalases of *S. schenckii*, structural features can now be modeled with accuracy. The sequence features of the three catalases encoded in the *S. schenkii* genome suggest that these enzymes may play different roles depending on the organism's morphological state as either free-living or as a pathogen. In Figure 1 Panel A, BLAST analysis shows that the main homologs of *S. schenckii* catalases are clustered (Figure 1 Panel B and C). The variation in catalytic residues poses the question of whether the catalases of *S. schenckii* have different kinetic parameters and may respond differently to oxidant agents and other molecules present in the media (see below).

The other aspect to consider with catalases is the conservation of structural features. In Figure 2, Panel A shows the previously high-resolution crystal structure reported for *N. crassa* catalases, which have been studied in detail (Díaz et al., 2009). Future research can be focused on structural comparisons with other fungal organisms and may ultimately lead to the study of the kinetic and structural features of other fungal catalases. As shown here, Cat1 and Cat2 of *S. schenckii* are small catalases, while Cat3 is a member of the large catalases.

In the case of catalase 1, the relevant BLAST hits are with catalases from Ascomycetes such as *Ophiostoma piceae*, *Diaporthe sp*., *Valsa mali*, *Hypoxylon sp*., among other plant pathogens (Figure 1 Panel A). Here, the phylogenetic distribution is wider than that observed for the other two catalases.

For catalase 2, the homology with BLAST hits is the lowest of the three catalases, and the highest proteins showing homology are derived from *Fusarium*, *Trichoderma*, *Aspergillus,* and *Penicillium* species. However, the homology found is lower than that observed with the other two catalases.

Catalase 3, ~~with~~ homology to catalases from ascomycete fungi such as *Coniochaeta sp*, *Thozetella sp*, *Podospora anserina,* and others with similar lifestyles, is strikingly similar to Catalase 1 from *N. crassa*. The most distant hit is with the bioluminescent basidiomycete *Mycena chlorophos*. Overall, this is consistent with the previous report of Román-Cansiano on identifying these enzymes and renders a potential specific role of each catalase while growing in a saprophytic stage or during the interaction with the host (Román-Casiano et al., 2021).

One interesting feature of these catalases is that the catalytic residues are not conserved, especially the catalytic triad Arg 87 (conserved), tryptophan 90 (not conserved, replaced by valine), and histidine 91 (conserved) (Zámocký et al., 2009; Díaz et al., 2009) (Figure 1 Panel C), which may have contrasting affinities for H_2O_2 or inhibitory molecules (Karakus, 2020).

In *S. schenckii*, the expression patterns of the catalase genes in transcriptomic data (Giosa et al., 2020) and http://sporothrixgenomedatabase.unime.it/) are as follows: the highest expressing enzyme in the yeast form is Cat 3 (ERT00986.1) at 7.38 log2FC. For Cat1 (ERS99939.1), it is 5.44 log2FC in the yeast form. Finally, Cat 2 (ERS95255.1) was not found in the transcriptome analysis between morphologies, consistent with the findings by Román-Casiano and colleagues (2021), where even in the presence of H_2O_2, ~~the~~ expression is low. However, the zymogram analysis shown by Román-Cansiano (2021) suggests that the three catalases are expressed, and in high H_2O_2 concentrations, Cat3 loses activity completely, and a decrease in ~~total~~ catalase activity is observed. This may impact the infection progression by limiting or blocking growth.

Overall, the catalase-encoding gene distribution is complex. Even with extensive genomic data, these enzymes' congruent analysis and evolutionary aspects have been carried out in fungi, especially in pathogenic fungi (Passardi et al., 2007). Biochemical data on these enzymes are also missing, particularly regarding H_2O_2 affinity, catalytic ~~velocity,~~ and inhibitors.

The structure of fungal catalases shows that the large and small subunit catalases contain well-defined domains (Figure 2 Panel A). The heme is deeply buried in the active site and is accessible via a 45 Å tunnel. Close inspection of the catalase models from *S. shcenckii* suggests that small subunit catalases are more structurally divergent from *N. crassa* homologs. Overall, the conserved residues are in the vicinity of the active site. Cat3 from *S. schenckii* shows a conserved structure compared to the well-defined *N. crassa* large subunit catalase (Figure 2).

Further analysis of the cumulative genomic data may shed light on the sequence and structural differences of catalases related to differences in catalysis and stability, subcellular localization, and turnover. A surprising role for catalases was found by Nava-Ramírez and Hansberg (2020), who demonstrated that the C-terminal domain of the large-size subunit catalase from *N. crassa* possesses chaperone activity that is absent in small subunit catalases. When this C-terminal domain is transferred to small subunit catalases, it functions as a chaperone as well, rendering a more stable enzyme not only for H_2O_2 but also for other stress conditions (Hansberg et al., 2022). ~~The~~ C-terminal domain originates from the fusion of the bacterial small subunit catalase and Hsp31 chaperone (Hansberg et al., 2022). The chaperone activity is closely related to the effect of ROS and the misfolding of proteins, rendering catalases a secondary tool for preventing cell damage. The structural features found in catalase 3 of *S. schenckii* may also possess this activity (Figure 2 Panel B, catalase 3), which is also relevant during exposure to innate immune cells.

The biochemical features of *S. schenckii* catalases and experimental determination of their structure are lacking. Additionally, their role in infection has not been studied in detail. The evidence suggests that these enzymes are relevant to oxidative stress, but further research is needed.

4 Future research

The study of both the free-living and the pathogenic lifestyle of *S. schenckii* is relevant to understanding dissemination and zoonosis. In the case of fungi that interact with plant hosts, such as *Trichoderma atroviride*, its genome encodes two catalase-peroxidases (http://peroxibase.toulouse.inra.fr/). ~~In the case of~~ *T. atroviride*, the role of these catalases has not been addressed, but KatG2 (TatKatG2) sequence analysis suggests that it is a secreted enzyme (Zámocký et al., 2009).

An important feature of oxidative stress and radical detoxifying enzymes is linked to cell damage in *T. atroviride*. Hernández-Oñate and colleagues (2012) described that NADPH oxidase-dependent ROS production is linked to development upon physical cell injury. H_2O_2 and oxylipins are signaling molecules shared in all kingdoms of life that respond to damage. Moreover, ~~C~~atalase 2 is downregulated in transcriptomic data, suggesting that H_2O_2 is a part of the signaling for injury repair and needs to accumulate in the hyphae; this remains an open question in the case of pathogenic fungi and the role of ROS in the differentiation process, cell damage and the regulation of cell death mechanisms.

Oxidative stress is linked to cellulose degradation and involves the generation of hydroxyl radicals via the Fenton reaction from the H_2O_2 produced by the lytic polysaccharide monooxygenases (LPMOs) secreted by fungi (Li et al., 2021; Castaño et al., 2018). ROS that are produced in this process also have a deleterious effect on antioxidant enzymes such as oxidases, glutathione S-transferases, and thioredoxins, which may increase cell damage by reducing antioxidant enzymes (Castaño et al., 2021), while glycoside hydrolases are adapted to such conditions. Taking the data from Román-Cansiano (2021) and the observation that cellulose degradation requires and exacerbates ROS production and antioxidant enzymes are sensitive to this environmental insult, it is tempting to test catalase activities in *Sporothrix* and other pathogenic fungi growing with cellulose as a carbon source and to test which catalase is more active or is resistant to oxidative stress during the free-living lifestyle of these organisms. For instance, it remains to be ~~seen~~ whether the expression of a β-glucosidase with transglycosylation and cellulase activities are involved in the in vivo cellulolytic complex of *S. shenckii* (Hernández-Guzmán et al., 2016).

The regulatory pathways for the antioxidant response are also diverse in fungi. The antioxidant counteracting transcription factors are also involved in virulence traits in plant pathogens (Singh et al., 2021), which is related to the role of ROS and cell damage (Hernández-Oñate et al., 2012). The varying lifestyle of *S. schenckii* poses the open question of how to cope with the various ROS stress encountered in this organism's ~~two~~ lifestyles.

To our surprise, little is known about the inhibition of fungal catalases. The canonical catalase inhibitors are sodium azide, hydroxylamine, potassium cyanide, salicylic acid (also a molecule involved in plant defense systems), metal ions, and 3-amino-1,2,3-triazol, but no quantitative or structural studies have been carried out with catalases from fungi. The best examples are either mammalian or bacterial purified enzymes (Ma et al., 2017).

One concerning setting is the activation of catalases; one study showed that metformin, a common anti-diabetic drug, activates catalase in a mouse model with tetrachloride-induced severe oxidative liver injury (Dai et al., 2014); thus, the detailed role of catalases in pathogenic fungi could lead to preventive actions in patients undergoing metformin treatment. Additional evidence of catalase activation is the role of the alkaloid piperine in enhancing its activity (Caceres et al., 2017). Another interesting catalase activator is vanillin and vanillic acid in animal models (Salau et al., 2020), suggesting that further research is needed to discover and use antifungal treatments.

The inhibition of catalases may require extensive experimental analysis for each fungal catalase. There are cases where catalases are inhibited with relatively harmless molecules derived from natural products such as tea catechins or plant flavonoids (Pal et al., 2014; Krych and Gebicka, 2013) or simply by ethanol (Temple and Ough, 1975). Another relevant aspect is the inhibition of catalase by natural means, such as targeting heme iron with molecules present in the respiratory burst, such as reactive nitrogen species. Heme binds molecules such as nitric oxide, cyanide, and hydrogen sulfide (Bieza et al., 2015; Milani et al., 2005); thus, exploring another hydrogen peroxide detoxifying enzyme, such as peroxidases, is relevant to the mechanism of invasion and survival ~~by~~ pathogenic fungi of mammalian and plant hosts.

Additionally, a collection of different compounds found in the plant *Jacquima macrocara* that inhibit the growth and spore germination of *Fusarium* ~~verticillioides~~ inhibits catalase activity completely at 1.25 mg/mL of the plant extract (Valenzuela-Cota el al., 2019). The repercussions of finding novel antimicrobial compounds that one of its targets is the antioxidant capacity of pathogenic fungi is worth exploring further, not only for human pathogens but also for veterinary purposes and phytopathogenic fungi.

Environmental hazards can also be of interest (Asemoloye et al., 2018). Asemoloye and colleagues (2018) found that crude ~~oil~~ induced catalases, laccases, and peroxidases in fungal organisms present in the rhizosphere. These results are relevant for the biodegradation of oil-derived molecules and strong selective pressure for fungi that, as demonstrated, require degrading enzymes such as laccase and an arsenal of antioxidant enzymes but are also strong selective pressure for pathogenic fungi with a free-living stage.

The redox state regulates the circadian response in fungi (~~L~~ang et al., 2022). Nevertheless, does this influence the pathogenic state of *Sporothrix* and other pathogenic fungi, such as *Metharizium,* in response to light? In particular, survival mechanisms during UV light exposure (Brancini et al., 2022) or the role of conidia formation and other biological aspects of cell differentiation, such as the outcome of light of different wavelengths, have been reported in *Metarhizium* (~~L~~as et al., 2020). On the other hand, cell differentiation in fungi depends on ROS, specifically for the formation of invasive structures such as the appressorium in *Magnaporthe oryzae* (~~Z~~ou et al., 2019). In vivo measurements of ROS during cell differentiation or invasion could shed light on the role of ROS in dimorphic pathogenic fungi.

All questions regarding the role of catalases and the antioxidant arsenal can be first assessed in alternative infection models, such as *Tenebrio molitor* (Lozoya-Pérez et al., 2021; de Souza et al., 2015). Using *T. molitor* as a model, relevant information can be obtained from gene knockouts or silencing of catalase genes.

Are other conditions relevant for catalase regulation? Recently, it was found that different species of *Sporothrix* show lower survival rates due to abnormal cell-wall composition during carbon and nitrogen starvation and are also linked to the virulence phenotype elicited by different members of the *Sporothrix* complex (Lozoya-Pérez et al., 2020). One interesting feature to explore is whether catalases and other antioxidant enzymes are downregulated during starvation, which may also reduce virulence.

Finally, do pathogenic fungi possess bifunctional catalases, which may be involved in the free-living lifestyle and have a pivotal role in host invasion? One such example is the bifunctional catalase MkatG1 in the locust-specific pathogen *Metarhizium acridum* (Keyhani et al., 2017). In this insect pathogen, catalase is induced during exposure to the cuticle and ~~appressorium formation~~. In the mutant lacking this catalase, germination and appressorium formation are reduced on locust wings ~~and~~ quinone/phenolic compounds, showing the relevance of this catalase/peroxidase enzyme in host invasion.

Overall, catalases offer the opportunity to revisit their role and can provide potential solutions for antifungal therapies. Linares and colleagues found that anticancer drugs enhance the activity of catalases in *C. albicans*, which could contribute to the concomitant infections of this pathogen among patients undergoing chemotherapy (Linares et al., 2006).

The case of the *Candida* genus is particularly relevant to the study of catalases and pathogenesis. Firstly, members of the *Candida* genus contain differences in their cell wall components, resulting in a differential recognition by the immune system (Navarro-Arias et al., 2019). Secondly, this genus shows a geographic-dependent prevalence and, thus, different phenotypes related to antifungal drugs and virulence determinant production (Ziccardi et al., 2015), rendering it a hot topic to analyze with other aspects such as catalase production. Finally, the relationship of some members of the *Candida* genus and higher expression levels of virulence factors, resistance to polyenes, azoles, and echinocandins, along with higher catalase expression, is part of the pathogenesis, as demonstrated for *Candida glabrata* (Figueiredo-Carvalho et al., 2017).

In the case of fungal pathogens, there are still several basic physiological aspects to be explored to fully assess ways of controlling fungal infections and reducing ~~treatment~~ resistance. Also, the study of clinical or specific geographical isolates will help to determine virulence and resistance to antifungal drugs (Ziccardi et al., 2015), which may be favored by higher catalase expression or diversity.

5 Conflict of Interest

Bernardo Franco is an Academic Editor for Peer J. The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

The authors conceived the review, revised and discussed the current literature, analyzed additional data, wrote the manuscript, and prepared the figures.

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611 **Figure legends**:

612 Figure 1. Sequence and structural features of *S. schenckii* catalases. Panel **A**, BLAST
613 analysis was used to identify the closest homologs for the three catalases of *S.*
614 *schenckii,* and 100 hits were downloaded and visually represented in pairwise identity
615 2D maps with Alignment Viewer (https://alignmentviewer.org/). In Panel A, pairwise
616 identity 2D maps are shown for the three catalases. The number of hits for catalase 1
617 (ERS99939.1) was 132. For ~~C~~atalase 2 (ERS95255.1), 177 hits were obtained, and for
618 ~~C~~atalase 3 (ERT00986.1), 140 hits were obtained. Catalase 2 shows lower homology
619 with the cognate orthologs than ~~C~~atalase 1 or 3. In Panel **B,** Sequence alignment and
620 phylogenetic analysis of the three catalases from *Neurospora crassa* and *S. shenckii*
621 (Phylogenetic analysis was conducted with MEGA version 11.0.13 (Tamura et al.,
622 2021)) In Panel **C**, Weblogo fragments representing the regions with the active site
623 residues from the sequence alignment between *N. crassa* and *S. sch~~n~~eckii* catalases.

Red arrows indicate conserved catalytic residues in all sequences, and blue arrows represent residues identified from the catalytic core but are not conserved in all catalases (data retrieved from Díaz et al., 2009).

Figure 2. Conserved structural features of *S. schenckii* catalases compared with *N. crassa* experimentally determined structures. Panel **A**, protein dimers are represented as ribbon and rainbow of *N. crassa* catalases. The PDB number is indicated. 1SY7 is the large subunit catalase/peroxidase, and 5WHS and 4BIM are the small subunit catalases. Relevant domains are indicated in the large subunit catalase, and heme is indicated with white arrows. Panel **B**, AlphaFold 2 models of the *S. schenckii* catalases and structural alignment with the three *N. crassa* catalases (RMSD 1.15). In blue is 1SY7, in green is 4BIM, in red is 5WHS, in yellow is catalase 1 (ERS99939.1), in light blue is catalase 2 (ERS95255.1), and in purple is catalase 3 (ERT00986.1). Conserved residues are indicated in magenta. Structural alignment was conducted with mTM-align (Dong et al., 2018)

Figure 1

Sequence and structural features of *S. schenckii* catalases.

Panel **A**, BLAST analysis was used to identify the closest homologs for the three catalases of *S. schenckii,* and 100 hits were downloaded and visually represented in pairwise identity 2D maps with Alignment Viewer (https://alignmentviewer.org/). In Panel A, pairwise identity 2D maps are shown for the three catalases. The number of hits for catalase 1 (ERS99939.1) was 132. For Catalase 2 (ERS95255.1), 177 hits were obtained, and for Catalase 3 (ERT00986.1), 140 hits were obtained. Catalase 2 shows lower homology with the cognate orthologs than Catalase 1 or 3. In Panel **B,** Sequence alignment and phylogenetic analysis of the three catalases from *Neurospora crassa* and *S. shenckii* (Phylogenetic analysis was conducted with MEGA version 11.0.13 (Tamura et al., 2021)). Panel **C**, Weblogo fragments representing the regions with the active site residues from the sequence alignment between *N. crassa* and *S. schneckii* catalases. Red arrows indicate conserved catalytic residues in all sequences, and blue arrows represent residues identified from the catalytic core but are not conserved in all catalases (data retrieved from Díaz et al., 2009).

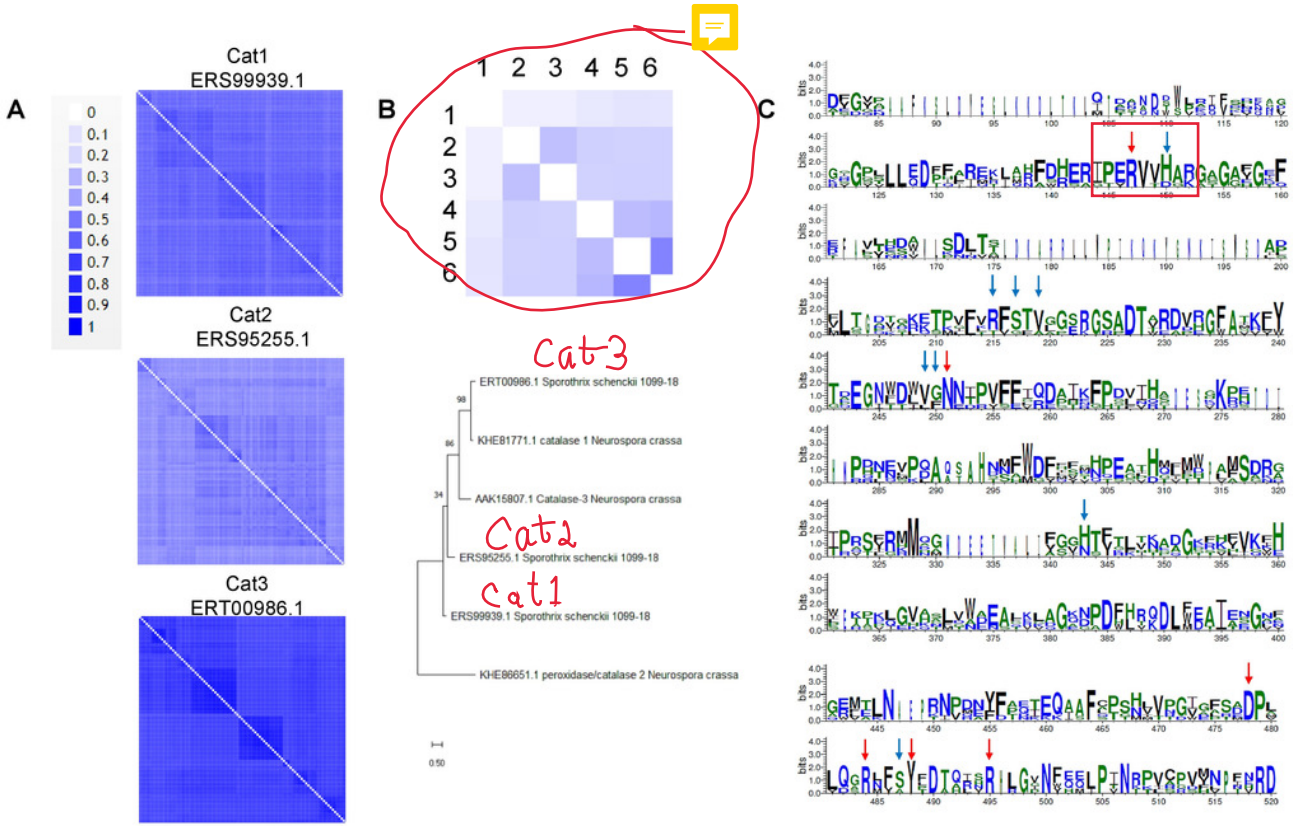
A
Cat1
ERS99939.1
Cat2
ERS95255.1
Cat3
ERT00986.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
B
1 2 3 4 5 6
1
2
3
4
5
6
Cat3
ERT00986.1 Sporothrix schenckii 1099-18
KHE81771.1 catalase 1 Neurospora crassa
AAK15807.1 Catalase-3 Neurospora crassa
Cat2
ERS95255.1 Sporothrix schenckii 1099-18
Cat1
ERS99939.1 Sporothrix schenckii 1099-18
KHE86651.1 peroxidase/catalase 2 Neurospora crassa
C

Figure 2

Conserved structural features of *S. schenckii* catalases compared with *N. crassa* experimentally determined structures.

Panel **A**, protein dimers are represented as ribbon and rainbow of *N. crassa* catalases. The PDB number is indicated. 1SY7 is the large subunit catalase/peroxidase, and 5WHS and 4BIM are the small subunit catalases. ~~Relevant~~ domains are indicated in the large subunit ~~catalase,~~ and heme is indicated with white arrows. Panel **B**, AlphaFold 2 models of the *S. schenckii* catalases ~~and~~ structural alignment with the three *N. crassa* catalases (RMSD 1.15). In blue is 1SY7, in green is 4BIM, in red is 5WHS, in yellow is catalase 1 (ERS99939.1), in light blue is catalase 2 (ERS95255.1), and in purple is catalase 3 (ERT00986.1). Conserved residues are indicated in magenta. Structural alignment was conducted with mTM-aling (Dong et al., 2018)