

CATALASE ACTIVITY OF FUNGAL STRAINS FROM THE GENERA *PENICILLIUM*, *ASPERGILLUS*, AND *TRICHODERMA*

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ABSTRACT:

The catalase activity of fungi is particularly important, playing an essential role in protecting cells against oxidative stress by decomposing hydrogen peroxides. This enzyme contributes to maintaining cellular homeostasis and supports the adaptation of microbes to stressful environmental conditions. In addition, the antioxidant capacity of fungal strains has relevant applications in biotechnology, bioremediation and the pharmaceutical industry, offering prospects for the use of these microorganisms in industrial processes and fundamental research. In this study, a screening of 23 fungal strains from the genera *Penicillium*, *Trichoderma* and *Aspergillus* was performed to evaluate their catalase capacity. The results showed a variability of catalase activity between strains, with maximum values recorded in *Penicillium* spp. 18 and *Trichoderma* spp. 12 strains, highlighting differences in the capacity to neutralize oxidative stress between genera.

Keywords: catalase, antioxidant activity, fungi, biotechnology.

1. INTRODUCTION

Fungi represent an important biotechnological resource, being used as bioproducers of metabolites and enzymes with industrial and biomedical applications (Bils, 2016; Ayuningtyas, 2021). Among the enzymes of major interest, catalase occupies a central place due to its essential role in antioxidant protection and cellular detoxification processes.

Catalase is an antioxidant enzyme that catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water and oxygen, preventing the accumulation of reactive oxygen species (ROS) and limiting oxidative stress. By regulating ROS levels, this enzyme contributes to maintaining cellular homeostasis and influences fundamental processes such as proliferation, apoptosis and metabolism. Alteration of catalase activity is associated with various metabolic and inflammatory conditions, and its therapeutic potential is being investigated in neurodegenerative, cardiovascular and inflammatory pathologies. At the same time, gene therapy strategies aimed at increasing catalase expression highlight its biological and applied importance (Rasheed, 2024; Gebicka, 2019).

From an industrial perspective, catalase is mainly obtained from mammalian liver and from the filamentous fungus *Aspergillus niger*, appreciated for its stability and high enzymatic yield. In parallel, microorganisms adapted to low temperatures (psychrophiles and psychrotrophs) represent an underexplored but promising source, due to their increased catalytic activity in the range of 0–20 °C and the reduced risk of microbiological contamination, characteristics relevant for industrial processes carried out at moderate or low temperatures (Gharaghani, 2022).

Catalase activity has also been demonstrated in species such as *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus nidulans*, *Aspergillus flavus* and *Aspergillus*

fumigatus, where it contributes to survival under oxidative conditions and adaptation to the host environment. However, for many other *Aspergillus* species, data on the regulation and expression level of catalase remain limited, which highlights the need for further research in this area (Aggez, 2022; Gharaghani, 2022).

The current interest in the valorization of fungal catalases is supported by their potential in sustainable industrial processes and in the development of enzymes adapted to extreme conditions. In particular, the exploration of psychrophiles and psychrotrophs for catalase biosynthesis is at an early stage, but offers relevant perspectives for expanding the biotechnological applications of these enzymes (El-Elimat, 2021; Gharaghani, 2022).

In conclusion, fungal catalases constitute an essential element of defense mechanisms against oxidative stress and represent an area of major interest from both a biological and biotechnological perspective; in this context, the aim of our research is to investigate and identify new fungal strains with increased catalase activity.

2. MATERIALS AND METHODS

Catalase activity was determined by the standard spectrophotometric method, based on the ability of hydrogen peroxide to react with ammonium molybdate, forming a stable-colored complex, quantifiable spectrophotometrically.

A quantity of 0.1 mL of metabolite solution and 2 mL of 0.03% H₂O₂ solution were introduced into the reaction mixture. After incubation for 10 minutes at room temperature, the reaction was stopped by adding 1 mL of 4% ammonium molybdate solution, which determines the formation of the colored complex. Catalase activity was expressed as a function of the variation of the optical density of the sample at a wavelength of 410 nm compared to the control (Komina, 2012; Titova, 2012).

Catalase activity was calculated using the formula:

$$\text{Activity CAT (U/mg protein)} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) \cdot V / v \cdot t \cdot \epsilon \cdot C_{\text{prot}}$$

where:

Abs_control – absorbance of the control sample

Abs_sample – absorbance of the analyzed sample

V – total reaction volume (mL)

v – sample volume (mL)

t – incubation time (min)

ε – extinction coefficient

C_prot – protein concentration in the sample (mg/mL)

Spectrophotometric determinations were performed, using cuvettes with an optical layer thickness of 1 cm, all samples were analyzed in triplicate to ensure reproducibility of the results, and the results were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The study of the catalase activity of fungi is of particular importance, given the essential role of the catalase enzyme in cellular protection against oxidative stress, as well as the application potential of these microorganisms in fields such as biotechnology, bioremediation and the pharmaceutical industry (Poljovka, 2023; Dishliyska, 2023).

As part of the research, a screening of 23 fungal strains belonging to the genera *Penicillium*, *Trichoderma* and *Aspergillus* was carried out with the aim of evaluating catalase activity.

For the screening, 8 strains belonging to the genus *Penicillium*, 3 strains from the genus *Aspergillus*, 8 strains from the genus *Trichoderma* were selected. Their selection was based on the previously demonstrated biotechnological interest, since, in preliminary experiments, the analysed strains showed increased levels of catalase activity, determined by express methods of enzymatic evaluation.

Within the genus *Penicillium* spp., catalase activity showed considerable variations compared to other genera, with values ranging between 123.9 and 430.4 mmol/min/mg protein. The lowest activity was recorded in strain *Penicillium* sp. 6 123.9861 mmol/min/mg protein, while strain *Penicillium* sp. 18 showed the highest enzymatic activity 430.42 mmol/min/mg protein. This variability suggests significant differences in the capacity to respond to oxidative stress, possibly correlated with adaptation to the specific conditions in the aquatic microhabitats of origin.

In the case of the genus *Aspergillus*, the catalase activity values were more homogeneous, ranging between 169 and 252 mmol/min/mg protein. The *Aspergillus* sp. 23 strain showed the highest activity 252.15 mmol/min/mg protein, closely followed by the *Aspergillus* sp. 22 strain 250.75 mmol/min/mg protein, while the *Aspergillus* sp.4 strain recorded a lower activity 169.8 mmol/min/mg protein. Compared to the *Penicillium* strains, the maximum level of catalase activity in the strains of the genus *Aspergillus* was lower, suggesting a relatively lower antioxidant capacity under the analyzed experimental conditions (Fig. 1).

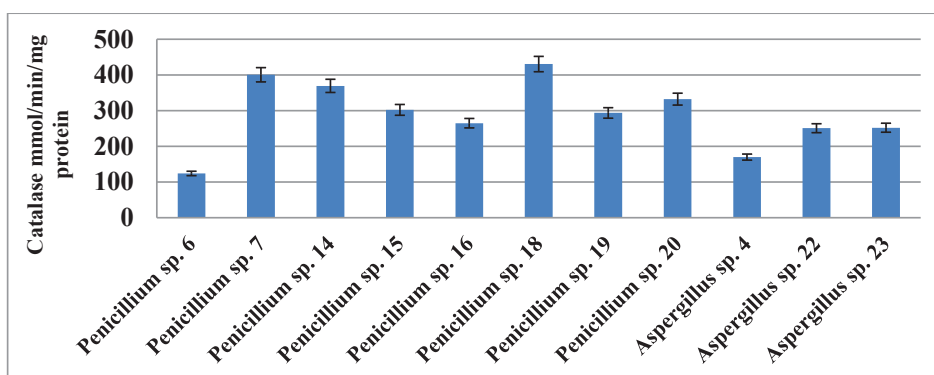


Figure 1. Catalase activity of *Penicillium* and *Aspergillus* fungal strains

Regarding strains belonging to the genus *Trichoderma*, catalase activity also revealed a pronounced metabolic variability, suggesting differences in the capacity to neutralize oxidative stress between isolates (Fig. 2).

The values recorded ranged from 118 to 342 mmol/min/mg protein. The lowest catalase activity was observed in *Trichoderma* sp. 9 strain 118.2 mmol/min/mg protein, closely followed by *Trichoderma* sp. 8 strain 122.84 mmol/min/mg protein. *Trichoderma* sp. 10, 11 and 13 strains showed relatively close values of 140–143 mmol/min/mg protein, indicating a moderate and relatively uniform level of enzymatic activity.

A higher level was revealed in the strains *Trichoderma* sp. 21 with values of 202 mmol/min/mg protein and the strain *Trichoderma* sp.17 228.8424 mmol/min/mg protein, which may reflect an increased capacity to adapt to conditions with higher oxidative potential. The strain *Trichoderma* sp. 12 is remarkable, which presented the highest catalase activity within this genus 342.2 mmol/min/mg protein, significantly exceeding the other isolates of the analyzed genus *Trichoderma*.

Compared to other previously studied genera, the maximum level of catalase activity in *Trichoderma* sp. 12 is comparable to the high values recorded in some *Penicillium* strains. At the same time, most strains of the genus *Trichoderma* fall within an intermediate range of enzymatic activity, suggesting a balanced antioxidant profile. This distribution of catalase activity indicates a specific intra-genus variability, which may reflect differential adaptations to oxidative stress conditions and highlights the selective potential of certain strains for biotechnological applications.

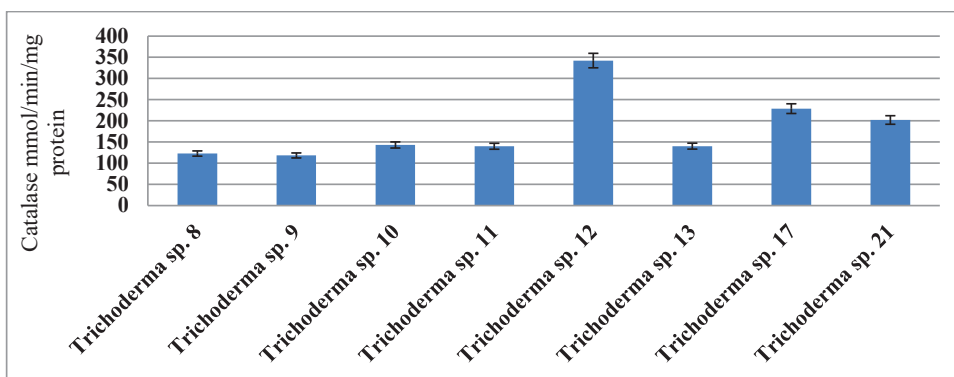


Figure 2. Catalase activity of micromycetes of the genus *Trichoderma*

The data obtained in the study indicate that fungal strains from the genera *Penicillium*, *Aspergillus* and *Trichoderma* are significant producers of catalase, which is consistent with observations reported in the literature. In particular, strains *Penicillium* sp. 18 and *Trichoderma* sp. 12 exhibited high catalase activities (430.42 and 342.2 mmol/min/mg protein, respectively), highlighting a strong antioxidant profile and supporting the potential of these genera for biotechnological applications.

The results obtained are in accordance with those reported by Aggez C. (2022), who highlighted the maximum catalase production by *Aspergillus fumigatus* after 7 days of cultivation (213 U/mL). Regarding the genus *Trichoderma*, studies conducted by Zapata-Sarmiento D. (2025) demonstrated that the response to oxidative stress induces an increase in the activity of catalase and peroxidases, thus highlighting the role of these enzymes in the adaptation and survival of fungi under challenging environmental conditions.

Furthermore, Koleva Z. et al. (2024) highlighted the interest in extracellular catalase, due to its biotechnological applicability and ease of purification, indicating that *Penicillium* constitutes a valuable source of catalase for industrial and biotechnological processes. These findings support our observations on the high activity of *Penicillium* sp. 18 strains, compared to other analyzed strains, and confirm their potential as efficient catalase producers.

4. CONCLUSIONS

As a result of the research, the strains *Penicillium* sp. 18 (430.42 mmol/min/mg protein) and *Trichoderma* sp. 12 (342.2 mmol/min/mg protein) were identified as producing catalase at significantly higher levels compared to the other analyzed strains. These results highlight the metabolic variability between isolates and suggest a superior capacity to neutralize oxidative stress in these strains. The selection of these microorganisms for catalase production demonstrates their biotechnological potential, indicating the possibility of their use as efficient producers of antioxidant enzymes.

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DECLARATIONS

The authors declare that they have no conflict of interest.

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REFERENCES

1. Ageze, C. A., & Karakus, Y. Y. (2022). Production, purification and characterization of catalase from *Aspergillus fumigatus*. *Journal of Microbiology & Biotechnology*, 7. <https://doi.org/10.23880/oajmb-16000246>
2. Ayuningtyas, E., Sibero, M., Elisabet, B. R. H., Nadia, F., Evan, Murwani., Retno, Z., Dewi, S., Wijayanti, D., Sabdono, A., Pringgenies, D., Radjasa, O., & Zhang, Z. (2021). Screening of extracellular enzyme from phaeophyceae-associated fungi. *IOP Conference Series: Earth and Environmental Science*. <https://doi.org/10.1088/1755-1315/750/1/012005>
3. Bills, G. F., & Gloer, J. B. (2016). Biologically active secondary metabolites from the fungi. *Microbiology Spectrum*, 4(6). <https://doi.org/10.1128/microbiolspec.FUNK-0023-2016>
4. Dishliyska, V., Stoyancheva, G., Abrashev, R., Miteva-Staleva, J., Spasova, B., Angelova, M., & Krumova, E. (2023). Catalase from the Antarctic fungus *Aspergillus fumigatus* I-9: Biosynthesis and gene characterization. *Indian Journal of Microbiology*, 63(4), 541–548. <https://doi.org/10.1007/s12088-023-01110-8>
5. El-Elimat, T., Raja, H. A., Figueroa, M., Sharie, A. H., Bunch, R. L., & Oberlies, N. H. (2021). Freshwater fungi as a source of chemical diversity: A review. *Journal of Natural Products*, 84, 898–916. <https://doi.org/10.1021/acs.jnatprod.0c01059>
6. Gebicka, L., & Krych-Madej, J. (2019). The role of catalases in the prevention/promotion of oxidative stress. *Journal of Inorganic Biochemistry*, 197, 110699. <https://doi.org/10.1016/j.jinorgbio.2019.110699>
7. Gharaghani, M., Jafarian, H., Hatami, M., Shabanzadeh, M., & Zarei Mahmoudabadi, A. (2022). Evaluation of catalase activity of clinical and environmental isolates of *Aspergillus* species. *Iranian Journal of Microbiology*, 14(1), 133–137. <https://doi.org/10.18502/ijm.v14i1.8815>
8. Koleva, Z., Abrashev, R., Angelova, M., Stoyancheva, G., Spasova, et al. (2024). A novel extracellular catalase produced by the Antarctic filamentous fungus *Penicillium rubens* III11-2. *Fermentation*, 10, 58. <https://doi.org/10.3390/fermentation10010058>
9. Komina, A. V., Korostileva, K. A., Gyrylova, S. N., Belonogov, R. N., & Ruksha, T. G. (2012). Interaction between single nucleotide polymorphism in catalase gene and catalase activity under the conditions of oxidative stress. *Physiological Research*, 61, 655–658. <https://doi.org/10.33549/physiolres.932333>
10. Titova, N.M., Subbotina, T.N. (2012) *Enzymology: Laboratory Workshop* (in Russian). Krasnoyarsk: Siberian Federal University. 60 p. <https://elib.sfu-kras.ru/handle/2311/63424>
11. Poljovka, A., Musil, M., Bednář, D., Chovanová, K., Bauerová-Hlinková, V., Bellová, J., Kohútová, L., Baráth, P., & Zámocký, M. (2023). Comparison of fungal thermophilic and mesophilic catalase–peroxidases for their antioxidative properties. *Antioxidants*, 12(7), 1382. <https://doi.org/10.3390/antiox12071382>
12. Rasheed, Z. (2024). Therapeutic potentials of catalase: Mechanisms, applications, and future perspectives. *International Journal of Health Sciences (Qassim)*, 18(2), 1–6. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10915913/>
13. Zapata-Sarmiento, D. H., Rodríguez-Hernández, A. A., Sepúlveda-Jiménez, G., & Rodríguez-Monroy, M. (2025). Tolerance and antioxidant response to heavy metals are differentially activated in *Trichoderma asperellum* and *Trichoderma longibrachiatum*. *PeerJ*, 13, e19016. <https://doi.org/10.7717/peerj.19016>