Iranian Journal of Microbiology
Volume 14 Number 1 (February 2022) 133-137

ORIGINAL ARTICLE

Evaluation of catalase activity of clinical and environmental isolates of *Aspergillus* species

Maral Gharaghani[1], Hadis Jafarian[1], Maryam Hatami[2], Mahboubeh Shabanzadeh[2], Ali Zarei Mahmoudabadi[1,2*]

[1]*Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*
[2]*Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

Received: January 2021**, Accepted:** November 2021

ABSTRACT

Background and Objectives: Catalases are a good scavenger of H_2O_2 which degrades hydrogen peroxide into water and oxygen. They are considered as a virulence factor that are present in both spores and hypha of fungi. There is limited data regarding catalase activity in *Aspergillus* species. This study aimed to assess the mycelial catalase activity of clinical and environmental isolates of *Aspergillus niger, A. tubingensis, A. flavus, A. luchuensis, A. piperis* and *A. terreus.*
Materials and Methods: Briefly, clinical and environmental *Aspergillus* species were used in the current study. Catalase activity was assessed for both groups of isolates including 13 *A. flavus* (12 clinical, 1 environmental), 13 *A. terreus* (8 clinical, 5 environmental), 26 *A. tubingensis* (13 clinical, 13 environmental), and 44 *A. niger* (25 environmental, 19 clinical) species. Fungal balls of mycelia were separated from the liquid culture and were crushed using homogenizer. The supernatants were collected and used for a catalase activity assay.
Results: Totally, in our study 98 *Aspergillus* including 45 environmental and 53 clinical isolates were assessed for catalase activity. High catalase activity was detected among environmental *Aspergillus* species (Mean= 1.62 mU/ml) and the mean of mycelial catalase activity among clinical *A. terreus* isolates was higher than environmental strains.
Conclusion: In summary, mycelial catalase activity varied among species and environmental isolates demonstrated higher catalase activity. Totally a significant difference was found between clinical and environmental *Aspergillus* isolates.

Keywords: Catalase; *Aspergillus*; Virulence factor; Clinical; Aspergillosis

INTRODUCTION

Catalases are the extracellular and intracellular enzymes existing in bacteria, fungi, plants, and animals. They are a haem-containing antioxidant enzyme and a good scavenger of H_2O_2 which degrades H_2O_2 into H_2O and O_2 and have therapeutics and industrial applications (1-3). Catalase is a putative virulence factor in yeasts, spores, and fungal hypha. Detoxification of hydrogen peroxide by catalase enzyme has been suggested as a way to overcome the host immune response. *In vitro* studies showed hydrogen peroxide produced by neutrophil play an essential role in the killing of fungal hyphae (4) and this process is blocked by the addition of a commercial catalase (5).

*Corresponding author: Ali Zarei Mahmoudabadi, Ph.D, Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran; Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Tel: +98-6133330074 Fax: +98-6133332036 Email: zarei40@hotmail.com

Aspergillus species are saprotrophic fungi that mostly occurring in the soil on decaying organic materials. Aspergillosis mostly occurs as an opportunistic infection in immunocompromised patients. Despite advances in antifungal drugs, aspergillosis remains a major health problem and rapidly grows in high-risk patients. Previous studies suggest that catalase A, primarily is a peroxisomal protein and a key antioxidant protein, is also found in cellular organelles such as mitochondria and the nucleus (6, 7). Catalase activity is mostly reported from *Candida albicans* (8), *Cryptococcus neoformans* (9), *Aspergillus nidulans* (10-12), *A. flavus* (13) and *A. fumigatus* (14, 15). However, there is limited data regarding catalase activity in other *Aspergillus* species. This study aimed to assess the mycelial catalase activity of clinical and environmental isolates of *A. niger, A. tubingensis, A. flavus, A. luchuensis, A. piperis* and *A. terreus.*

MATERIALS AND METHODS

Isolates and identification. Clinical *Aspergillus* species (53 isolates) were previously isolated from patients with otomycosis, onychomycosis, and environmental *Aspergillus* species (45 isolates) were previously isolated from air samples. Identification of isolates was assessed according to morphological characteristics and sequencing of the β-Tubulin gene (16, 17) (Table 1). Preserved isolates in sterile distilled water, were cultured on Sabouraud dextrose agar (Scharlau, Spain) and incubated at ambient temperature for one week.

Determination of catalase activity. Catalase activity was assessed for 98 isolates of *Aspergillus* species including 53 clinical and 45 environmental isolates. Isolates were grown in 1% yeast extract medium (Merck, Germany) in a shaker incubator at 100 rpm for 3 days. After incubation, fungal balls of mycelia were separated from the culture broth by filtration using Whatman filter paper. Harvested mycelia washed twice with 0.05 M Tris-HCl (pH=7.5) and dried in 200 µl acetone. One gram of dried mycelia was resuspended in 500 µl of 10 mM Tris-HCl (PH=7.8) containing acid-washed glass beads. Cell suspensions were crushed a SpeedMill PLUS Homogenizer (Analytikjena, Germany). After centrifugation at 100g for 15 min, the supernatants were collected and used for a catalase activity assay. Catalase activity was determined using a Catalase Assay Kit (Navand Salamat, Iran). After that, the microplate was read by Elisa reader (BioTek, USA) at 540 nm. Finally, the catalase standard curve was drawn based on a serial dilution of the standard catalase solution (13).

Statistical analysis. All data were analyzed using SPSS 22.0 and statistically, significance ($p < 0.05$) was calculated. Therefore, a non-parametric t-test was used to analyze the data.

RESULTS

Overall, in our study 98 *Aspergillus* isolates were tested for catalase activity. These strains were selected from environmental (n=45) and clinical (n=53) sources. The standard curve of catalase activity has been shown in Fig.1. As shown in Fig. 1, according to the regression coefficient (R2= 0.98) curve was linear.

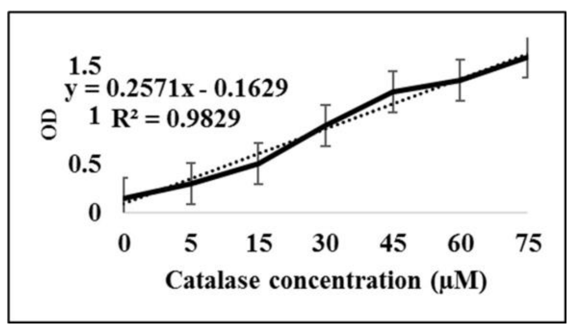

Fig. 1. The standard curve of catalase activity according to the kit method, as the regression coefficient (R^2= 0.98) curve was linear

Table 1. Clinical and environmental isolates of *Aspergillus* used in this study

Isolates sources		*A. flavus*	*A. terreus*	*A. tubingensis*	*A. niger*	*A. luchuensis*	*A. piperis*	Total
Clinical	Otomycosis	12	1	13	19	1	--	53
	Onychomycosis	--	7	--	--	--	--	
Environmental		1	5	13	25	--	1	45
Total		13	13	26	44	1	1	98

In the present study, all *Aspergillus* isolates were showed catalase activity but enzymatic secretion varied among species. High catalase activity was detected among environmental *Aspergillus* species (Mean = 1.62 mU/ml). Still, the mean of mycelial catalase activity among clinical *A. terreus* isolates was higher than environmental strains. Also, as shown in table 2 statistical analysis revealed that among all clinical and environmental *Aspergillus* species only in *A. niger* isolates difference was significant (p= 0.005844, p< 0.05). Likewise, a significant difference was found between clinical and environmental *Aspergillus* isolates (p=0. 025813, p< 0.05).

DISCUSSION

Aspergillus species are saprophytic fungi that able to grow on different substrates and secreted a variety of metabolites and enzymes (10). Catalase secretion is one of these enzymes that have been identified in *Aspergillus* species and produced during invasive disease. Several reports have detected that catalase activity among this genus protects them from cellular damage to the host (10, 18). However, few studies have focused on the secretion of catalase in different clinical species of *Aspergillus* (13). Our study showed that mycelial catalase activity was detected among different species of *Aspergillus* isolates. Besides, based on our finding a significant difference was found in catalase activities between clinical and environmental *Aspergillus* isolates. As a result, the average catalase secretion in environmental isolates was higher than in clinical isolates. However, Rouein et al. reported higher mycelial catalase activity in clinical isolates of *A. flavus* and *A. fumigatus* than environmental isolates (13).

Shibuya et al. have believed that mycelial catalases in *Aspergillus* transiently protect it from the reactions of host defence (19) and the catalase activity of mycelial form is greater than conidial form in *Aspergillus* (13, 20). Gallin et al. believed that catalase activity is contributing to the pathogenicity of invasive aspergillosis, especially in immunocompromised patients (21). Besides, Paris et al, indicated that the inactivation of catalase secretion in *A. fumigatus* strains resulted in reduced virulence in mice (20). In our study, only clinical strains of *A. terreus* had shown an increased level of catalase activity compared to environmental strains. More data are available in catalase secretion by fungi, industrial properties and their activity in literatures (18, 22, 23). However, there is no more data that compared the catalase activity in clinical and environmental isolates of *Aspergillus*. Generally, similar to other studies, our clinical and environmental strains were secreted variable amount of catalase. However, we found that clinical isolates have a higher catalase activity than environmental isolates.

A recent study suggests that catalase is involved in Aflatoxin B1 biosynthesis (22). Also, Wang et al. showed that catalase activity is an essential factor

Table 2. Catalase activity among different clinical and environmental *Aspergillus* species

***Aspergillus* species**	**No.**	**Catalase activity**			**p-value**
		Lowest	**Highest**	**Mean**	
A. niger C	19	0.18	2.46	1.34	p = 0.005844.
A. niger E	25	0.97	3	1.88	significant at p < 0.05
A. tubingensis C	13	0.06	2.76	1.46	p = 0.54681.
A. tubingensis E	13	0.49	2.94	1.66	not significant at p < 0.05
A. flavus C	12	0.38	2.84	1.43	-
A. flavus E	1	0.33	-	-	-
A. terreus C	8	0.18	0.85	0.65	p = 0.809634.
A. terreus E	5	0.44	0.69	0.57	not significant at p < 0.05
A. luchuensis C	1	0.69	-	-	-
A. piperis E	1	1.28	-	-	-
Total C isolates	53	0.06	2.84	1.28	P = 0.025813.
Total E isolates	45	0.33	3	1.62	significant at p < 0.05

C: Clinical, E: Environmental

in fungi for adaptation to environmental stress (18). Therefore, the role of catalase activity in environmental isolates seems to be better growth. In the present study, different species of *Aspergillus* could secret catalase enzyme and this rate was highest in the *Aspergillus niger* species complex. It is important to note that the presence of melanin in this complex can play a role similar to the catalase enzyme (24).

CONCLUSION

In summary, mycelial catalase activity varied among species and environmental isolates demonstrated higher catalase activity. Totally a significant difference was found between clinical and environmental *Aspergillus* isolates. Since catalase activity in *Aspergillus* isolates is associated with their pathogenesis, this study can help to understand the pathogenesis of different species of *Aspergillus* in various forms of its disease.

ACKNOWLEDGEMENTS

Authors would like to thank Ahvaz Jundishapur University of Medical Sciences for the financial support of this study (Grant number OG: 9959).

REFERENCES

1. Scibior D, Czeczot H. [Catalase: structure, properties, functions]. *Postepy Hig Med Dosw (Online)* 2006;60:170-180.
2. Grigoras AG. Catalase immobilization - A review. *Biochem Eng J* 2017;117:1-20.
3. Day BJ. Catalytic antioxidants: a radical approach to new therapeutics. *Drug Discov Today* 2004;9:557-566.
4. Urban CF, Nett JE. Neutrophil extracellular traps in fungal infection. *Semin Cell Dev Biol* 2019;89:47-57.
5. Warris A, Ballou ER. Oxidative responses and fungal infection biology. *Semin Cell Dev Biol* 2019;89:34-46.
6. Petrova VY, Rasheva TV, Kujumdzieva AV. Catalase enzyme in mitochondria of *Saccharomyces cerevisiae*. *Electron J Biotechnol* 2002;5:11-12.
7. Skoneczny M, Rytka J. Oxygen and haem regulate the synthesis of peroxisomal proteins: catalase A, acyl-CoA oxidase and Pex1p in the yeast *Saccharomyces cerevisiae*; the regulation of these proteins by oxygen is not mediated by haem. *Biochem J* 2000;350:313-319.
8. Linares CE, Giacomelli SR, Altenhofen D, Alves SH, Morsch VM, Schetinger MR. Fluconazole and amphotericin-B resistance are associated with increased catalase and superoxide dismutase activity in *Candida albicans* and *Candida dubliniensis*. *Rev Soc Bras Med Trop* 2013;46:752-758.
9. Giles SS, Stajich JE, Nichols C, Gerrald QD, Alspaugh JA, Dietrich F, et al. The *Cryptococcus neoformans* catalase gene family and its role in antioxidant defense. *Eukaryot Cell* 2006;5:1447-1459.
10. Calera JA, Sanchez-Weatherby J, Lopez-Medrano R, Leal F. Distinctive properties of the catalase B of *Aspergillus nidulans*. *FEBS Lett* 2000;475:117-120.
11. Scherer M, Wei H, Liese R, Fischer R. *Aspergillus nidulans* catalase-peroxidase gene (cpeA) is transcriptionally induced during sexual development through the transcription factor StuA. *Eukaryot Cell* 2002;1:725-735.
12. Nekiunaite L, Arntzen MØ, Svensson B, Vaaje-Kolstad G, Abou Hachem M. Lytic polysaccharide monooxygenases and other oxidative enzymes are abundantly secreted by *Aspergillus nidulans* grown on different starches. *Biotechnol Biofuels* 2016;9:187.
13. Rouein S, Ghasemi F, Badiee P. Compare catalase activity between *Aspergillus flavus* and *A. fumigatus*, isolated from clinical and environmental specimens. *Jundishapur J Microbiol* 2020;13(8):e103634.
14. Boysen JM, Saeed N, Wolf T, Panagiotou G, Hillmann F. The peroxiredoxin Asp f3 acts as redox sensor in *Aspergillus fumigatus*. *Genes (Basel)* 2021;12:668.
15. Goetz KE, Coyle CM, Cheng JZ, O'Connor SE, Panaccione DG. Ergot cluster-encoded catalase is required for synthesis of chanoclavine-I in *Aspergillus fumigatus*. *Curr Genet* 2011;57:201-211.
16. Hivary S, Fatahinia M, Halvaeezadeh M, Zarei Mahmoudabadi A. The potency of luliconazole against clinical and environmental *Aspergillus nigri* complex. *Iran J Microbiol* 2019;11:510-519.
17. Moslem M, Zarei Mahmoudabadi A. The high efficacy of luliconazole against environmental and otomycosis *Aspergillus flavus* strains. *Iran J Microbiol* 2020;12:170-176.
18. Wang ZL, Zhang LB, Ying SH, Feng MG. Catalases play differentiated roles in the adaptation of a fungal entomopathogen to environmental stresses. *Environ Microbiol* 2013;15:409-418.
19. Shibuya K, Paris S, Ando T, Nakayama H, Hatori T, Latge JP. Catalases of *Aspergillus fumigatus* and inflammation in aspergillosis. *Nihon Ishinkin Gakkai Zasshi* 2006;47:249-255.
20. Paris S, Wysong D, Debeaupuis JP, Shibuya K, Philippe B, Diamond RD, et al. Catalases of *Aspergillus fumigatus*. *Infect Immun* 2003;71:3551-3562.

21. Gallin JI, Alling DW, Malech HL, Wesley R, Koziol D, Marciano B, et al. Itraconazole to prevent fungal infections in chronic granulomatous disease. *N Engl J Med* 2003;348:2416-2422.
22. Yun Y, Lu Z, Yang J, Liang T, Xiao G, Qiao Y, et al. Electrochemical analysis of specific catalase activity during development of *Aspergillus flavus* and its correlation with aflatoxin B1 production. *Food Chem* 2021;337:127978.
23. Zhu Z, Yang M, Bai Y, Ge F, Wang S. Antioxidant-related catalase CTA1 regulates development, aflatoxin biosynthesis, and virulence in pathogenic fungus *Aspergillus flavus*. *Environ Microbiol* 2020;22:2792-2810.
24. Romsdahl J, Blachowicz A, Chiang AJ, Singh N, Stajich JE, Kalkum M, et al. Characterization of *Aspergillus niger* isolated from the international space station. *mSystems* 2018;3(5):e00112-18.