Indian J Microbiol (Oct–Dec 2016) 56(4):445–450
DOI 10.1007/s12088-016-0593-5

ORIGINAL ARTICLE

Study on Antioxidant Enzymatic Activities of *Trichosporon asahii*

Yangmei Zhang[1] · Haitao Li[1] · Rongya Yang[1] · Congmin Wang[1]

Received: 20 February 2016 / Accepted: 30 April 2016 / Published online: 7 May 2016
© Association of Microbiologists of India 2016

Abstract Superoxide dismutase (SOD) and catalase are considered the most important antioxidant enzymes which protect fungus from the oxidant damage of reactive oxygen species. In this study, we collected 44 strains of *Trichosporon asahii* (*T. asahii*) from different sources and investigated their SOD and catalase activities. The results showed that the SOD and catalase activities of Clinical group were significantly higher than those of Environment group ($p < 0.01$). The SOD and catalase activities of *T. asahii* in Internal passage group went up gradually after passage in mice, and were significantly higher in 5th generation of Internal passage group ($p < 0.05$). The SOD and catalase activities of Fluconazole-resistant group strains also increased after resistant induction, and the SOD and catalase activities were significantly higher in the 10th generation of Fluconazole-resistant group ($p < 0.05$). This implied that *T. asahii* has stronger antioxidant ability. The strains of *T. asahii* from different sources have different antioxidant abilities, which mainly manifest in the difference of antioxidant enzymatic activities. Clinical group strains have the strongest antioxidant capacity; Internal passage group strains and Fluconazole resistant group strains better; Environmental group strains the lowest. These results also suggested that the antioxidant defensive response of *T. asahii* might be relevant to its infection mechanism and drug resistance mechanism.

Yangmei Zhang and Haitao Li have contributed equally to this work.

✉ Rongya Yang
yangrongya1958@sina.cn

[1] General Hospital of Beijing Military Command PLA, No. 5, Nanmen Cang, Dongcheng District, Beijing 100007, China

Keywords *Trichosporon asahii* · Catalase · Superoxide dismutase · Reactive oxygen species

Introduction

Reactive oxygen species (ROS) mainly include hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), superoxide anion ($O_2^{\cdot-}$) and so on [1]. Given that they could react with nucleic acids, lipid and proteins, ROS were recognized that they may nearly destroy many cell functions of organisms [1]. Many studies (including bacterium and fungus) have demonstrated that ROS could be produced by phagocytes [1] and antifungal drugs [2] during their killing process. For organisms, they have taken shape a series of ways to escape or repair these oxidant damage to survive from the deleterious ROS [1]. Among them, the antioxidant enzymes [particularly catalase, superoxide dismutase (SOD), peroxidase, glutathione systems] were well studied and related to the virulence or pathogenicity of fungus directly or indirectly [3–9].

Previous studies have shown that SOD and catalase were the most important antioxidant enzymes that work to diminish the oxidant damage of ROS [3–9]. The two antioxidant enzymes have been proved extensively in the studies of common fungus including *Candida albicans* (*C. albicans*), *Aspergillus fumigatus* (*A. fumigatus*), *Cryptococcus neoformans* (*C. neoformans*). Hwang et al. [3] and Martchenko et al. [4] proved that SOD1 and SOD5 were necessary for the virulence of *C. albicans* when infecting mice, respectively. The catalase of *C. albicans* may also enhance its pathogenicity to human neutrophils [5]. Similar to *C. albicans*, the SOD of *A. fumigatus* may have some role as a virulence factor when defending against neutrophil and phagocyte [6], whereas its mycelial catalase could only protect this fungus from the host for a while [7].

In *C. neoformans*, its Cu, ZnSOD helped to protect fungal cells from macrophages, but was not a virulence factor as well as its catalase [8, 9].

In recent years, the incidence of disseminated trichosporonosis caused by *Trichosporon asahii* increases obviously, accounting for 5–10 % of deep infection of fungus in patients [10]. Moreover, *T. asahii* is becoming more and more resistant to antifungal drugs [10]. Once *T. asahii* caused disseminated or systemic infection in the body, its mortality could be more than 80 % [11], which making the therapy become more harder. Therefore, it will be of great significance to explore the infection mechanism and pathogenic mechanism of *T. asahii* from the perspective of antioxidant defense response. In our previous studies, Zong et al. [12] found that three different oxidants (diamide, H_2O_2 and menadione) induced different levels of oxidative damage to *T. asahii*. Among them, the killing effect of menadione was the strongest, diamide better, H_2O_2 the least [12]. In addition, the higher its concentration was, the stronger its killing effect would be [12]. Therefore, we hypothesize that *T. asahii* also has basic antioxidant defense system. Since *T. asahii* could invade the host and cause serious infections [10], we infer that there may be a connection between its pathogenicity and their capacity to resist or evade the attack of host immune system. To test this hypothesis, we collected 44 *T.asahii* strains from different sources, and investigated their SOD and catalase activities, then compared and analyzed the results. This will contribute to further study of antioxidant mechanism and infection mechanism of *T. asahii*.

Materials and Methods

Strains and Sources

There were 44 strains of *T. asahii* collected and they were divided into four groups according to their sources. Environmental group: including 3 strains, CBS8904, CBS7137, CBS8520 (CBS-KNAW, The Netherlands). Clinical group: including 9 strains, CBS2479; BZP07001, BZP07002, BZP07003, BZP07004, BZP09001, BZP09002, BZP07005, BZP07005^R (General Hospital of Beijing Military Area Command, PLA). Internal passage groups: including 20 strains CBS2479, CBS8904, CBS7137, CBS8520 and their five generations (CBS2479P1-5, CBS8904P1-5, CBS7137P1-5, CBS8520P1-5). Fluconazole-resistant groups: including 12 strains CBS247, CBS8904 and their selected generations (CBS2479 F6, F9, F10, R7, R8, R9; CBS8904F6, F9, F10, R7, R8, R9). The strains above were confirmed to be AS2.2174 (API 20cAUX bioassay and ITS sequence analysis). The *Candida parapsilosis* ATCC22019 (Peking University First Hospital, China) was used as a control strain.

Induction of Internal Passage Strains

Strains CBS2479, CBS8904, CBS7137, CBS8520 were selected to passage in mice and extended from 1 to 5 generations in turn [13]. Then isolated from kidney tissue and cultured on potato dextrose agar (PDA, Merck KGaA, Germany).

Induction of Fluconazole-Resistant Strains

The strains CBS2479, CBS8904 were cultured on PDA that contained fluconazole with stepwise increasing concentrations to induce highly resistant strains. The resistant strains CBS2479^R, CBS8904^R [14] were then cultured on fluconazole-free PDA and passaged in turn to obtain the reply strains. We selected the mid and late stage of the induction strains.

Fungal Cells and Enzyme Extracts Preparation

The preparation was employed based on the assay of Linares et al. [15]. The strains were cultured on PDA for 48 h at 35 °C. 1 ml volume of each cell suspension (contained about 1.5×10^8 cfu/ml *T. asahii* and 0.9 % sterile saline) was transferred to 50 ml Sabouraud Glucose Broth (SDB), and incubated (37 °C, 150 r/min, 48 h). After centrifugation, the cells were washed by sterile water for 3 times, then the cell mat was prepared. They were resuspended by potassium phosphate buffer (PH 7.0, 50 mmol/l), and added 0.5 g glass beads (Sigma, USA). Before the centrifugation, the suspensions above were shaking strongly for 6 times, then the supernatant was collected for the enzymatic assays [15].

Enzymatic Assays

Total SOD activity assay kit, catalase activity assay kit and total protein quantitative assay kit (Biological engineering of Nanjing Jian Cheng, China) were employed to determine SOD and catalase activities following the instructions provided by the manufacture. Repeated three times.

Statistics

SPSS 13.0 statistical software was employed for calculations.

Results

In this study, the SOD activity of the *C. parapsilosis* ATCC22019 was 41.289 U/mg protein (U/mg), its catalase activity was 4.208 U/mg. Table 1 showed SOD activity of Environmental group of *T. asahii* CBS8904, CBS7137, CBS8520 were 3.828, 3.46, 4.75 U/mg, the average was

Table 1 Antioxidant enzymatic activities of Environmental group and Clinical group of *T. asahii*

Groups	Strains	Sources	SOD (U/mg prot)	Catalase (U/mg prot)
Environmental group	CBS 8904	Corn	3.828	45.833
	CBS 7137	Soil	3.460	42.610
	CBS 8520	Corruption leaves	4.750	57.082
Clinical group	CBS 2479	Nail	6.221	61.956
	BZP07001	Skin	6.226	85.250
	BZP07002	Sputum	16.112	145.071
	BZP07003	Sputum	16.826	164.552
	BZP07004	Urine	8.804	78.330
	BZP07005	Liver	9.598	81.052
	BZP07005R	Skin	8.152	112.140
	BZP09001	Sputum	11.988	123.986
	BZP09002	Sputum	10.164	142.740

The data are the mean of triplicate experiments. U: unit of SOD or catalase. The SOD and catalase activities of Clinical group were significantly higher than those of Environment group ($p < 0.01$)

4.01 ± 0.66 U/mg; their catalase activity were 45.833, 42.61, 57.082 U/mg, and the average was 48.51 ± 7.60 U/mg. The SOD activity of Clinical group strains ranged from 6.221 to 16.826 U/mg, the mean value was 10.45 ± 3.87 U/mg, the catalase activity ranged from 61.956 to 164.552 U/mg, the average was 110.56 ± 35.77 U/mg.

In Table 2, the two antioxidant enzymatic activities of strains CBS2479, CBS8904, CBS7137, CBS8520 gradually increased after continuous passage in mice for 5 generations. Compared with each parental strain, the SOD activity of descendants of the strain CBS2479 was 0.98-, 1.16-, 1.63-, 1.70-, 2.35-fold higher, and its catalase activity was 1.18-, 1.24-, 1.25-, 1.23-, 1.44-times greater. Until the 5th generation, the SOD activity of other 3 environmental strains CBS8904, CBS7137, CBS8520 were 2.05-, 1.45-, 1.5-fold greater than their parental strain, while their catalase activities were 1.34-, 1.55-, 1.21-times higher.

In Table 3, the antioxidant enzymatic activity of *T. asahii* CBS2479, CBS8904 went up with the increasing of generation induced by Fluconazole. Contrast to each parental strain, the SOD activity of CBS2479 that induced the 6th, 9th, 10th generations were 1.40-, 1.44-, 1.47-fold superior than before, their CAT activity were also 1.32-, 1.36-, 1.40-fold higher. However, the SOD activities of strains CBS2479 and CBS8904 gradually returned to normal levels in the late stage of reply induction as well as catalase.

Discussion

In this study, we observed that both of enzymatic activities (SOD and catalase) in Clinical group of *T. asahii* were significantly higher ($p < 0.01$) than those of Environment group (Table 1; Fig. 1a, b). The mean SOD activity of the clinical group strains was 10.45 ± 3.87 U/mg, and its mean catalase activity was 110.56 ± 35.77 U/mg. The average SOD activity of Environmental group was 4.01 ± 0.66 U/mg, and its average catalase activity was 48.51 ± 7.60 U/mg. In contrast to Environment group, the SOD activity of Clinical group was about 2.6 times higher, while its catalase activity was about 2.3 times greater. Besides, the antioxidant enzymatic activities of these isolated strains from different clinical sources also manifested certain differences. Given that different strains had different antioxidant capacity, mainly reflected in the activities of antioxidant enzymes, we suggested that the *T. asahii* clinical group stains may have higher antioxidant capacity than the environment group strains. According to previous studies, we also obtained that the mean SOD activity of Clinical group strains of *T. asahii* (10.45 ± 3.87 U/mg) was equivalent to that of *Cryptococcus* (12 ± 0.5 U/mg) [16], and below that of *Candida dubliniensis* (27.87 ± 20.82 U/mg) [15], significantly lower than that of *C. albicans* (151.8 ± 73.27 U/mg) [15]. The mean catalase activity of *T. asahi* Clinical group strains (48.51 ± 7.60 U/mg) was significantly higher than that of *A. fumigatus* (1.75 ± 0.75 U/mg), *Aspergillus terreus* (3.5 ± 0.8 U/mg) [17]. Hence, we hypothesized that the antioxidant ability of *T. asahii* clinical group strains may be close to *Cryptococcus*, but lower than that of *Candida*, significantly higher than that of *Aspergillus*.

These data also demonstrated that the two enzymatic activities in the Internal passage group of strains (CBS2479, CBS8904, CBS7137, CBS8520 and their generations) were obviously promoted ($p < 0.05$) after passage (Table 2, Fig. 1c, d). Prior to passage, the mean SOD activity of strains was 4.56 ± 1.23 U/mg, the mean catalase activity was 51.87 ± 9.15 U/mg. To extend the 5th generation, the SOD activity increased to 8.65 ± 4.15 U/mg, and the catalase

Table 2 Antioxidant enzymatic activities of Internal passage group of *Trichosporon asahii*

Enzyme (U/mg prot)	Strains	Before passage	Generations				
			P1	P2	P3	P4	P5
SOD	CBS2479	6.221	6.100	7.194	10.132	10.598	14.610
	CBS8904	3.828	4.288	4.240	5.164	7.182	7.854
	CBS7137	3.460	2.490	4.970	5.833	5.023	5.016
	CBS8520	4.750	3.265	4.857	7.571	8.660	7.115
Catalase	CBS2479	61.956	72.992	77.034	77.431	76.186	89.045
	CBS8904	45.833	52.702	58.355	60.830	63.433	61.418
	CBS7137	42.610	42.270	50.916	59.775	65.858	66.025
	CBS8520	57.082	58.664	57.196	65.749	60.964	68.964

The data are the mean of triplicate experiments. U: unit of SOD or catalase. The SOD and catalase activities of Internal passage group strains went up gradually after passage in mice, and was significantly higher in 5th generation of Internal passage group ($p < 0.05$)

Table 3 Antioxidant enzymatic activities of Fluconazole-resistant group of *T. asahii*

Enzyme (U/mg prot)	Strains	Before induction	Generations					
			F6	F9	F10	R7	R8	R9
SOD	CBS2479	6.221	8.683	8.978	9.126	5.499	4.432	4.121
	CBS8904	3.828	4.378	6.845	6.921	3.543	3.704	2.937
CAT	CBS2479	61.956	82.085	84.469	86.738	58.190	61.598	65.448
	CBS8904	45.833	48.160	63.595	74.128	23.997	25.562	28.844

The data are the mean of triplicate experiments. U: unit of SOD or catalase. The SOD and catalase activities of Fluconazole-resistant group strains increased after resistant induction, and were significantly higher in the 10th generation of Fluconazole-resistant group ($p < 0.05$). The antioxidant enzymatic activity of strains after reply induction gradually returned to normal levels

activity risen to 71.36 ± 12.19 U/mg, increasing 90 and 34 %. In addition, the mean catalase activity of the Internal passage group was higher than that of the Environment group ($p < 0.05$). These data suggested that once *T. asahii* invaded the host, its antioxidant defensive mechanism may be stimulated or induced through the interaction with the host immune systems, finally achieved that the antioxidant enzyme activities raised to different extent together with increase of its oxidation resistance [1]. Many studies have demonstrated that SOD and catalase played an important protective role in clearing up or cutting off the production of ROS from macrophages or neutrophils [3–9]. In *A. fumigatus*, Diamond and Clark [18] found that the catalase could protect hyphae from myeloperoxidase system of neutrophils. So, we speculated that the antioxidant enzymes of *T. asahii* played a certain role in protecting strains from oxidative damage of host immune systems, even may contribute to rescuing from the attack of host immune systems, further leading to the formation of infection as well.

At the same time, we proved that the two enzyme activities of CBS2479 and CBS8904 went up with the increasing of generation induced by Fluconazole (Table 3; Fig. 1e, f). Before drug-treatment, the mean SOD activity was 5.02 ± 1.69 U/mg, and the mean catalase activity was 53.89 ± 11.40 U/mg. Until the 10th generation of Fluconazole-resistant induction, its SOD activity added to 8.02 ± 1.56 U/mg, and the catalase activity risen to 80.43 ± 8.92 U/mg, which increasing by 60, 49 % ($p < 0.05$). Furthermore, the two enzymatic activities of the 10th generation strains in Fluconazole-resistant group were higher than those of Environmental group ($p < 0.05$). For reply induction strains, their antioxidant enzymatic activities gradually returned to normal levels. At the 9th generation of reply induction, the mean SOD activity of strains was 3.53 ± 0.84 U/mg, the mean catalase activity was 47.15 ± 25.88 U/mg. These results implied that fluconazole may simulate or induce the expression of antioxidant enzymatic activity of *T. asahii*, leaving the antioxidant capacity of these strains further promoted [15]. Because that the activities of SOD and catalase in *T. asahii* could go down to normal level without fluconazole exposure, we suggested that the induction of antioxidant enzymatic activity may be regulatory [1]. There were other antifungal agents that also could induce antioxidant stress response except fluconazole and amphotericin B [15]. Hoehamer et al. [2] confirmed that the expression of the proteins involved in antioxidant mechanism elevated after the exposure of the azole (ketoconazole) and echinocandin

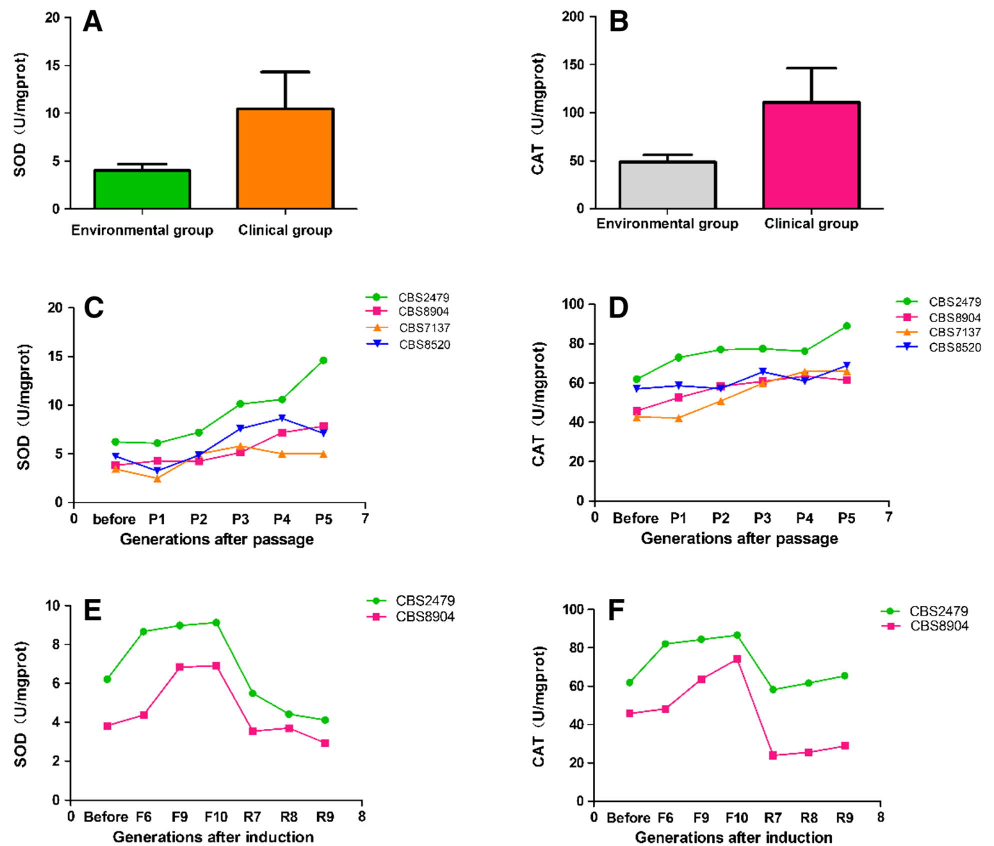

Fig. 1 Antioxidant enzyme *SOD* (**a**, **c**, **e**) and catalase (**b**, **d**, **f**) activities of different sources of *T. asahii*. **a**, **b** The SOD and catalase activities of Clinical group were significantly higher than those of Environment group ($p < 0.01$). **c**, **d** The SOD and catalase activities of Internal passage group strains went up gradually after passage in mice, and were significantly higher in 5th generation of Internal passage group ($p < 0.05$). **e**, **f** The SOD and catalase activities of Fluconazole-resistant group strains increased after resistant induction, and were significantly higher in the 10th generation of Fluconazole-resistant group ($p < 0.05$). The antioxidant enzymatic activities of strains after reply induction gradually returned to normal levels

(caspofungin). Blum et al. [17] pointed out that *Aspergillus terreus* who had inherent resistance to amphotericin B was closely related with its higher catalase activity and had no obvious correlation to its cell wall components and lipid peroxidation level when compared with the *A. fumigatus*. Consequently, we inferred that fluconazole might also cause a certain degree of oxidative damage to *T. asahii* during the killing process. Meanwhile, the induced antioxidant enzymes of *T. asahii* may be one of fluconazole-resistance mechanism, which required to further research.

Moreover, our results also suggested that there was some relationship between the two enzymes SOD and catalase in *T. asahii* after internal passage and fluconazole resistance induction that they had the same variation tendency, which is in accordance with Linares et al. [15].

In conclusion, this investigation proved that *T. asahii* has stronger antioxidant defensive mechanism as well as most of other fungus [3–9]. The antioxidant capacity of *T. asahii* differed according to their sources, mainly reflected in the activities of antioxidant enzymes. Among them, Clinical group strains have the strongest antioxidant capacity; Internal passage group strains and Fluconazole-resistant group strains better; Environmental group strains the lowest. Furthermore, the antioxidant enzymes (SOD and catalase) in *T. asahii* could be activated or induced by the exposure of host defensive system and fluconazole. The antioxidant defensive response of *T. asahii* might be relevant to its infection mechanism and drug-resistance mechanism. However, this aspect still needed to further explore.

Acknowledgments This research was supported by Project No. BWS11J059 of Army “Twelve Five” of China. Special thanks also should go to Xiufeng Han and Zhaoxia Guo who have put considerable time and effort into the supply of strains in the Internal passage group and Fluconazole-resistant group.

Compliance with Ethical Standards

This article does not contain any studies with human participants performed by any of the authors. All animal experiments were performed and supervised with the approval of the local ethical committee and all the experiments were performed according to the National (China) Institutes of Health Guide for the Care and Use of Laboratory Animals.

Conflict of Interest There are no conflicts of interest for this article.

References

1. Temple MD, Perrone GG, Dawes IW (2005) Complex cellular responses to reactive oxygen species. Trends Cell Biol 15:319–326. doi:10.1016/j.tcb.2005.04.003
2. Hoehamer CF, Cummings ED, Hilliard GM, Rogers PD (2010) Changes in the proteome of *Candida albicans* in response to

azole, polyene, and echinocandin antifungal agents. Antimicrob Agents Chemother 54:1655–1664. doi:10.1128/AAC.00756-09
3. Hwang CS, Rhie GE, Oh JH, Huh WK, Yim HS, Kang SO (2002) Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of *Candida albicans* against oxidative stresses and the expression of its full virulence. Microbiology 148:3705–3713. doi:10.4050/JAHS.48.71
4. Martchenko M, Alarco AM, Harcus D, Whiteway M (2004) Superoxide dismutases in *Candida albicans*: transcriptional regulation and functional characterization of the hyphal-induced SOD5 gene. Mol Biol Cell 15:456–467. doi:10.1091/mbc.E03-03-0179
5. Wysong DR, Christin L, Sugar AM, Robbins PW, Diamond RD (1998) Cloning and sequencing of a *Candida albicans* catalase gene and effects of disruption of this gene. Infect Immun 66:1953–1961
6. Holdom MD, Hay RJ, Hamilton AJ (1995) Purification, N-terminal amino acid sequence and partial characterization of a Cu, Zn superoxide dismutase from the pathogenic fungus *Aspergillus fumigatus*. Free Radic Res 22:519–531. doi:10.3109/10715769509150324
7. Paris S, Wysong D, Debeaupuis JP, Shibuya K, Philippe B, Diamond RD, Latgé JP (2003) Catalases of *Aspergillus fumigatus*. Infect Immun 71:3551–3562. doi:10.1128/IAI.71.6.3551-3562.2003
8. Cox GM, Harrison TS, McDade HC, Taborda CP, Heinrich G, Casadevall A, Perfect JR (2003) Superoxide dismutase influences the virulence of *Cryptococcus neoformans* by affecting growth within macrophages. Infect Immun 71:173–180. doi:10.1128/IAI.71.1.173-180.2003
9. Giles SS, Stajich JE, Nichols C, Gerrald QD, Alspaugh JA, Dietrich F, Perfect JR (2006) The *Cryptococcus neoformans* catalase gene family and its role in antioxidant defense. Eukaryot Cell 5:1447–1459. doi:10.1128/EC.00098-06
10. Kushima H, Tokimatsu I, Ishii H, Kawano R, Shirai R, Kishi K, Hiramatsu K, Kadota J (2012) Cloning of the lanosterol 14-α-demethylase (ERG11) gene in *Trichosporon asahii*: a possible association between G453R amino acid substitution and azole resistance in *T. asahii*. FEMS Yeast Res 12:662–667. doi:10.1111/j.1567-1364.2012.00816.x
11. Gross JW, Kan VL (2008) *Trichosporon asahii* infection in an advanced AIDS patient and literature review. AIDS 22:793–795. doi:10.1097/QAD.0b013e3282f51ecc
12. Zong LN, Li HT, Yong RY, Ao JH, Wang WL, Zhu H, Cong L, Wang CM (2012) Experimental study on oxidant sensitivity of *Trichosporon asahii*. J Pract Dermatol 5:65–70. doi:10.3969/j.issn.1674-1293.2012.02.001 **(in Chinese)**
13. Han XF, Li HT, Yang RY, Zhang YM, Tian YL, ZhouJF YD (2015) The change of morphology and antifungal susceptibility in *Trichosporon asahii* by in vivo passage and in vivo induction. J Pract Dermatol 8:6–10. doi:10.11786/sypfbxzz.1674-1293.20150103 **(in Chinese)**
14. Guo ZX, Li HT, Yang RY, Zhu H, Wang CM, Liao Y, Xia ZK (2013) In vitro induction and stability evaluation of fluconazole resistance in *Trichosporon asahii*. Chin J Dermatol 46:41–44. doi:10.3760/cma.j.issn.0412-4030.2013.05.013 **(in Chinese)**
15. Linares CE, Giacomelli SR, Altenhofen D, Alves SH, Morsch VM, Schetinger MR (2013) 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 46:752–758. doi:10.1590/0037-8682-0190-2013
16. Dias AL, Brigagão MR, Colepicolo P, Siqueira AM, Silva EG, Paula CR (2006) Superoxide dismutase in *Cryptococcus neoformans* varieties *gattii*, *grubi*, and *neoformans*. Mem Inst Oswaldo Cruz 101:107–109. doi:10.1590/S0074-02762006000100021
17. Blum G, Perkhofer S, Haas H, Schrettl M, Würzner R, Dierich MP, Lass-Flörl C (2008) Potential basis for amphotericin B resistance in *Aspergillus terreus*. Antimicrob Agents Chemother 52:1553–1555. doi:10.1128/AAC.01280-07
18. Diamond RD, Clark RA (1982) Damage to *Aspergillus fumigatus* and *Rhizopus oryzae* hyphae by oxidative and nonoxidative microbicidal products of human neutrophils in vitro. Infect Immun 38:487–495