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Light-based antifungal strategy for the control of *Candida auris*

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Abstract: *Candida auris* is a pathogen that has been attracting worldwide focus due to its high resistance to conventional drugs. This work evaluated the photodynamic inactivation mediated by two phenothiazine dyes against the CBS 10913 strain. 2022 the Author(s)

1. Introduction

Fungal infections by *Candida auris* are currently a serious and emerging problem, due to its high resistance to conventional antifungals with confirmed cases in over 35 countries and a mortality rate of 30-72%. PhotoDynamic Therapy (PDT) involves the use of a photoactive drug and light to produce reactive oxygen species (ROS), which kill microbial cells by oxidative stress. There is no evidence in the literature that PDT promotes microbial resistance. Furthermore, this therapy is equally effective in inactivating microorganisms resistant to conventional antimicrobials [1,2]. Herein, we evaluated the photodynamic inactivation of *C. auris* mediated by two phenothiazine dyes and the production of ROS following PDT.

2. Methodology

The strain used in this study was CSB 10913, cultivated for 48 h in SDA, subsequently in SD broth for another 24 h. The suspension was centrifuged and washed with PBS and its final concentration was adjusted to 10^7 CFU/mL. The photosensitizers (PSs) used were methylene blue (MB) (100, 50, 25 and 12.5 µM) and 1,9- dimethyl methylene blue (DMMB) (3, 1.5, 0.75 and 0.375 µM). Briefly, 100 µL of fungal suspension was added to the wells of a 96-well plate and incubated with 100 µL of PBS (light and control groups) and MB or DMMB (dark groups). The treated groups were incubated with the respective PS in the different concentrations for 10 min. Thereafter, the plates were irradiated with a LEDBox (λ= 660 nm) at doses of 30, 20 and 10 J/cm^2. Control and dark groups were kept protected from light for the same period of time. After this procedure, the yeast suspensions were serially diluted in PBS and 10 µL of each dilution were added to a Petri dish with SDA and incubated for 24 h for CFU counting. To measure ROS, the highest concentrations of DMMB and MB were incubated with the probe DCFH-DA 10 µM and irradiated with 30 J/cm^2. H_2O_2 10 µM was used as a positive control. After 3 h, the plate was read on a spectrophotometer (Exc: 485 nm and Em: 538 nm). Experimental data were analyzed through the Mann-Whitney test using the GraphPad Prism 7.04 software. The level of significance was set at $p < 0.05$.

3. Results

Figure 1 exhibits the fungal viability for DMMB and MB for different PS concentrations and light doses. The lowest light dose (10 J/cm^2) was able to completely eradicate *C. auris* when the highest concentration of DMMB (3 µM) was used. Interestingly, 20 and 30 J/cm^2 were equally effective in reducing fungal viability for the highest DMMB concentrations (1.5 and 3 µM). In contrast, MB-mediated PDT promoted a complete eradication of *C. auris* only when the highest light dose (30 J/cm^2) was combined with the highest concentration of MB (100 µM).

ROS production is showed in figure 2. As expected, the highest concentrations of PS generated more ROS than the lowest concentrations. Similar levels of ROS were noticed for 3 µM of DMMB and 100 µM of MB. However, 1.5 and 3 µM of DMMB promoted similar killing under 30 J/cm^2, even though ROS production was significantly lower for 1.5 µM.

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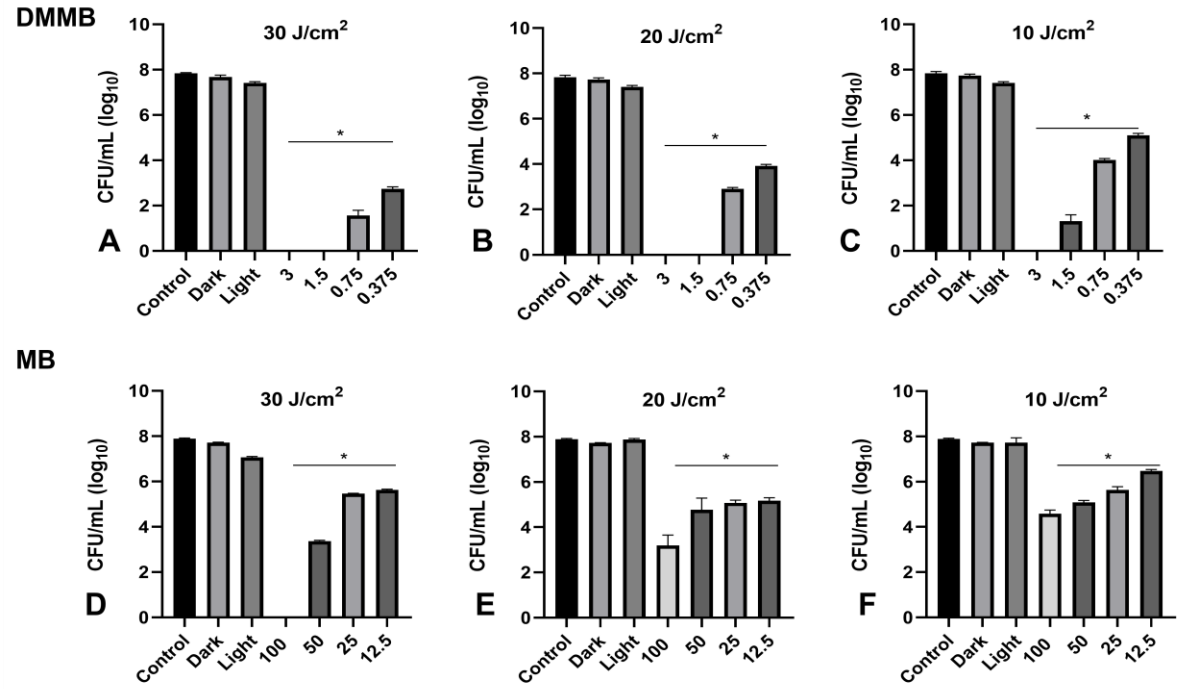

Figure 1: Mean values ± standard deviation of the fungal viability following PDT. DMMB (A,B,C), MB (D,E,F).

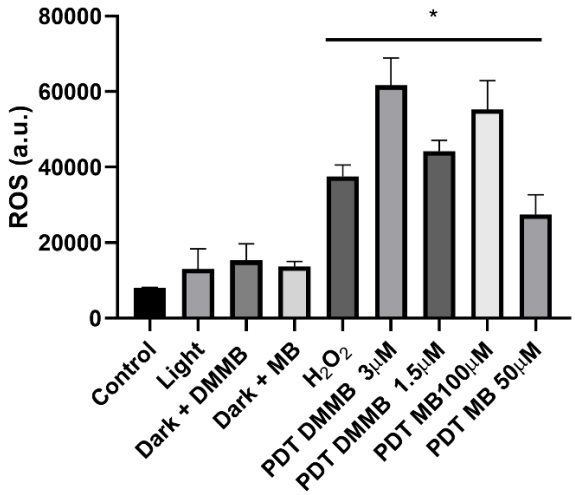

Figure 2: Mean values ± standard deviation of the production of reactive oxygen species.

4. Conclusions

Taken together, our results demonstrate that PDT can be used to fight *C. auris.* Additionally, DMMB was more effective than MB. Other mechanisms than ROS generation seems to be behind DMMB- mediated PDT of *C. auris*.

5. References

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