Photodynamic inactivation can fight biofilms of *Candida albicans* with upregulated ABC transporters.

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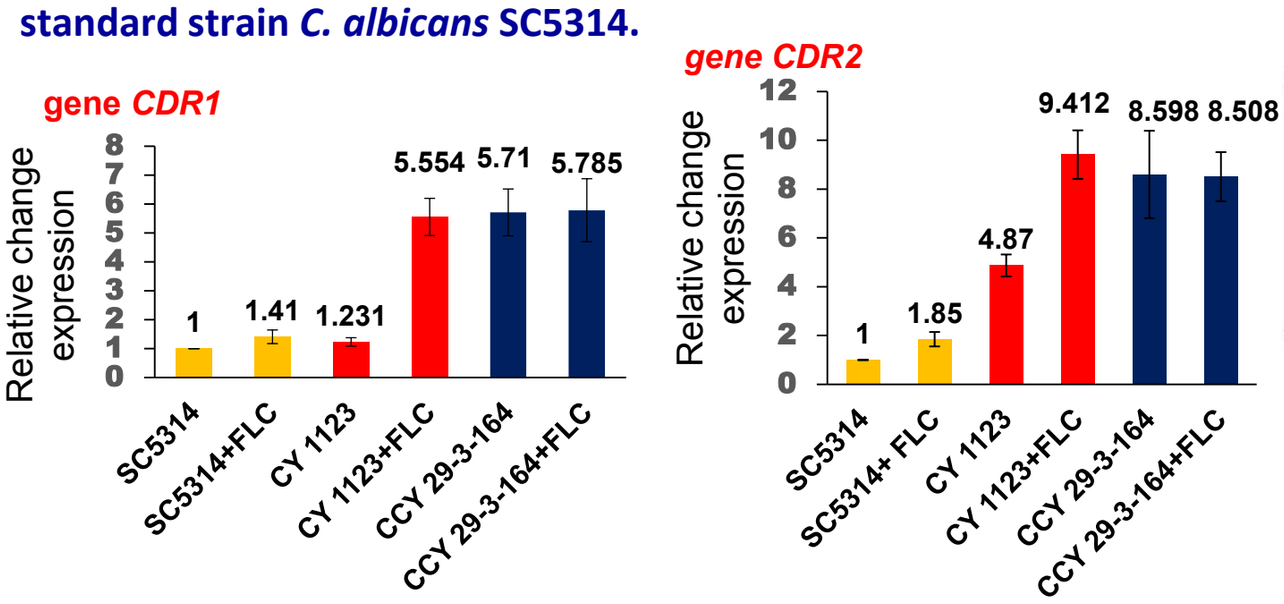
Introduction

The expression of the efflux pumps (Cdr1p, Cdr2p and Mdr1p) is the most frequent resistance mechanism in *Candida albicans* planktonic culture. However, this resistance mechanism is also characteristic for the early phase of biofilm development. Limitations in the eradication of microbial biofilms by conventional therapy are motivation for searching for new options such as photodynamic inactivation (PDI). This approach is effective due to production of reactive oxygen species after irradiation of photoactive compounds by suitable light source.

The main aim of this study was to learn whether PDI employing methylene blue (MB) will be efficient on biofilms formed by 2 *C. albicans* clinical isolates resistant to fluconazole (FLC) with determined CDR and MDR resistance compared to the susceptible standard strain *C. albicans* SC5314.

Materials and Methods

The clinical isolates of *C. albicans* CY 1123 and CCY 29-3-164 resistant to FLC (CLSI, M27-S4, 2012, Etest) and the standard strain *C. albicans* SC5314 were involved in the PDI study. Regulation of the genes: *CDR1, CDR2*, and *MDR1* was studied by qPCR in planktonic culture. The quantification of gene expression was analyzed using $2^{-\Delta\Delta CT}$ with respect to housekeeping gene *ACT1*. The relative change in expression was calculated with respect to the control sample of *C. albicans* SC5314 normalized to 1. In experiment with PDI, 1 mM MB was selected as the photosensitizer irradiated with red laser (output power 190 mW/cm², wavelength 660 nm). Protocol is described in the Figure 1. Prior this experiment, toxicity assay on *Galleria mellonella* was performed (Figure 2). In CLSM, samples were stained with calcofluor white (excitation/emission 355/433 nm) and propidium iodide (excitation/emission 535/617 nm) in order to evaluate an effectiveness of PDI on the biofilm structure and cell survival.



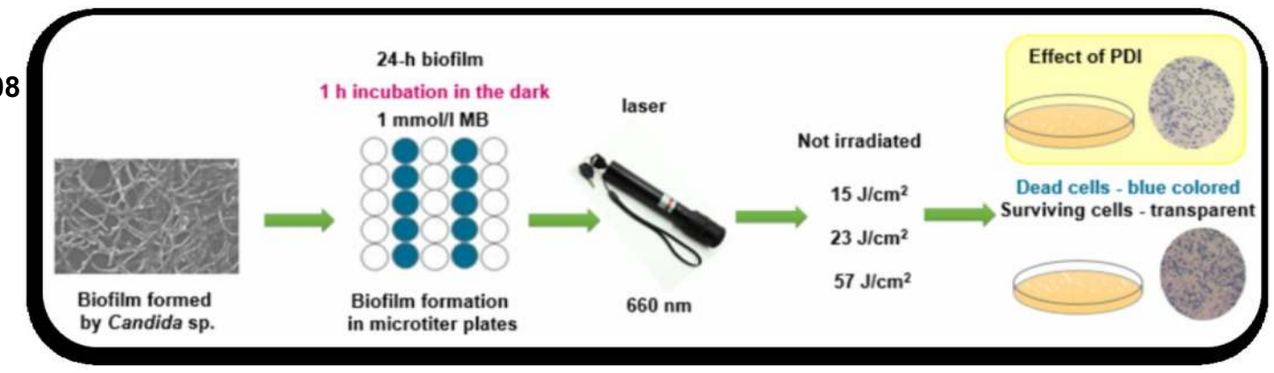
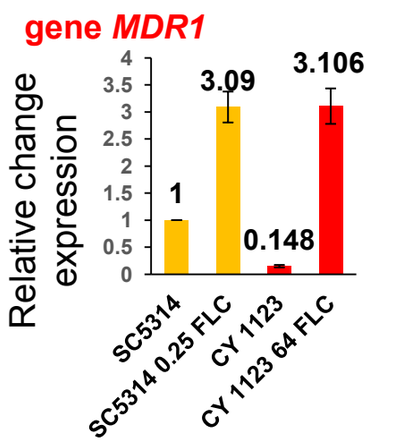
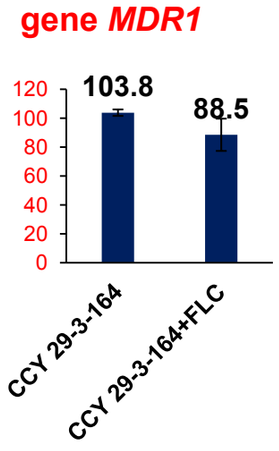
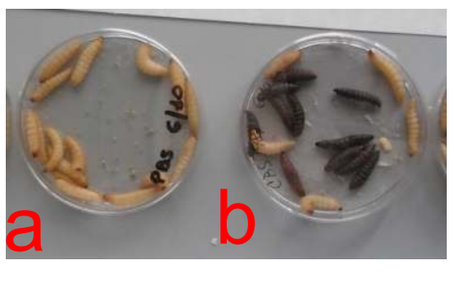


Fig.1 Illustration of PDI by laser on *Candida* biofilm with 1-h pre-incubation in the presence of MB.

Fig.3 shows a regulation of the efflux genes *CDR1, CDR2,* and *MDR1.* The standard strain SC5314 was also tested after exposure to subinhibitory concentration of FLC (0.25 µg/ml; the second yellow column). The FLC-resistant strains CY 1123 and CCY 29-3-164 were cultivated in the presence of FLC (64 µg/ml; the second red and blue column). The strain CY 1123 showed marked upregulation of all tested genes in the presence of FLC, but, gene coding for Cdr2 was most contributing one. Moreover, this gene was continuously upregulated. The strain CCY 29-3-164 proved only overregulation of the *CDR* genes, but stable high upregulation of the gene *MDR1* was observed.







Toxicity of 1 mM MB was tested on *G. mellonella*. Fig.2 documents surviving larvae (a) in contract to the dead ones that are black (b). Fig.2 (c) represents surviving larvae after administration of 1 mM MB estimated in the end of experiment (9th day). Results confirmed that 1 mM MB was not toxic for tested system.

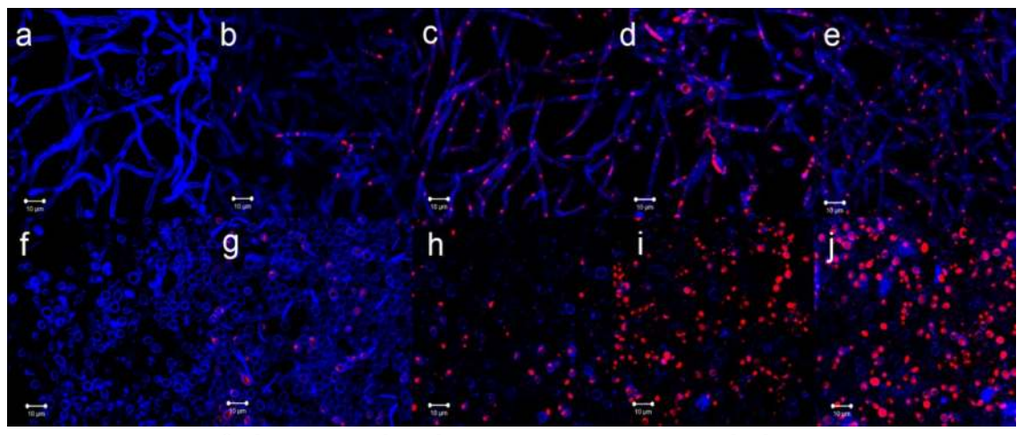
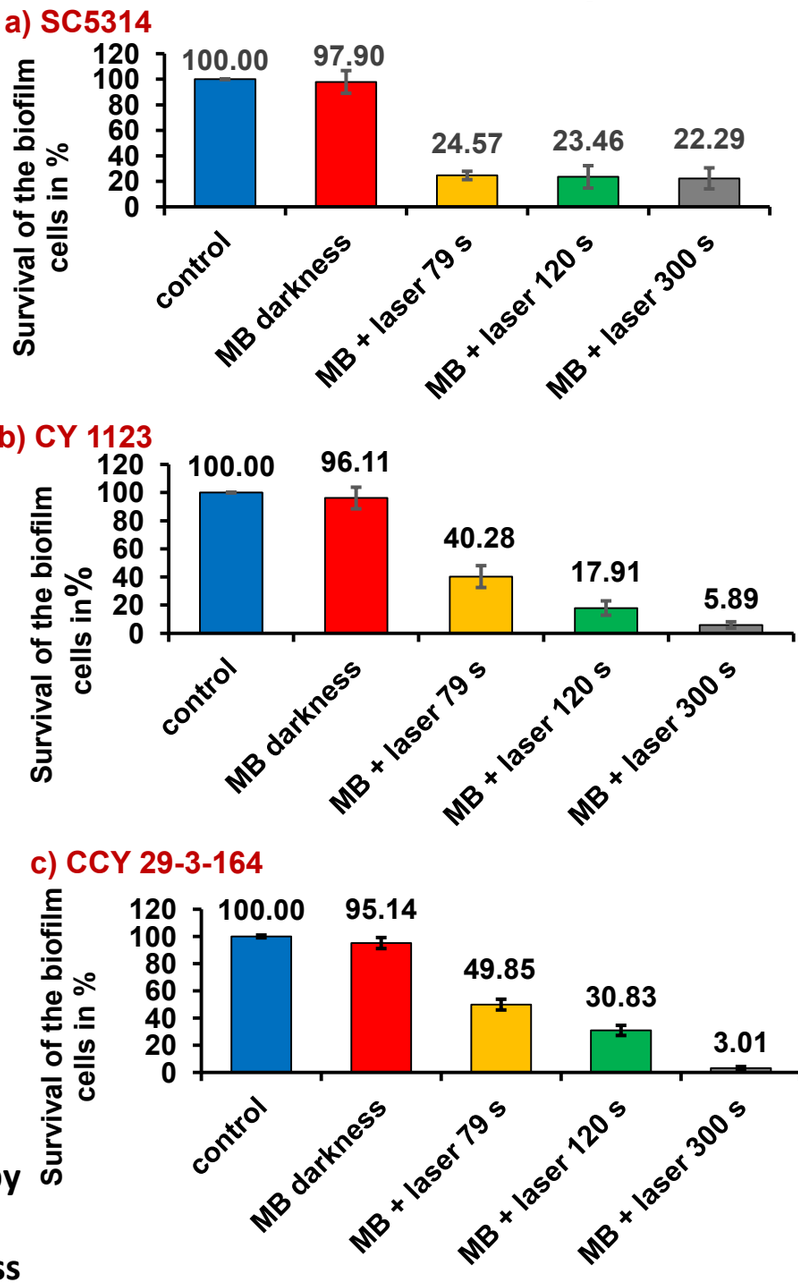


Fig.5 CLSM of the standard strain SC5314 and the FLC-resistant strain CY 1123 incubated in the presence of FLC stained with CW and PI; *C. albicans* SC5314 (a – e) and *C. albicans* CY 1123 (f – j): control biofilm without MB (a, f), 1 mM MB in the darkness (b, g), 1mM MB and laser irradiation for 79 s (c, h), 1mM MB and laser irradiation for 120 s (d, i), 1mM MB and laser irradiation for 300 s (e, j). Proportion of red dead cells after PDI indicates inhibitory effect of MB irradiated by laser. Bars represent 10 µm.

Fig.4 documents an effectiveness of PDI employing MB (1 mM) illuminated by red laser on biofilm formed by the standard strain *C. albicans* SC5314 (a) and the FLC-resistant strains of *C. albicans* (b, c). The period of irradiation was 79, 120, and 300 s corresponding to total delivered energies of 15, 23, and 57 J/cm². PDI effectively eradicated biofilm cells; neither CDR nor MDR resistance influenced susceptibility. Additionally, effect of PDI was proved not only on biofilm composed of the yeasts, but also that manifested dominant mycelial form.



Conclusions

- **PDI was effective on biofilm formed by the *C. albicans* strains resistant to FLC, and resistance mediated by efflux transporters did not play a significant role in the susceptibility of biofilm to PDI**
- **employment of a laser in PDI enabled a short period of irradiation while maintaining the high effectiveness of PDI**

Acknowledgement

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