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Research Paper

In vitro photodynamic therapy of *Candida albicans*, the cause of vulvovaginal candidiasis, is enhanced by *Bacillus* and *Enterococcus* probiotics

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ABSTRACT

Background: *Candida albicans* is the primary cause of vulvovaginal candidiasis, a worldwide health concern for women. The use of supplemental methods, such as antimicrobial photodynamic therapy (aPDT) and probiotics, was promoted by the ineffectiveness of the existing antifungal drugs.
Methods: This study examines the combined effects of probiotics (*Bacillus* and *Enterococcus* isolated from the fermented pickles) and PDT (using red laser (655 nm, 18 J/cm^2) as a light source and methylene blue dye (30 mg/mL) as a photosensitizer) on the *in vitro* virulence activity of *C. albicans* including growth, biofilm formation, antifungal resistance, biofilm elimination, and biofilm dispersion.
Results: The probiotic strains demonstrated a higher resistance to PDT compared to the fungal cell. *Bacillus* and *Enterococcus* enhanced the antifungal effects of PDT on planktonic *Candida* cells in both pre-PDT and post-PDT interactions. The inhibition of biofilm formation by PDT was improved upon interaction with *Bacillus* (70 %) and *Enterococcus* (58 %). The eradication of *Candida* biofilm using PDT was increased after a combination with *Bacillus* (67 %) and *Enterococcus* (46 %). The nystatin resistance of the fungal biofilm following PDT treatment was decreased from (μg/ml) 25 to 6.25 due to the interaction with both probiotic strains. Fungal cell dispersion from the biofilm after PDT treatment diminished by 18 % and 25 % in the presence of *Bacillus* and *Enterococcus* strains. *Galleria mellonella* mortality was significantly changed following the PDT of the fungi/probiotic-injected larvae.
Conclusions: This synergistic activity suggests the use of probiotics/PDT as a supplemental treatment for vulvovaginal candidiasis.

1. Introduction

Candida albicans is a polymorphic fungus that asymptomatically inhabits the oral, gastrointestinal, and vaginal systems. Disruption of body homeostasis due to factors such as antibiotic use, immune system dysfunction, microbiome dysbiosis, and changes in mucocutaneous integrity can lead to tissue invasion and biofilm formation, resulting in superficial and systemic candidiasis [1]. Biofilm formation, a complex arrangement of surface-attached cells, facilitates a sequential process that encompasses hypha development, accumulation of extracellular matrix components, immune evasion, expression of virulence genes, and antibiotic resistance [2]. The infection can ultimately disseminate via the dispersal of yeast cells from the mature biofilm. Vulvovaginal candidiasis (VVC), an infection of the vaginal mucosa and vulva, is a prevalent manifestation of superficial fungal infections (SFIs) primarily attributed to *Candida albicans*. Approximately 70–75 % of women experience this disease at least once in their lifetimes, while recurrent vulvovaginal candidiasis (RVVC), defined as four or more episodes within a year, impacts 5 % to 8 % of adult women [3]. The standard treatment approach for vaginal candidiasis involves either topical or systemic administration of several antifungal drugs, notably azoles. Conventional antifungal therapy is generally effective against candidiasis; nevertheless, both topical (itching and burning) and oral (nausea, vomiting, chills, disorientation, neutropenia, and hepatotoxicity) medications may induce various side effects [4]. Prolonged antibiotic therapy is constrained by antifungal resistance and a rising prevalence of VVC caused by non-*albicans Candida*, which exhibit greater resistance to treatment. Since VVC remains a public health concern, innovative

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(complementary) treatment strategies are of interest. Probiotics and antimicrobial photodynamic therapy (aPDT) represent two novel therapeutic approaches for the management of vaginal candidiasis.

Probiotics are live microorganisms that provide health benefits to the host when administered in sufficient quantities. The modification of the vaginal microbiome, especially a decrease in lactic acid bacteria, is a primary factor in the commensal-pathogen transition of *Candida*; hence, probiotics have been explored for the prevention and treatment of vulvovaginal candidiasis [5]. While *Lactobacillus* and *Bifidobacterium* are the most recognized probiotics, the probiotic capabilities of other microbial genera have also been evaluated. Recent studies have investigated the health benefits of spore-forming *Bacillus* [6] and high-acid-adapted *Enterococcus* [7]. Probiotics demonstrate antifungal effects via lowering the vaginal pH, enhancing the host immune response, strengthening the mucosal defensive barrier, and generating antimicrobial substances [8]. Recent data indicate probiotics may inhibit the formation of pathogenic biofilms [9].

Photodynamic therapy (PDT) relies on the interaction of light energy of a certain wavelength with photosynthesizers (PS) to produce superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), and singlet molecular oxygen ($^1O_2$). Reactive oxygen species (ROS) engage with various cellular biomolecules, leading to permanent biological damage [10]. While antibiotic resistance does not pertain to the efficiency of photodynamic therapy, and resistance to PDT is infrequent among pathogens, aPDT shows significant promise for treating localized infections, including superficial (vulvovaginal) candidiasis [11].

Photodynamic therapy has shown enhancement through several combinatorial techniques by antibacterial agents, inorganic salts, nanoparticles, and physical modalities [12]. Although both probiotics and PDT are relevant in the treatment of *Candida* infections, limited research exists regarding their synergistic effects. There is also restricted evidence about the effects of PDT on non-pathogenic (probiotic) microorganisms. This study initially evaluated the effects of PDT on *Bacillus* and *Enterococcus* probiotic strains and its impact on *Candida albicans*. Secondly, the probiotic strains were utilized to enhance *in vitro* photodynamic therapy against *C. albicans* growth, biofilm formation, antifungal resistance, biofilm elimination, and biofilm disposal.

2. Methods and materials

2.1. Microbial cells and growth conditions

The C. albicans strain was previously isolated and characterized from women with clinical signs of VVC [13]. Yeast cells were cultivated aerobically in YPD (Yeast extract–Peptone–Dextrose) broth, including (W/V): 0.5 % yeast extract, 1 % peptone, and 1 % glucose at 37 °C. The *Bacillus* (1630F) and *Enterococcus* (7C37) strains were previously isolated from fermented pickles and grown in nutrient broth (NB, Merck, Germany) at 37 °C [14]. All media were solidified with 1.5 % (w/v) agar as required [15].

2.2. Photosensitizer and light source

This study utilized methylene blue (MB) as the photosensitizer. A 2 mM stock solution of MB (Sigma) was prepared in sterile phosphate buffer saline (PBS) and stored in the dark at 4 °C no longer than two weeks before use. The stock solution was filtered through a 0.22 μm polycarbonate membrane and further diluted in PBS to achieve the desired concentration. Microbial cells were incubated with varying PS concentrations (15–150 mg/mL) in the dark for 10 min prior to laser irradiation. Following pre-treatment, samples were washed with PBS to remove excess dye. The light source comprised a laser diode (λ = 655 nm) (Noorgostar, Iran). The distance from the laser apparatus to the exposure samples was 1.0 cm, yielding a coverage spot size of 0.5 mm, along with a power density of 100 mW/cm^2 and an energy density of 18 J/cm^2 after 180 s of irradiation. The same energy density was used for all PS concentrations.

2.3. Photodynamic inactivation of candida albicans, Bacillus sp. 1630F, and Enterococcus sp. 7C37

To examine the aPDT at varying PS concentrations, *C. albicans, Bacillus*, and *Enterococcus* cells were cultured overnight in YPD broth and NB at 37 °C. The cultures were centrifuged at 10,000 x g for 10 min and washed with sterile PBS solution. The fungal and bacterial cell pellets at a standard cell concentration (10^6 cells mL^{-1}), were re-suspended in a fresh PBS solution containing varying amounts of methylene blue (15–150 mg/mL). PS pretreatment and PDT were conducted according to the standard procedure (Section 2.2). One hundred microliter aliquots of the desired dilution were inoculated onto plates containing YPD/NA media and incubated for 48 h at 37 °C. After incubation, the colony-forming units per milliliter (CFU/mL) were quantified, and cell viability was assessed by comparison to control samples that were not exposed to PDT. The relative probiotic survival rate (RPSR) was established to determine the PS concentration that exerts the most significant inhibitory impact on *Candida* while minimally affecting probiotics:

$$\text{RPSR } (\%) = \frac{\text{P viability} - \text{C viability}}{\text{P viability}} \times 100 \quad (1)$$

P and C viability refers to the viability of probiotic and *Candida* cells at each PS concentration.

2.4. Photodynamic inactivation of C. albicans in the interaction with Bacillus and Enterococcus

The survival of planktonic *C. albicans* cells, biofilm formation, and biofilm eradication were investigated following the fungal interactions with *Bacillus* and *Enterococcus* before (pre-) and after (post-) PDT. The augmentation of aPDT in every experiment was determined as follows:

$$\text{PDT enhancement } (\%) = \frac{X1 - X2 - X3}{X1} \times 100 \quad (2)$$

X_1 and X_2 represent the effects of PDT on *C. albicans* either alone or in combination with probiotics, whereas X3 denotes the effect of probiotics on *Candida* viability in the non-illuminated trial.

2.4.1. Viability of planktonic cells

For assessment of cell viability of pre-PDT interaction, a fresh suspension of the overnight probiotics and *Candida* cells (1000 μL of 10^6 CFU/mL) was co-incubated in YPD broth for 2 h, washed with PBS solution, and then subjected to the standard PDT procedure (Section 2.2). To evaluate the post-PDT interaction between *Candida* and probiotics, the fungal cell suspension received PDT treatment and then centrifuged at 10,000 g for 10 min, washed with sterile PBS, supplemented by the fresh media containing probiotic cells (10^6 CFU/mL), and incubated for a further 2 h. The single fungal suspension subjected to PDT and the dual fungal/probiotic suspension that was not illuminated, were used as the control in every experiment. To determine *Candida* viability, 100 μL aliquots of the desired dilution were inoculated onto YPD agar plates and incubated for 48 h at 37 °C. Following incubation, the *Candida* colony-forming units per milliliter (CFU/mL) were quantified for both single and dual *Candida* suspensions. Scanning electron microscopy (SEM; LEO 435 VP) was employed to investigate the cell morphological change following the PDT treatment [13]. The accumulation of photosensitizers in the fungal cells was quantified following the spectrophotometric measurement of the residual methylene blue outside the cells [16].

2.4.2. Biofilm formation

To investigate the biofilm formation in the pretreatment model, a mixed suspension of *Candida* and probiotic (1000 μL of 10^6 CFU/mL

each) was subjected to the PDT. The mixed cell suspension was centrifuged at 10,000 g for 10 min, washed with sterile PBS, supplemented by 2 ml fresh YPD medium, transferred to the 12-well polystyrene plates, and incubated for 48 h at 37 °C. Following incubation, the supernatant was discarded, and the wells were washed twice with PBS solution to remove unbounded cells. The *Candida* cells were carefully scraped and quantified by the standard plate count method (Section 2.3). The post-PDT treatment was conducted similarly, with the single fungal culture being initially subjected to PDT, followed by the addition of probiotics to establish a biofilm after 48 h of incubation at 37 °C. The single and dual biofilms were assessed based on the colony development on YPD agar.

2.4.3. Biofilm eradication

In the pre-PDT experiment, a single and dual (with probiotics) *Candida* biofilm was formed using the standard procedure (Biofilm formation section). The mature biofilms were subjected to the PDT treatment, and the viability of the attached *Candida* cells was subsequently assessed according to the prescribed procedure. For the post-PDT technique, a single *Candida* biofilm was formed, and the mature biofilm was subjected to the PDT treatment. After illumination, the wells were washed twice with PBS solution and supplemented with fresh media containing probiotics suspension (10^6 CFU/mL) or only YPD media (control), then incubated for a further 24 h. The viability of attached *Candida* cells was assessed according to the standard protocol (Biofilm formation section).

2.4.4. Biofilm dispersal

The single- and dual- species (with Bacillus and Enterococcus) Candida biofilms were developed in 12-well polystyrene plates as previously outlined. The mature biofilms were subjected to the PDT. The wells were gently washed with PBS solution and stuffed with fresh sterile YPD. The medium was aspirated during incubation periods of 0, 6, 12, 18, or 24 h at 37 °C. The number of Candida disseminated cells was determined by counting CFUs. The biofilm dispersion of Candida cells not exposed to PDT was assessed as a control.

2.5. Antifungal susceptibility

The antifungal susceptibility of C. albicans in single and dual biofilms (with Bacillus and Enterococcus) exposed to the PDT was evaluated against nystatin (Sigma Aldrich, Germany). Biofilm development and PDT treatment were conducted according to the above procedures. The supernatant was aspirated, wells were washed with PBS, and two-fold serial dilutions of nystatin prepared in YPD broth medium were added to wells. The adhered biofilm was manually scraped off after 24 h of incubation at 37 °C, and the viability of fungus cells was measured by counting colony-forming units (CFUs). The antibiotic susceptibility of Candida biofilm not subjected to the PDT was also determined as a control.

2.6. Galleria mellonella survival assay

The suspension (10^6 CFU/mL) of both Candida and probiotic cells in PBS solution was produced. The cells were injected into the last left proleg of larvae (third instar) using a 10 ml Hamilton syringe. Four distinct groups (each comprising ten larvae) were included: a) non-PDT-treated larvae injected with C. albicans; b) PDT-treated larvae injected with a single C. albicans; c) PDT-treated larvae injected with Candida/Bacillus mixed suspension; d) PDT-treated larvae injected with Candida/Enterococcus mixed suspension. The *in vivo* PDT treatment of larvae was performed according to the approach outlined by Merigo et al. [17]. The larvae were reared at 30 °C following PDT treatment, and survival was assessed daily for seven days. The larvae were considered dead when they did not respond to physical stimulation.

2.7. Data analysis

All experiments were performed at least three times. The standard error of the mean was calculated and presented as error bars in the figures. One-way analysis of variance and *t*-test were conducted using SPSS version 16 software (IBM Co.) to determine the significance of results (p-value less than 0.05).

3. Results

3.1. Effects of PDT on Bacillus and Enterococcus strains

To assess the relative aPDT effect on probiotics in relation to *Candida* cells, we established the relative probiotic survival rate (RPSR), wherein a higher value indicates a more pronounced inhibitory effect on fungi compared to bacteria. Fig. 1 illustrates that both bacterial probiotics exhibited more resistance to PDT than *C. albicans* (except at 120 and 150 mg/mL PS concentrations for *Bacillus*), and *Enterococcus* exhibited more resistance to PDT than *Bacillus*. The highest RPSR value was seen at a methylene blue concentration of 30 mg/mL for both *Enterococcus* (53 %) and *Bacillus* (43 %), which was used in the subsequent experiments.

3.2. Effects of PDT on C. albicans in the single and dual cultures

Fig. 2a illustrates the growth suppression of planktonic *C. albicans* in both single and dual cultures subsequent to the PDT. In the pre-PDT experiment, co-culturing fungal and probiotic cells for 2 h prior to PDT resulted in a 32 % (*Bacillus*) and 43 % (*Enterococcus*) increase in *Candida* inactivation, compared to the PDT treatment of a single fungal culture. When single *Candida* cells received PDT and were subsequently combined with probiotics (post-PDT), the growth inhibition was enhanced by 20 % (*Bacillus*) and 30 % (*Enterococcus*) compared to the control. Fig. 2b depicts the enhancement of biofilm inhibition in the dual culture compared to the single *Candida* culture. *Bacillus* and *Enterococcus* achieved 70 % and 58 % more PDT inhibition of biofilm formation in the pre-PDT experiment, whereas the biofilm inhibition was 15 % and 30 % improved in the post-PDT experiments. Fig. 2c illustrates the eradication of biofilm following the PDT treatment. In the pretreatment experiment (pre-PDT), when the mixed bacteria/fungi biofilm aligned with the PDT, biofilm elimination was enhanced by 67 % and 46 % by *Bacillus* and *Enterococcus*, respectively. Once the initially illuminated single *Candida* biofilm was treated with probiotics (post-PDT), the biofilm eradication was improved by 42 % and 28 % with *Bacillus* and *Enterococcus*, respectively. The morphological analysis of C. albicans by scanning electron microscopy revealed a notable alteration following PDT treatment (Fig. 3). Prior to illumination, the cells were intact, smooth, and regularly shaped. Nonetheless, fungal cells when treated with PDT displayed several morphological alterations, including shrinkage and indentation. The most severe changes in cell morphology have been observed in the presence of the *Bacillus* (Fig. 3c).

3.3. Effects of PDT on cell dispersion from single and dual biofilms

The dispersion of fungal cells from the 24-hour single- and dual-formed biofilms after the PDT treatment was assessed for a further 24 h (Fig. 4). In the single-species biofilm, *Candida* cells dispersed 1.3 times less after the PDT treatment. Upon exposure of the dual fungal/probiotic biofilm to PDT, *Candida* dispersion was diminished by 1.5 (*Bacillus*) and 1.7 (*Enterococcus*) times relative to the control group.

3.4. Effect of PDT on nystatin susceptibility

The concentration of nystatin required to suppress the growth of a single fungal biofilm cell was 50 μg mL^{-1}. The nystatin resistance of *C. albicans* was reduced twofold (MIC 25 μg mL^{-1}) if the fungal biofilm was initially exposed to PDT. Photodynamic treatments of dual *Candida*-

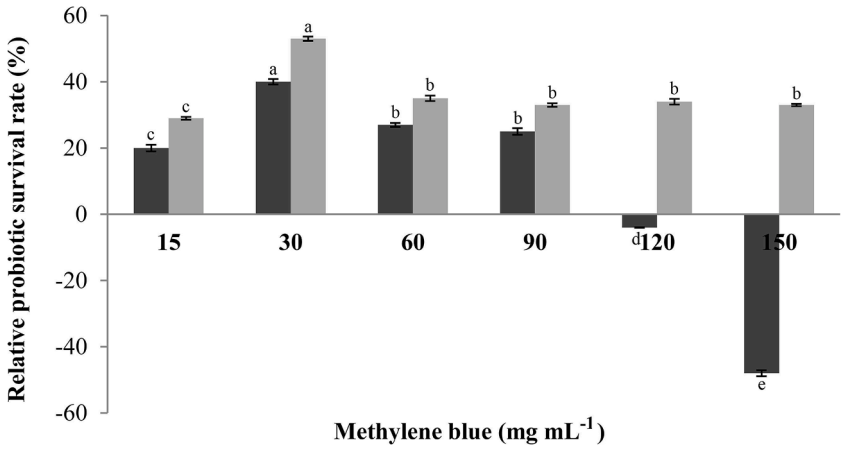

Fig. 1. Probiotic relative survival rate after photodynamic inactivation at different photosensitizer concentrations. Black column, *Bacillus*; gray column, *Enterococcus*.

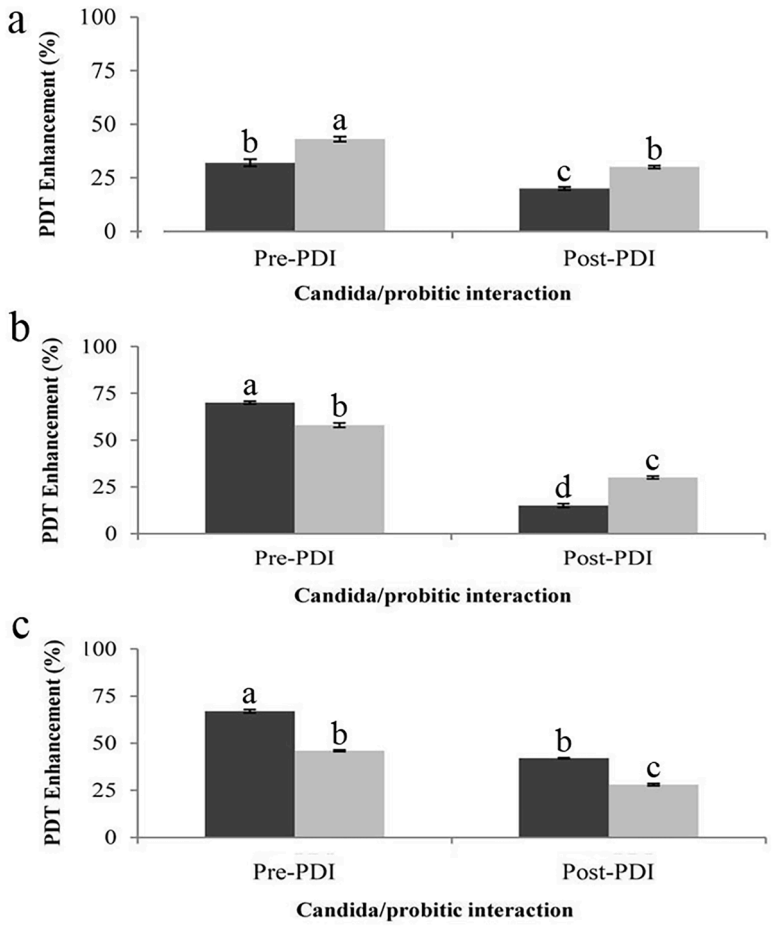

Fig. 2. *Candida albicans* photodynamic inactivation promotes by probiotic strains. a, planktonic growth inhibition; b, biofilm formation inhibition; c, biofilm eradication. Black column, *Bacillus*; gray column, *Enterococcus*.

probiotic biofilm reduced its resistance to less than 6.25 μg mL^{-1} antibiotic (Fig. 5).

3.5. Effects of PDT on Galleria mellonella mortality in single and dual strain injection

To determine the pathogenicity of single and dual microbial infections followed by the PDT treatment, third-instar *Galleria mellonella* were injected with *C. albicans*. The larvae died significantly faster when only infected with *C. albicans*, with 50 % mortality within 6 days. PDT decreased mortality, resulting in the death of only 15 % of larvae. The survival rate attained 100 % in the larvae injected with mixed fungal/bacterial suspension followed by PDT treatment (Fig. 6).

4. Discussion

Photodynamic therapy relies on the photosensitization of the (microbial) cells through the incorporation of exogenous chemicals referred

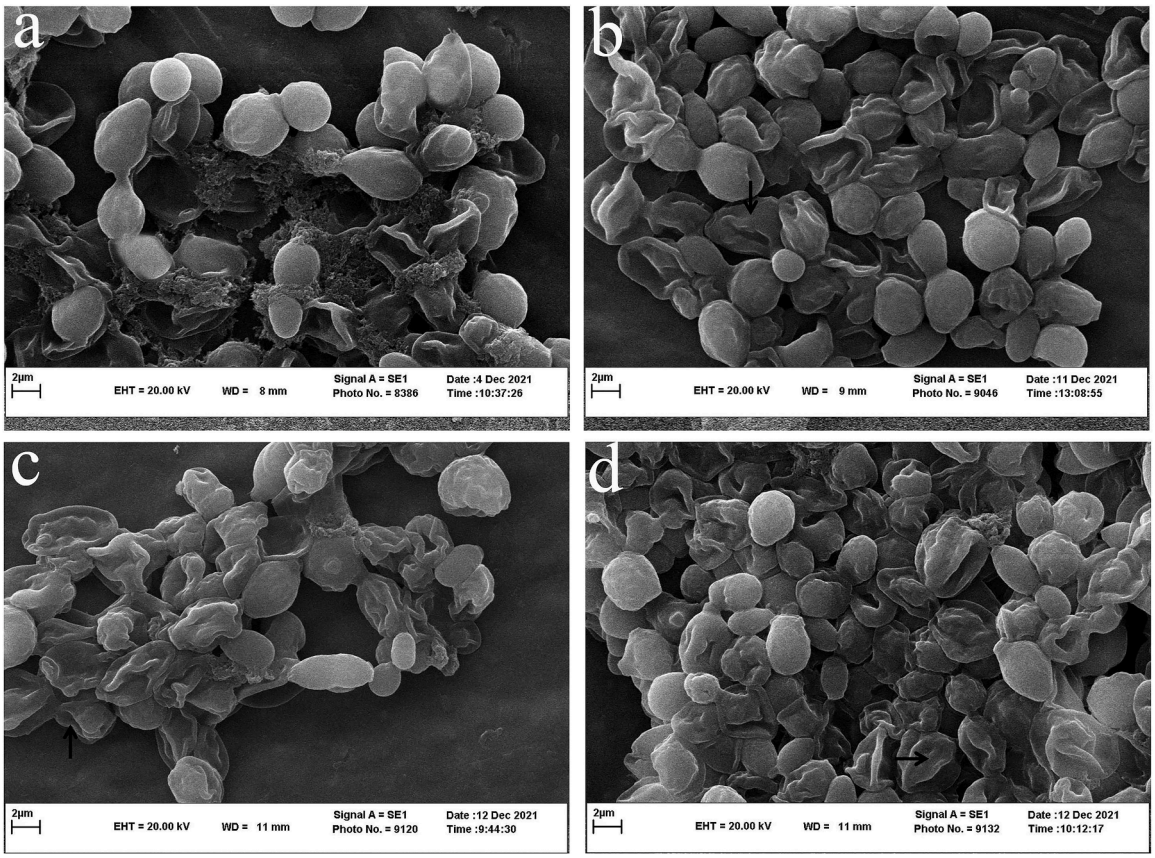

Fig. 3. Scanning electron micrographs of Candida albicans. a, single non-PDT-treated culture; b, single PDT-treated culture; c, dual *Candida/Bacillus* PDT-treated culture; d, dual *Candida/Enterococcus* PDT-treated culture. Arrows indicate morphological alterations including shrinkage and indentation.

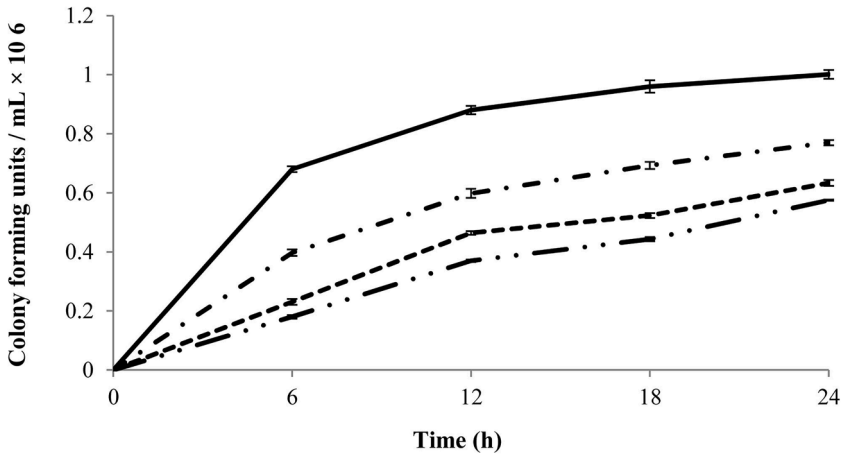

Fig. 4. Dispersion of *Candida albicans* cells from the biofilms. Solid line: single fungal biofilm; dash-dot line: single fungal biofilm treated by PDT; dash line: dual fungal/*Bacillus* biofilm treated by PDT; dash-dot-dot line: dual fungal/*Enterococcus* biofilm treated by PDT.

to as photosensitizers (PSs). Methylene blue, a cationic thiazine dye, is noted for its low toxicity, affordability, and commercial availability and was utilized in this study [18]. We used the same energy density for all PS concentrations, and in this condition, 30 mg/ml methylene blue showed the optimum PDT results. However, it should be noticed that the optimum PS concentration could be changed if variable radiation intensities were used. Probiotics exhibited more resistance to PDT than *C. albicans.* While the wavelength of the photosensitizer stimulation, laser power output, and irradiation duration remained constant throughout every experiment, the effects of photodynamic inactivation may differ among strains due to variations in intracellular PS concentration and subsequent oxidative stress conditions. Consistent with this theory, it has been established that gram-positive bacteria are more easily killed by PDT than gram-negative species, most likely because the PS generally accumulates more effectively in gram-positive bacteria than in gram-negative bacteria and fungi [19]. Nevertheless, numerous publications indicate that cationic dyes effectively penetrate the cell envelope of fungi, and there is growing evidence of their phototoxic efficacy against gram-negative bacteria and fungal cells [20, 21]. A notable characteristic of probiotics is their significant antioxidant activity. Intracellular carotenoid content protects the (microbial) cell from photoinactivation [22]. We anticipated that the superior capacity

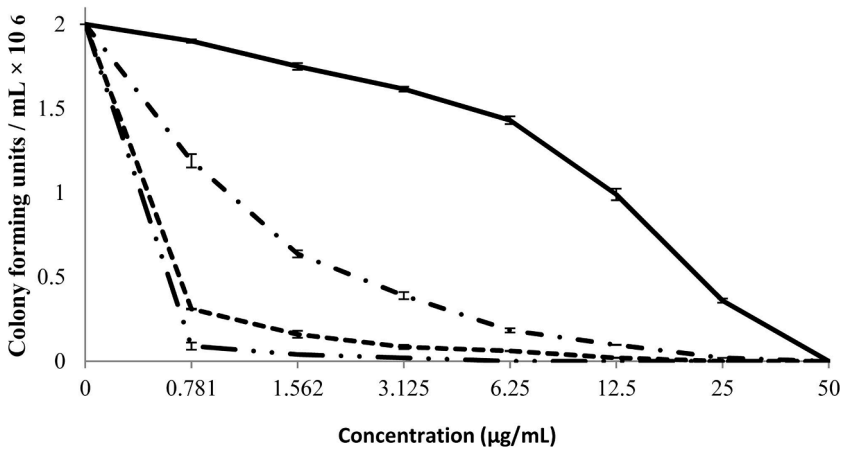

Fig. 5. *Candida albicans* growth in the presence of various nystatin concentrations. Solid line: single *Candida* cells; dash-dot line: single *Candida* cells after PDT; dash line: *Candida* in dual culture with *Bacillus* after PDT; dash-dot-dot line: *Candida* in dual culture with *Enterococcus* after PDT.

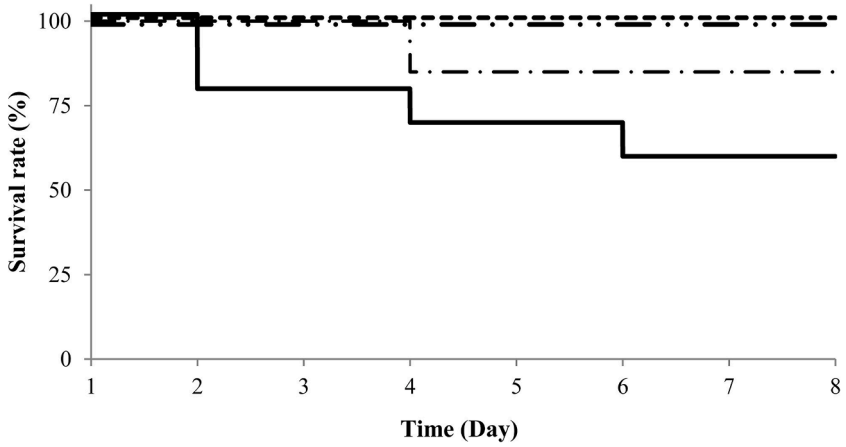

Fig. 6. Kaplan–Meier survival curves of *G. mellonella* larvae after infection. Solid line: larvae were injected by single *C. albicans*; dash-dot line: larvae were injected by single *C. albicans* treated by PDT; dash line: larvae were injected by dual fungal/*Bacillus* cells treated by PDT; dash-dot-dot line: larvae were injected by dual fungal/*Enterococcus* cells treated by PDT.

of *Bacillus* and *Enterococcus* strains to scavenge intracellular ROS can mitigate the effects of PDT against these cells compared to *Candida albicans*. High radiation intensities have been documented to entirely suppress the growth of certain *Enterococcus* [23] and *Bacillus* [24] strains; therefore, the application of radiation with lower energy density in probiotic/PDT strategy can effectively battle pathogenic fungus while conserving benign strains.

Probiotics enhanced the antimicrobial effects of PDT on *C. albicans*. Fungal cells uptake increased amount of methylene blue when they were co-incubated with *Bacillus* (1.2 times) and *Enterococcus* (1.4 times). An increase in intracellular PS can result in heightened oxidative stress, consequently intensifying the effects of PDT [10]. Probiotics have been documented to generate compounds that impair the integrity of the fungal cell, leading to modification in membrane permeability and cell shape [8]. Madduri and co-workers proved that *Bacillus* strains produce lipopeptides that effectively promote membrane permeabilization in *Candida glabrata* [25]. We predict the utilized probiotic strains might increase *Candida* porosity, resulting in greater accumulation of PS and subsequent radicals.

Inhibition of biofilm formation by PDT was enhanced in the dual probiotic/fungal culture compared to the single *Candida* culture. It was documented PDT illumination resulted in the downregulation of quorum sensing (QS) gene expression, which is essential for biofilm formation [26]. The interference of quorum sensing signals was also reported in the pathogen/probiotic interaction [27]. Therefore, PDT and probiotics may synergistically diminish fungal cell-to-cell communication, resulting in an enhanced reduction of biofilm formation. The potential of PDT to eliminate certain microbial biofilms was demonstrated in some previous studies [28]. In contrast to these reports, such as 60 min, 13.68 J/cm2 [29] and 30 min, 9.36 J/cm^2 [30], the combination of photodynamic therapy and probiotics was effective in eradicating biofilm with reduced exposure time and lowered energy intensity.

Cell deformation was noticed in several species, including Escherichia coli, Staphylococcus aureus, and Candida albicans when exposed to the PDT treatments [31,32]. We showed that changes are more severe in the probiotic/PDT treatment that may affect cell porosity and intracellular PS accumulation. Biofilm dispersal is the final stage of biofilm development, in which yeast cells detach from the biofilm to spread the infection. Biofilm dispersion may occur via an active or passive process. Previous studies suggested that PDT could damage the structurally important elements of the biofilm by the creation of highly reactive oxygen species, leading to the loss of its integrity and subsequent dispersal (passive mode) [33]. Quorum sensing (QS) signals are recognized to regulate multiple stages of biofilm development, including its dispersion. Both probiotics and PDT can disrupt fungal cell-to-cell signaling [34]; thus it is unsurprising that PDT-probiotic may reduce biofilm dispersal, as seen in this study (active mode). The efficacy of antifungal agents must be improved because of widespread resistance to antibiotics. The combined use of PDT and nystatin proved to be more effective than nystatin alone in the management of different candidiasis

[35]. We demonstrated the synergistic effects of probiotics, PDT, and nystatin on the inhibition of *Candida* cell growth. All probiotics, PDT and nystatin may influence fungal cell permeability [36,37], hence promoting the synergistic intracellular accumulation of photosensitizer (PS) and antibiotic, making it pertinent for the therapeutic management of superficial candidiasis. *Galleria mellonella* is a promising *in vivo* insect model utilized to assess *Candida* virulence and investigate its pathogenesis [38]. The mortality of larvae after infection with a specific dose is generally employed as the principal parameter to assess the virulence potential of *Candida* species [39]. In line with our results, Merigo et al. [17] indicate that the survival rate of *G. mellonella* larvae infected with *C. albicans* and various dye photosensitizers was markedly enhanced by PDT relative to the control group.

5. Conclusion

The study explored the impact of pre- and post-PDT interactions between *C. albicans* and two probiotic strains, *Bacillus* and *Enterococcus*, to improve the photodynamic inactivation (using a red laser (18 J/cm^2, 655 nm) as a light source and methylene blue dye (30 mg/ml) as a photosensitizer) of fungal cells. Probiotic cells were more resistant to PDT than *C. albicans*. Both bacterial strains could enhance PDT effects against the *in vitro* pathogenicity of *Candida*, including planktonic cell viability, biofilm formation, biofilm eradication, biofilm dispersion, and antibiotic resistance. Additional studies may contribute to the development of an innovative combined PDT/probiotic strategy as a supplementary therapeutic choice for superficial fungal infections like oral and vulvovaginal candidiasis.

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CRediT authorship contribution statement

Zeinab Ghanbari: Writing – original draft, Methodology, Investigation. **Ali Makhdoumi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

References

[1] R. Gaziano, S. Sabbatini, C. Monari, The interplay between *Candida albicans,* vaginal mucosa, host immunity and resident microbiota in health and disease: an overview and future perspectives, Microorganisms 11 (2023) 1211, https://doi.org/10.3390/microorganisms11051211.
[2] C. Tsui, E.F. Kong, M.A. Jabra-Rizk, Pathogenesis of *Candida albicans* biofilm, Pathog. Dis. 74 (4) (2016) ftw018, https://doi.org/10.1093/femspd/ftw018.
[3] V. Sustr, P. Foessleitner, H. Kiss, A. Farr, Vulvovaginal Candidosis: current concepts, challenges and perspectives, J. Fungi (Basel) 6 (4) (2020) 267, https://doi.org/10.3390/jof6040267.
[4] M. Satora, A. Grunwald, B. Zaremba, K. Frankowska, K. Żak, R. Tarkowski, K. Kułak, Treatment of vulvovaginal candidiasis-an overview of guidelines and the latest treatment methods, J. Clin. Med. 12 (16) (2023) 5376, https://doi.org/10.3390/jcm12165376.
[5] A. Barrientos-Durán, A. Fuentes-López, A. de Salazar, J. Plaza-Díaz, F. García, Reviewing the composition of vaginal microbiota: inclusion of nutrition and probiotic factors in the maintenance of eubiosis, Nutrients 12 (2) (2020) 419, https://doi.org/10.3390/nu12020419.
[6] F.M.F. Elshaghabee, N. Rokana, R.D. Gulhane, C. Sharma, H. Panwar, *Bacillus* as potential probiotics: status, concerns, and future perspectives, Front. Microbiol. 8 (2017) 1490, https://doi.org/10.3389/fmicb.2017.01490.
[7] E.J. Im, H.H. Lee, M. Kim, M.K. Kim, Evaluation of enterococcal probiotic usage and review of potential health benefits, safety, and risk of antibiotic-resistant strain emergence, Antibiotics (Basel) 12 (8) (2023) 1327, https://doi.org/10.3390/antibiotics12081327.
[8] R. Vazquez-Munoz, A. Dongari-Bagtzoglou, Anticandidal activities by *Lactobacillus* species: an update on mechanisms of action, Front. Oral Health 2 (2021) 689382, https://doi.org/10.3389/froh.2021.689382.
[9] A. Barzegari, K. Kheyrolahzadeh, S.M. Hosseiniyan Khatibi, S. Sharifi, M.Y. Memar, S.Z. Vahed, The battle of probiotics and their derivatives against biofilms, Infect. Drug Resist. 13 (2020) 659–672, https://doi.org/10.2147/IDR.S232982.
[10] N. Alvarez, A. Sevilla, Current advances in photodynamic therapy (PDT) and the future potential of PDT-combinatorial cancer therapies, Int. J. Mol. Sci. 25 (2) (2024) 1023, https://doi.org/10.3390/ijms25021023. PMID: 38256096; PMCID: PMC10815790.
[11] X. Wu, Y. Hu, Photodynamic therapy for the treatment of fungal infections, Infect. Drug Resist. 15 (2022) 3251–3266, https://doi.org/10.2147/IDR.S369605.
[12] R. Youf, M. Müller, A. Balasini, F. Thétiot, M. Müller, A. Hascoët, U. Jonas, H. Schönherr, G. Lemercier, T. Montier, T. Le Gall, Antimicrobial photodynamic therapy: latest developments with a focus on combinatory strategies, Pharmaceutics 13 (12) (2021) 1995, https://doi.org/10.3390/pharmaceutics13121995.
[13] Y. Farrokhi, B. Al-Shibli, D.F. Al-Hameedawi, Z. Neshati, A. Makhdoumi, *Escherichia coli* enhances the virulence factors of *Candida albicans*, the cause of vulvovaginal candidiasis, in a dual bacterial/fungal biofilm, Res. Microbiol. 172 (4–5) (2021) 103849, https://doi.org/10.1016/j.resmic.2021.103849.
[14] S. Talebi, A. Makhdoumi, M. Bahreini, M.M. Matin, H.S. Moradi, Three novel *Bacillus* strains from a traditional lacto-fermented pickle as potential probiotics, J. Appl. Microbiol. 125 (3) (2018) 888–896, https://doi.org/10.1111/jam.13901.
[15] O. Erkmen, Preparation of media and sterilization techniques, in: O. Erkmen, Laboratory Practices in Microbiology, Academic Press, 2021, pp. 3–18.
[16] A. Taraszkiewicz, G. Szewczyk, T. Sarna, K.P. Bielawski, J. Nakonieczna, Photodynamic inactivation of *Candida albicans* with imidazoacridinones: influence of irradiance, photosensitizer uptake and reactive oxygen species generation, PLoS One 10 (6) (2015) e0129301, https://doi.org/10.1371/journal.pone.0129301.
[17] E. Merigo, S. Conti, T. Ciociola, C. Fornaini, L. Polonelli, G. Lagori, M. Manfredi, P. Vescovi, Effect of different wavelengths and dyes on *Candida albicans: in vivo* study using *Galleria mellonella* as an experimental model, Photodiagnosis, Photodyn. Ther. 18 (2017) 34–38, https://doi.org/10.1016/j.pdpdt.2017.01.181.
[18] I. Khan, K. Saeed, I. Zekker0, et al., Review on methylene blue: its properties, uses, toxicity and photodegradation, Water 14 (2) (2022) 242, https://doi.org/10.3390/w14020242.
[19] F.F. Sperandio, Y.Y. Huang, M.R. Hamblin, Antimicrobial photodynamic therapy to kill Gram-negative bacteria, Recent Pat. Anti-Infect. Drug Discov. 8 (2) (2013) 108–120, https://doi.org/10.2174/1574891x113089990012.
[20] B. Sharma, V. Thakur, G. Kaur, G.R. Chaudhary, Efficient photodynamic therapy against gram-positive and gram-negative bacteria using rose Bengal encapsulated in metallocatanionic vesicles in the presence of visible light, ACS Appl. Bio Mater. 3 (12) (2020) 8515–8524, https://doi.org/10.1021/acsabm.0c00901.
[21] X. Wu, Y. Hu, Photodynamic therapy for the treatment of fungal infections, Infect. Drug Resist. 15 (2022) 3251–3266, https://doi.org/10.2147/IDR.S369605.
[22] T.N. Demidova, M.R. Hamblin, Effect of cell-photosensitizer binding and cell density on microbial photoinactivation, Antimicrob. Agents Chemother. 49 (6) (2005) 2329–2335, https://doi.org/10.1128/AAC.49.6.2329-2335.2005.
[23] M. Rubilar-Huenchuman, C. Ortega-Villanueva, I.A. González, C.E. Palavecino, The effect of photodynamic therapy on *Enterococcus* spp. and its application in dentistry: a scoping review, Pharmaceutics 16 (6) (2024) 825, https://doi.org/10.3390/pharmaceutics16060825.
[24] L. Dong, J. Qin, L. Tai, K. Mou, X. Liao, F. Chen, X. Hu, Inactivation of *Bacillus subtilis* by curcumin-mediated photodynamic technology through inducing oxidative stress response, Microorganisms 10 (4) (2022) 802, https://doi.org/10.3390/microorganisms10040802.
[25] M. Madduri, S.M. Rudramurthy, U. Roy, Two promising natural lipopeptides from *Bacillus subtilis* effectively induced membrane permeabilization in *Candida glabrata*, Acta Biochim. Pol. 71 (2024) 11999, https://doi.org/10.3389/abp.2024.11999.
[26] N. Tang, S. Yuan, Y. Luo, A.J. Wang, K. Sun, N.N. Liu, K. Tao, Nanoparticle-based photodynamic inhibition of *Candida albicans* biofilms with interfering quorum sensing, ACS Omega 8 (4) (2023) 4357–4368, https://doi.org/10.1021/acsomega.2c07740.
[27] M.K. Salman, J. Abuqwider, G. Mauriello, Anti-quorum sensing activity of probiotics: the mechanism and role in food and gut health, Microorganisms 11 (3) (2023) 793, https://doi.org/10.3390/microorganisms11030793.
[28] D. Wang, E. Kyere, F. Ahmed Sadiq, New trends in photodynamic inactivation (PDT) combating biofilms in the food industry-a review, Foods 10 (11) (2021) 2587, https://doi.org/10.3390/foods10112587.
[29] B. Chen, J. Huang, H. Li, Q.H. Zeng, J.J. Wang, H. Liu, Y. Pan, Y. Zhao, Eradication of planktonic *Vibrio parahaemolyticus* and its sessile biofilm by curcumin-mediated photodynamic inactivation, Food Control 113 (2020) 107181, https://doi.org/10.1016/j.foodcont.2020.107181.
[30] B. Chen, J. Huang, Y. Liu, H. Liu, Y. Zhao, J.J. Wang, Effects of the curcumin-mediated photodynamic inactivation on the quality of cooked oysters with *Vibrio parahaemolyticus* during storage at different temperature, Int. J. Food Microbiol. 345 (2021) 109152, https://doi.org/10.1016/j.ijfoodmicro.2021.109152.
[31] C. Rodríguez-Cerdeira, E. Martínez-Herrera, G. Fabbrocini, B. Sanchez-Blanco, A. López-Barcenas, M. El-Samahy, E.R. Juárez-Durán, J.L. González-Cespón, New applications of photodynamic therapy in the management of candidiasis, J. Fungi (Basel) 7 (12) (2021) 1025, https://doi.org/10.3390/jof7121025.
[32] R. Cahan, N. Swissa, G. Gellerman, Y. Nitzan, Photosensitizer-antibiotic conjugates: a novel class of antibacterial molecules, Photochem. Photobiol. 86 (2) (2010) 418–425, https://doi.org/10.1111/j.1751-1097.2009.00674.x.
[33] W.C. de Melo, P. Avci, M.N. de Oliveira, et al., Photodynamic inactivation of biofilm: taking a lightly colored approach to stubborn infection, Expert Rev. Anti Infect. Ther. 11 (7) (2013) 669–693, https://doi.org/10.1586/14787210.2013.811861.
[34] Z. Fekrirad, N. Kashef, E. Arefian, Photodynamic inactivation diminishes quorum sensing-mediated virulence factor production and biofilm formation of *Serratia*

marcescens, World J. Microbiol. Biotechnol. 35 (2019) 191, https://doi.org/10.1007/s11274-019-2768-9.

[35] Q. Hu, T. Li, J. Yang, Y. Peng, Q. Liu, N. Liu, Efficacy of photodynamic therapy in the treatment of oral candidiasis: a systematic review and meta-analysis, BMC Oral Health 23 (1) (2023) 802, https://doi.org/10.1186/s12903-023-03484-z.

[36] H. Kinoshita, M. Yoshioka, F. Ihara, T. Nihira, Cryptic antifungal compounds active by synergism with polyene antibiotics, J. Biosci. Bioeng. 121 (2016) 394–398, https://doi.org/10.1016/j.jbiosc.2015.08.003.

[37] L.M. Baltazar, A. Ray, D.A. Santos, P.S. Cisalpino, A.J. Friedman, J.D. Nosanchuk, Antimicrobial photodynamic therapy: an effective alternative approach to control fungal infections, Front. Microbiol. 6 (2015) 202, https://doi.org/10.3389/fmicb.2015.00202.

[38] I.D. Jacobsen, *Galleria mellonella* as a model host to study virulence of *Candida*, Virulence 5 (2) (2014) 237–239, https://doi.org/10.4161/viru.27434.

[39] S. Gago, R. García-Rodas, I. Cuesta, E. Mellado, A. Alastruey-Izquierdo, *Candida parapsilosis, Candida orthopsilosis*, and *Candida metapsilosis* virulence in the non-conventional host *Galleria mellonella*, Virulence 5 (2) (2014) 278–285, https://doi.org/10.4161/viru.26973.