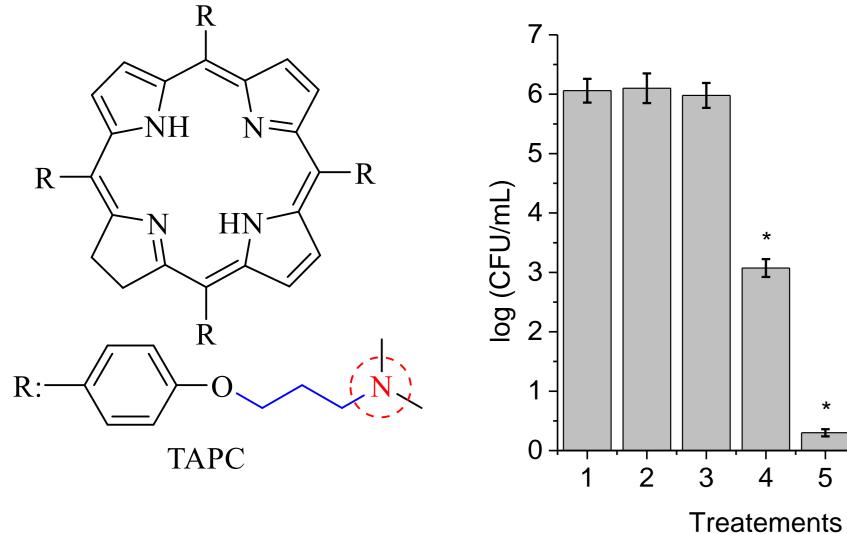
Photodynamic effect of 5,10,15,20-tetrakis[4-(3-N,N-dimethylaminopropoxy)phenyl]chlorin towards the human pathogen *Candida albicans* under different culture conditions

Paula Cordero, Darío D. Ferreyra, María G. Alvarez and Edgardo N. Durantini

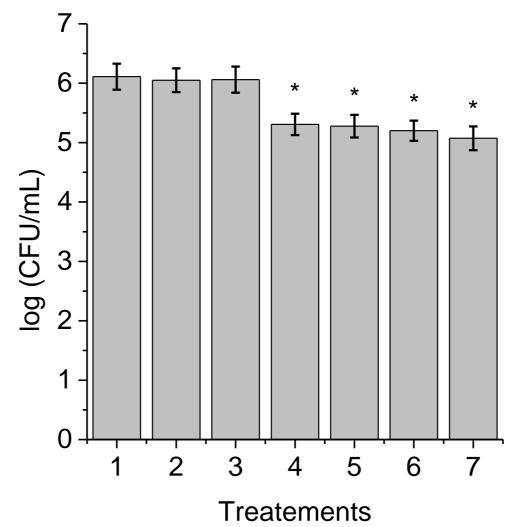
IDAS-CONICET, Departamento de Química, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Ruta Nacional 36 Km 601, X5804BYA Río Cuarto, Córdoba, Argentina. Correspondence: edurantini@exa.unrc.edu.ar

For over a hundred years, it has been known that light and dyes can kill microorganisms. Although this therapy was overshadowed by antibiotics, increased resistance of microorganisms to these compounds has restarted research to develop new antimicrobial strategies. In this way, photodynamic inactivation (PDI) has shown be effective to eliminate microorganisms. This method is founded on the addition of a photosensitizer (PS) that rapidly binds to microbial cells. Under aerobiosis, excitation of the PS with visible light of an appropriate wavelength produces reactive oxygen species (ROS), which can react with the biomolecules into de cells. Thus, this photocytotoxic effect lead to the death of microbes.

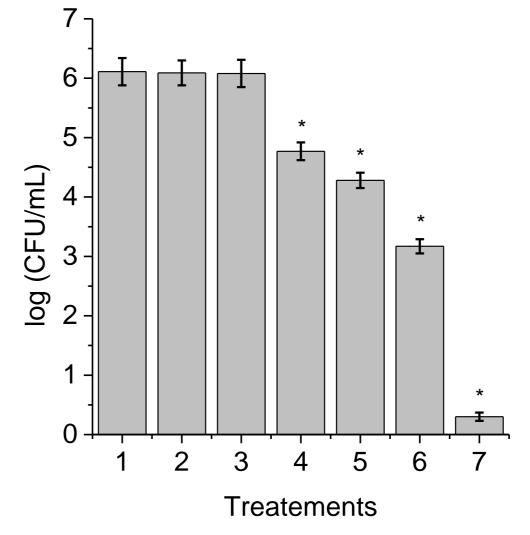
Survival of *C. albicans* incubated with 2.5 μ M TAPC and irradiated with white light (90 mW/cm²)



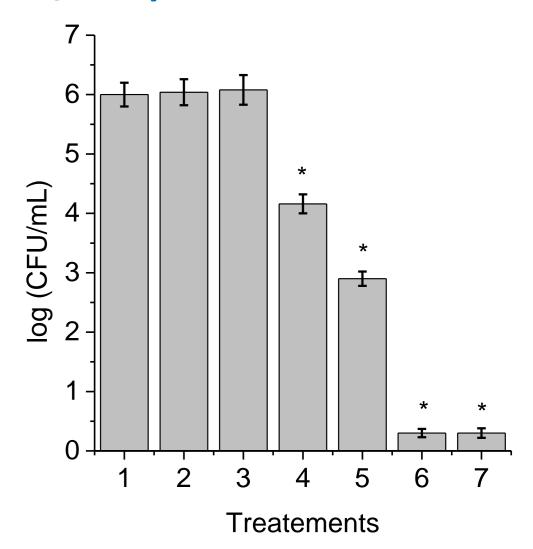
1) dark control; 2) irradiated control; 3) cells treated with TAPC in dark; 4) cells treated with TAPC and 2 min irradiation; 5) cells treated with TAPC and 5 min irradiation; 6) cells treated with TAPC and 15 min irradiation; 7) cells treated with TAPC and 30 min irradiation



1) dark control treated with NaN₃; 2) irradiated control treated with NaN₃; 3) cells treated with NaN₃ and TAPC in dark; 4) cells treated with NaN₃ and TAPC and 2 min irradiation; 5) cells treated with NaN₃ and TAPC and 5 min irradiation; 6) cells treated with NaN₃ and TAPC and 15 min irradiation; 7) cells treated with NaN₃ and TAPC and 30 min irradiation

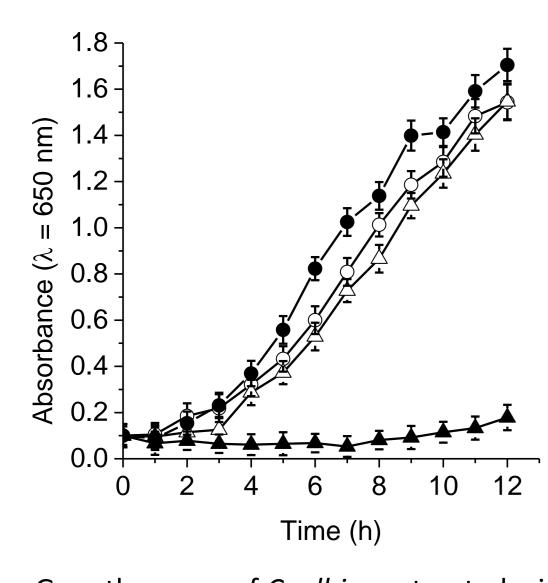


1) dark control treated with D-mannitol; 2) irradiated control treated with D-mannitol; 3) cells treated with D-mannitol and TAPC in dark; 4) cells treated with D-mannitol and TAPC and 2 min irradiation; 5) cells treated with D-mannitol and TAPC and 5 min irradiation; 6) cells treated with D-mannitol and TAPC and 15 min irradiation; 7) cells treated with D-mannitol and TAPC and 30 min irradiation



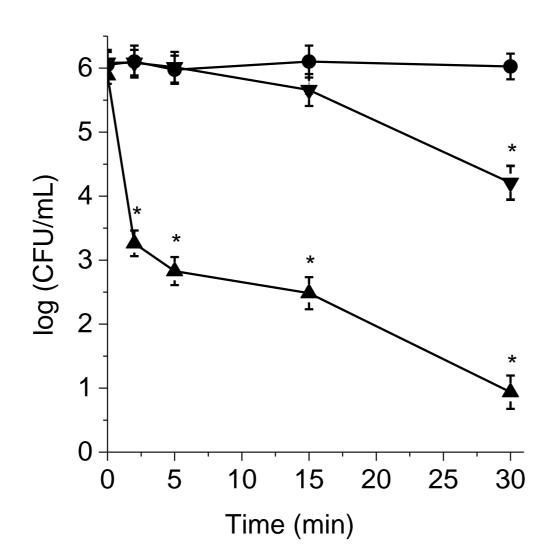
1) dark control treated with DMSO; 2) irradiated control treated with DMSO; 3) cells treated with DMSO and TAPC in dark; 4) cells treated with DMSO and TAPC and 2 min irradiation; 5) cells treated with DMSO and TAPC and 5 min irradiation; 6) cells treated with DMSO and TAPC and 15 min irradiation; 7) cells treated with DMSO and TAPC and 30 min irradiation

Photosensitized effect of TAPC on the growth of *C. albicans* cells



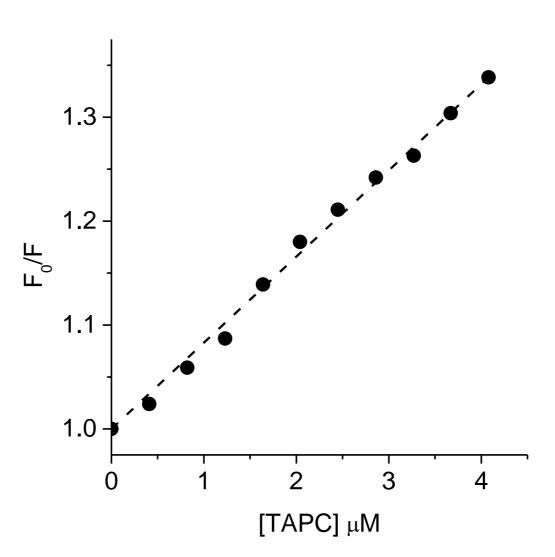
Growth curves of *C. albicans* treated with 5 μ M TAPC (\blacktriangle) and exposed to white light in SB at 37 $\,^{\circ}$ C. Controls: untreated cells in dark (O), untreated cells irradiated (\bullet), cells treated with 5 μ M TAPC in dark (\triangle).

Photoinactivation of *C. albicans* pseudohyphae



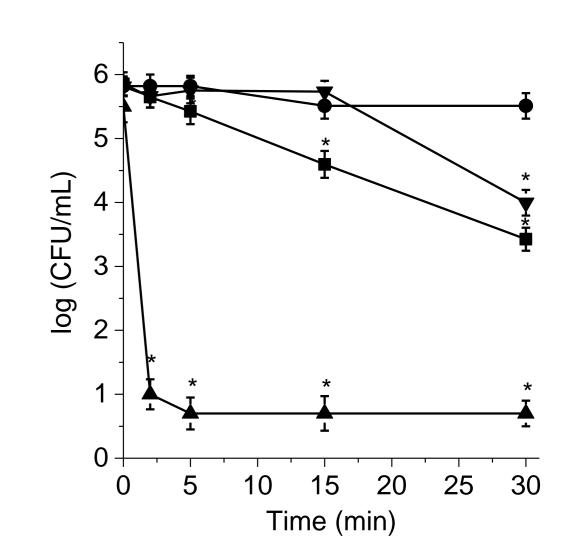
Survival curves of pseudohyphae of *C. albicans* incubated with 5 μ M TAPC in PBS (\clubsuit) and in HS (\blacktriangledown) for 30 min at 37 $^{\circ}$ C in dark and exposed to visible light for different irradiation times. Control pseudohyphae of *C. albicans* untreated with TAPC and irradiated (\bullet), pseudohyphae of *C. albicans* treated with 2.5 μ M TAPC in PBS in dark (\triangle) and in HS in dark (∇)

Interaction of TAPC with BSA



Stern-Volmer plot of TAPC quenching of BSA (3 x 10-5 M) in water ($\lambda_{\rm exc}$ = 295 nm, $\lambda_{\rm em}$ = 340 nm). $K_{\rm SV}$ = 8.29x10⁴ M⁻¹ and $k_{\rm q}$ = 8.29 x10¹² s⁻¹ M⁻¹

Inactivation of planktonic cells in presence of BSA



Survival curves of *C. albicans* incubated with 5 μ M TAPC in PBS (\blacktriangle) and containing 1% (\blacksquare) and 4.5% BSA (\blacktriangledown) for 30 min at 37 $^{\circ}$ C in dark and exposed to white light for different irradiation times. Control of *C. albicans* untreated with TAPC and irradiated (\bullet), *C. albicans* treated with 5 μ M TAPC in PBS in dark (\triangle)

Conclusions. Photokilling of *Candida albicans* sensitized by 5,10,15,20-tetrakis[4-(3-*N*,*N*-dimethylaminopropoxy)phenyl]chlorin (TAPC) was studied in under different culture conditions. Planktonic yeast suspensions in PBS treated with 5 mM TAPC were eliminated after an irradiation of 5 min with white light. The addition of reactive oxygen species scavengers showed that singlet molecular oxygen was involved in the photoinactivation. Also, a contribution of type I mechanism was detected in the yeast inactivation. Under growth conditions, *C. albicans* was detained in presence of TAPC and white light irradiation. Moreover, photocytotoxic activity was evaluated in *C. albicans* pseudohyphae. A reduction of 5 log and 1.5 log were found in pseudohyphae survival suspended in PBS and human serum (HS), respectively. This difference was assigned to a decrease in the binding of the photosensitizer to the pseudohyphae in the serum. Therefore, the photoinactivation of *C. albicans* cells in PBS was investigated in the presence of albumin (bovine serum albumin, BSA). The interaction of TAPC with this protein produced a reduction in the photoinactivation. These studies allow determined the appropriated conditions to eradicate *C. albicans* under different culture conditions.