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Effect of photodynamic inactivation on virulence factors of different strains of *Staphylococcus aureus*

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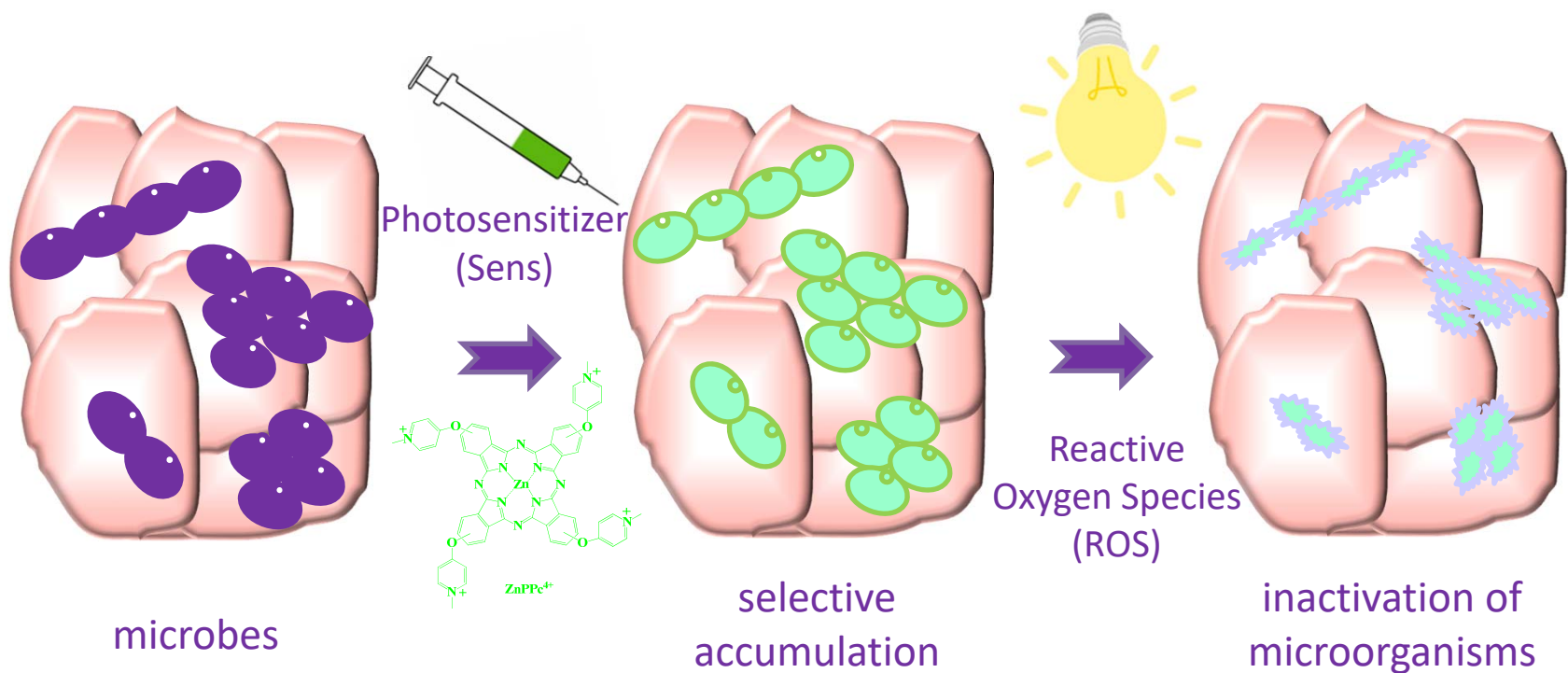


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Effect of photodynamic inactivation on virulence factors of different strains of *Staphylococcus aureus*

Graphical Abstract



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Abstract: The dramatic increase of bacteria resistant to antibiotics makes it unlikely that the discovery of new classical drugs will solve this problem permanently. Therefore, new antimicrobial approaches are needed to treat antibiotic-resistant *S. aureus* infections. Photodynamic inactivation (PDI) emerges as a photochemotherapeutic approach with applications in antimicrobial therapy. The PDI process is based on the combined use of light, oxygen, and a photosensitizing (PS) agent. These three components interact to generate reactive oxygen species (ROS), which are cytotoxic and irreversibly damage the vital components of microbial cells, causing death. In this work, the photoantimicrobial effect of zinc(II) 2,9,16,23-tetrakis[4-(*N*-methylpyridyloxy)]phthalocyanine (ZnPPc⁴⁺) on different *S. aureus* strains was evaluated, comparing its action on several virulence factors (hemolysin, lipase and lecithinase activity and mannitol fermentation) before and after PDI. Finally, the ability to produce biofilms pre- and post-treatment was analyzed. The results indicate that ZnPPc⁴⁺ is an effective PS to photoinactivate different strains of planktonic *S. aureus*, at low concentration and light doses, and to significantly reduce the number of bacteria that are part of biofilm. Therefore, PDI may become a promising alternative therapy, not only to control the reproduction of pathogenic microorganisms, but also to reduce the effects of virulence factors that may remain after PDI.

Keywords: bacteria; biofilms; photodynamic inactivation; phthalocyanine; virulence factors.



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Introduction

The misusing and overusing of antibiotics due to:

- ◆ Increase in the consumption of antibiotics.
- ◆ Inadequate prescription of antibiotics.
- ◆ Self-medication and overuse.
- ◆ Failure of some patients to complete the treatments.
- ◆ Widespread use of antibiotics in livestock feed.



Antibiotics Resistance

When bacteria become resistant to antibiotics, common infections will no longer be treatable

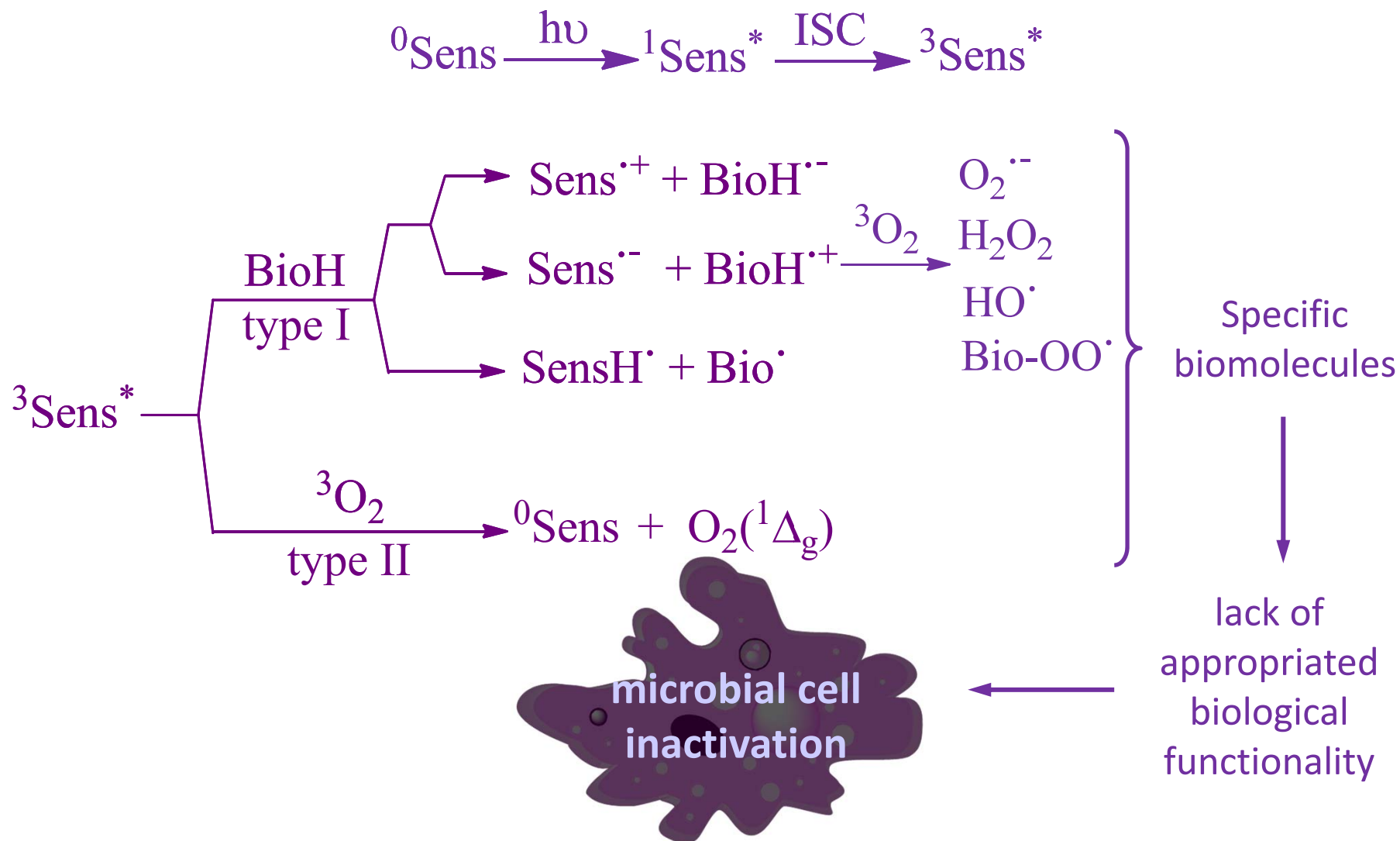
Search for new effective antimicrobial therapies



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Introduction



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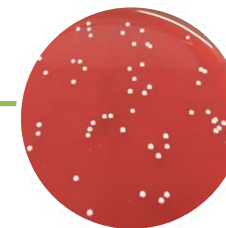
Introduction

Virulence Factors

Staphylococcus aureus has more than 40 virulence factors

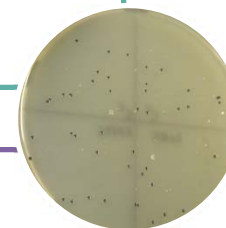
β -hemolysin

- Lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent.



Lipase

- Catalyzes hydrolysis of lipids in Baird-Parker medium. Appear an iridescent film in and immediately surrounding colonies, visible by reflected light.

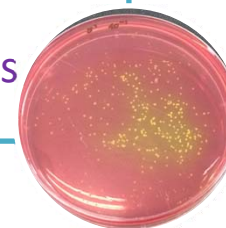


Lecithinase

- A precipitate zone is evidenced in Baird-Parker medium surrounding the colonies.

Mannitol fermentation

- Coagulase-positive *staphylococci* produce colonies with bright yellow zones in mannitol salt agar plates.



Biofilm



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Introduction

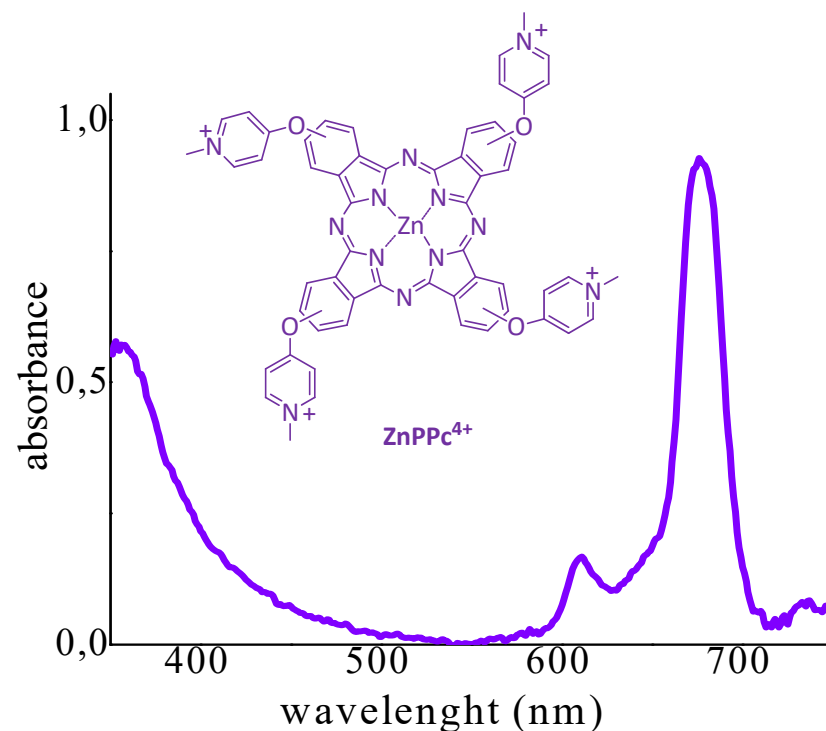
Strains

S. aureus ATCC 25923
Reference strain

S. aureus DM1
Isolated from hospital
infection

S. aureus DM2
Isolated from bovine
mastitis infection

Photosensitizer



PS	λ_{abs} (nm)	λ_{em} (nm)	ϵ (M ⁻¹ cm ⁻¹)	Φ_{F}	Φ_{Δ}
ZnPPc ⁴⁺	680	686	1.10x10 ⁵	0.22	0.59



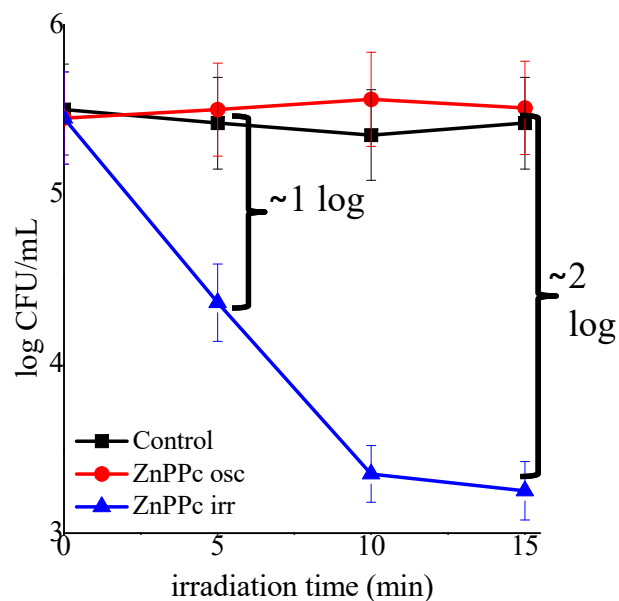
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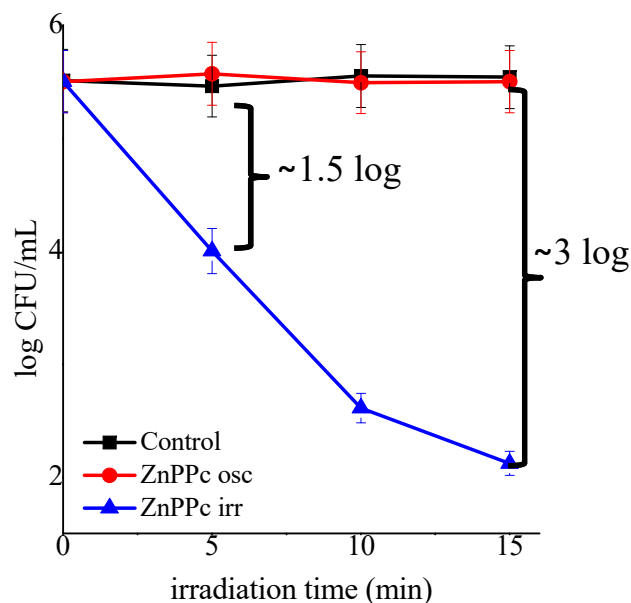
Results and discussion

PDI of planktonic *S. aureus* strains

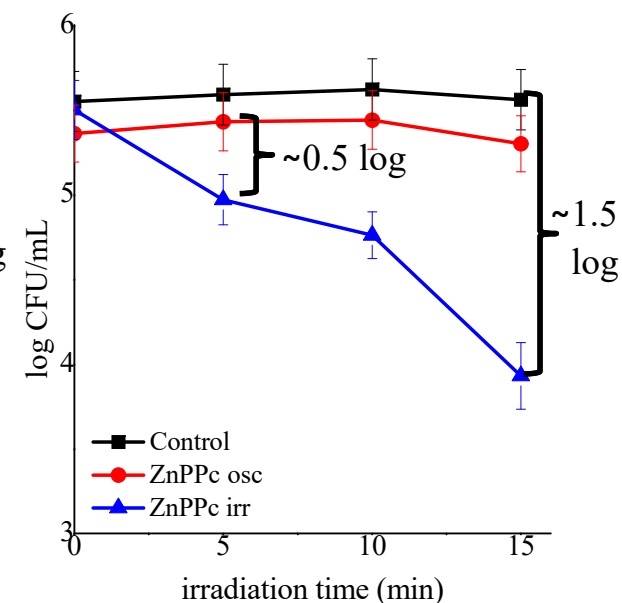
S. aureus ATCC 25923
ZnPPc⁴⁺ = 0.5 μ M



S. aureus DM1
ZnPPc⁴⁺ = 25 nM



S. aureus DM2
ZnPPc⁴⁺ = 25 nM



- PDI was effective against all strains at very low concentrations of ZnPPc⁴⁺ and light doses.
- Differences in susceptibility to photodynamic treatment were observed.

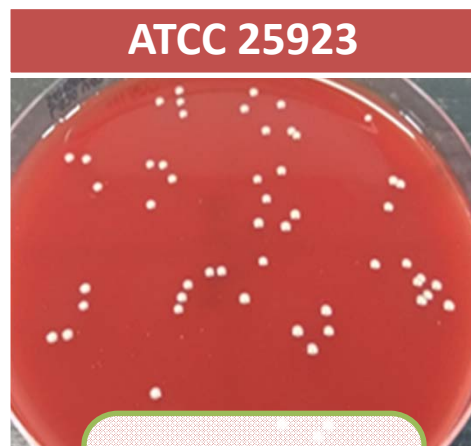


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Results and discussion

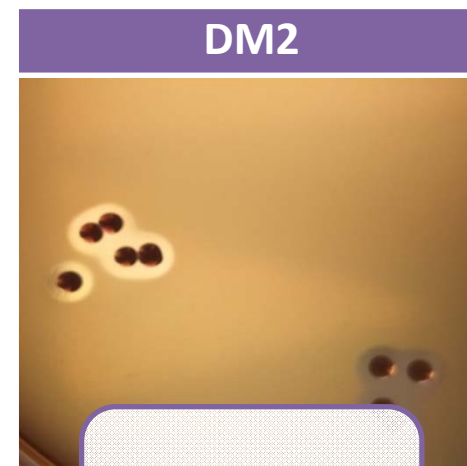
✓ β -hemolysin



Presence of hemolysis before and after PDI.



Presence of hemolysis before and after PDI.



Presence of hemolysis before and after PDI.

- All strains showed large, creamy, yellowish, smooth-edged and convex colonies and no changes after PDI treatment.

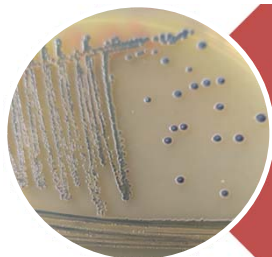


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Results and discussion

✓ Lipase and lecithinase activity

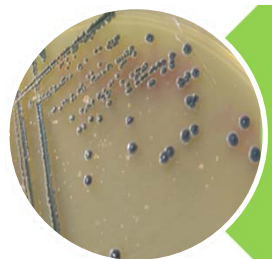


ATCC 25923

Tellurite reduction to tellurium

Presence of lipase activity

Absence of lecithinase activity

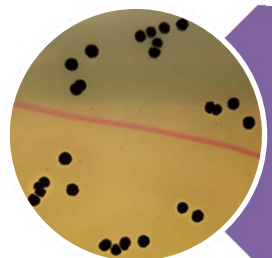


DM 1

Tellurite reduction to tellurium

Presence of lipase activity

Absence of lecithinase activity



DM 2

Tellurite reduction to tellurium

Presence of lipase activity

Absence of lecithinase activity



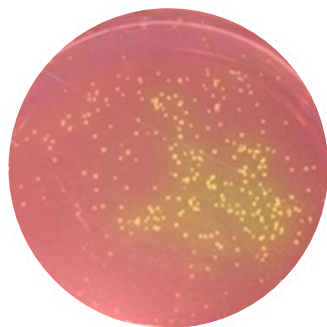
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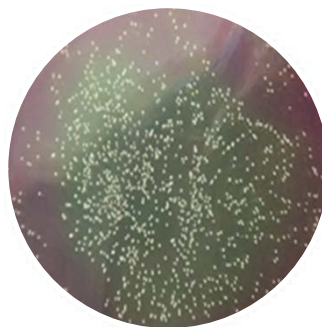
Results and discussion

✓ Mannitol fermentation

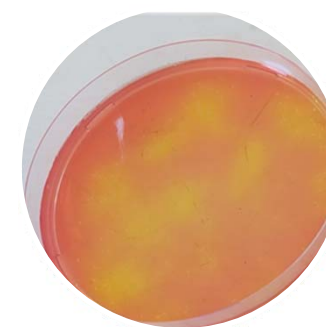
S. aureus ATCC 25923
ZnPPc⁴⁺ = 0.5 μ M



S. aureus DM1
ZnPPc⁴⁺ = 25 nM



S. aureus DM2
ZnPPc⁴⁺ = 25 nM



- All strains presented yellow colonies with halo before and after PDI treatment.
- *S. aureus* ATCC 25923 and DM1 showed differences in colony size after PDI treatment.



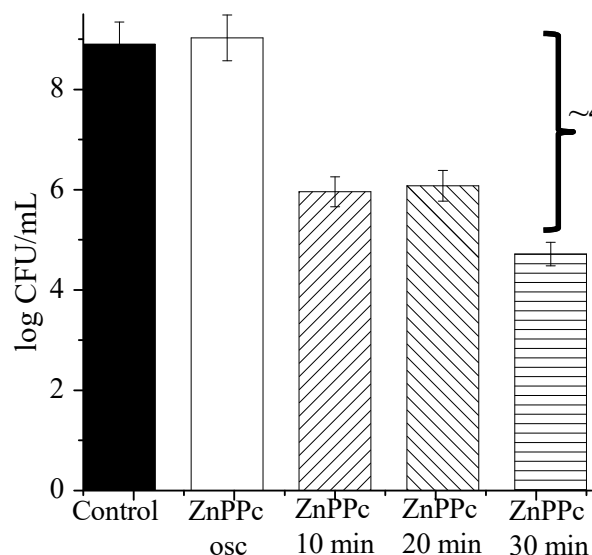
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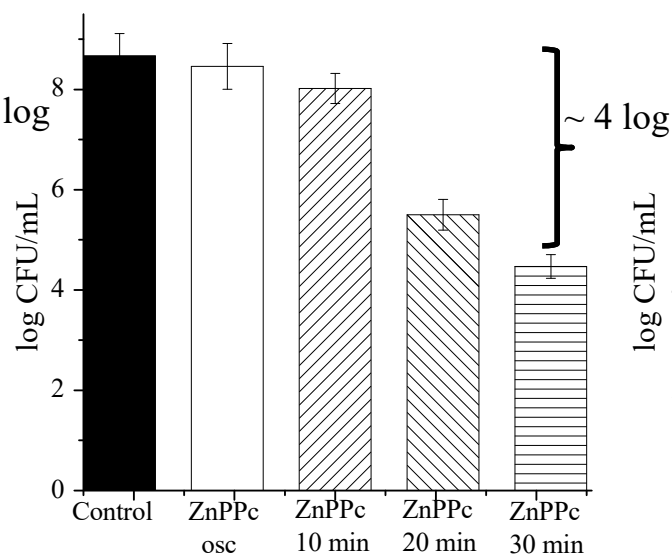
Results and discussion

PDI of *S. aureus* biofilms

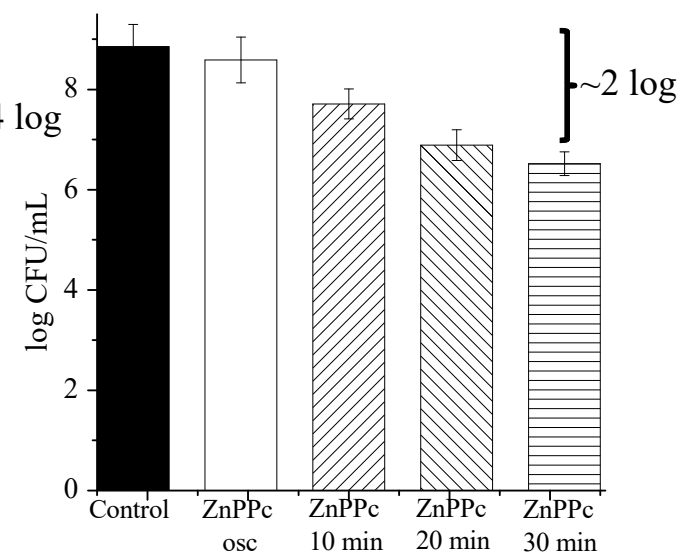
S. aureus ATCC25923
ZnPPc⁴⁺ = 1 μ M



S. aureus DM1
ZnPPc⁴⁺ = 50 nM



S. aureus DM2
ZnPPc⁴⁺ = 0.1 μ M



- Biofilm cell count of the three strains progressively decreases as the irradiation times increase.
- In all cases, a considerable decrease in the number of cells was obtained.



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Conclusions

- ZnPPc⁴⁺ was not toxic in absence of light at the concentrations tested for any of the three *S. aureus* strains.
- PDI was effective for all planktonic *S. aureus* strains.
- *S. aureus* DM1 showed the greatest sensitivity. *S. aureus* ATCC 25923 was the most resistant strain to PDI.
- ZnPPc⁴⁺ proved to be a very efficient photosensitizer, since low concentrations and short illumination times cause good inactivation rates in the three strains analyzed.
- The phenotypic expression of enzymes β -hemolysin, lipase and lecithinase was not affected by PDI, at the ZnPPc⁴⁺ concentrations and light doses studied.



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Conclusions

- A phenotypic effect was observed on strain DM1 after PDI. A smaller size of colonies respect to control culture was found after 15 min irradiation.
- The ability to ferment mannitol was maintained in all the cultures that underwent the photodynamic treatment.
- PDI by ZnPPc⁴⁺ was effective on biofilms of the three *S. aureus* strains.
- ZnPPc⁴⁺ significantly reduce the number of bacteria that are part of biofilm in all strains tested, at low concentration and light doses.
- PDI using ZnPPc⁴⁺ may become a promising alternative therapy, not only to control the reproduction of pathogenic microorganisms, but also to reduce the effects of virulence factors that may remain after PDI.



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