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Evaluation of different quantification methods to determine the photodynamic inactivation of bacterial biofilms

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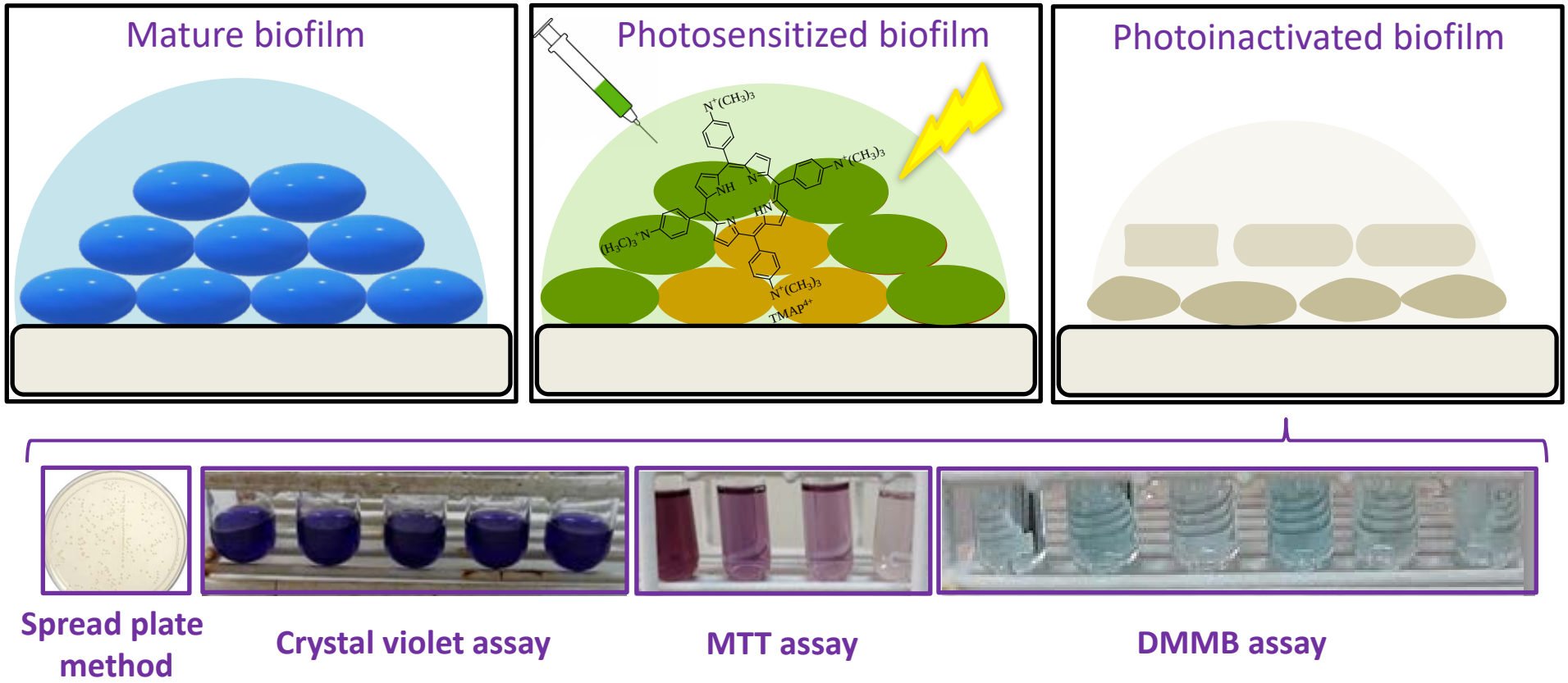
Evaluation of different quantification methods to determine the photodynamic inactivation of bacterial biofilms

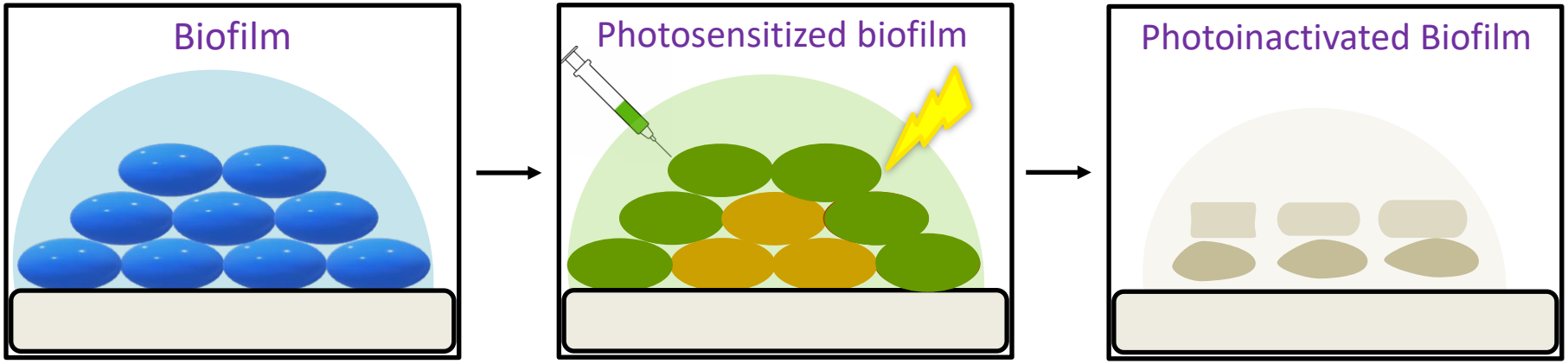
Graphical Abstract



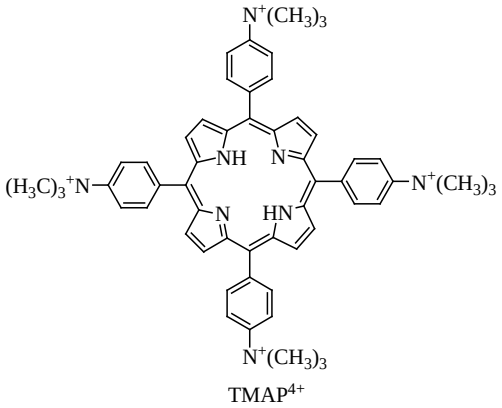
Abstract: Biofilms are a crucial factor in persistent infections. This global health threat has stimulated interest in the development of effective antimicrobial drugs and therapies. Photodynamic Inactivation (PDI) uses a photosensitizer and light to produce reactive oxygen species under aerobic conditions, which damage cellular components, inducing the death of microorganisms. In this work, the photodynamic action of 5,10,15,20-tetra (4-*N,N,N*-trimethylammoniophenyl) porphyrin ($TMAP^{4+}$) on the Gram positive *Staphylococcus aureus* and the Gram negative *Escherichia coli* biofilms was quantified by different methods. First, the plate count technique showed that PDI is more efficient on *S. aureus* than *E. coli* biofilm, and the photodynamic effect increases with irradiation time. The crystal violet staining did not allow distinguishing significant differences between biofilms with or without treatment of both bacteria. On the other hand, the MTT method showed that there was a decrease in the number of viable cells as the irradiation time increased for both strains. Finally, DMMB staining determined that the amount of matrix present in the biofilm decreased as fluence increased, being more evident in *S. aureus* than in *E. coli*. Increasing knowledge about the PDI target on biofilms allows the design of more specific control strategies for its efficient eradication.
Keywords: bacteria - biofilms - photodynamic inactivation - porphyrin - quantification methods

Introduction

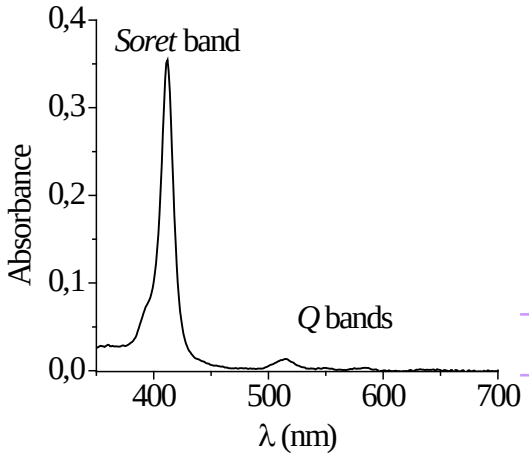
Photodynamic inactivation of biofilms



Photosensitizer



TMAP^{4+}



Bacteria

Gram positive — *Staphylococcus aureus* ATCC 25923

Gram negative — *Escherichia coli*

λ_{abs}(nm)	λ_{em}(nm)	ε ($M^{-1}cm^{-1}$)	Φ_F	Φ_Δ
412	644	1,78x10^5 [a]	0,12[b]	0,65[b]

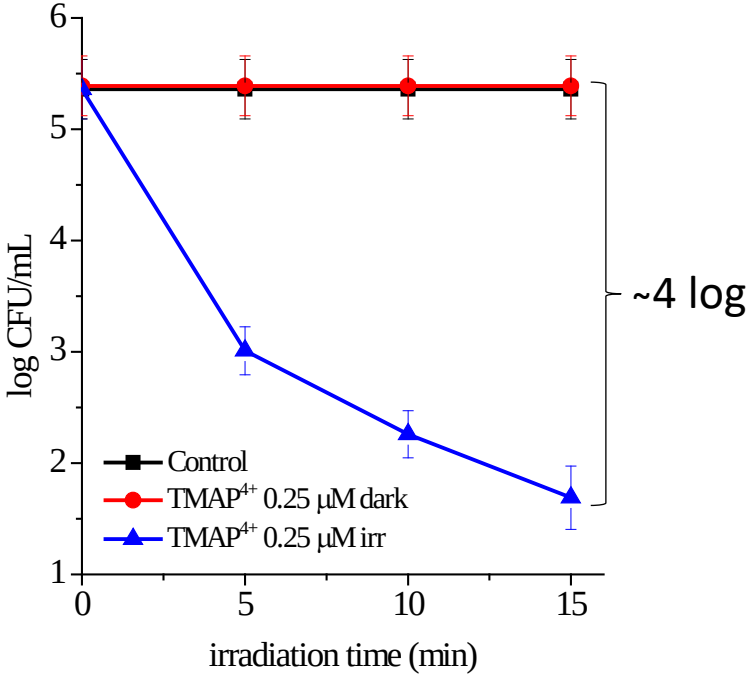
[a] water, [b] DMF

Results and discussion

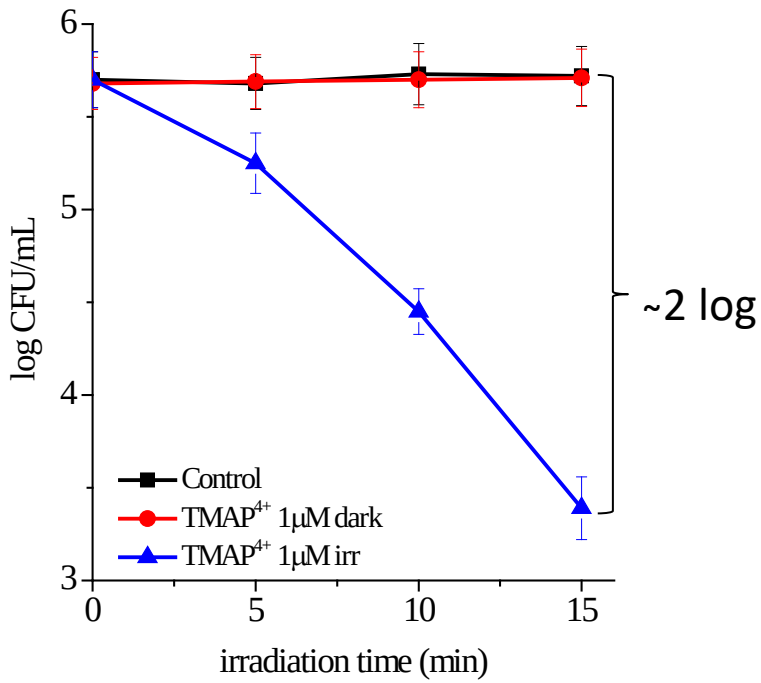
Spread plate method

Planktonic

S. aureus



E. coli



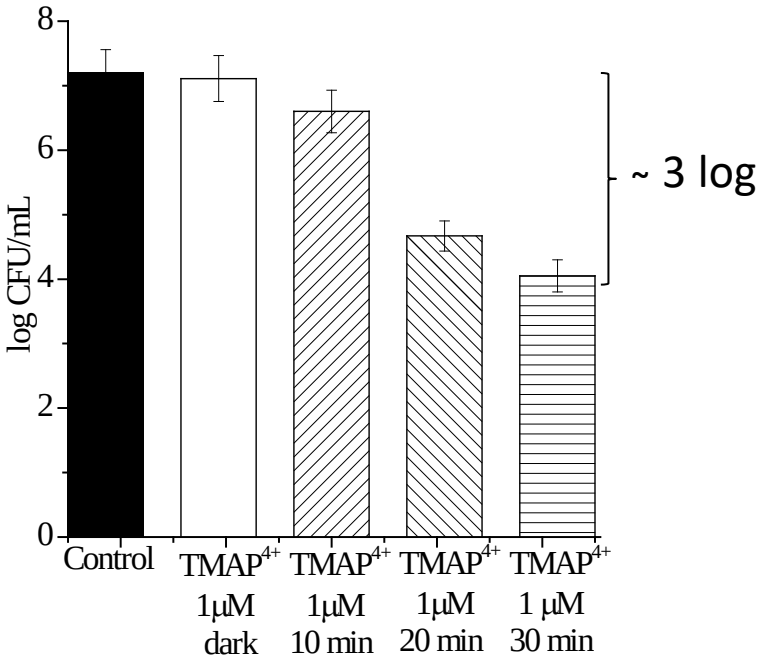
PDI of both planktonic bacteria was possible with low concentrations of TMAP and light doses

Results and discussion

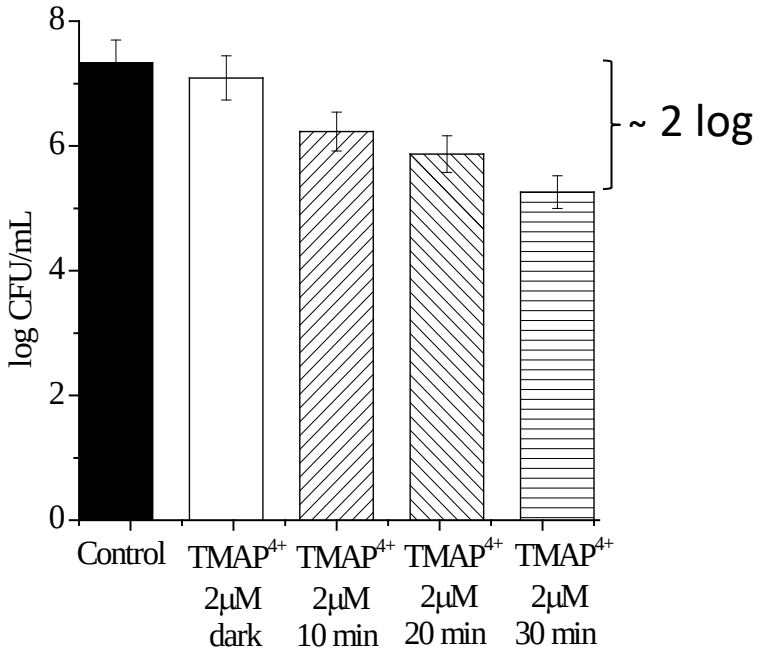
Spread plate method

Biofilm

S. aureus



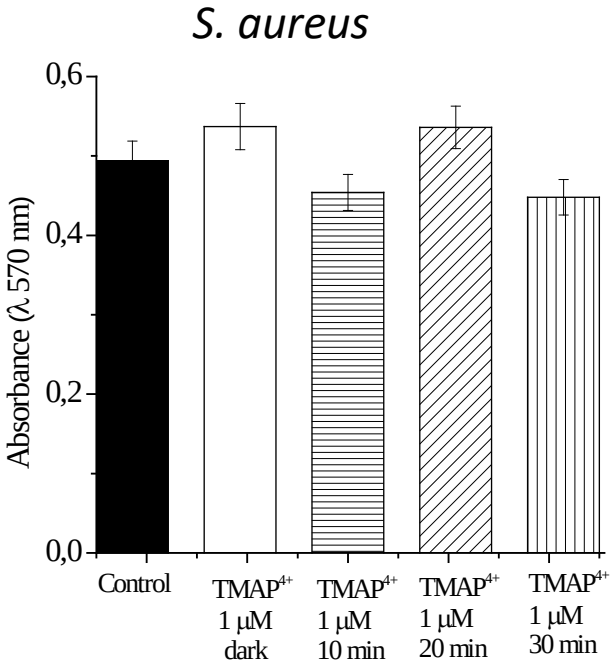
E. coli

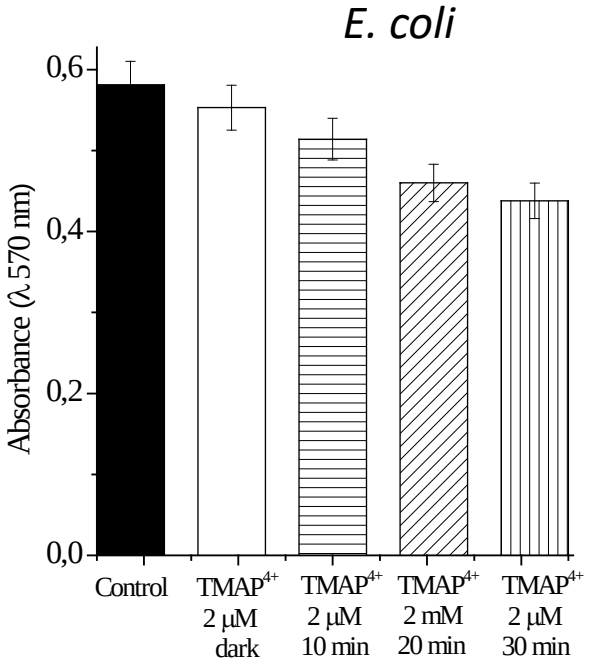


PS concentrations and light doses must be higher to get a PDI similar to that obtained in planktonic cultures.

Results and discussion

Crystal violet assay





No significant differences were found between the control and treated biofilms. This is because this assay stain both living and dead cells and the extracellular matrix that surrounds them.

Results and discussion

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) assay

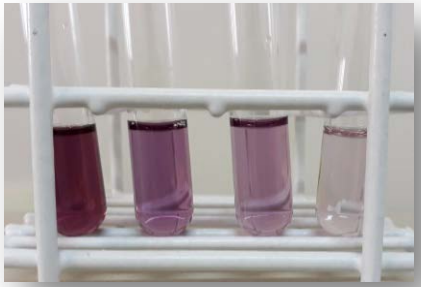
S. aureus

	Absorbance 550 nm	
Control	0.609	
Dark Control	0.600	
Wash Control	0.569	
10 min irradiation	0.193	68,3 %
20 min irradiation	0.118	80,6 %
30 min irradiation	0.070	88,5 %

E. coli

	Absorbance 550 nm	
Control	0.174	
Dark Control	0.125	
Wash Control	0.100	
10 min irradiation	0.058	66,7 %
20 min irradiation	0.056	67,8 %
30 min irradiation	0.050	71,3 %

Decrease in cell viability in irradiated biofilms as the time of exposure to light increases.



Results and discussion

1,9-Dimethyl-Methylene Blue (DMMB) assay

S. aureus

	Absorbance 620 nm	
Control	0.250	
Dark control	0.180	
Wash control	0.134	
10 min irradiation	0.165	34,0 %
20 min irradiation	0.133	46,8 %
30 min irradiation	0.102	59,2 %

E. coli

	Absorbance 620 nm	
Control	0.242	
Dark control	0.246	
Wash control	0.180	
10 min irradiation	0.167	31,0 %
20 min irradiation	0.160	33,9 %
30 min irradiation	0.139	42,6 %

The combination of $TMAP^{4+}$ and white light induces a decrease in the exopolysaccharide matrix, increasing its destruction as the irradiation times lengthen.

Conclusions

- Spread plate method: Longer irradiation times and PS concentrations were required to produce an efficient inactivation in biofilms similar to that achieved in planktonic state in both strains.
- CV Assay: This technique cannot be used to quantify the effect of PDI because the dye stains total biomass.
- MTT Assay: A decrease in viable cells was observed as the time of exposure to light increased in both bacterial strains.
- DMMB Assay: The amount of extracellular matrix present in the biofilm decreases with increasing irradiation times.
- $TMAP^{4+}$ is an effective cationic PS for the eradication of both Gram positive and negative bacterial biofilm and therefore, PDI can be considered as an interesting alternative therapy for the treatment of bacterial biofilms infections.

Acknowledgments