An in-vitro study on photodynamic inactivation of methicilin-sensitive and -resistant *Staphylococcus aureus*

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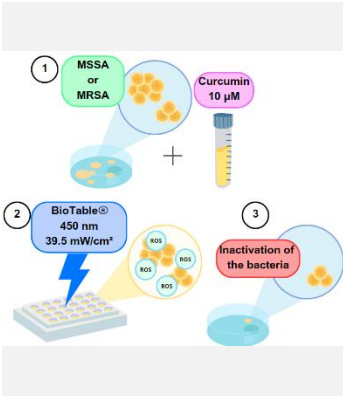

This study assessed the performance of curcumin as a photosensitizer (PS) in the photodynamic inactivation (PDI) of bacteria, acting against Staphylococcus aureus (MSSA) and its methicillin-resistant strain (MRSA). Tests were carried out in-vitro, relying on a blue-light light emitting diode (LED) source, and showed that MRSA required a much higher energy dose for inactivation, which still did not reach the same log-reduction as the sensitive strain did. PDI is an emerging approach against antibiotic resistant bacteria (ARB) and a detailed assessment of operational conditions may point to optimal parameters against the studied targets, leading to an effective technique in the process wastewater disinfection, and advanced water treatment.

Introduction

The photodynamic process consists of the production of reactive oxygen species (ROS) via type I mechanism, and singlet oxygen, by type II mechanism, when a PS is excited by specific wavelengths of light in the presence of molecular oxygen [1]. PDI is the application of such process aiming at cell death for infection control, a common approach in medical and clinical settings. PDI has also gained attention in environmental applications, as in vector control and wastewater disinfection [2, 3], which invites for research in current challenges in the field, e.g., the threat of ARB spread [4]. Curcumin, a vegetal-based and edible PS, along with its degradation photoproducts, does not cause ecotoxicity [5, 6], raising as a potential PS in water and wastewater applications. In this context, this study aimed to provide a preliminary assessment of the performance of synthetic curcumin irradiated by blue light in MSSA and MRSA PDI.

Material and Methods

Experiments were performed in-vitro against a commercial MSSA strain (ATCC® 25923), and MRSA (clinical isolate), adapting peer protocol (Sammarro Silva *et al.* 2023). Tests were carried out in multi-well plates illuminated by a blue-LED light source for photochemistry and photobiology assays (Biotable® 450 nm, 39.5 mW/cm²). Curcumin powder was commercially obtained (PDT Pharma, Brazil), and the stock solution was prepared in ethanol 99.7%, then work solutions (2x) were diluted in sterile distilled water, leaving ethanol at a negligible concentration. Initial delivered fluence was 5 J/cm². Given that no inactivation was obtained against MRSA, we increased fluence by 10-fold, irradiating suspensions at 50 J/cm². Viable colonies were quantified by growth in brain-heart-infusion agar incubated at 37 °C.

Results and Discussion

Figure 1 shows the log-reduction observed for MSSA and MRSA and indicate that PDI leads to a more prominent effect on the antibiotic sensitive strain. This encourages research towards answering why ARB shows resistance to PDI as a disinfection method.

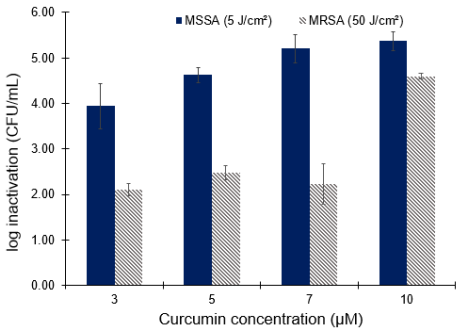

Figure 1. . Photodynamic inactivation values of *S. aureus* methicillin-sensitive and resistant strains using curcumin under blue light at different irradiation doses (5 J/cm² for MSSA and 50 J/cm² for MRSA). Error bars display the standard deviation (n=4).

Given that higher fluence also refers to longer light exposure, we believe that some PS uptake must be occurring in MRSA during illumination. This would respond to why no concentration-dependent effect was verified for the methicillin-resistant strain at 5 J/cm² and only at 50 J/cm² an increase in inactivation was verified (10 µM curcumin). In fact, mechanisms

of antimicrobial resistance include limiting uptake of substance, modification of a molecule target, and active efflux of a substance [8], and all of these may be extrapolated to a PS molecule. In terms of performance, however, though no complete inactivation was found, the process brings a promising perspective on applications in water and wastewater PDI. Next steps refer to a screening of operational conditions, which will shed light onto optimal parameters for PDI applications against ARB in wastewater disinfection, and advanced water treatment, as well as explore how antibiotic resistance mechanisms interfere with non-selective inactivation technologies such as PDI.

Conclusions

Here we showed that blue light-mediated PDI is effective against *S. aureus* when curcumin is applied as a PS. However, operational conditions differ, suggesting energy dose as a limiting factor for satisfactory log-reductions in MRSA. It should be noted that PS uptake may be taking place during this process, so substance internalization in ARB may also affect performance of PDI, requing a detailed assessment of operational conditions to encompass such microrganisms in efficient disinfection.

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