

**TITLE:** STANDARDIZATION OF PROTEIN EXTRACTION AFTER ANTIMICROBIAL PHOTODYNAMIC THERAPY MEDIATED BY ROSE BENGAL AGAINST *Staphylococcus aureus*

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**ABSTRACT:**

Antimicrobial photodynamic therapy (aPDT) is a promising tool to control bacterial contamination. During aPDT, bacteria are killed by reactive oxygen species generated for a visible light absorbing photosensitizer. Rose bengal (RB) is an anionic dye in the xanthen family and reported as efficient photosensitizer in aPDT. There are few researches that evaluated the dynamics of action of RB against microorganisms. In this sense, the proteomic methods can be used for investigating the mechanistic aspects of the photosensitizer action in bacteria. For proteomics analyses is necessary standardize the extraction of proteins in order to obtain a sufficient quantity for later identification. Thus, this study aimed extraction proteins of *Staphylococcus aureus* ATCC 25923 after aPDT mediated by RB for protein analysis. The tests were performed with *S. aureus* cultured in 30 ml of brain heart infusion broth (BHI) overnight at 37°C. The cultures were centrifuged at 45000 rpm for 5 min, washed three times and resuspended in 0.85% saline solution. The bacterial inoculum was standardized in spectrophotometer at 625 nm to contain approximately  $10^8$  cells per ml for use in photodynamic inactivation assays. Bacterial suspension (279 ml) was treated with RB at concentration 10 nmol/l and irradiated by green LED (0.17 J/cm<sup>2</sup>). Positive control (bacteria and phosphate buffered saline), photosensitizer control (bacteria and RB without light) and light control (bacteria exposed to LED in the absence of photosensitizer) have also been tested. The samples were filtered with membrane filter of 0.22 µm. Then, lysis buffer and sonicator were used to break the bacterial cells and the samples were centrifuged (4800 rpm for 10 min). The quantification of protein was performed by Bradford protein assay. The protein extraction and quantification were realized in triplicate and medium values found were 587, 294, 323 and 595 µg/ml, for sample treated with RB, positive control, photosensitizer control and light control, respectively. The results demonstrated than the protein extraction process was efficient to follow with the identification in mass spectrometry.

**Keywords:** Photochemotherapy, photosensitizing agents, Rose Bengal, Proteomics