**TITLE:** ROSE BENGAL-BASED PHOTOSENSITIZATION REDUCES *Salmonella* Typhimurium BIOFILMS

**AUTHORS:** SILVA, A. F.; SANTOS, A. R.; SILVA, L. G. Z.; FREITAS, C. F.; TREVISAN, D.A.C; CAMPANERUT-SÁ, P. A. Z.; MIKCHA, J.M.G.

**INSTITUTION:** UNIVERSIDADE ESTADUAL DE MARINGÁ, MARINGÁ, PR (AVENIDA COLOMBO, 5.790, CEP 87020-900, MARINGÁ-PR, BRAZIL).

**ABSTRACT:**

The impact of foodborne diseases, especially infection caused by *Salmonella* spp., is a worldwide concern. An estimated 93.8 million cases of gastroenteritis are caused by *Salmonella* spp. each year, with 155 000 deaths worldwide, representing a considerable problem in both developing and developed countries. *Salmonella* spp. biofilms have attracted the attention of food processing industries because they are a continuing source of food contamination. However, the recently applied methods for the inactivation of microorganisms are not always efficient and ecologically friendly. A promising tool for the control of microbial biofilms in food is Photodynamic Inactivation (PI), which consists of the administration of a photosensitizing substance that is fixed in the bacterium and then irradiated with light. This photosensitizer (PS) in the presence of molecular oxygen and light with adequate wavelength generates reactive oxygen species that induce cellular damage and inactivate the microorganisms. This study evaluated the effect of photodynamic inactivation of *Salmonella* Typhimurium biofilm, using rose bengal dye as PS and green LED light source. An overnight culture of *S.* Typhimurium ATCC 14028 diluted in Trypticase Soy Broth ($10^7$ CFU /mL) was transferred to 24-well plates and incubated at 35ºC for 48h. After biofilm formation, the medium was replaced by PS solution at concentrations ranging from 10 to 1000 µmol/L. After 30 min of incubation in the absence of light, the plates were irradiated for 30 min. The effect of PS without irradiation, and the effect of light irradiation without PS were also evaluated. Following treatments, cells were detached using an ultra-sonic bath, diluted, seeded in Tryptone Soy Agar and incubated at 35 °C for 24 hours. Biofilm cells were not reduced after exposure only to light source or only to PS, indicating that both conditions do not present antibiofilm. The number of viable cells recovered after 48h of biofilm formation reached up to 7.6 $\log_{10}$ CFU/$cm^2$. Treatment with the lowest concentration evaluated (10 µmol/L) slight reduced the number of cells attached ($p > 0.05$). When the PS were tested at 50, 100, 250, 500 and 750 µmol/L, the number of viable cells decreased by ~3.6 $\log_{10}$ CFU/$cm^2$ ($p<0.05$). Surprisingly, the higher concentration tested did not reduced the number of biofilm cells, probably because of the auto-aggregation process of the compound at higher concentration. The data obtained indicated that the number of *S.* Typhimimurium biofilm cells were reduced after photodynamic inactivation, using the green LED light source and the bengal rose photosensitizer, which make this technology a potential tool for biofilm control.

**Keywords:** Bengal rose, biofilm, photoinactivation, *Salmonella* Typhimurium.

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