TITLE: ANTIMICROBIAL ACTIVITY OF CHITOSAN/RHAMNOLIPID NANOPARTICLES AGAINST *Salmonella* Enteritidis

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ABSTRACT:

The failures in current antimicrobial treatments together with the incidence of foodborne infections increase the need for new approaches to control bacterial growth. Additionally, the consumers trends for natural and sustainable products stimulate food industry to replace many synthetic or animal-based products by alternatives that follow the principles of green chemistry. The development of nanoparticles composed of natural biopolymers and biosurfactants has emerged as a promising strategy for applications in the food industry. In this study, the antimicrobial activity of chitosan solution, rhamnolipid solution and chitosan/rhamnolipid nanoparticles was evaluated against *Salmonella* Enteritidis ATCC 13076. Chitosan was extracted from squid pens and prepared by desproteinization, deacetylation and depolymerization. The chitosan obtained had a molecular weight of 6.3×10^3 g mol^{-1} and 10.19% of deacetylation degree. A 0.5 mg mL^{-1} chitosan solution was prepared in 0.5 mol L^{-1} acetic acid. A 0.5 mg mL^{-1} solution of rhamnolipid was obtained by dissolution of commercial rhamnolipid (25% Rhamnolipid Inc.) in water. Chitosan/rhamnolipid nanoparticles were prepared by mixing chitosan and rhamnolipid at 1:1 (v/v) ratio with addition of 3.0 mL of sodium tripolyphosphate (TPP) aqueous solution (0.5 mg mL^{-1}) under constant stirring. The minimum inhibitory concentration (MIC) was determined using the microbroth dilution technique and minimum bactericidal concentration (MBC) was also evaluated. Chitosan solution inhibited bacterial growth showing a MIC of 20 µg mL^{-1} and MBC of 160 µg mL^{-1} whereas, the bacteria was resistant to rhamnolipid at the concentration tested. The nanoparticles showed a MIC (chitosan/rhamnolipid) of 20/23 µg mL^{-1} and MBC of 80/92 µg mL^{-1}. Therefore, the MBC value was lower than that obtained for the chitosan solution, suggesting a synergistic effect. The results of this study demonstrated that the combination of chitosan/rhamnolipid in nanoparticles can be a potential source for the development of new selective antimicrobial agents to control this important bacterial pathogen.

Keywords: chitosan, rhamnolipid, nanoparticles, *Salmonella* Enteritidis.

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