TITLE: Effects and influences of nisin nanoemulsion in *Staphylococcus aureus* virulence factors

AUTHORS: FURLANETTO, A.; ALVES, F. C. B.; PEREIRA, A. F. M.; SANI, A. A.; RICCARDI C. S.; FERNANDES JÚNIOR, A.

INSTITUTION: INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU, UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO", BOTUCATU, SP (R. Prof. Dr. Antônio Celso Wagner Zanin, 250 - Distrito de Rubião Junior - Botucatu/SP)

ABSTRACT:

Staphylococcus aureus is a Gram-positive bacterium responsible for causing various diseases in humans, which in addition to being considered pathogenic, has raised concerns about the existence of strains resistant to current antibacterials. Bacterial resistance to current antimicrobials reaches alarming levels and is therefore a challenge to be overcome with the possibility of using new alternatives such as nisin, an important antimicrobial peptide. The use of nanotechnology for antimicrobial compounds (eg nanoemulsions and nanoparticles) shows a potential enhancement of the antimicrobial properties of nisin. Thus, the objective study was to verify the possibility of nanogold nanoencapsulation and to quantify the inhibitory action against five strains of *Staphylococcus aureus* of ATCC, of which four were enterotoxin producers and one biofilm producer. Five nisin nanoemulsions were prepared with different percentages of Tween 20 and Span 80 under ultrasonic effect and their droplet size was measured, being the smallest droplet size presented at 98 nm and the largest at 249 nm. The macroscopy, microscopic and stability tests of nanoemulsions were also made. Gold nanoparticles (AuNPs) were synthesized with gold chloride trihydrate (HAuCl4 $3H_2O$) by magnetic stirring, with the addition of sodium citrate and, subsequently, nisin was incorporated by magnetic stirring as well, forming nanoparticles coupled to nisin (AuNPs-Nis). The absorbance and the wave-length value of AuNPs and AuNPs-Nis were calculated. The Minimum Inhibitory Concentration (MIC) was performed by Resazurin Microtiter Assay (REMA) with the five different nisin nanoemulsions and nisin tested alone. The nanoemulsions presented expected appearance and stability, that is, the larger the droplets, the more opaque the solution. As for the antibacterial activity, the two nisin nanoemulsions with the largest droplets presented better MIC values. The lowest nanoemulsion MIC was against the enterotoxin A producing strain, with a value of 7.8 µg/mL, while MIC of nisin was 125 µg/mL against the same strain. The color change from HAuCl4 3H2O from yellow to dark red, together with the wavelength value of 524 nm, showed the AuNPs and AuNPs-Nis have formed. Thus, nanotechnology applied in microbiology has shown great potential against pathogenic bacteria, and has been used to increase the action of antimicrobial agents, as verified in this study using nisin with active agent.

Keywords: Antibacterial peptides, bacterial resistance, nanotechnology

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