

Title: EFFECT OF NISIN ON GROWTH AND PROTEASE PRODUCTION OF PSYCHROTROPHIC BACTERIA ISOLATED OF MILK

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Abstract:

The spectrum of action of nisin is limited to Gram-positive bacteria, while Gram-negative bacteria exhibit natural resistance, due to the presence of outer membrane, especially the lipopolysaccharide layer, which prevent the diffusion of bacteriocin molecules to your site of action. The activity of nisin on Gram-negative bacteria is dependent of treatments that destabilize the outer membrane. However, under specific environmental conditions, as low temperature and low or median pH, nisin has shown inhibitory activity on *Salmonella*. In these conditions, the effect on others Gram-negative bacteria is still poorly understood, especially on psychrotrophic microorganisms. In his work, we evaluated the effect of nisin on growth and protease production of *Pseudomonas fluorescens* and *Serratia liquefaciens* under low temperature. For this, two strains psychrotrophic isolated of milk were used: *P. fluorescens* L227 and *S. liquefaciens* L53. The cultures were previously grown in Luria Bertani broth (LB) at 30 °C for 24 h, inoculated at cellular density of 10⁶ CFU /mL in LB broth added of nisin (200 AU/mL), and incubated at 10 °C. In different time intervals, the cell viability was determined by microdrops technique on plate count agar (PCA). The plates were incubated at 30 °C during 8-12 h. After 72 h of treatment, the cells were collected by centrifugation at 10,000 g for 10 min, and the protease activity of supernatant evaluated using azocasein as substrate. The presence of nisin partially inhibited the growth of *P. fluorescens* and *S. liquefaciens*, with better efficiency on *P. fluorescens*. Although the effect on growth has been small, the protease activity demonstrated a significant reduction. These results demonstrated that under specific conditions, nisin alone demonstrated activity on psychrotrophic Gram-negative bacteria, possibly due to the interference of temperature in the composition of the outer membrane.

Keywords: bacteriocins, biocontrol, Gram-negative bacteria.

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