

Produção de nisina em leite desnatado diluído por *Lactococcus lactis* subsp. *lactis* ATCC 11454 em biorreator

Arauz, LJ Nisin production in diluted skimmed milk utilizing *Lactococcus lactis* subsp. *lactis* ATCC 11454 in bioreactor. São Paulo, SP. 2011 [Tese de Doutorado - Área de Concentração: Tecnologia de Fermentações - Faculdade de Ciências Farmacêuticas da Universidade de São Paulo - FCF/USP]. Orientadora: Prof^ª. Dr^ª. Thereza Christina Vessoni Penna

Nisin is a natural antimicrobial peptide produced by *Lactococcus lactis* subsp. *lactis* ATCC 11454 during its exponential growth phase. The bacteriocin is used as natural food preservative due to its antimicrobial activity against Gram-positive bacteria and outgrowth of spores. This property allows its application in numerous fields (pharmaceutical, veterinary and cosmetic).

The aim of this work was to study the bacterial growth kinetics of *L. lactis* and respective nisin production in bioreactor, using diluted skimmed milk as an inexpensive medium. During the production, the consumption of sugar and protein, lactic acid formation and nisin adsorption on the producer strain cells were evaluated. Pre-cultivation with 10^7 UFC.mL⁻¹ of *L. lactis* were expanded in a 2 L bioreactor containing 25% diluted skimmed milk in water (1.5 L, pH 6.7). The assays were performed at 30 °C for 52 hours, varying agitation and airflow rate: (i) 200 rpm (0.0, 0.5, 1.0 and 2.0 L.min⁻¹) and (ii) 100 rpm (0.0, 0.5 L.min⁻¹).

Nisin activity was evaluated through diffusion assays using *Lactobacillus sakei* ATCC 15521 as sensitive strain. The best nisin concentration (62.68 mg.L⁻¹ or 2511.89 AU.mL⁻¹), was achieved at 16 hours, 200 rpm and with no airflow rate ($k_L a = 5.29 \times 10^{-3} \text{ h}^{-1}$). The quantity of nisin adsorbed by the producer cells were low (6.8 -15.1%) when compared to the quantity released in the supernatant. These results showed that diluted skimmed milk supported cell growth and growth-associated nisin. Preliminary assays of lyophilization (biopreservation) and purification by chromatography of nisin produced in bioreactor were performed. Lyophilization presented a loss of nisin activity (24.8%) while purification by hydrophobic interaction chromatography with Butyl-Sepharose column recovered 40% of the activity, showing that both processes can be applied to the bacteriocin.

Keywords. Lantibiotic, antimicrobial, bacteriocin, food preservative, lactic acid bacteria.

Tese Disponível no endereço eletrônico:
<http://www.teses.usp.br/teses/disponiveis/9/9134/tde-05082011-171618/>
E-mail: lucianaja@usp.br