

TITLE: Isolation and identification of lactic acid bacterium which produces bacteriocin-like inhibitory substances (BLIS) for fermentation in skim milk and soy milk

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ABSTRACT: Lactic-acid bacteria (LAB) are currently classified as part of the Gram-positive bacteria group which produce high added value bioproducts for the food industry through their fermentation process. The lactic acid is the largest metabolite produced by these bacteria even though other compounds, namely acetic acid, ethanol, aromas (diacetyl and ketone), polysaccharides, specific proteases or bacteriocins can also be produced. Bacteriocins are peptides described in the literature for their antimicrobial properties against certain bacteria, protozoan, fungi and viruses. In this work, bacteria were isolated from directly collected milk in Man, Rogosa and Sharpe (MRS) and M17 agar. The isolate that showed an inhibition halo against *Listeria innocua* CLIST 2052 (FioCruz, Rio de Janeiro, Brazil) was selected for identification via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) and 16S rRNA gene sequencing. MALDI-TOFMS results revealed that the isolate belongs to the *Lactococcus* genus (Biotyper 3.1 score of 1.769). Furthermore, 16S rRNA gene sequencing showed 99% identity to *Lactococcus lactis*. The antimicrobial compound situated on the cell-free supernatant (CFS) treated with trypsin, papain or pepsin (1mg/ml) did not exhibit an inhibition zone, which demonstrates the composite's proteinaceous nature. The CFS was precipitated with 40% ammonium sulfate and had its antimicrobial activity improved, being analyzed in tricine-SDS-PAGE to better visualize the peptides. *L. lactis* cells ($\sim 10^6$ CFU/mL) were cultivated in pasteurized 10% skim milk and soy milk at 37°C for 22 hours. The acidification activity of each sample was monitored by Cinac system (Ysebaert, Frépillon, France). Cells grown in soy milk showed V_{\max} (-10^3 pH units/min): 2.76 ± 0.1 and pH_{final} : 4.69 ± 0.04 h whereas *L. lactis* cultivated in skim milk displayed V_{\max} (-10^3 pH units/min): 1.44 ± 0.2 and pH_{final} : 6.36 ± 0.021 h after 22 hours of fermentation. Nevertheless, bacterial counts were quite similar between the ones cultivated in skim milk (2.75×10^8 CFU/mL ± 0.83) and in soy milk (3.5×10^8 CFU/mL ± 1.1). These results indicate that despite the fact that the acidification profile between skim milk and soy milk were different, the final bacterial viability were quite similar. It could be explained due to the different compounds present on these matrix.

Keywords: Bacteriocin-like inhibitory substances, Lactic-acid bacteria, Cinac, skim milk, soy milk

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