

TITLE: *In vitro* antagonistic activity of lactic acid bacteria isolated from Minas artisanal cheeses against *Brucella abortus*

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ABSTRACT:

Brucellosis is a zoonotic disease of worldwide distribution mainly associated with animal contact or to the consumption of unpasteurized milk and dairy products. Minas artisanal cheeses are produced with raw milk and endogenous starter cultures, mainly lactic acid bacteria (LAB), which influence on cheese taste and quality. Thus, the aim of this study was to evaluate the *in vitro* antimicrobial activity of strains of LAB isolated from Minas artisanal cheeses against *Brucella abortus*. A total of 18 LAB strains (*Lactobacillus plantarum* n = 11, *Pediococcus acidilactici* n= 1, *Lactobacillus rhamnosus* n = 4 and *Lactobacillus brevis* n = 2), isolated from Minas Artisanal cheeses, produced in three regions of Minas Gerais State, Brazil (Canastra, Campos das Vertentes and Araxá), were tested. For detection of antimicrobial activity 10³ CFU of each LAB strain was inoculated in 30 mL of Man-Rogosa-Sharpe (MRS) broth and incubated at 37°C for 24h. Following the incubation, the number of CFU per supernatant was determined and the cultures were centrifuged. The pH of each supernatant was measured and sterilized by filtering through 0.22 µm filters. Prior to sterilization, half of the supernatant was neutralized to the pH 7 with NaOH. Both pH-adjusted and unadjusted supernatants were co-incubated in tryptose broth with 10³ CFU of *B. abortus* 2308, *B. abortus* 544 and *B. abortus* 2683 (field strain - isolated from a bovine milk sample) at 37°C, in 5% CO₂, for 24h. After incubation, 100 µL of the broths were plated on tryptose agar and incubated at 37°C, in 5% CO₂, for 72 h. *Escherichia coli* ATCC 25922 was used as control. All analyses were performed in three repetitions, each one with two replicates. The LAB supernatants showed reductions in pH from 3.84 to 6.2, depending on the LAB strain and concentration of LAB in the final supernatant, which ranged from 10⁵ to 10⁹ CFU /mL. In this way, 13 LAB strains reduced pH of the medium below 4.65 and were able to totally inhibit all *B. abortus* strains tested. In contrast, it was observed that the pH-neutralized supernatants of all LAB strains did not suppress the growth of any *B. abortus* strain tested. Inhibition results strongly suggest that the organic acids and the reduced pH produced by the metabolism of LAB were responsible for the inhibition of *B. abortus* growth. The result of the present study shows that some tested LAB can contribute to the sanitary quality of artisanal cheeses and may be an alternative to increase the security and conservation of foods elaborated with raw milk.

Keywords: raw milk cheese, milk microbiota, brucellosis, food security

Development Agency: CNPq, Fapemig and Capes