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Detection of Potentially Pathogenic Non-Tuberculous Mycobacteria in Artisanal Coalho Cheese from the State of Paraiba, Northeast Brazil

Sarti ECFB[1*], Rodrigues RA[2], Duarte LFC[2], Bacanelli GM[1], Lilenbaum W[3] and Araújo FR[4]

[1]*Biotechnology and Biodiversity, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil*

[2]*Veterinary Sciences, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil*

[3]*Laboratory of Veterinary Bacteriology, Fluminense Federal University (UFF), Niterói, Rio de Janeiro, 24210030, Brazil*

[4]*Embrapa Catlle, Campo Grande, MS, 79106-550, Brazil*

[*]**Corresponding author:** Sarti ECFB, Biotechnology and Biodiversity, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, 790770900, Brazil, Tel: 05567999881947; E-mail: elainebaez@hotmail.com

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Abstract

The artisanal Coalho cheese is one of the most consumed dairy products in some regions of Brazil, especially in the Northeastern. Because it is minimally ripened, it should be produced using heat-treated milk, however, its manufacture commonly uses raw milk. Reports of contamination of Coalho cheese with pathogenic bacteria are increasing, including *Mycobacterium* spp. Non-tuberculous mycobacteria (NTM) are emerging pathogens that cause infections in humans. This study describes the identification of viable NTM in artisanal Coalho cheese in the State of Paraíba, Northeastern, Brazil. On hundred samples of artisanal Coalho cheese, produced without sanitary inspection, were collected at street markets in the regions of Borborema, Agreste and Mata, and cultured in Stonebrink medium. Isolates were analyzed by PCR for hsp65 of *Mycobacterium* spp. and the DNA amplicons were sequenced. The resulting sequences were submitted to identity search by Blastn (NCBI). Colonies were isolated in 5/100 (5%) samples of Coalho cheese, which were positive in the PCR for hsp65. The resulting DNA consensus sequences showed similarity to hsp65 from *Mycobacterium fortuitum* (100% identity), *Mycobacterium novocastrense* (99% identity), *Bifidobacterium crudilactis* (98% identity), *Kocuria rhizophila* (98% identity) and *Kocuria palustris* (91% identity).The consumption of artisanal Coalho cheese from street markets represents a risk for human health, due to the possibility of transmission of non-tuberculous mycobacteria and other actinobacteria potentially pathogenic. This study reinforces the need for the establishment of public policies to prevent the commercialization of Coalho cheese, produced without sanitary inspection.

Keywords: Non-tuberculous mycobacteria; *M. fortuitum*; *M. novocastrense*; *B. crudilactis*; *K. rhizophila*; *K. Palustris*; Coalho cheese; Microbiological culture

Introduction

The artisanal Coalho cheese is one of the most consumed dairy products in some regions of Brazil, especially in the Northeastern region [1]. Because it is minimally ripened, it should be produced using heat-treated milk, however, its manufacture commonly uses raw milk [2]. Besides, Coalho cheese also may be contaminated with pathogens during handling, packaging, and storage [3].

The genus *Mycobacterium* includes a wide range of organisms, including pathogens of the *M. tuberculosis* complex, which cause tuberculosis in human and domestic/wild-life animals; opportunistic or potential pathogens and saprophytic species [4]. Non-tuberculous mycobacteria (NTM) are emerging causes of human diseases, and this group of mycobacteria has been increasingly reported as primary pathogens causing pulmonary and extrapulmonary infections [5,6].

Contamination of cheese with mycobacteria was described in different countries [1,7-9]. In some regions of Brazil, such as the State of Paraíba, the prevalence of bovine tuberculosis is unknown [10]. This fact, associated with the habit of producing artisanal cheeses with raw milk [2] and with inadequate hygiene practices, suggests the hypothesis of contamination of these products by mycobacteria and the risk of transmission to humans.

This study had the objective to evaluate the presence of viable mycobacteria in the artisanal Coalho cheese from street markets in the State of Paraíba, Northeastern Brazil.

Materials and Methods

One hundred samples (150 g) of artisanal Coalho cheese, produced without sanitary inspection, were collected randomly from July to December 2016, in street markets of the regions of Borborema, Agreste and Mata, State of Paraíba. These samples were placed in sterile plastic bags, frozen and sent to the Embrapa Beef Cattle, State of Mato Grosso do Sul. In the laboratory, 5 g samples of the cheese were mixed with 5 mL of 0.85% sterile saline, and the homogenized in 2 mL tubes containing 1.4 mm ceramic beads, on a MagNALyser Instrument (Roche). The homogenates were transferred to 50 ml sterile tubes, and 40 ml of 0.85% sterile saline were added, vortexed for 10 seconds. After decontamination by the sodium lauryl sulfate protocol, samples were cultured in Stonebrink's medium up to 90 days, with weekly observations.

To identify the isolates, a PCR targeting hsp65 of *Mycobacterium* spp. was performed [11]. Subsequently, the amplicons were purified using ExoSAP, and sequenced in duplicate, using the Big Dye Terminator Kit Cycle Sequencing (version 3.1, Applied Biosystems,

Foster City, USA). The resulting consensus DNA sequences were subjected to identity search through Blastn (NCBI).

Results and Discussion

Isolates positive in the PCR for hsp65 were detected in 5/100 (5%) samples of Coalho cheese. The resulting consensus DNA sequences showed similarity to hsp65 from *Mycobacterium fortuitum* (sequence ID: MF280110.1 , e-value: 1e-166, identities: 330/330 [100%]) *Mycobacterium novocastrense* (sequence ID: HM807282.1, e-value: 0.0, identities: 376/380 [99%]), *Bifidobacterium crudilactis* (sequence ID: LN849256.1, e-value: 2e-157, identities: 325/333 [98%]), *Kocuria rhizophila* (sequence ID: CP022039.1, e-value 0.0, identities: 379/387 [98%]) and *Kocuria palustris* (sequence ID: CP012507.1, e-value: 4e-143, identities: 335/367 [91%]).

The Coalho cheese is one of the main cheese types that is consumed in Northeastern Brazil. The Coalho cheese was proposed to be a functional food, among other aspects, based on the antimicrobial activity of its water-soluble peptides against *Enterococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* [12]. Nevertheless, high levels of occurrence of *Salmonella* and coagulase-positive *staphylococci* were found in this type of cheese [13]. The growth predictions under the temperature, pH, and water activity conditions in commercial Coalho cheese samples also indicated that this food has pH and water activity characteristics that allow the growth of *E. coli*, *Listeria monocytogenes*, *Salmonella*, and *Staphylococcus aureus* [3].

With regards do mycobacteria, in Northeast Brazil, there are reports of Coalho cheese contaminated with viable *Mycobacterium avium* subspecies *paratuberculosis* in the State of Piauí [1] and with the presence of *M. bovis* DNA, in the State of Pernambuco [8].

In this study, we did not find *M. bovis* in the cultures. Nevertheless, NTM were found in two samples (*M. fortuitum* and *M. novocastrense*) and other actinobacteria in three samples (*Bifidobacterium crudilactis*, *Kocuria rhizophila* and *Kocuria palustris*.

In Brazil, *M. fortuitum* was found in raw milk samples from the States of São Paulo [14] and Paraná [15]. This species can cause diseases not only in immunosuppressed patients [5], but also skin infection [16] and lung infection [6] in immunocompetent patients.

Mycobacterium novocastrense was first described in a skin granulation on a child's hand [17]. This rapid-growing NTM was also described in pulmonary infection from an HIV-infected patient [18] and in respiratory samples from patients with suspect of pulmonary tuberculosis [19]. With regards to isolation on food, this species was detected in raw milk from the State of São Paulo [14].

Although the genus *Bifidobacterium* spp. is considered a probiotic organism, two cases of mixed pyogenic infections by this microorganism have been recently reported [20].

The species *K. rhizophila* and *K. palustris* are part of the microbiota of the skin and oropharynx, being isolated from samples of environmental and animal origin. Clinical cases of infections by *K. rhizophila* and *K. palustris* are increasingly being described: identified *K. palustris* in a patient with peripheral ulcerative keratitis while [21] and in duodenal mucosa from a patient with Celiac Disease [22]; identified *K. rhizophila* in a central catheter of a child with sepsis [23].

Conclusions

The consumption of artisanal Coalho cheese from street markets represents a risk for human health, due to the possibility of transmission of non-tuberculous mycobacteria and other actinobacteria potentially pathogenic. This study reinforces the need for the establishment of public policies to prevent the commercialization of Coalho cheese, produced without sanitary inspection.

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