
Mycobacteria research in water buffalo milk

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Milk is an important nutritional source to man, but it can also carry pathogenic mycobacteria, especially *Mycobacterium bovis*. In this study, we tried to evaluate the classical technique (culture) and molecular technique PCR (*Polymerase Chain Reaction*) and PRA (*PCR - Restriction Enzyme Analysis*) in the isolation and identification of mycobacteria from water buffalo's milk samples. First of all, it was standardized an efficient PCR method for *M. bovis* identification from milk. In this sense, a known number of *M. bovis* AN5 were inoculated in the milk that was submitted to a serial dilution. Then, two different PCR protocols were done to evaluate the threshold of *M. bovis* detection. Two pair of primers were tested, one to detect *Mycobacterium* spp (INS1 e INS2) and other to detect *M. bovis* (JB21 e JB22). Dilutions were also submitted for culture using Stonebrink medium and incubated at 37°C/90 days, to determine

the number of bacilli in the milk. After this, 23 samples of milk collected from water buffalos (*Bubalus bubalis*), 7 PPD+ and 16 PPD-, were analyzed by PCR technique and culture (Stonebrink medium) to detect the presence of *M. bovis*. Isolation of other mycobacteria were done by culture (Lowenstein-Jensen medium) and identified by mycolic acids and PRA. The results of PCR showed that the protocol using primer pair INS1/ INS2 was positive until 10⁻³ dilution (800 CFU/mL). The protocol with primers JB21/JB22 was more sensitive and specific, detecting *M. bovis* until 10⁻⁴ dilution (80 CFU/mL). *M. bovis* could not be identified by PCR and isolated in culture from milk samples of water buffalo. Other species of mycobacteria were identified as: *M. flavescens* (1 sample), *M. simiae* (3 samples) and *M. intracellulare* (1 sample). The last two species are considered pathogenic mycobacteria to human.

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e-mail: clesojunior@uol.com.br

Immunological activity evaluation of *Alchornea* spp in vitro on the production of hydrogen peroxide, nitric oxide and tumor necrosis factor- α by murine macrophages

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The use of natural resources as treatment and healing for diseases is as old as the human species. However, most of all plant species were not investigated chemistry or biologically. Many plants used in the traditional medicine modulate the immunological response. The immune system is a remarkably

adaptive defense system that has evolved in vertebrates to protect them from invading pathogenic microorganisms and cancer. Macrophages play an important role in this system because they are cells capable to secrete many biological active products such as reactive nitrogen and oxygen species and