

Title: PROTEOMIC ANALYSIS IN THE IDENTIFICATION OF ENTEROBACTERIA ISOLATED FROM MILK LINE PRODUCTION

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Abstract:

Environmental mastitis causes economic losses to the bovine culture production system and can be associated with bacteria in the milking environment. The major environmental mastitis pathogens are enterobacteria and its reliable identification is essential for controlling the disease. The great variability of biochemical characteristics of Enterobacteriaceae species makes it difficult to perform an accurate phenotypic identification. Thus proteomics technique like Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) has been studied in order to optimize the microbiological diagnosis. While biochemical techniques performance takes place in 24 hours up to 6 days period, MALDI-TOF allows identification of standardized species in a few hours. This study was conducted to characterize the Enterobacteriaceae species isolated from milk, fecal samples and water by conventional methods and by MALDI-TOF MS. Milk samples from 31 lactating cows were collected in three consecutive weeks in winter and spring seasons of 2014, totaling 186 samples. Also it was collected to analyze the environment fecal samples from each cow and water samples from five different supplies points of the property: well, pond, tap, drinking fountain and stream. Phenotypic identification yielded 48 enterobacteria isolates. Pure colonies were taken to Laboratory of Medical Microbiology Research, Institute of Microbiology Paulo Goes (UFRJ) for identification by MALDI-TOF, Bruker. The prevalent specie in milk samples was *Escherichia coli* 73% (35/48), followed by *Klebsiella oxytoca*, *Enterobacter cloacae*, *Citrobacter freundii* and *K. pneumoniae* 4% each one (2/48). In fecal samples the prevalence of *E. coli* was 96% (70/73) as expected and was also isolated *K. pneumoniae* in 4% (3/73). The prevalence of *E. coli* in water samples was 38% (6/16), followed by *Raoultella ornithinolytica* 25% (4/16), *C. diversus*, *E. cloacae*, *E. gergoviae*, *K. oxytoca*, *E. asburiae*, *Salmonella* sp. 6% each (1/16). The proteomic analysis confirmed 88.23% of the result of the phenotypic identification. Besides phenotypic identification being so laborious and time consuming it also has basis in Enterobacteriaceae identification where slightly biochemical variations can provide false results. MALDI-TOF proved to be an efficient tool for distinguishing Enterobacteriaceae species. Also, it has high potential for routine automated analysis, allowing the identification of isolates on a large scale.

Key words: Mastitis, enterobacteria identification, mass spectrometry.

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