

Title: DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE) IN THE DETECTION OF BACTERIA CAUSING BOVINE MASTITIS

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Abstract:

Mastitis is a multifactorial inflammation of mammary gland and *Staphylococcus* spp. is the most common cause of bacterial bovine mastitis. Detection of subclinical mastitis is based on California Mastitis Test (CMT) and Somatic Cells Counting (SCC) otherwise these tests do not allow the identification of the etiology and the suitable treatment. For a reliable diagnosis it is necessary to submit milk samples to laboratorial analysis comprised of bacterial culture and identification which is time consuming and laborious. Thus, molecular techniques have been applied to improve mastitis diagnosis. The present study evaluated the bacterial population from mastitis positive teats and healthy teats of the same cows through Denaturing Gradient Gel Electrophoresis (DGGE). Moreover, bacteria were isolated from samples on blood agar medium and the identification was confirmed by MALDI TOF-MS (Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry). Ten animals presenting simultaneously healthy and subclinical mastitis teats detected by CMT were selected. Two samples of each quarter were collected. *Staphylococcus aureus* was isolated from six animals. After extraction of bacterial community DNA from samples, universal primers were used to amplify by PCR (Polymerase Chain Reaction) the V3 region of 16S rDNA of bacteria. PCR products were separated by Denaturing Gradient Gel Electrophoresis (DGGE). The DGGE gels showed banding patterns of sufficient complexity and variability to investigate differences in bacterial communities from mastitis teats samples compared to healthy teats. Samples from eight animals DGGE presented unique operational taxonomic units (OTUs) in subclinical mastitis teats not presented in healthy ones. Samples from two animals did not present differences electrophoretic profile but a higher intensity of some bands in the gel of subclinical mastitis samples was detected. DGGE is an appropriate technique to point out differences between bacterial populations from healthy and mastitis udder quarters.

Keywords: Bovine mastitis, diagnostic, electrophoresis

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