ABSTRACTS

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Direct Polymerase Chain Reaction Detection of *Staphylococcus aureus* and *Streptococcus agalactiae* in Raw Milk

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Staphylococcus aureus and *Streptococcus agalactiae* can cause both clinical and sub-clinical mastitis in dairy cows. These bacteria can survive and multiply on the skin of teat and in the udder. Therefore, these organisms can be transmitted from cow to cow during milking. The polymerase chain reaction (PCR) method was developed for the direct and rapid detection of *Staphylococcus aureus* and *Streptococcus agalactiae* in raw milk samples collected from individual cows. A set of primers based on the nucleotide sequences of *Staphylococcus aureus femA* gene was used for the detection of *Staphylococcus aureus*, and a pair of primers complementary to the conserved region of group B streptococcus 16S ribosomal RNA gene was used for the detection of *Streptococcus agalactiae*. In sensitivity tests, the detection limits of 50 CFU of *Staphylococcus aureus*, and 1 CFU of *Streptococcus agalactiae* per ml of raw milk were obtained when raw milk samples inoculated with the bacteria were homogenized (1:10 dilution) in Brain Heart Infusion (BHI) and pre-enriched for 3 hours at 37°C. A single primer pair designed from the sequence data of eubacterial 16S rRNA was also used to provide a positive control for the amplification reactions. By using a multiplex PCR strategy, coamplification of *Staphylococcus aureus femA* determinant (686-bp fragment), *Streptococcus agalactiae* 16S rRNA gene (544-bp fragment), and the conserved region of bacterial 16S rRNA (789-bp fragment) was achieved. Raw milk samples from 66 cows of 15 herds were examined using either a single primer PCR set or the multiplex PCR assay. There was 100% agreement between the results obtained using the two PCR detection methods, and 100% agreement when compared to traditional culture techniques.