Detection of the Major Mastitis-Causing Pathogens by a Rapid and High-Throughput Molecular Assay

Nathalie Bissonnette,

Agriculture and Agri-Food Canada, 2000 College Road - P.O. Box 90, Sherbrooke (Quebec) J1M 1Z3
E-Mail :nathalie.bissonnette@agr.gc.ca

Mastitis is the most costly disease for dairy producers worldwide. One key component of better control of this disease is identification of the causative bacterial agent during udder infections in cows. Mastitis is complex, however, given the diversity of pathogens that must be identified. Development of a rapid and efficient bacterial species identification tool is thus necessary.

This study was conducted to demonstrate the feasibility of bacterial DNA extraction for the automated molecular detection of major mastitis-causing pathogens directly in milk samples to complement traditional microbiological identification. The detection procedure includes specific genomic DNA amplification by multiplex PCR for each species, separation by capillary electrophoresis, and laser-assisted automated detection. The specificity of the primers was assessed with a panel of bacteria representing mastitis-negative control species. The extraction protocol comprised multiple steps, starting with centrifugation for fat removal followed by heating in the presence of Chelex 100 to trap divalent ions. The analytical sensitivity of spiked milk samples was 500 cfu/mL for all species tested. The overall diagnostic sensitivity (95.4%) and specificity (97.3%) were determined in a double-blind randomized assay by processing 172 clinical milk samples with microbiological characterization as the gold standard. Extraction and detection procedures were designed and optimized to achieve detection in a respectable time frame, at a reasonable cost, and with a high throughput capacity. The high-throughput recording of mastitis-causing pathogens by the devised assay can now be applied to milk monitoring schemes for eventual integration into a pathogen-detection scheme for commercial dairy cattle.

Implications: The proposed DNA-based assay is designed to achieve simultaneous detection, at a reasonable cost and in a respectable time frame, for the following species in the same milk sample: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis*, *Strep. agalactiae*, *Strep. dysgalactiae*, and *Klebsiella spp*. (including *K. oxytoca* and *K. pneumoniae*).