

## **Bacterial Profiling and Development of Molecular Diagnostic Assays for Detection of Bacterial Pathogens Associated with Bovine mastitis**

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**Abstract :** For the identification of bovine mastitic pathogen, an economical, rapid and sensitive molecular diagnostic assay is developed by PCR multiplexing of gene and pathogenic species specific DNA sequences. The multiplex PCR assay is developed for detecting nine important bacterial pathogens causing mastitis Worldwide. The bacterial species selected for this study are *Streptococcus agalactiae*, *Streptococcus dysagalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus haemolyticus*, *Staphylococcus chromogenes* *Mycoplasma bovis* and *Staphylococcus epidermidis*. A single reaction assay was developed and validated by 27 reference strains and further tested on 276 bacterial strains obtained from culturing mastitic milk. The multiplex PCR assay developed here is further evaluated by applying directly on genomic DNA isolated from 200 mastitic milk samples. It is compared with bacterial culturing method and proved to be more sensitive, rapid, economical and can specifically identify 9 bacterial pathogens in a single reaction. It has detected the pathogens in few culture negative mastitic samples. Recognition of disease is the foundation of disease control and prevention. This assay can be very helpful for maintaining the udder health and milk monitoring.

**Keywords :** multiplex PCR, bacteria, mastitis, milk

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