Characterizing Staphylococci from Mammary Quarters Coinfected with another Mastitis Agent

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Introduction:

Mastitis is a major economic burden on dairy farms. Subclinical mastitis causes an elevation in milk somatic cell count (SCC). Dairy farmers are paid a premium for milk with a low SCC. Continuing improvements in mastitis management have led to the increasing prevalence of mastitis pathogens once referred to as minor pathogens, particularly the coagulase negative staphylococci (CNS). In some herds CNS may contribute up to 50% of the bulk milk SCC and they are the most frequently isolated bacteria from the bovine mammary gland. Furthermore, recent investigations at MU have shown that CNS can be commonly isolated from mammary quarters coinfected with another mastitis pathogen. This study aims to determine whether certain CNS species are found more frequently in association with another mastitis pathogen than others.

Preliminary Results:

Staphylococcal isolates were streaked on half of a 5% blood agar plate and grown for 24 hours at 37 C.

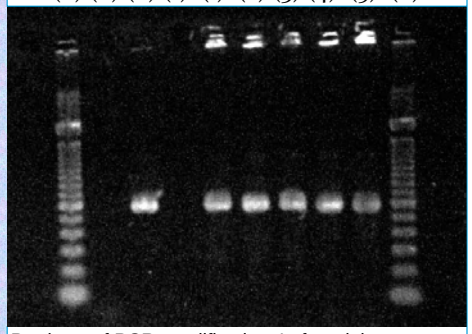
Coagulase positive (top) and coagulase negative (bottom) tests. Coagulase negative samples fail to gel the plasma.

Materials & Methods:

Mammary quarter foremilk samples were collected from all lactating cows on the MU Foremost dairy monthly for 17 months and mammary quarters coinfected with CNS and another mastitis pathogen were identified. Infected quarters possessing both a CNS and another pathogen were selected using a standardized method.

Selected isolates were grown on 5% blood agar at 37ºC for 24 hours and checked for purity. The bacteria were lysed and the *rpo*B gene was amplified via polymerase chain reaction (PCR) (Drancourt & Raoult, 2002). A gel was run to confirm presence of amplified *rpo*B gene. Successfully amplified DNA products were purified using a commercial kit (Invitrogen).

Purified isolates will be sequenced at the MU DNA core. Gene sequences will be compared with an online database (Megablast). Species identity will be assigned based on 95% homology with a published sequence and less than 5% difference from the second most similar species.



Products of PCR amplification. Left to right: molecular weight ladder (L), dH2O negative control, positive control (+), negative control (-), amplicons from five CNS isolates (1-5), and another ladder.

Over 200 isolates stored in 1.5mL tubes await sequencing for this study.

Discussion:

Data will be analyzed to determine whether certain species of CNS are more likely associated with a coinfection with another pathogen. Results of this study will enhance our understanding of CNS mastitis and potentially guide future management strategies for subclinical mastitis.