



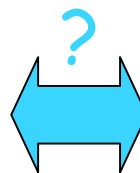
# Bovine mastitis pathogens: individual cow milk samples vs bulk milk samples



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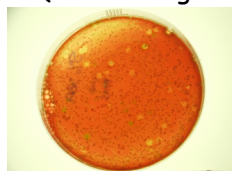
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**Aim:** To investigate whether the strain-types of *Staphylococcus aureus* and *Streptococcus uberis* obtained from bulk milk samples (BMS) reflect the strain-types present in individual milk samples (IMS).

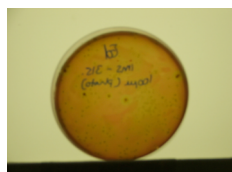


**Methods:** Composite milk samples from 143 individual cows and two bulk milk samples were collected from The University Dairy Herd.

Culture and isolation of *S. aureus* and *S. uberis* (bacteriological techniques)

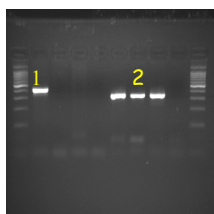


Sheep blood agar plate

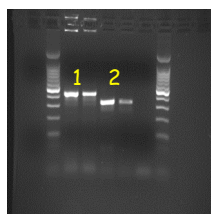


Edwards plate

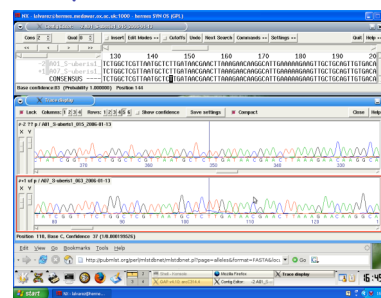
PCR to confirm species identity (Phuektes *et al.* 2003)



Type Colonies (chromosomal DNA) Type colonies (boiled cell suspensions)  
1. *S. aureus*  
2. *S. uberis*



Multilocus sequence typing (MLST): an unambiguous characterization of bacterial isolates



• ***Staphylococcus aureus*:** 15 suspect *S. aureus* isolates were obtained from the 143 IMS; 11 of these isolates were confirmed using the species-specific PCR. Characterisation of these *S. aureus* isolates by MLST and processing of BMS are currently being undertaken.

• ***Streptococcus uberis*:** 124 and 15 suspect *S. uberis* isolates were primarily obtained from the 143 IMS and one of the BMS, respectively; 6 isolates from IMS, previously confirmed by species-specific PCR, 13 isolates from the BMS, and a *S. uberis* type colony were characterised by MLST. No full allelic profile was obtained for any of the isolates (no amplification for all the seven loci). Only one isolate from IMS and 8 from BMS were successfully sequenced. The data suggested that all the bulk milk isolates have the same sequence type, while the isolate from the IMS appeared to have a different sequence type.

**Current work** to address the following questions:

- **What is the optimal protocol to use prior to species PCR?** Different phenotypic techniques are being incorporated to our protocol.
- **How could we maximize the specificity and sensitivity of the species PCR?** Species PCR is being optimised and 16S-23S PCR using universal primers will be run parallel as a control procedure.
- **How could we obtain a full allelic profile when using MLST?** Amplification of target genes is currently being optimised using type colonies as control.

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