Characterization of naturally occurring and induced teat skin colonization with *Staphylococcus chromogenes* in dairy heifers

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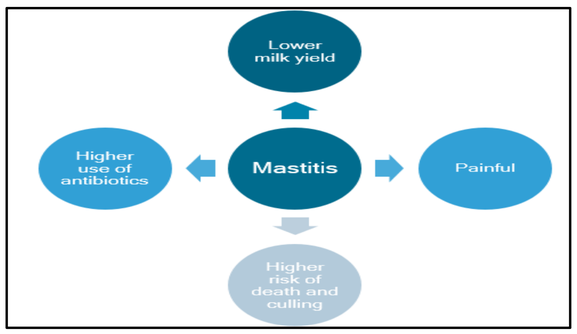
Introduction

- Mastitis is defined as inflammation of the mammary gland.
- Mastitis is often caused by two groups of organisms, *Staphylococcus aureus* and Non-*aureus* staphylococcal (NAS) species.
- Intramammary infections (IMI) found in dairy heifers are commonly caused by NAS species, frequently *Staphylococcus chromogenes.*
- *Staphylococcus chromogenes* is linked to subclinical and mild clinical mastitis.
- *Staphylococcus chromogenes* may also be protective against major mastitis pathogens, however this may be strain specific.
- Induced teat colonization may help test protective properties, however methods for this still need to be determined.

Figure 1: A mastitis informational diagram showing the various impacts of mastitis.



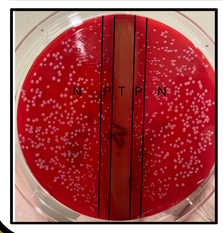


Figure 2: This figure is a picture of the novel isolate *S. chromogenes* 304 (SC304), showing the isolates *in vitro* ability to inhibit the growth of *S. aureus.* Figure depicts zones of inhibition, including total growth inhibition (T), partial growth inhibition (P) and no growth inhibition (N)

Objectives

1.) Collect and characterize *Staphylococcus chromogene*s isolates from the teat ends of breeding age heifers to evaluate natural strains of this bacterial species present on the farm.

2.) Formulate a teat dip solution to enable inoculation of the teat ends of breeding age heifers with a novel strain of *Staphylococcus chromogenes* (SC304).

3.) Utilize MALDI-TOF and RAPD PCR strain typing methods to identify and distinguish SC304 from the natural occurring strains identified on heifer teat ends over time.

Hypotheses

1.) The strain typing methods selected for this study will be able to distinguish the naturally occurring *Staphylococcus chromogenes* strains from the strain selected for the teat inoculation study, SC304.

2.) After inoculation, SC304 will be able to be identified on heifer teats over time.

Methodology

Objective 1. Collect and characterize teat end isolates

- A group of breeding aged heifers from Foremost Dairy Research Center were enrolled.
- Samples were collected from two randomly selected teat ends from each enrolled heifer.
- Teat end samples were collected by rubbing sterile swabs back and forth on each teat end to collect teat skin bacteria.
- Samples were plated on Columbia Blood Agar (CBA) and Mannitol Salt Agar (MSA) and grown for 24 and 48 hrs at 37°C.
- At each time point, samples were evaluated and species that phenotypically resemble *Staphylococcus* were sub-cultured.
- MALDI-TOF MS was used to speciate isolates.

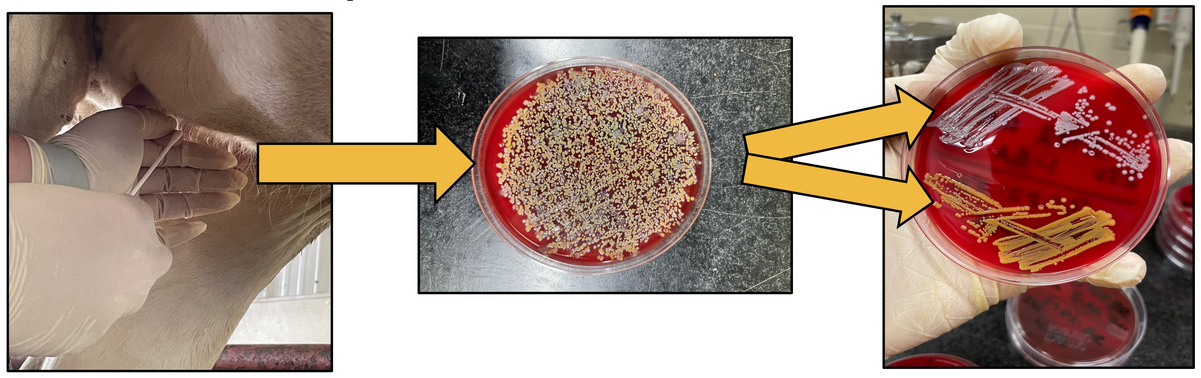


Figure 3: Representation of methodology used for Objective 1, starting on the left with teat skin swabbing of the heifer, to the middle CBA plate of the teat skin swab culture results which was sub-cultured into two distinct *Staphylococcus* species in the picture on the right.

Objective 2: Formulation of teat dip solution and challenge of quarters

- First, SC304 was grown by inoculating onto CBA and incubated at 37°C for 24 hrs.
- Next, 3-4 colonies of SC304 were transferred into 250 mL of Tryptic Soy Broth (TSB) and incubated at 37°C until the mid-log phase of bacterial growth.
- Culture concentration were checked using a spectrophotometer and a dilution series was plated on CBA to allow for concentrations to be determined.
- Once methodology was consistently achieving appropriate concentrations, heifers were enrolled for the dip trial. Heifers were enrolled if they were Holstein, under 90 days pregnant and had an apparently healthy mammary gland.
- Heifers were dipped with SC304 for two consecutive days. Dipping was done after teats were cleaned with a dry cloth. After dipping, heifers were kept in the chute for 5 minutes to allow the dip to dry.

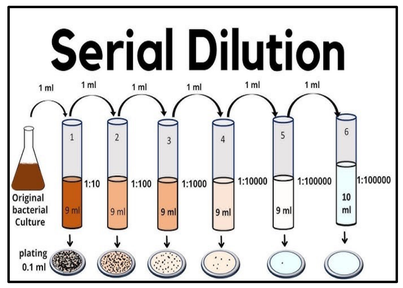
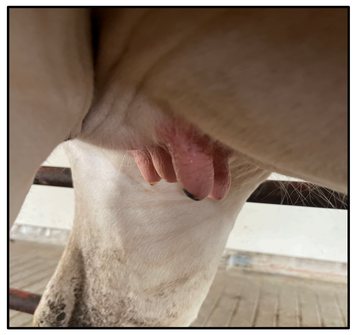


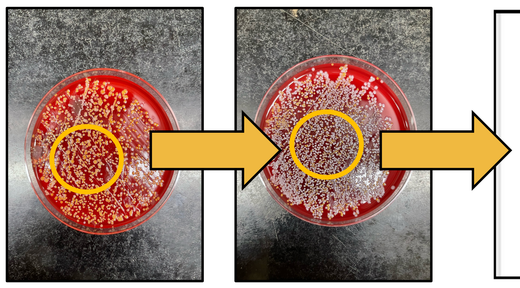
Figure 4: Representation depicting the formulation of the teat dip solution. Specifically, the broth bacterial concentration being checked by running the plate dilution series and spectrophotometer measurements.

Figure 5: Heifer teat ends immediately after being dipped with SC304.



Objective 3: Strain identification over time

- Post challenge, teats were swabbed through the same process as described above.
- Teats were swabbed on days 1,3, 7, 14, and 28 post challenge.
- Teat swabs were plated on CBA and incubated for 24 hours at 37°C and evaluated.
- Analysis of the plates included selection of 4 colonies that were phenotypically similar to SC304.
- MALDI-TOF tube extraction method was used to identify SC304, which had been added to the MALDI database.
- All isolates were banked to allow for future confirmation of strain identification use RAPD PCR.



Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Staphylococcus chromogenes MUUHL_304	2.52	169126916
2 (+++)	Staphylococcus chromogenes MUUHL-VT-S42	2.27	126886188
3 (+++)	Staphylococcus chromogenes ISU 34368 ISUV	2.09	46126

Figure 6: Photographic representation depicting the progression of methods used for objective 3. The left picture is a pre-challenge teat swab sample plated on CBA and the middle picture is a post-challenge teat swab sample, in which it can be noted that a higher percentage of white colonies can be seen on the post challenge plate. Our challenge strain has a white colony color, atypical for *S. chromogenes*, which is more typically yellow in color. On the far right is an example of the MALDI results obtained in which the isolates collected post challenge match with our challenge isolate, SC304.

Results

Objective 1.

- 26 teats from 13 heifers were swabbed.
- 7 staphylococcal species, including 170 *S. chromogenes* isolates were identified from teat swab

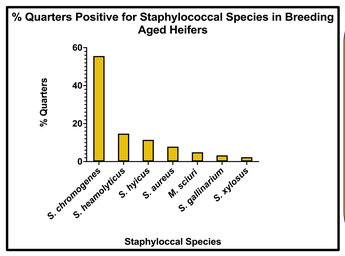


Figure 7: A graph displaying the various staphylococcal species that were identified on heifer teat ends in this study. Overall, 7 different staphylococcal species were identified from these samples.

Objective 2.

- Mid-log growth phase for SC304 was identified at **6 hours incubation** at **37°C.**
- Spectrophotometer measurements at the mid-log growth phase were consistent, with a median of **OD_{600} 0.321 (range: 0.3-0.375).**
- Dilution plate series as a means of confirmation of broth concentrations were consistent with **a median of 1.25 x 10^5 CFU/mL (range of 1.0 x 10^5 – 1.7 x 10^5 CFU/mL).**

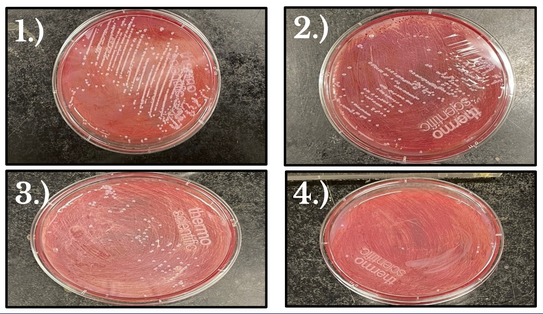


Figure 8: A picture series in numerical order of a successful dilution series specifically, **1 x 10^5 CFU/mL**

Objective 3.

- SC304 was successfully identified on teat ends on Day 1, 3, and 7 post challenge. Both MALDI-TOF and RAPD typing methods were successful in distinguishing SC304.

Post challenge Day	% Quarters SC304 Positive
Day 1	75% (15/20)
Day 3	60% (12/20)
Day 7	35% (7/20)

Figure 9: A data table displaying the quarters that were positive for SC304 over time. Among the SC304 positive quarters, the challenge isolate was successfully identified among the naturally occurring *S. chromogenes* isolates.

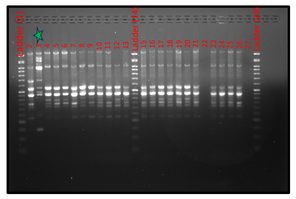


Figure 10: A picture of a RAPD PCR gel electrophoresis showing the difference in SC304 (lane 3) compared to naturally occurring isolates (lanes 4-27). This shows that this strain typing method can distinguish SC304 from the naturally occurring *S. chromogenes* collected from the study farm.

References

- Kerro Dego, O., P. A. Pacha, B. E. Gillespie, and G. M. Pighetti. 2020. Experimental *Staphylococcus aureus* Mastitis Infection Model by Teat Dipping in Bacterial Culture Suspension in Dairy Cows. Animals (Basel) 10(5).
- https://www.amstewardship.ca/faast-reviews/dairy-industry/types-of-mastitis/
- Serial Dilution Picture-RBR Life Science Youtube

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