Antibacterial activity characterization of *Staphylococcus chromogenes* isolates originating from dairy cattle.

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Introduction

Mastitis is one of the most important diseases among dairy cattle.

Non-aureus staphylococci (NAS) have become the most common bacteria isolated from subclinical mastitis cases in dairy cows around the world.

Staphylococcus chromogenes is the most common NAS species identified among bovine milk samples.

Although, the overall impact on udder health is unknown. Some studies have demonstrated protective properties of *S. chromogenes* against somatic cell count elevations.

Antimicrobial peptides, known as bacteriocins, may play a role in mediating the potential protective effects of *S. chromogenes.*

No previous work has evaluated the antibacterial activity or presence of bacteriocin associated genes among a large collection of *S. chromogenes* isolates.

Objective

Identify and characterize *S. chromogenes* isolates that can inhibit *in vitro* growth of *Staphylococcus aureus.*

Materials & Methods

Phenotypic characterization:

A banked collection of *S. chromogenes* isolates was used, including isolates originated from:

- Quarter level milk samples (n = 112)
- Bulk tank samples (n = 131)
- Teat swabs (n = 131)
- Used bedding (n = 4)

Selected isolates were plated on Columbia Blood Agar (CBA) and incubated for 24hr at 37^{o}C.

Isolate concentration was standardized to 0.5 MacFarland Standard and a single center streak was plated on 2 CBA plates (**Figure 1**).

After 24 hr of incubation at 37^{o}C, the agar was flipped and all *S. chromogenes* center streaks were crossed streaked against two different *S. aureus* strains (ATCC 29740 and ATCC 29213).

After 24 hours of additional incubation, phenotypic growth inhibition was categorized as **complete inhibition, partial inhibition, or no inhibition**.

Materials & Methods (Continued)

Genotypic characterization:

DNA extraction was completed using Qiagen DNeasy Blood and Tissue kit.

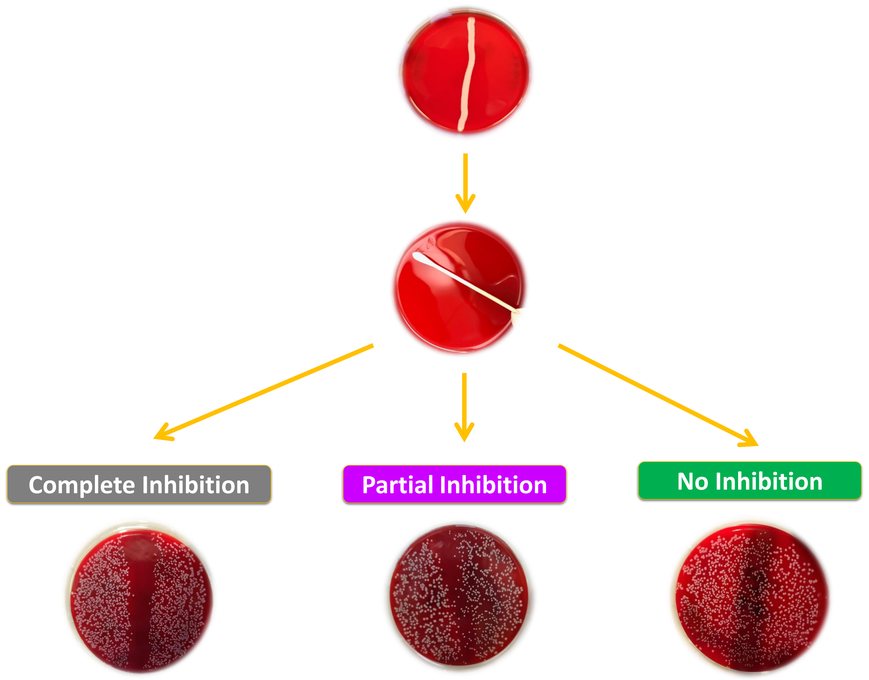
PCR amplification of target genes was conducted using previously identified bacteriocin primers (**Table 1**).

PCR products were visualized using 1% agarose gel electrophoresis and UV transillumination.

Bacteriocins	Gene	Primer	Amplicon size	Reference
Auerocin A70	aurABCD	CCTTATAACTTCGAATGCT AAATATTAACAAGAGAAA	525	Netz et al., 2001
Aureocin A53	aucA	GAAGTTGTGAAAACTATTA CATAAAACAAAGAGCCAAAGT	322	Netz et al., 2002
Staphylococcin C55	sacaA and sacbA	AGCGTGGTGATTCTTATG TCTGATTTATTTAGTTCTGGATA	499	Navaratna et al., 1998
Pep5	pepA	AGAGGAGGTGGTTATATATG TGAGTTCCATGCCCAGTG	427	Ersfeld-Dressen et al., 1984
Epidermin	epiA	GGAGTGTTTAAAATGGAAGC CCTTTTCCCAGTCTATTTTG	431	Allgaier et al., 1986
Epilancin K7	elkA	CTCAAAAGAGTGATTTAAGTCCGC CCACCAGTAATATTGCAACCGC	115	van de Kamp et al., 1995
Epcidin 280	eciA	CGGAGGGATATATTATGG CAATCACTACTATTGACAATCAC	195	Heidrich et al., 1998
Nukacin ISK-1*	nukA	AGGAGGTAACAAACATGG CCCCTTTTTATGAACAACAAG	195	Ceotto et al., 2010
Bsa	bsaA2	TTAACAGCAGAAGCTATTAAAACTACCAG ATGGAAAAAGTTCTTGATTTAGACG	144	Daly et al., 2010

Table 1. A list of primers selected for this study. All selected primers have been previously characterized to amplify bacteriocin genes originating from staphylococcal species. *Denoted primers used to date in this study.

Figure 1. Diagram demonstrating laboratory process used to determine *in vitro* antibacterial activity of *S. chromogenes* isolates. First, *S. chromogenes* was plated on one side of the CBA plate. After incubation, the plate was flipped and *S. aureus* was plated. After additional incubation, the plates were checked for *S. aureus* growth patterns.

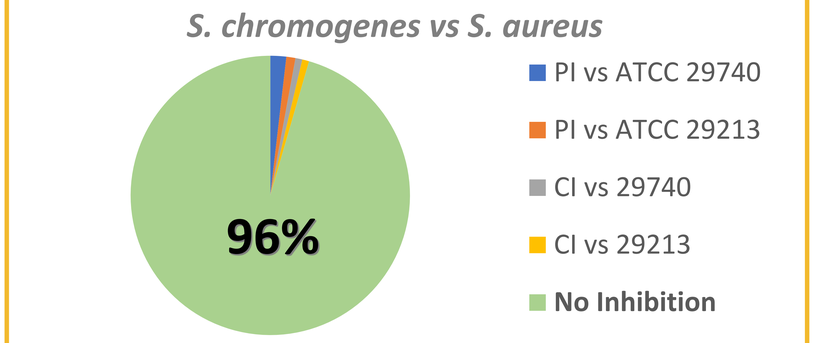


Results

Overall, 11(2.91%) isolates displayed partial growth inhibition against one strain of *S. aureus* and 2(0.53%) isolates displayed complete inhibition against both *S. aureus* strains (**Figure 2**).

To date, 202 isolates have been tested for the presence of the *nukA* gene via PCR with results visualized via gel electrophoresis. There has been no evidence of bacteriocin gene presence among tested isolates.

Figure 2. Chart illustrating that 96% of all *S. chromogenes* isolates did not show evidence of *in vitro* inhibition when tested against two *S. aureus* strains (ATCC 29740 and ATCC 29213). Partial inhibition (PI) and complete inhibition (CI) were identified among a few tested isolates.



Conclusions

In vitro phenotypic growth inhibition of *S. aureus* is rare among *S. chromogenes* isolates. To date, there has been no evidence of *S. chromogenes* specific bacteriocin associated genes identified in the isolate collection. Although, it is important to note that the study is currently ongoing. The long-term goal of the study is to determine if antibacterial activity identified among these unique and rare isolates of *S. chromogenes* could be used to protect cows from major mastitis causing pathogens.

Acknowledgements

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