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Antimicrobial Peptides of Bovine non-*aureus* Staphylococci

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Objectives

- 1) Determine the inhibitory capability of 441 non-*aureus* staphylococci (NAS; often named coagulase-negative staphylococci) isolates from 26 different species against a bovine *Staphylococcus aureus* and human multi-drug resistant *S. aureus* (MRSA)
- 2) Determine the presence of bacteriocin gene clusters from 441 NAS whole genome sequences

Background

- Non-*aureus* staphylococci (NAS), a group of approx. 50 species, are the most commonly isolated bacteria from the bovine udder
- NAS may have a protective effect against infection of the udder by major mastitis pathogens
- Bacteriocins are likely the cause of inhibition, they are antimicrobial peptides produced by bacteria
- They have a strong potential to be utilized in a variety of fields, from veterinary medicine to food production

Materials and Methods

1) Inhibitory Activity:

- 441 NAS isolates from the Canadian Bovine Mastitis and Milk Quality Research Network (CBMQRN) tested for inhibition against bovine *S. aureus* using a cross-streaking method
- Only NAS that inhibited *S. aureus* were tested against MRSA



2) Genome mining for bacteriocins

- 441 NAS were whole genome sequenced (WGS) and analyzed for bacteriocin clusters using:

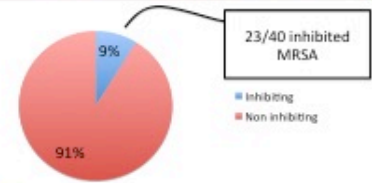
- 1) antiSMASH: a software designed for secondary metabolite identification
- 2) BLAST: genomes were searched for any bacteriocin-associated genes contained in the Class I, Class II, and Class III databases from BAGEL (<http://bagel.molgenrug.nl/index.php/bacteriocin-database>)
- 3) Spine and AGent: to compare genomes of the inhibiting NAS with closely related genomes of non-inhibiting NAS of the same species, according to the phylogenetic trees of each species



Results

1) Inhibition

- 40/441 NAS inhibited *S. aureus*
- 23/40 NAS inhibited MRSA



Left: *S. epidermidis* inhibition zone of *S. aureus*.
Right: *S. aureus* control plate

2) Genome mining for bacteriocins

- 105 bacteriocin gene clusters were identified in 95 isolates from 16 different NAS species
- Bacteriocin gene clusters were spread throughout the NAS phylogeny
- 21.5% NAS could potentially produce bacteriocins

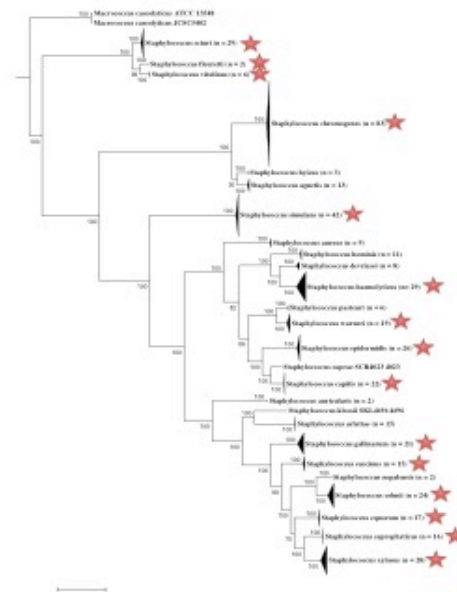


Fig 1. Distribution of bacteriocin biosynthetic gene clusters distributed in NAS species isolated from Canadian dairy cows shown on the phylogenetic tree from Naushad *et al* (2017). Bacteriocin gene clusters are indicated with a star.

Conclusions/Implications

- NAS bacteriocins are able to inhibit *S. aureus* and MRSA *in vitro*
- NAS are a rich source of potential bacteriocins
- Identified bacteriocins may lead to novel antimicrobials to be used for the prevention and treatment of infections with Gram-positive pathogens, which will improve udder health and decrease the economic impact of mastitis