Bacteriocin structural gene shuffling reveals multiple diverse structural gene homologues in the *S. bovis/S. equinus* complex pangenome.

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Introduction: Using a combination of genome analysis and laboratory experiments, we discovered shuffling of bacteriocin structural genes in *Streptococcus infantarius* and *Streptococcus gallolyticus. Streptococcus gallolyticus* LL009 produces gallocin D, which is distinct from gallocin A produced by other *S. gallolyticus* strains. *S. gallolyticus* gene clusters share a high degree of gene synteny while the structural genes are highly variable. This prompted further investigation into all sequenced strains in the *Streptococcus bovis/Streptococcus equinus* complex (SBSEC) to determine the incidence and organisation of bacteriocin genes within this taxonomic group.

Methods: Whole genome sequencing led to the *in silico* identification of gallocin D. Gallocin D and infantaricin A peptides were synthesized and studied for activity. All sequences of the SBSEC were downloaded and analysed for bacteriocin potential using Bagel4. Bacteriocin operons were manually annotated for predicted peptides and aligned using ClustalO, entire operons were compared using MAUVE.

Results: Gallocin D is a narrow spectrum two component bacteriocin with mature peptides of 3343Da and 3019Da. These peptides were synthesized and display activity against VRE strain EC300 with a MIC value of 1.56 µM (Hill et al. 2020). Screening the SBSEC pangenome identified sixty-seven areas of interest (AOIs). These contained 166 predicted structural peptides, comprised of both class I and class II bacteriocins. The AOIs were aligned and grouped into six clusters based on gene synteny. Individual AOIs contained between two and fifteen structural genes.

Discussion: The operons containing multiple bacteriocin structural genes display remarkable diversity in their predicted mature peptide and signal sequences, however in some cases different bacteriocins shared almost identical leader sequences. It is tempting to speculate that the possession of such a variety of bacteriocin structural genes within this bacterial group offers competitive advantages to the producers in different microbial niche.