

## **The bacteriocinogenic potential of the Hungate1000 culture collection of rumen isolated micro-organisms.**

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**Introduction:** The Hungate1000 collection is a catalogue of rumen isolates. Bacteriocins are secondary metabolites with antibacterial activity and roles in niche clearing, colonisation resistance and spatial segregation. Production is considered a probiotic trait. Using in silico methods gives insights into the mechanisms and ecology of bacteriocin production. This study aimed to identify putative novel bacteriocin biosynthetic gene clusters (BGCs) from the rumen microbiome.

**Methods:** BGCs were predicted using Antismash and functionally annotated using InterPro. BGCs were classified on core biosynthetic machinery within the predicted operon, and distribution was overlaid on a phylogenetic tree. Propeptides were predicted using homology to existing peptides, key motifs within short open reading frames (sORF), a Neuripp score, and the genomic context of the sORF. Peptides were aligned using ClustalO, and regions of synteny were analysed using progressiveMauve and Easyfig.

**Results:** A total of 1072 BGCs were predicted across 349 genomes (85.1%); of these, 525 are putative RiPP/bacteriocin BGCs. The most abundant BGC determined were putative ranthipeptide BGCs, mainly among Clostridia. Lanthipeptides were predicted across multiple genera, with class II lanthipeptides the most diverse and large subset predicted. The majority of the BGCs identified belonged to Firmicutes, with the remainder among Proteobacteria, Actinobacteriota, Spirochaetota and Bacteroidota.

**Discussion:** In silico screening identified putative novel bacteriocin BGCs, including natural nisin variants. We hypothesise that screening the rumen microbiome for antimicrobial peptides may help find in situ solutions to reducing ruminant methane production whilst increasing animal feed efficiency. The abundance and diversity BGCs highlight the potential of the rumen as a reservoir for novel antimicrobial peptides for potential use in the agricultural, food and medical settings.