Psychrophilic *Clostridium estertheticum* complex and *Romboutsia* as a potential source for novel bacteriocins

Background:
The food industry has shifted its focus to the development of preservatives from natural sources as an alternative to chemical preservatives in response to increased consumers' demand for foods containing only natural additives. Bacteriocins, which are small antimicrobial peptides produced by numerous bacterial species, are among the natural compounds being applied to meet these demands. Anaerobic bacteria have attracted attention as novel sources of antimicrobial compounds.

Genome mining to detect novel bacterocins in *Clostridium estertheticum*

Targeted genome mining reveals the psychrophilic *Clostridium estertheticum* complex as a potential source for novel bacteriocins, Including cesin A and estercticin A

https://www.frontiersin.org/articles/10.3389/fmicb.2021.801467/full

Insight: Twenty novel bacteriocin biosynthetic gene clusters (BBGC), which were classified into eight (six antibiotics' and two sactipeptides) distinct groups, were discovered in 18 genomes belonging to *C. estertheticum* (*n* = 12), *C. tagluense* (*n* = 3) and genomospecies2 (*n* = 3). MS/MS analysis revealed that *C. estertheticum* CF004 produces cesin A, a short natural variant of nisin, and HRMS indicated the production of a novel sactipeptide named estercticin A.

Class I bacteriocins (lantibiotics)- new natural nisin variants

Cesin, a short natural variant of nisin, displays potent antimicrobial activity despite lacking two essential C-terminal macrocycles

https://pubmed.ncbi.nlm.nih.gov/37754751/

Insight: Recently, we discovered a short natural variant of nisin, cesin, in *Clostridium estertheticum*. Unlike other natural nisin variants, cesin lacks the two terminal macrocycles forming the pore forming domain; the effects of which are unknown. The current study aimed at elucidating the consequential effect of lacking the essential pore forming domain on the antimicrobial activity of cesin. Following heterologous expression of cesin in *Lactococcus lactis*, the lantibiotic demonstrated a broad and potent antimicrobial profile comparable to that of nisin. Determination of its mode of action using lipid II and lipoteichoic acid binding assays linked the potent antimicrobial activity to lipid II binding and electrostatic interactions with teichoic acids. Fluorescence microcopy showed that cesin lacks pore forming ability in its natural form and after addition of the two C-terminal macrocycles from nisin. Stability tests have shown that this lantibiotic is highly stable against different pH and temperature conditions, but it is denatured by trypsin. A bioengineered analogue, cesin R/G however overcame the trypsin denaturation. Using *Listeria monocytogenes*, we showed that DltA and Ddl, which are involved in cell wall modification and synthesis, respectively, confer intrinsic resistance to cesin. This study has revealed that cesin is a novel nisin variant that kills target bacteria by inhibiting cell wall synthesis without pore formation.

Rombocin, a short stable natural nisin variant, displays selective antimicrobial activity against *Listeria monocytogenes* and employs dual mode of action to kill the strains

https://pubmed.ncbi.nlm.nih.gov/38194633/

Insight: The use of nisin to prevent contamination of foodborne pathogens, such as *Listeria monocytogenes*, during meat processing has received considerable attention. However, the application of nisin has been limited, e.g. its susceptibility to proteolytic degradation and instability at neutral pH. To address those limitations, we identified rombocin A, a novel short nisin variant, through genome mining of *Paenibacillus*. Unlike other natural nisin variants, rombocin A contains only four stable macrocycles. Using a novel nisin-controlled expression system combined with nisin modification machinery (NisBTC), we heterologously expressed fully modified rombocin A in food safe strain *Lactococcus lactis* and demonstrated its selective antimicrobial activity against pathogenic *L. monocytogenes* and potent activity against multidrug-resistant strains. Rombocin A retains lipid II binding activity and impairs membrane function, but lacks pore-forming ability. Stability tests have confirmed its high stability against different pH, temperature, and enzyme digestion. With its gene-encoded nature, rombocin A is amenable to bioengineering to generate novel derivatives. Further mutation studies led to the identification of rombocin K, a

mutant with enhanced bioactivity. Our findings suggest that rombocin is a promising candidate for use as a food preservative bacteriocin and/or as a drug development agent.