Actifensin – a novel antimicrobial from sheep

TEAGASC researchers have discovered a novel bacteriocin from sheep faeces with activity against a broad range of food and gut pathogens.

The World Health Organisation (WHO) considers antibiotic resistance to be one of the foremost challenges to global public health, food security and development (WHO, 2020). Antibiotic resistance occurs naturally but is accelerated by overuse and misuse in both humans and animals. In response to this growing crisis, other potential biocontrol agents such as bacteriocins, bacteriophages and other microbial metabolites have been put forward as an alternative to antibiotics.

Bacteriocins are small, antimicrobial, post-translationally modified peptides that are produced by bacteria. They are typically defined as "narrow spectrum", in which they target members of the same species, or "broad spectrum", whereby they are active against bacteria of other species or genera (O'Connor *et al.*, 2020). This potential for target specificity is another advantage over traditional antibiotics, which can have deleterious effects on the entire microbiota (Dethlefsen, *et al.*, 2011).

In silico genome mining methods have shown that the gut microbiome is a rich source of bacteriocin-producing bacteria (Walsh *et al.*, 2015). *Actinomyces* are Gram-positive non-spore forming anaerobes commonly isolated from the gastrointestinal tract of humans and other animals. In humans they are also found in the oral cavity where they play a role in plaque formation. On the Longlife project, we have isolated a strain of *Actinomyces ruminicola* that produces a novel, broad-spectrum bacteriocin called actifensin (Sugrue *et al.*, 2020).

Structure of actifensin

A. ruminicola DPC 7226 was isolated from sheep faeces. The antimicrobial activity of the strain was first identified by screening against the acid-tolerant indicator strain, *Lactobacillus delbrueckii* subsp. *bulgaricus* LMG 6901. Further analysis of the bacterial cells and the cell-free supernatant by high-performance liquid chromatography (HPLC) and matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) revealed a mass of approximately 4 kDa responsible for this activity. Treatment with proteinase K resulted in a loss of activity, indicating that the antimicrobial was proteinaceous in nature.

A combination of N-terminal sequencing of the purified peptide and genome analysis of *A. ruminicola* DPC 7726 identified a small (69 amino acid) open reading frame, which encodes the antimicrobial peptide, designated *afnA*. Encoded homologues of the AfnA peptide were identified in 14 other strains of *Actinomyces*, as well as in a number of eukaryote genomes, including fungi and arthropods. These homologues of *afnA* encode defensins, ubiquitous and ancient

antimicrobial peptides in eukaryotes that play a key role in innate immunity. *AfnA* therefore represents a new family of defensin-like bacteriocins and was thereafter named actifensin.

Antimicrobial activity of actifensin

Actifensin was classified as having a broad spectrum of inhibition, demonstrating antimicrobial activity against Gram-positive bacteria such as *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Bacillus*, other *Actinomyces* spp., and *Clostridium* spp. Notably, actifensin inhibited pathogens such as *Clostridioides difficile*, vancomycin-resistant *Enterococcus* and methicillin-resistant *Staphylococcus*, bacteria that represent a significant challenge to the medical field due to their antibiotic resistance. Weak inhibition was also demonstrated against the common food-borne pathogens *Listeria monocytogenes* and *Listeria innocua*.

Future prospects for actifensin and other defensin-like bacteriocins

Actifensin demonstrated a number of traits that would be advantageous from a food industry perspective. Notably, the peptide is heat stable, retaining its antimicrobial activity after 30 minutes at 100°C. It is also quite potent, with a minimum inhibitory concentration of 0.76 µM against *Streptococcus agalactiae* and *C. difficile*. It is also notable that the homologues of *afnA* identified in other species and strains of *Actinomyces* had a low level of similarity. This diversity in the amino acid sequence (52 % on average) suggests that this family of defensin-like bacteriocins may provide a broad structural basis on which to deliver and design new broad-spectrum antimicrobials for treatment of animal and human infections.

Conclusion

The discovery of actifensin and other defensin-like bacteriocins represents new possibilities in the field of biocontrol and an opportunity to reduce our reliance on antibiotics. Actifensin's broad spectrum of activity and physical properties may have widespread applications in both food and health.
This work and the image in **Figure 1** featured on the cover of the *Journal of Bacteriology* in May 2020.

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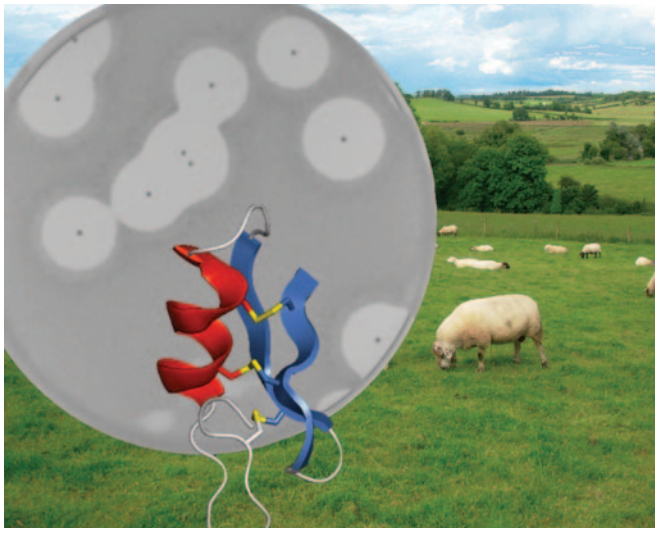

FIGURE 1: This research featured on the cover of the Journal of Bacteriology in May 2020.

Sugrue, I., O'Connor, P.M., Hill, C., Stanton, C. and Ross, R.P. (2020). '*Actinomyces* produces defensin-like bacteriocins (actifensins) with a highly degenerate structure and broad antimicrobial activity'. *Journal of Bacteriology*, 202 (4): e00529-19.
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