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Antimicrobial antagonists against food pathogens: a bacteriocin perspective

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Efforts are continuing to find novel bacteriocins with enhanced specificity and potency. Traditional plating techniques are still being used for bacteriocin screening studies, however, the availability of ever more bacterial genome sequences and the use of *in silico* gene mining tools have revealed novel bacteriocin gene clusters that would otherwise have been overlooked. Furthermore, synthetic biology and bioengineering-based approaches are allowing scientists to harness existing and novel bacteriocin gene clusters through expression in different hosts and by enhancing functionalities. The same principles apply to bacteriocin producing probiotic cultures and their application to control pathogens in the gut. We can expect that the recent developments on bacteriocins from Lactic Acid Bacteria (LAB) described here will contribute greatly to increased commercialisation of bacteriocins in food systems.

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Introduction

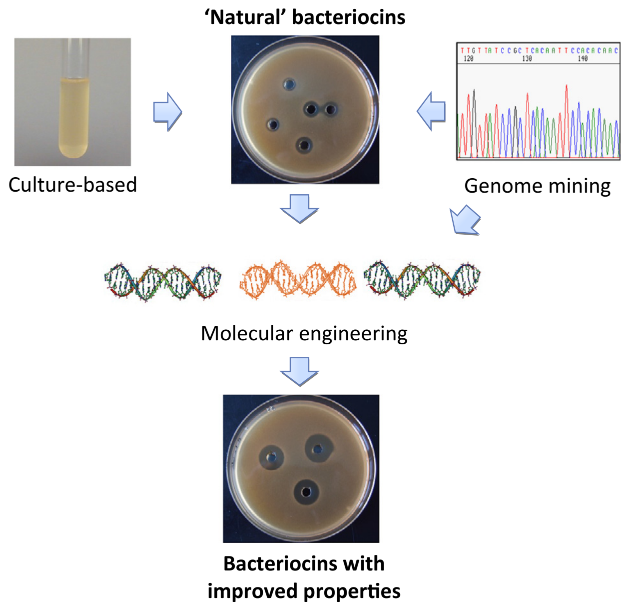
Consumer awareness of the effect of diet on health has led to a demand for minimally processed foods in which chemical preservatives are replaced by more natural alternatives. Traditionally foods were preserved by (LAB), natural constituents of fermented foods, which confer their preservative effects by the production of lactic acid, hydrogen peroxide and small peptides known as bacteriocins. Bacteriocins are active against a number of genera (broad spectrum) or particular species (narrow spectrum) [1–3] and are very diverse, varying in size, structure and specificity. The fact that many bacteriocins are produced by food-grade LAB and possess potent antimicrobial activity means that they are ideally suited to controlling food spoilage and pathogenic bacteria [4,5,6•].

Bacteriocins can be broadly divided into two classes: class I, of which the lantibiotics (post-translationally modified peptides containing unusual amino acids) are the best-known example and class II, containing unmodified peptides [7]. Their mode of action is likely driven by the primary structure of the bacteriocin with membrane permeabilisation being a very common theme. The producing culture is protected by the production of specific immunity proteins and the low levels of resistance detected so far makes them desirable alternatives to antibiotics [6•]. Their main advantage over chemical preservatives is their ability to preserve without affecting the sensory qualities of the food while adhering to the demand for natural preservatives. The ideal bacteriocin should be potent at low concentrations, active against a range of spoilage and pathogenic organisms, innocuous to the host and economical to produce [8]. These antimicrobials can be introduced into a food through incorporation of the bacteriocin-producing strain into the food product (most commonly in fermented foods), the generation and use of a bacteriocin-containing fermentate or as a more concentrated bacteriocin-containing food preservative. Currently only two bacteriocins are being used commercially as food preservatives: nisin produced by *Lactococcus lactis* (marketed as Nisaplin and under other brand names) has been used commercially for 50 years [9] and carnocyclin A (marketed as Micocin) a circular bacteriocin produced by *Carnobacterium maltaromaticum* UAL307 is an approved biopreservative in the US and Canada developed to inhibit *Listeria monocytogenes* in ready-to-eat meat (RTE) products [10]. This review focuses predominantly on bacteriocins as antimicrobial antagonists and efforts to develop them as viable food biopreservatives (see Figure 1).

The continuing search for novel bacteriocins

A primary focus of bacteriocin research is identifying novel bacteriocins and bacteriocin-producing strains for specific applications. The general consensus is that the bacteriocin/bacteriocin-producer that is best suited to controlling a problematic spoilage/pathogenic microorganism will often be one that is found in the same environmental niche. This is based on the expectation that bacteriocins provide an advantage to competitors

Figure 1



Enhancement of bacteriocin functionality using genome mining and molecular engineering techniques.

fighting for scarce resources in a particular environment. A prime example relates to *Weissella hellenica* QU 13, isolated from a barrel in which Japanese pickles are fermented, which was found to produce two leaderless bacteriocins, weissellicin Y, homologous to the class IId Enterocin L50A and L50B, and weisselicin M. In the latter case, it is notable that this novel broad spectrum class IId antimicrobial is effective against *Bacillus coagulans*, a known contaminant of pickle fermentations. Thus, strain QU 13 is a good example of a fermentation-associated isolate which has the potential to be employed to control an undesirable microbial contaminant [11]. *Lactococcus garvieae* is a pathogen affecting farmed and fresh fish from marine and freshwaters and is also considered an emerging zoonotic pathogen. Garvicin A, a novel class IIb bacteriocin produced by the human isolate *L. garvieae* 21881, inhibits other *L. garvieae* stains and has potential to treat or prevent *L. garvieae* infections. More specifically, it is suggested that the purified bacteriocin in combination with probiotic LAB would be useful in the fight against *L. garvieae* infections [12]. Another *L. garvieae* strain, a fermented pork sausage isolate *L. garvieae* BCC 43578, produces garvieacin Q, a novel class IId bacteriocin active against other *L. garvieae* and *L. monocytogenes* [13]. The ability to control *L. monocytogenes* is a particularly highly sought-after trait and it is thus notable that enterocin W, a two component lantibiotic produced by *Enterococcus faecalis* NKR-4-1 isolated from *pla-ra* Thai fermented fish [14], exhibits activity against this pathogen. Given that *Staphylococcus aureus* is also a major concern for the food industry, it is interesting that bactofencin A, a cationic disulphide bond-containing bacteriocin similar to eukaryotic defensins, is active against *S. aureus*. In addition to the unusual nature of this bacteriocin, it is notable that its producer, the porcine isolate *Lactobacillus salivarius* DPC6502, does not contain a classical immunity-like gene, but instead encodes a *dltB* homologue that confers resistance [15]. While the examples provided above relate to strains that produce a single bacteriocin, it should be noted that the production of multiple bacteriocins by a single strain can be advantageous as the various bacteriocins are likely to have different modes of action, thereby extending the spectrum of inhibition and reducing the likelihood of development of resistance. The genome of *Enterococcus faecium* NKR-5-3, isolated from *pla-ra* Thai fermented fish, encodes five enterocins, NKR-5-3 — A, B, C, D and Z and produces at least four of them, that is, NKR-5-3 — A, B, C and D. Enterocin NKR-5-3C was confirmed to be a class IIa bacteriocin which exhibits potent antilisterial activity. The other bacteriocins are proposed to represent different classes but further investigations are required to establish this definitively [16•,17].

The particular expansion in numbers of circular bacteriocins

Although previously regarded as being rare, the discovery of circular bacteriocins has become more common in recent years. This is notable as these bacteriocins are thought by some to have the potential to form the next generation of biopreservatives as a consequence of their stability and activity. Indeed, gassericin A, garvicin ML, lactocyclin Q and leucocyclin Q produced by LAB inhibit a range of Gram-positive bacteria including food spoilage bacteria and food pathogens [18]. The remarkable stability and activity of these bacteriocins is attributed to their head to tail cyclisation which confers the bacteriocins with increased protease and heat resistance [19,20•]. Garvicin ML is a recently discovered circular bacteriocin produced by *L. garvieae* DCC43 isolated from a Mallard duck which inhibits *L. garvieae* [21]. Leucocyclin Q, produced by a Japanese pickle isolate *Leuconostoc mesenteroides* TK41401, is particularly active against *B. coagulans* which, as noted above, is a major pickle food spoilage organism [22]. Studies relating to the mode of action of these, and indeed other, bacteriocins continue to also attract attention. Notably, in this regard, Liu *et al.* [23] recently noted that sublethal doses of carnocyclin A induced an adaptation response in *L. monocytogenes* 08-5923 by affecting genes responsible for cell wall biosynthesis and metabolic function maintenance.

New studies relating to the use of bacteriocins as part of a hurdle approach to preservation

Bacteriocins can become more effective biopreservatives when used in combination with other antimicrobial