SERIES ON ANTIBIOTIC ALTERNATIVES

Bacteriocins — a viable alternative to antibiotics?

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Abstract | Solutions are urgently required for the growing number of infections caused by antibiotic-resistant bacteria. Bacteriocins, which are antimicrobial peptides produced by certain bacteria, might warrant serious consideration as alternatives to traditional antibiotics. These molecules exhibit significant potency against other bacteria (including antibiotic-resistant strains), are stable and can have narrow or broad activity spectra. Bacteriocins can even be produced *in situ* in the gut by probiotic bacteria to combat intestinal infections. Although the application of specific bacteriocins might be curtailed by the development of resistance, an understanding of the mechanisms by which such resistance could emerge will enable researchers to develop strategies to minimize this potential problem.

Probiotics
Live microorganisms that confer a health benefit on the host when administered in adequate amounts.

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It could be argued that the identification and development of antibiotic therapy represents the most significant scientific achievement of the twentieth century in terms of an impact on human morbidity and mortality. Unfortunately, several problems have arisen that limit these initial benefits and cast doubt on how useful antibiotics will prove to be in the twenty-first century. Pathogens have emerged that are resistant to single, and subsequently multiple, antibiotics. Moreover, there is a shortage of new families of antibiotics that could potentially compensate for resistance to existing antibiotics, in part owing to the high costs and risks associated with developing and using such products[1,2]. Finally, it has become clear that the administration of broad-spectrum antibiotics can lead to 'collateral damage' to the human commensal microbiota, which has several key roles in host health[3–5]. The potential association between the use of broad-spectrum antibiotics and the increasing incidence of atopic and autoimmune diseases is a particular cause for concern[3,4].

As a consequence, there is a need for the development of new antimicrobials that can be used in clinical settings. Alternatives that have been investigated include plant-derived compounds[6], bacteriophages and phage lysins[7], RNA-based therapeutics[8], probiotics[9], and antimicrobial peptides from a variety of sources[10]. One option that can no longer be ignored is a subgroup of antimicrobial peptides known as bacteriocins. These are small, bacterially produced, ribosomally synthesized peptides that are active against other bacteria and against which the producer has a specific immunity mechanism[11]. Bacteriocins are a heterogeneous group and are usually classified into peptides that undergo significant post-translational modifications (class I) and unmodified peptides (class II) (BOX 1; TABLE 1). Many bacteriocins have a high specific activity against clinical targets (including antibiotic-resistant strains), have mechanisms of action that are distinct from current chemotherapeutic products and, given their proteinaceous nature, are amenable to gene-based peptide engineering. Although several broad-spectrum bacteriocins exist that can be used to target infections of unknown aetiology, potent narrow-spectrum bacteriocins have also been identified that can control targeted pathogens without negatively affecting commensal populations[12,13]. It should be noted that the emergence of resistant bacteria is still a possibility, albeit one that might be minimized through a detailed understanding of bacteriocin mechanisms of action and through peptide engineering.

In this Review, we highlight the key traits of bacteriocins which suggest that these compounds represent potential alternatives to antibiotics. When using the term bacteriocins, we refer specifically to small peptide antimicrobials and thus do not discuss the clinical potential of larger proteins such as the bacteriolysins, colicins and pyocins. Bacteriocins have also been investigated with respect to their potential in animal health[14], in marine environments[15] and in enhancing food safety and quality[11], but these applications have been reviewed previously and are not addressed here.

Benefits of bacteriocins

Bacteriocins have many properties which suggest that they are viable alternatives to antibiotics. These include their potency (as determined *in vitro* and *in vivo*), their low toxicity, the availability of both broad- and

Box 1 | **Classification of bacteriocins**

Several approaches have been taken to classify bacteriocins. One, which is used to classify the bacteriocins of lactic acid bacteria (LAB), divides these peptides into class I peptides, which undergo post-translational modification, and class II peptides, which are largely unmodified (or undergo modest modification; for example, the formation of disulphide bridges, circularization or the addition of *N*-formylmethionine)[11]. This system proposes that larger proteins be removed from the bacteriocin category. Similarly, the antimicrobials that are ribosomally synthesized by Gram-negative organisms can be divided into small peptides, such as microcins, and larger proteins, such as colicins. The microcins have previously been divided on the basis of the presence (class I) or absence (class II) of significant modification[150]. We argue that the designation bacteriocin should be retained for peptide antimicrobials, and thus, ribosomally synthesized antimicrobial proteins are not covered in this Review.

In addition to subdividing bacteriocins on the basis of their modifications, further subdivisions exist. In the case of modified bacteriocins, a comprehensive nomenclature for ribosomally synthesized, post-translationally modified peptides (RiPPs) has recently been proposed[151]. Given that the RiPPs include modified bacteriocins, this nomenclature is also adopted here. Thus, class I (modified) bacteriocins can be subgrouped as lantibiotics[152], linaridins[118], proteusins[25], linear azole- or azoline-containing peptides[153], cyanobactins (including patellamide-like and prenylated, anacyclamide-like cyanobactins)[67], thiopeptides[154], lasso peptides[155], sactibiotics[156], bottromycins[157], glycocins[158,159], and modified microcins that do not belong to other subgroups (for example, microcin C7-C51)[160] (TABLE 1). In some of these families, peptides with antimicrobial activity are rare (linardins) or not well characterized (cyanobactins).

The unmodified or circular (class II) bacteriocins can be divided into five groups that correspond to the four subclasses of unmodified LAB bacteriocins and one of the subclasses of unmodified microcins. These subclasses are peptides that contain a YGNGV motif (in which N represents any amino acid; the class IIa peptides); two-peptide bacteriocins (class IIb peptides); circular bacteriocins (class IIc peptides); unmodified, linear, non-pediocin-like, single-peptide bacteriocins that do not belong to other subclasses (class IId peptides); and the microcin E492-like bacteriocins (class IIe peptides; formerly known as the class IIb microcins). We propose that subclass IIc should be expanded to include the unmodified anacyclamides[161] and that subclass IId should incorporate certain microcins, such as microcin V and microcin S[89,150]. Although subclass IIe peptides are categorized within the class II bacteriocins, it should be noted that peptides such as microcin E492 might carry a siderophore-type post-translational modification[71].

narrow-spectrum peptides, the possibility of *in situ* production by probiotics and the fact that these peptides can be bioengineered.

In vitro *potency*. The potency of bacteriocins against clinically important pathogens varies both across and within the various peptide subclasses. In general, the class I bacteriocins, including lantibiotics and thiopeptides, are most active against Gram-positive pathogens. Lantibiotics, such as nisin, planosporicin, Pep5, epidermin, gallidermin, mutacin B-Ny266, lacticin 3147 and actagardine (and their bioengineered derivatives) exhibit notable *in vitro* activity against clinically important pathogens such as *Streptococcus pneumoniae*, staphylococci (including methicillin-resistant *Staphylococcus aureus* (MRSA)), vancomycin-resistant enterococci (VRE), various mycobacteria, *Propionibacterium acnes* and *Clostridium difficile*[16]. Thiopeptides are also predominantly active against Gram-positive pathogens. Thiopeptides exhibit highly potent *in vitro* activity, but their commercial development has been hampered by their poor solubility. Notable thiopeptides include nocathiacin I and derivatives[17], philipimycin[18], GE2270 A[19] and the thiomuracins[20]. GE2270 A has also been modified to create LFF571 (Novartis) and exhibits potent activity against *C. difficile* and most other Gram-positive organisms (with the exception of bifidobacteria and lactobacilli)[21]. Among the other modified bacteriocins, the sactibiotic thuricin CD has been found to be particularly potent against *C. difficile*[12], and another sactibiotic, subtilosin A, displays a narrow spectrum of activity against *Enterococcus faecalis*, *Streptococcus pyogenes* and *Listeria monocytogenes*[22], as well as *Gardnerella vaginalis*[23]. Although the glycocin sublancin 168 does not seem potent enough to justify commercial applications[24], it may be that other glycocins will be identified or created that show greater potential. The proteusin polytheonamide A has been found to be active at microgram-per-millilitre concentrations against some, albeit non-pathogenic, Gram-positive strains[25], whereas bottromycin A2 exhibits potent activity against MRSA and VRE[26].

There are also many examples of unmodified, class II bacteriocins with potent antimicrobial activity against Gram-positive targets. This includes some class IIa bacteriocins, which are active against *L. monocytogenes*[27] and other Gram-positive pathogens[28]. Class IIc peptides such as the enterococcal bacteriocin (enterocin) AS-48 have been investigated predominantly with a view to use in non-clinical applications, but do nonetheless possess antimicrobial activities, which suggests that other applications might be worth pursuing[29]. Several class IId bacteriocins — for example, epidermicin NI01 — have also provided interesting *in vitro* results[30].

Although there are many examples of bacteriocins with substantial activity against Gram-negative bacteria *in vitro*, the generally held view is that bacteriocins exhibit less potential as chemotherapeutics for infections with Gram-negative organisms. Bacteriocins produced by Gram-negative bacteria — normally termed microcins, despite representing different classes of bacteriocin (BOX 1) — typically show the greatest potential in this regard. It should be noted that they often display a narrow spectrum of activity and that there have been few studies in which specific activities have been assessed. In relation to the modified microcins, notable observations include the activity of microcin C7-C51 (MccC7-C51) against at least some strains of *Escherichia*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Shigella*, *Proteus* and *Yersinia* spp.[31], the activity of the lasso type bacteriocin MccJ25 against some strains of *Escherichia* and *Salmonella* spp.[32,33], and the activity of the linear azole-containing peptide MccB17 against a wide range of Gram-negative bacteria, including *Escherichia*, *Citrobacter*, *Klebsiella*, *Salmonella*, *Shigella* and *Pseudomonas* spp.[34,35]. With respect to the unmodified microcins, it has been established that MccV[36] and MccL[37] (from subclass IId), and MccE492 (REF. 38), MccM[39] and MccH47 (REF. 39) (from subclass IIe) all exhibit activity against at least some Gram-negative targets.

Although lantibiotics are generally thought to have poor activity against Gram-negative organisms, purified lantibiotics such as nisin and epidermin have been found to kill some of these bacteria[40,41]. The sactibiotic

Table 1 | **Class I and II bacteriocins**

Group	Distinctive feature	Examples
Class I (modified)		
MccC7-C51-type bacteriocins	Is covalently attached to a carboxy-terminal aspartic acid	MccC7-C51
Lasso peptides	Have a lasso structure	MccJ25
Linear azole- or azoline-containing peptides	Possess heterocycles but not other modifications	MccB17
Lantibiotics	Possess lanthionine bridges	Nisin, planosporicin, mersacidin, actagardine, mutacin 1140
Linaridins	Have a linear structure and contain dehydrated amino acids	Cypemycin
Proteusins	Contain multiple hydroxylations, epimerizations and methylations	Polytheonamide A
Sactibiotics	Contain sulphur–α-carbon linkages	Subtilosin A, thuricin CD
Patellamide-like cyanobactins	Possess heterocycles and undergo macrocyclization	Patellamide A
Anacyclamide-like cyanobactins	Cyclic peptides consisting of proteinogenic amino acids with prenyl attachments	Anacyclamide A10
Thiopeptides	Contain a central pyridine, dihydropyridine or piperidine ring as well as heterocycles	Thiostrepton, nocathiacin I, GE2270 A, philipimycin
Bottromycins	Contain macrocyclic amidine, a decarboxylated carboxy-terminal thiazole and carbon-methylated amino acids	Bottromycin A2
Glycocins	Contain *S*-linked glycopeptides	Sublancin 168
Class II (unmodified or cyclic)		
IIa peptides (pediocin PA-1-like bacteriocins)	Possess a conserved YGNGV motif (in which N represents any amino acid)	Pediocin PA-1, enterocin CRL35, carnobacteriocin BM1
IIb peptides	Two unmodified peptides are required for activity	ABP118, lactacin F
IIc peptides	Cyclic peptides	Enterocin AS-48
IId peptides	Unmodified, linear, non-pediocin-like, single-peptide bacteriocins	MccV, MccS, epidermicin NI01, lactococcin A
IIe peptides	Contain a serine-rich carboxy-terminal region with a non-ribosomal siderophore-type modification	MccE492, MccM

Mcc, microcin.

subtilosin A is another unusual example of a modified bacteriocin that is produced by a Gram-positive organism and has activity against some Gram-negative bacteria[22]. Finally, there are rare examples of class IIa bacteriocins that are produced by Gram-positive organisms and exhibit activity against Gram-negative bacteria[42].

It should be noted that the effectiveness of individual bacteriocins could be further enhanced through combination with other antimicrobials or membrane-active substances. Although there have been few studies in this area, nisin showed synergistic activity with the antibiotics polymyxin E and clarithromycin against *Pseudomonas aeruginosa*[43] and with ramoplanin and other non-β-lactam antibiotics against many strains of MRSA and VRE[43,44]. Other combinations that have produced interesting results include the membrane-permeabilizing peptide $(KFF)_3K$ with MccJ25 against *Salmonella enterica* subsp. *enterica* serovar Typhimurium[45], the class IIa enterocin CRL35 with several antibiotics against *L. monocytogenes*[46], and the sactibiotic subtilosin A with glycerol monolaurate, lauric arginate or ε-poly-L-lysine (or with a combination of two of these compounds) against bacterial vaginosis-associated pathogens[47].

In vivo *activity against pathogens.* Although *in vitro* studies have highlighted the potential value of bacteriocins as alternatives to antibiotics, it is crucial to assess this activity in more clinically relevant circumstances. Of the different categories of bacteriocins described above, the lantibiotics and thiopeptides have been most extensively investigated from this perspective. For example, lantibiotics have been shown to control or prevent the growth of staphylococci and/or enterococci in and on catheter tubing[48]. Moreover, nisin has been shown to target *S. pneumoniae* and to be 8–16 times more active than vancomycin in an intravenous regimen[49]. Similarly, a naturally occurring nisin variant, nisin F, effectively controls *S. aureus in vivo* when incorporated into bone cement[50], inhibits the growth of the pathogen in the respiratory tract of rats when administered intranasally[51] and briefly suppresses the growth of this species in the intraperitoneal cavity[52]. Moreover, the lantibiotic B-Ny266 was found to be active *in vivo* against *S. aureus* in a mouse model of intraperitoneal infection, having a median effective dose (ED_{50}) comparable to that of vancomycin[53]. Similar studies have revealed that the ED_{50} values of the lantibiotic mersacidin are even lower than those for vancomycin[54] and that mersacidin is effective when treating MRSA harboured in the nasal

Median effective dose
The amount of an antimicrobial that is required to produce a specific effect in half an animal population.

cavity[55], despite the fact that the *in vitro* activity of this bacteriocin is not particularly notable[56]. Finally, planosporicin had a good efficacy in a mouse model of *S. pyogenes*-induced septicaemia[57].

The efficacy of thiopeptides for systemic applications was originally thought to be restricted by their insoluble nature. However, philipimycin, thiazomycin and nosiheptide have given impressive results when assessed using a mouse model of *S. aureus* infection[18,19,58]. Several semi-synthetic analogues of thiopeptides have also been generated that address the issue of solubility. These include compound 19, an amide derivative of a nocathiacin; compound 19 dramatically outperformed the antibiotics linezolid and vancomycin with regard to reducing *S. aureus* survival in a systemic mouse infection and in mouse thigh models of MRSA infections, respectively[59]. Furthermore, LFF571, a derivative of the thiopeptide GE2270A, was more effective than vancomycin in an experimental model of primary and relapsing *C. difficile* infection[60].

Other than research assessing the lantibiotics and thiopeptides, many of the other relevant studies relate to the class IIa bacteriocin peptides. In various investigations, the administration of such bacteriocins has provided protection from *L. monocytogenes* infection in mice[61,62]. Furthermore, liposomes containing the class IIa bacteriocin E50-52 were found to inhibit the intracellular growth of *Mycobacterium tuberculosis* and prolong the survival of mice in an acute-tuberculosis model[63].

In vivo studies with purified peptides from other subclasses of bacteriocins are rare, although it is notable that, when tested in a mouse model of intraperitoneal infection with *Salmonella enterica* subsp. *enterica* serovar Newport, treatment with the lasso peptide MccJ25 significantly decreased pathogen numbers in the liver and spleen compared with those in control mice[64].

Low toxicity. Another benefit of many bacteriocins is their low oral toxicity for the treated host. Indeed, many bacteriocins produced by lactic acid bacteria, in particular, have been consumed in fermented foods for millennia. By virtue of its wide-scale use as a food preservative, nisin has been the focus of particular attention in this regard. The lack of toxicity of nisin and other lantibiotics has been demonstrated in several studies[57,65]. It should be noted that the *Enterococcus* spp.-associated cytolysin (a lantibiotic) does exhibit cytotoxic activity, but it is the only lantibiotic thus far to be shown to have this property[66]. By contrast, antibacterial activity among cyanobactins is rare, whereas cytotoxic activity is common[67].

There have been few studies in which the cytotoxicity of unmodified, class II bacteriocins has been tested. Although pediocin PA-1 (when used at 10–20 μg per ml) displayed some cytotoxicity against Vero monkey kidney cells and simian virus 40-transfected human colon cells[68], other class IIa bacteriocins, such as the carnobacteriocins BM1 and B2, displayed no significant cytotoxicity against Caco-2 (human epithelial colorectal adenocarcinoma) cells, even when used at concentrations 100-fold higher than those required for antimicrobial activity[69]. At low and intermediate concentrations, the class IId peptide MccE492 induced biochemical and morphological changes typical of apoptosis, and at higher concentrations (>20 μg per ml), a necrotic phenotype was observed[70]. However, it has been suggested that this phenotype could be exploited such that MccE492 could be used as an antitumour agent[71].

Broad- and narrow-spectrum bacteriocins. There are many bacteriocins that exhibit broad-spectrum antimicrobial activity. As with broad-spectrum antibiotics, this is an attractive trait, as it allows us to target infections of unknown aetiology. However, broad-spectrum antibiotics are known to damage the commensal human microbiota, so there is an appreciation of the value of using narrow-spectrum antimicrobials in specific circumstances.

C. difficile-associated diarrhoea (CDAD) is a particularly appropriate example of such a circumstance, in that in this case the disease often results from, and is treated with, antibiotics that can modulate the resident gut microbiota. *C. difficile* can competitively benefit from antibiotic-induced disruption of the microbiota and can then flourish in this altered environment. The subsequent growth and toxin production by *C. difficile* results in CDAD, which requires further antibiotic treatment. This can address the acute problem, but can also lead to further disruption of the commensal population, and the disease often recurs[72]. Screening the gut for narrow-spectrum bacteriocins that target *C. difficile* led to the discovery of the sactibiotic thuricin CD, which is produced by *Bacillus thuringiensis*[12]. Thuricin CD was found to exhibit antimicrobial activity comparable to the activities of the antibiotics vancomycin and metronidazole (both of which are used to treat CDAD in the clinic) in a model of the human distal colon. Importantly, however, thuricin CD did not significantly alter the composition of the commensal microbiota, whereas both vancomycin and metronidazole brought about a dramatic increase in the abundance of organisms of the phylum Proteobacteria at the expense of organisms of other phyla[73] (FIG. 1).

There have been numerous other efforts to identify and develop antimicrobials with narrow-spectrum activity against *C. difficile*. One outcome of these efforts is a semi-synthetic derivative of the lantibiotic actagardine[13]. Furthermore, another semi-synthetic peptide, thiopeptide LFF571, is active against *C. difficile*, but does not exhibit as narrow an antimicrobial spectrum as thuricin CD and the actagardine derivative; nonetheless, the relatively low activity of LFF571 against lactobacilli and bifidobacteria has been highlighted[21]. Similarly, among the unmodified bacteriocins, the class IIa bacteriocin pediocin PA-1, which effectively treats mice infected with *L. monocytogenes*[74], has been shown to be inactive against most gut bacteria *in vitro* and *in vivo*[75,76].

Such narrow-spectrum activity could also be beneficial at other sites of infection. For instance, the activity of subtilosin A against the vaginal pathogen *G. vaginalis* and the lack of subtilosin A activity against probiotic *Lactobacillus* spp. isolates[77] suggest that further investigations to test the ability of this peptide to treat vaginosis are merited. Finally, although the narrow-spectrum

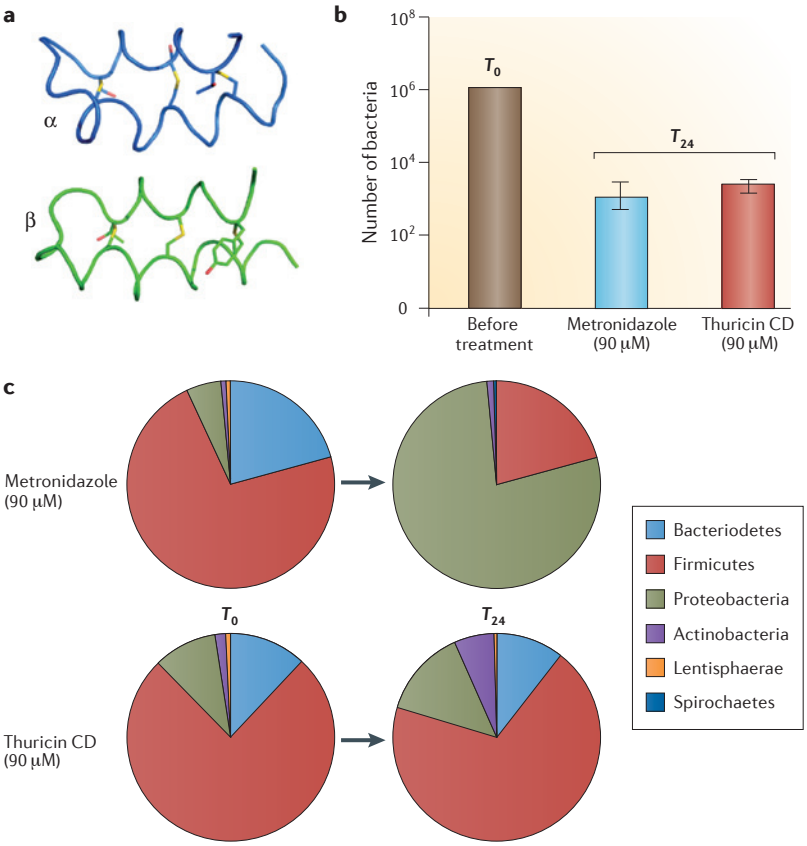

Figure 1 | **Narrow-spectrum activity of thuricin CD.** **a** | The structure of thuricin CD, a two-peptide sactibiotic, showing the α and β components (Protein Data Bank accession 2L9X and 2LA0). **b** | Thuricin CD and the antibiotic metronidazole have a comparable effect on *Clostridium difficile* after 24 hours (T_{24}) in a human colon infection model[73]. **c** | The effect of both metronidazole and thuricin CD on the gut microbiota[73]. Although thuricin CD has a minimal effect on the composition of the gut microbiota (depicted here at the phylum level), metronidazole markedly alters the balance of these microbial populations.

activity of many bacteriocins produced by Gram-negative bacteria is noteworthy, in some instances the spectrum of activity might be too low to justify commercial exploitation.

Potential for the use of probiotic-produced bacteriocins. Some bacteriocins benefit from the fact that, in addition to being administered by standard methods, they have the potential to be produced at the site of infection by probiotic bacteria. Many gut bacteria have previously been shown to require bacteriocin production for gut colonization. For example, when a MccV producer and a non-producing derivative were co-administered to mice, the non-producing strain could not colonize and was thus eliminated from the large intestine[78]. With respect to probiotic gut microorganisms, the production of a bacteriocin has long been considered to be a beneficial trait (for reviews, see REFS 79,80), but the evidence supporting this theory has often been indirect or circumstantial.

Previous studies had reported the significant inhibition of *L. monocytogenes* and enterohaemorrhagic *Escherichia coli* in mice[81,82] by the bacteriocin-producing strain *Lactobacillus casei* str. LAFTI L26, as well as the beneficial impact of administering *Lactobacillus johnsonii* str. La1 supernatant to children to control *Helicobacter pylori* colonization[83]. It is also interesting to note that when a five-strain probiotic mixture was successfully used to control *S.* Typhimurium-induced diarrhoea in pigs[84], the only bacteriocin-producing strain, *Lactobacillus salivarus* str. DPC 6005, dominated over co-administered strains, both in the ileal digesta and in the mucosa. However, the specific contribution of bacteriocin production to pathogen control in this particular case was not established[85]. To address this issue, it is necessary to use controls in the form of isogenic non-bacteriocin-producing mutants for comparison. This was achieved when oral administration of *L. salivarius* str. UCC118, which produces the class IIb bacteriocin ABP118, was shown to control *L. monocytogenes* infection in mice: this protective effect was abolished when a non-bacteriocin-producing derivative of *L. salivarius* str. UCC118 was administered[86] (FIG. 2). Moreover, an *L. monocytogenes* strain that is immune to ABP118 could successfully establish an infection even when the bacteriocin-producing *L. salivarius* str. UCC118 was present[86]. Recent investigations using *L. salivarius* str. UCC118 have also revealed that the operon for ABP118 synthesis is significantly upregulated following adhesion of the bacterium to epithelial cells, possibly because adhesion causes a sufficiently high local concentration of bacteria to trigger quorum sensing-mediated induction of ABP118 production[87].

Similarly, *Pediococcus acidilactici* str. MM33, which produces the class IIa bacteriocin pediocin PA-1, has been shown to be more successful at controlling VRE than the mutant *P. acidilactici* str. MM33A, which does not produce the bacteriocin; compared with the addition of this non-producer mutant, addition of the producer strain resulted in a much greater reduction in pathogen numbers (by 1.85 $\log_{10}$ colony-forming units per gram) 3 days post-infection[88]. Finally, *in vitro* studies highlighted that the production of the class IId bacteriocin MccS by *E. coli* str. G3/10 (one of six *E. coli* strains present in the probiotic Symbioflor 2) inhibited the adherence of enteropathogenic *E. coli* (EPEC) to epithelial cell lines, and this effect was not observed when using an EPEC strain that heterologously expressed the gene conferring immunity to MccS[89].

With respect to the oral cavity and the oral pathogen *Streptococcus mutans*, a strain that produces the bacteriocin mutacin 1140 has been developed to control plaque formation (SMaRT Replacement Therapy, developed by Oragenics). In human trials testing a non-pathogenic *S. mutans* strain that had been engineered to eliminate lactate dehydrogenase production to ensure that the bacterium did not contribute to plaque formation[90], this SMaRT strain was found to exclude other *S. mutans* strains. It is noteworthy that the SMaRT strain was retained by some individuals for up to 14 years after a single inoculation[91]. Similarly, it has been demonstrated that the bacteriocin-producing *Streptococcus salivarius* str. K12 can control several plaque-forming and halitosis-causing bacteria[92], and bacteriocin-producing

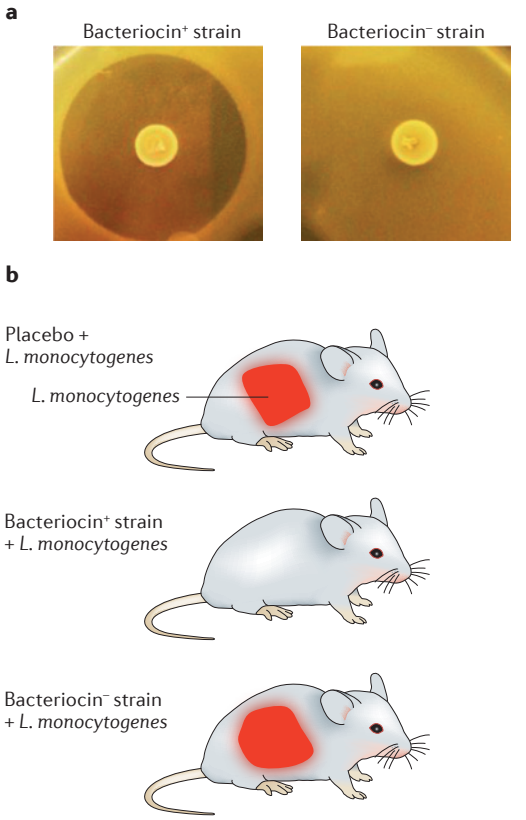

Figure 2 | **Probiotic delivery of bacteriocins.** The bacteriocin-producing probiotic *Lactobacillus salivarius* str. UCC118 (bacteriocin$^+$) or a non-bacteriocin-producing isogenic mutant (bacteriocin$^-$) were the focus of a particular study[86]. **a** | There is a zone of inhibition (that is, an area where bacterial growth has been prevented) around the bacteriocin$^+$ strain but not around the bacteriocin$^-$ strain when grown with *Listeria monocytogenes* on agar. **b** | Mice were administered (fed) a placebo (no bacterium), the bacteriocin$^+$ strain or the bacteriocin$^-$ strain before oral infection with luciferase-tagged *L. monocytogenes*. 30 minutes after infection, no light could be detected in the bacteriocin$^+$-fed animals, but significant light was detected in the control mice and those fed the bacteriocin$^-$ strain.

S. salivarius strains have also shown promise in controlling *S. pyogenes*-associated pharyngitis[93]. Several studies have reported that bacteriocin-producing probiotics can control microorganisms associated with vaginosis[94], but the specific contribution of *in vivo* bacteriocin production to controlling this condition has yet to be assessed.

The ability of probiotics to produce bacteriocins *in situ* may become an even more attractive trait in the future as culture-independent, next-generation DNA sequencing-based approaches continue to facilitate a more comprehensive analysis of the human microbiota, and the human gut microbiota in particular. These investigations have highlighted the importance of a 'balanced' gut microbiota and have led to unexpected revelations regarding the contribution of microorganisms to many diseases, including coeliac disease, obesity and diabetes[95,96]. The application of probiotic-produced bacteriocins to target the specific bacterial populations that are associated with chronic or acute diseases will allow researchers to definitively establish disease causality. These antimicrobials could also be used to target these as-yet-unculturable pathobionts for therapeutic applications. This theory was the foundation for a recent proof-of-concept study[97] testing the potential of *L. salivarius* str. UCC118 to control weight gain in mice. The authors found that the strain producing ABP118 controlled weight gain more successfully than its isogenic non-producing counterpart in animals fed a high fat diet. However, this effect was transient, and eventually the difference in weight between the two groups of animals decreased to below significant levels[97]. Thus, further investigations are required to identify a probiotic–bacteriocin combination that will contribute to weight management over a longer period. More recently, ABP118-producing and non-producing *L. salivarius* strains have been shown to have different effects on the gut microbiota of non-obese pigs and mice[98], further highlighting the key importance of bacteriocin production with respect to the ability of a probiotic to influence gut microbial populations.

Evidence has also recently emerged regarding the effect of bacteriocins on the immune system. Specifically, two studies[99,100] identified a number of *Lactobacillus plantarum* str. WCFS1 genes, many of which are involved in bacteriocin production or secretion, that seem to influence the immune response by dendritic cells and peripheral blood mononuclear cells, respectively. However, a detailed discussion of these findings is beyond the scope of this Review.

Bacteriocins are amenable to bioengineering. Owing to their peptide nature (that is, because they are directly encoded by genes), bacteriocins are often more amenable to engineering than classical antibiotics. Engineering of bacteriocins can be carried out by bacteriocin gene manipulation, can involve *in vitro* harnessing of the biosynthetic enzymes required for peptide production and/or can rely on partial or complete chemical synthesis of the antimicrobial. In most cases, the engineered peptides have been important for furthering our understanding of the fundamentals of bacteriocin activity and structure–function relationships. However, there is also an increasing number of engineered peptides that exhibit enhanced functionalities (activity and/or stability) which make them more attractive from a clinical perspective.

For example, bioengineered derivatives of the lantibiotics nisin, actagardine and nukacin ISK-1, as well as derivatives of lacticin 481 that have been generated *in vitro*[101], exhibit enhanced specific activity against Gram-positive and/or Gram-negative targets (for a review, see REF. 102); synthetic forms of lactocin S are more stable than their natural counterpart[103]; and nisin–vancomycin hybrids are active against VRE[104]. In the case of thiopeptides, semi-synthetic derivatives have been generated that have increased water solubility, including several nocathiacin derivatives[59] and GE2270 A[105]. An interesting variant of the linear azole-containing peptide MccB17, containng an extra oxazole moiety,

Pathobionts
Microbial components of the gastrointestinal tract that have the potential to cause disease.

Isogenic
Pertaining to a microbial strain derivative: identical to the parental strain except for a defined mutation.

has been isolated and found to be 1.5 times as active as the standard MccB17 variant without the extra moiety[106]. Moreover, three MccC7-C51-like compounds containing a terminal aspartic acid, glutamic acid or leucine have been chemically generated and shown to function in a manner similar to the wild-type, bacterially produced peptide (which contains a terminal aspartic acid and inhibits aspartyl-tRNA synthetase), but to inhibit aspartyl-, glutamyl- or leucyl-tRNA synthetases, respectively, suggesting that new MccC-like peptides that target any one of the 20 tRNA synthetases could be produced[107]. Numerous class IIa derivatives have also been generated. This topic has been recently reviewed[108], and highlights of this work include the production of derivatives with a broadened spectrum, enhanced stability[109,110], increased cell binding[111] or increased trypsin resistance[112].

The development of strategies to engineer bacteriocins has also provided researchers with the means to access the many apparently silent bacteriocin gene clusters that have been identified through the *in silico* inspection of bacterial genomic and metagenomic DNA (for a review, see REF. 113). Such *in silico* approaches have uncovered the existence of a large variety of novel gene clusters potentially encoding uncharacterized lantibiotics[114,115], thiopeptides[116,117], linardins[118], glycocins[119], cyanobacterial bacteriocins[120] and class II bacteriocins[121]. The recent harnessing of lantibiotics encoded within the genomes of *Geobacillus* spp. and *S. pneumoniae* indicate the potential benefits of such strategies[122,123].

Mechanism of action

Bacteriocins have been found to have many distinct mechanisms of action (FIG. 3) that differ from those of antibiotics. These mechanisms can be broadly divided into those that function primarily at the cell envelope and those that are active primarily within the cell, affecting gene expression and protein production.

Cell envelope-associated mechanisms. Nisin and several lantibiotics, in addition to some class II bacteriocins, target lipid II[124,125]. Lipid II is a key intermediate in the peptidoglycan biosynthesis machinery within the bacterial cell envelope and is also the target of the antibiotic vancomycin. Importantly, nisin and other bacteriocins bind lipid II at a site distinct from the vancomycin-binding site and thus retain activity against vancomycin-resistant Gram-positive pathogens[126]. Thus, by targeting lipid II, these molecules inhibit peptidoglycan synthesis, and for some this is the sole mechanism of action. Other lantibiotics can also use lipid II as a docking molecule to facilitate the formation of pores in the cell membrane, resulting in a loss of membrane potential and, ultimately, cell death[124,125].

Other bacteriocins also damage or kill target cells through pore formation in the cell membrane. For example, class IIa peptides and some other class II bacteriocins (such as lactococcin A[127] and microcin E492) bind to the cell envelope-associated mannose phosphotransferase system (Man-PTS), which then leads to pore formation. In the case of the class IIe peptide microcin E492, the bacteriocin is first recognized by FepA, CirA or Fiu (all of which are iron siderophore receptors) in the outer membrane and then passes through this membrane, via a mechanism that depends on the outer-membrane receptor TonB[128], en route to forming pores in the inner membrane. It is also thought that the proteusin polytheonamide A functions through a mechanism that involves pore formation, but precisely how this occurs and whether a receptor is involved have yet to be elucidated[25].

A smaller subset of lantibiotics, such as cinnamycin and related peptides, function by binding phosphatidylethanolamine in cell membranes and, in turn, inhibiting the enzyme phospholipase A2 (REF. 129). Finally, large-conductance mechanosensitive channel (MscL) is crucial for sublancin 168 activity, but it is not known whether this protein serves as a direct target for the glycocin or as a gate of entry to the cytoplasm[130].

Inhibition of gene expression and protein production. Bacteriocins can kill their target cells by interfering with DNA, RNA and protein metabolism. For example, MccB17 passes through the outer membrane via the porin OmpF and is transferred across the inner membrane in a manner that is dependent on SbmA (an inner-membrane peptide transporter). The bacteriocin then functions by inhibiting DNA gyrase-mediated DNA supercoiling, thereby interfering with DNA replication[131].

The lasso bacteriocin MccJ25 is recognized by the iron siderophore receptor FhuA at the outer membrane and requires TonB and SbmA at the inner membrane to enter the cell. After entering the cell, MccJ25 inhibits transcription by blocking the secondary channel of RNA polymerase [132]. In the case of MccC7-C51, passage through the inner layer of the *E. coli* cell wall occurs via the YejABEF transporter[133], after which the bacteriocin is processed by one of the many broad-specificity cytoplasmic aminopeptidases of the bacterium[134] to generate a modified aspartyl-adenylate[135]. This, in turn, inhibits aspartyl-tRNA synthetase, thus blocking mRNA synthesis.

Nocathiacins, thiostrepton, thiazomycin and several other thiopeptides target the bacterial ribosome, binding the 23s rRNA of the 50S ribosomal subunit[136]. Bottromycins function by blocking aminoacyl-tRNA binding to the 50S ribosome[26]. Other thiopeptides, such as GE2270 A, bind the bacterial chaperone elongation factor Tu (EF-Tu) to inhibit protein synthesis[136].

Resistance

For any antimicrobial under investigation with a view to clinical applications, the potential emergence of resistant pathogens is an issue that must be addressed. Bacteriocins have not been extensively used in a clinical setting, so our understanding of this potential threat for these antimicrobials has been revealed primarily through laboratory-based research.

Possible resistance mechanisms have been identified for those bacteriocins that function primarily by targeting the cell envelope. For example, studies indicate that enhanced resistance to lipid II-targeting lantibiotics

Siderophore
A low-molecular-mass compound that binds ferric iron extracellularly to form a stable chelate for transport of iron into the cell.

Porin
A large protein that crosses a cellular membrane and acts as a pore through which molecules can diffuse.

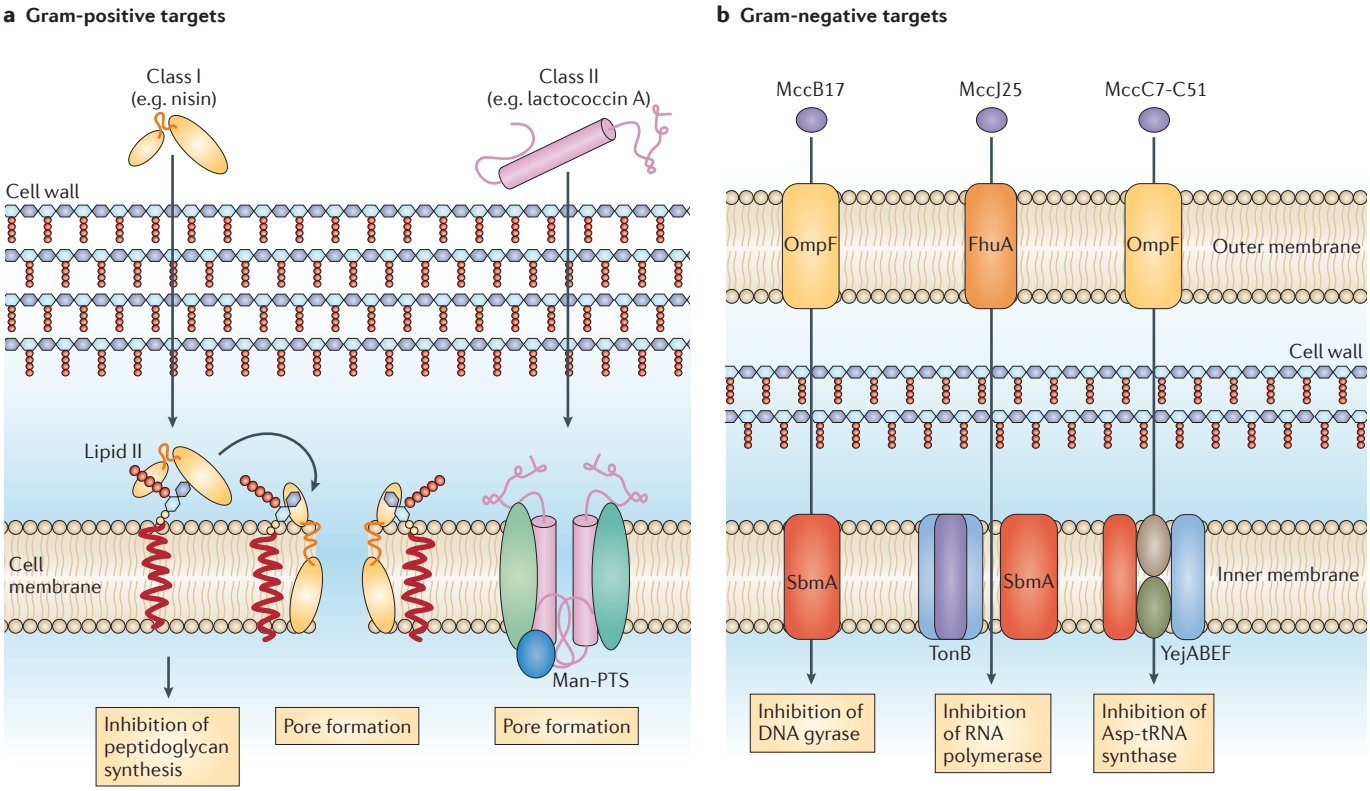

Figure 3 | **Mechanism of action of representative bacteriocins. a** | Some bacteriocins, and in particular many of those that inhibit Gram-positive bacteria, function by targeting the cell envelope. Some class I bacteriocins inhibit lipid II on the cell membrane, thereby abrogating peptidoglycan synthesis. Other bacteriocins form pores to inhibit or kill their target bacterium. For example, class II bacteriocins such as lactococcin A bind to the pore-forming receptor mannose phosphotransferase system (Man-PTS). Nisin and some other class I bacteriocins both inhibit peptidoglycan synthesis and form pores. Other class I peptides, such as the thiopeptides and bottromycins, control Gram-positive bacteria by targeting translation (not shown). **b** | Many bacteriocins that inhibit Gram-negative bacteria (and thus need to be transported through the outer and, in many cases, inner membranes before functioning) control their target bacteria by interfering with DNA, RNA and protein metabolism. For example, microcin B17 (MccB17) inhibits DNA gyrase, MccJ25 inhibits RNA polymerase, and MccC7-C51 inhibits aspartyl-tRNA synthatase. There are also exceptions, such as MccE492, that function through pore formation.

could emerge as a consequence of reduced accessibility to the receptor (as is the case in *S. aureus* with intermediate resistance to vancomycin[126]) or other changes in cell envelope composition[33,137,138]. A reduction in or loss of expression of cell envelope-associated receptors might also be an issue in the clinic and has been particularly notable in the laboratory in strains exhibiting resistance to Man-PTS-targeting class II bacteriocins[139]. It should be noted that there is a second category of resistant mutants in which the Man-PTS system is expressed normally, but the underlying mechanism of resistance to Man-PTS-targeting class II bacteriocins has yet to be determined for these mutants[139].

Research has also revealed that resistance to bacteriocins that have intracellular targets could arise through mutations in the genes encoding the bacteriocin targets. Specifically, cells become resistant to MccJ25 owing to certain mutations in the RNA polymerase subunit genes (altering the secondary channel of the polymerase)[140], to MccB17 as a consequence of point mutations in the DNA gyrase-encoding gene[141], and to ribosome-targeting thiopeptides because of mutations in the genes encoding ribosomal protein L11 or the GTPase-associated region of the bacterial ribosome[142].

Another potential concern that could limit the deployment of bacteriocins in clinical practice is immune mimicry. This term is used to describe the resistance that occurs in non-bacteriocin-producing strains which possess bacteriocin immunity genes, or immunity as a consequence of producing a closely related bacteriocin[143]. Proteolytic cleavage of bacteriocins is another potential route through which resistance could occur. This phenomenon has been observed in lactococci that are resistant to nisin[144] and in bacteria that are protected against MccC7-C51 as a consequence of containing MccC7-C51 self-immunity protein (MccF) or orthologous serine peptidases[145]. It has also been speculated that the proteolytic activity of YqeZ could be responsible for the protection provided by the *yqeZyqfAB* operon against sublancin 168 in bacilli[146].

These observations remind us of the need for vigilance when deploying such potent bacterial inhibitors. In some cases, bacteriocin resistance arises at a sufficiently low rate to allow commercialization of the peptide in its

natural form. In other cases, knowledge of the potential resistance mechanisms, such as those described above, could be crucial for minimizing the emergence of resistance when clinical applications commence. Potential solutions include the further derivatization of bacteriocins to create compounds that can bind receptors even in bacteria in which receptor gene mutations have occurred, the modification of peptides to reduce their sensitivity to proteases[147] or the use of bacteriocins in combination with other bacteriocins or antimicrobials with distinct mechanisms of action.

Conclusions

Many bacteriocins have properties which suggest that they could be of value in clinical settings. However, to date, the primary focus for use of these bacteriocins has been on animal, rather than human, health. Existing commercial examples include the use of thiostrepton in combination therapy ointments to treat dermatological indications in domestic animals and the use of nisin as the active agent in the mastitis prevention product Wipe Out (ImmuCell Corporation).

A lack of sufficient investment has been a significant problem with respect to the medical application of bacteriocins. Notably, however, there is some evidence to suggest that this issue is finally being addressed, as several bacteriocins are now being developed with a view to human applications. These bacteriocins include many thiopeptides, such as LFF571 (derived from GE2270 A), as well as Mu1140-S (a synthetic form of the lantibiotic mutacin 1140) (Oragenics) and NVB302 (a semi-synthetic derivative of the lantibiotic deoxyactagardine B)[148]. In addition to these synthetic bacteriocins, the natural producer of mutacin 1140 has been engineered for use in preventing tooth decay (SMaRT Replacement Therapy). There have also been efforts to scale up the production of the lantibiotic lancovutide with a view to treating non-infectious indications, including cystic fibrosis and dry eye syndrome (in trials with AOP Orphan Pharmaceuticals), and microbisporicin is being developed as an injectable therapy to control multidrug-resistant Gram-positive pathogens[149]. It is also important to note that several commercially produced probiotics synthesize bacteriocins. Although often the bacteriocins in question are uncharacterized and their activity spectra are unknown, there are exceptions to this rule, such as the bacteriocins produced by the BLIS K12 probiotics used in oral hygiene products.

Ultimately, the clinical application of bacteriocins will depend on our understanding of their mechanisms of action and on the development of strategies to prevent or curtail resistance in the future. The production of engineered bacteriocins with value-added properties continues to provide considerable cause for optimism, but this will need to be matched by the development of processes that allow these peptides to be produced at a sufficient scale and quality and, crucially, by further investments in clinical trials to determine or substantiate *in vivo* efficacy. Nonetheless, given the vast array of different bacteriocins available, their diverse structures and mechanisms of action and, importantly, the adaptability of the peptides and of their cognate biosynthetic machinery with respect to peptide engineering, it is surely a question not of 'if', but rather 'when' these peptides will have widespread use in clinical settings.

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