Review

Bacteriocins and their position in the next wave of conventional antibiotics

Veronica L. Caver a, Timothy D. Arthur a, Dimitri Kashtanov b, Michael L. Chikindas b,∗

a Department of Biochemistry and Microbiology, Rutgers State University, 76 Lipman Drive, New Brunswick, NJ 08901, USA
b School of Environmental and Biological Sciences, Rutgers State University, 65 Dudley Road, New Brunswick, NJ 08901, USA

A R T I C L E   I N F O
Article history:
Received 23 February 2015
Accepted 15 July 2015

Keywords:
Bacteriocins
Antimicrobial peptides
Antibiotic targets
Synergy
Future medicine

A B S T R A C T
Micro-organisms are capable of producing a range of defence mechanisms, including antibiotics, bacteriocins, lytic agents, protein exotoxins, etc. Such mechanisms have been identified in nearly 99% of studied bacteria. The multiplicity and diversity of bacteriocins and the resultant effects of their interactions with targeted bacteria on microbial ecology has been thoroughly studied and remains an area of investigation attracting many researchers. However, the incorporation of bacteriocins into drug delivery systems used in conjunction with, or as potential alternatives to, conventional antibiotics is only a recent, although rapidly expanding, field. The extensive array of bacteriocins positions them as one of the most promising options in the next wave of antibiotics. The goal of this review was to explore bacteriocins as novel antimicrobials, alone and in combination with established antibiotics, and thus position them as a potential tool for addressing the current antibiotic crisis.

© 2015 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.

1. Introduction

The overwhelming increase in antibiotic resistance is presently recognised as a global crisis and as such requires the immediate attention of the pharmaceutical industry, academia and government institutions [1]. The increasing rate of bacterial resistance and the inability to discern mechanisms of inhibiting them impedes the rate of antibiotic discovery. Antibiotic resistance is not new; it is a phenomenon that has been documented since the discovery of penicillin [2,3]. The current wave of resistance is problematic because the rate at which resistance is occurring is equivalent to the ubiquity of resistant pathogens. For more information on resistance mechanisms, the authors suggest the review by Cotter et al. [4]. Furthermore, there are additional complications of antibiotic overuse such as killing of healthy microbiota and environmental contamination that cause immediate and prolonged ecological issues.

Both novel substances and innovative methods are constantly being evaluated to address the rapid spread and development of drug-resistant infections in nosocomial settings. Although the next step is uncertain, it is clear that a viable alternative is necessary to ensure an efficacious paradigm shift that can stymie the epidemic of resistance. The dimensions of the antibiotic crisis have been discussed in reviews [1,5]. These articles deftly describe the dimensions that have led to this crisis but do not offer substantive information into alternatives.

Antimicrobial peptides have been in the forefront of antibiotic alternative research for decades, but their usefulness has failed to be substantively explored. In particular, bacteriocins, antimicrobial peptides of bacterial origin, are positioned as potentially significant contributors to the paradigm shift owing to the wide variety of commercially available formats. In addition, many groups are actively researching and developing existing and novel bacteriocins and bacteriocin-like substances.

This review aimed to position bacteriocins as possible alternatives to conventional antibiotics or, perhaps, as novel nature-derived stressors that can be used in formulations with synergistically acting antibiotics as complementary agents. The latter approach is in agreement with the strategy proposed by the National Center for Complementary and Integrative Health of the National Institutes of Health (NCCIH NIH).

2. A brief introduction and history of bacteriocins

Bacteriocins were first identified in 1925 and are defined as ribosomally synthesised, proteinaceous substances that inhibit the growth of closely related species through numerous mechanisms [6,7]. Production of these proteins is widespread among bacterial species and it is suggested that virtually all bacterial
species synthesise bacteriocins [8,9]. Such production is made possible by relatively simple biosynthetic machineries that are often associated with elements such as plasmids and conjugative transposons [10,11]. This process is further simplified by the fact that associated genes are often clustered on plasmids, chromosomes or transposable elements. This ubiquity posits bacteriocins as highly appealing.

There have been multiple classifications for bacteriocins. This controversy has led to such divisions as ‘true bacteriocins’ such as colicins, and those more recently discovered from *Lactobacillus* spp. [12] and other lactic acid bacteria (LAB). Whilst colicins are group structured [13], bacteriocins from LAB have undergone several classifications from being placed into four groups [14] to more recent groupings. There are even more subclassifications based specifically on the taxonomy of the producer micro-organism, such as those synthesised by enterococci [15]. The classification system used in this review divides bacteriocins by modification and size; bacteriocins of Gram-positive micro-organisms, such as those produced by LAB, identified as class I, undergo post-translational modifications, whilst class II undergo either no or minimal modifications. In addition, bacteriocins >10kDa are parsed into a third class [12]. Bacteriocins from Gram-negative bacteria are divided [16,17] into small peptides, such as microcins, and large peptides, such as colicins [18–20]. Further subdivisions exist within these broader categories, including instances of homology in motifs [21].

Currently, there is extensive research performed on bacteriocins, especially as the US Food and Drug Administration (FDA) regulates their usage as a food preservative. As of 2012, 62 genera encompassing 195 bacterial species are considered as microbial food cultures with a history of safe use for fermentation purposes [22]. Given this considerable endeavour, various targets and efficacies have been determined, further strengthening their position in the next wave of therapies.

### 3. Bacteriocins utilise some of the conventional drug targets

Current methods of identifying novel antibiotics generally fall into one of two categories, synthetic chemical efforts or isolation of new natural products. Examples of more recent synthetic chemical efforts include high-throughput screening of chemical libraries and targeted structure-guided experiments [23,24]. In addition, there are groups devoted to isolating and screening various natural and nature-derived sources.

Conventional antibiotics fall into five major categories with respect to their targets. These targets include: (i) bacterial peptidoglycan/cell wall disruption; (ii) protein biosynthesis; (iii) folate biosynthesis; (iv) DNA replication and transcription; and (v) disruption of the bacterial membrane [17,25–30].

These are considered the major clinically validated antibacterial targets. Bacteriocins are capable of inhibition of four of these pathways as well as some novel pathways. To illustrate the versatility of bacteriocin targets, we have expanded the conventional list of targets. Fig. 1 displays targets of both antibiotics and bacteriocins, their general location, and examples of each capable of inhibition of these targets.

Bacteriocins can inhibit closely related bacterial species, spores and have even shown instances of fungicidal activity [9,31]. In comparison, antibiotics have been generally regarded as more broad-spectrum with numerous side effects [1]. The side effects and increased incidence of bacteriocin resistance are two topics that require further research. Bacteriocins are effective against four of the aforementioned clinically relevant antibiotic targets. Some bacteriocins have been studied in vivo and were successful in inhibiting the targeted pathogens (for review see [27]). These findings support the necessity for further clinical investigation.

### 3.1. Inhibition of cell wall biosynthesis

Antibiotics that target cell wall biosynthesis include those in the β-lactam group. A recent decrease in β-lactam efficacy against common nosocomial infections has prompted the need for viable alternatives. Pathogens have developed β-lactam-degrading enzymes such as carbapenemases and penicillinases [32]. In light of this trend, there is great potential for the integration of bacteriocins, specifically lantibiotics, into new therapies. Despite this potential, the prevalence of lantibiotic resistance can increase when organisms are exposed to subinhibitory levels of lantibiotics for extended periods of time [33,34]. The cell wall is widely regarded as an excellent target for the development of novel technologies as its synthesis is highly conserved across pathogens and is absent from mammalian cells [34,35]. Furthermore, the cell wall is critical to overall bacterial survival in that it regulates cellular integrity and morphology, particularly in cases of internal osmotic pressure fluctuations. Therefore, prevention of cell wall biosynthesis is a critical target [35].

Current studies in *Escherichia coli* and *Bacillus subtilis* have indicated MreB, a bacterial actin homologue, as critical for maintenance of shape, and penicillin-binding proteins (PBPs) as enzymatic regulators. MreB rotates for maximum uniform distribution of peptidoglycan insertion sites, and subsequent motion is dependent on the availability of these subunits [36]. PBPs, particularly PBP2, are responsible for covalent cross-linking of glycan strands during growth [37]. Current antibiotics target cell wall synthesis at four different stages of peptidoglycan development: (i) inhibition of the synthesis of lipid II; (ii) inhibition of the undecaprenyl carrier lipid; (iii) binding of lipid II; and (iv) binding and blocking of the active sites of PBPs [3]. Some examples of antibiotics that target these sites include penicillins, glycopeptides, carbapenems, monobactams and cephalosporins [30]. The coupling of MreB motion and PBP2 regulation appears highly conserved among bacterial species [36–38]. Further analysis of how bacteriocins can affect MreB and/or PBP2 could prove extremely beneficial given this high level of conservation.

Nisin A, produced by *Lactococcus lactis*, one of the most frequently referenced bacteriocins, possesses multiple modes of action. This lantibiotic docks to lipid II, a membrane-bound precursor of the cell wall, and inhibits cell wall synthesis. In addition, following lipid II docking, pore formation by nisin molecules arranged as pore-forming ‘units’ can be induced, which rapidly kills cells. At high quantities, this process can be divided into two stages, with the first being bacteriostatic and the second bactericidal [3,33,38]. Nisin has also been found to act as a lytic agent [39]. Nisin is known to effectively inhibit numerous Gram-positive bacteria, leading to its usage in the food industry [40–42]. Similarly, nukacin ISK-1, produced by *Staphylococcus warneri*, inhibits cell wall synthesis by binding lipid II but it has not been shown to induce pore formation [43–45]. This bacteriocin has been shown to be potent for the treatment of meticillin-resistant *Staphylococcus aureus* (MRSA) biofilms [43]. It has been found that ring A is responsible for binding lipid II [45]. *Microbispora* sp. strain ATCC-PTA-5024 produces the lantibiotic NAI-107, which also binds to lipid II leading to inhibition of vancomycin-resistant enterococci and MRSA [46,47]. Other lantibiotics of interest include lactacin 481, a tricyclic lantibiotic that contains a lipid II-binding motif but inhibits PBP1b-catalysed peptidoglycan formation [48,49].

The aforementioned bacteriocins show great promise in preventing cell wall biosynthesis by binding lipid II. Future research may be directed towards reconstruction studies in which there is a better understanding of the molecular mechanism of development and the linkage of MreB, PBPs and lipid II. Understanding how these interact could potentially indicate novel antimicrobial targets.
3.2. Inhibitory and destructive effects on DNA structure

During DNA replication, positive supercoil relaxation results in a superhelical tension that facilitates the movement of polymerases down the open frame. This is an ideal and safe target because there are differences in the structure of DNA gyrase between eukaryotic and prokaryotic organisms [17]. Bacterial DNA gyrase is targeted through competitive inhibition of the ATPase active site on the GyrB subunit and by binding and preventing decatenation of replicating DNA. Quinolones represent a large group of antibiotics that target DNA gyrase.

Microcin B17, originally isolated from E. coli, has been found to have a decatenation mechanism of action [17]. Its structural features position it as potentially useful in DNA gyrase inhibition but it is currently not suitable for human usage. Microcin B17 represents an important mechanism of action that, using the motif data, could help in the design of engineered bacteriocins (the importance of which is discussed later in this review).

3.3. Inhibition of protein synthesis

Protein production is another critical cellular process frequently targeted by antibiotics. Often, protein synthesis is inhibited at the formation of the 30S initiation complex, the 70S ribosomal subunit or during the elongation process [50,51]. Inhibition that at each of these specific stages leads to a shortened or malformed protein and eventual cellular death. Antibiotics that target this include lincosamides such as clindamycin and lincomycin [52] as well as aminoglycosides such as amikacin and streptomycin. These antibiotics act by binding to the bacterial 30S ribosomal subunit, which inhibits translocation of the peptidyl-tRNA from the A site to the P site. This induces a misreading of mRNA and cells incapable of synthesising proteins [52].

Specifically, colicins E3, E4 and E6 and cloacin DF13 show 16S rRNase activity [54,55]. DNase and RNase mechanisms of action have been identified in the colicin family (E2, E7, E8 and E9; and D, E3, E4, E5, E6 and cloacin DF13, respectively) [19,54–58]. Members of the E-group endonuclease colicins have the H–N–H motif and bind the BtuB/Tol translocation machinery in order to cross the outer membrane [56,57]. These bacteriocins cleave the 16S rRNA at the 3′-end of the coding sequence, which inhibits translation [18,54,57]. It does so by accumulation of sequential impaired decoding events that result in low occupancy at the A site and inability to elongate the peptide past the first few codons. In short, it cleaves stop codons into the A site. It decreases the stability of the codon recognition complex, slowing aminoacyl–tRNA accommodation at the A site [18,54].

Similar to the RNase colicins are the tRNases (colicin D and E5), which act by accelerating the exhaustion of tRNA in the cytoplasmic pool and limiting protein synthesis [20]. Ogawa et al. demonstrated that tRNA<sup>35</sup>, tRNA<sup>36</sup>, tRNA<sup>37</sup> and tRNA<sup>38</sup> molecules are digested by colicin D and E5 in the susceptibility order of tRNA<sup>35</sup> > tRNA<sup>36</sup> > tRNA<sup>37</sup> > tRNA<sup>38</sup> [20]. Colicin D has also been shown to have a similar ribonuclease activity to that of E3 [59,60].

Outside of the colicin family, the bacteriocin carocin S2, produced by Pectobacterium carotovorum subsp. carotovorum, has been shown to have a ribonuclease mode of action against P. carotovorum SP33 [61].

3.4. Disruption of bacterial membrane integrity

Membrane-targeted antibiotics include β-lactams that disrupt the synthesis of the peptidoglycan layer of bacterial cell membranes. These antibiotics have been in use for decades and are the most susceptible to resistance. Various mutations of PBPs allow pathogens to evade β-lactam antibiotics.

A number of antimicrobial proteins use lipid II as a docking site, one of the classic examples being nisin. Other bacteriocins that dock to lipid II include Bac-GM17, PlnE/F, PlnJ/K, PepS, epidermin and geobacillin I. Bac-GM17 is produced by Bacillus clausii strain GM17 and is found to be both heat- and pH-stable (between pH 3 and pH 9). It was noted as having a bactericidal mode of action...
against numerous Gram-positive and Gram-negative bacteria as well as a fungistatic mode of action against *Candida tropicalis* K2 CIP203 [49]. Pep5, produced by *Staphylococcus epidermidis* 5, and epidermin, produced by *S. epidermidis* Tu3298, inhibit the growth of *S. epidermidis* on silicon catheters [62,63]. Geobacillin I, produced by *Geobacillus thermoleovorans* NC80-2, is structurally and functionally similar to nisin [25]. Four plantaricins (Pn I, F, J and K) have been identified as having anti-*Candida* activity [64].

Not all bacteriocins that cause membrane damage bind to lipid II. Dysgalactacin, produced by *Streptococcus dysgalactiae* subspp. *equi-similis* strain W2580, binds to membrane-bound glucose and/or the mannose phosphotransferase system (man-PTS). Once dysgalactacin has docked to the man-PTS it disrupts the cytoplasmic membrane by causing an efflux of potassium ions (K⁺), which dissipates the membrane potential as seen in *Streptococcus pyogenes* [65]. Lactococcin A and B and some *Listeria*-active pediocin-like bacteriocins similarly bind man-PTS [66]. Currently, one of the exact targets of man-PTS has been identified as Elh<sup>Man</sup> for *Listeria monocyotogenes* inhibition by mesentericin Y105 [67]. Further research is necessary to identify the exact targets of man-PTS. The staphylococcin aureocin A53 causes significant carboxyfluorescein leakage from acidic liposomes at high concentrations [68,69]. This damage does appear to result from a specific pore formation. Similarly, aureocin A70 also causes non-specific membrane permeabilisation and has been found to be active against *L. monocytogenes* [68,70].

Bacteriocins that act on the targeted cells by forming pores in their membranes do not always dock to either lipid II or man-PTS. Lactocin Q, produced by *L. lactis* QU 5, forms toroidal pores due to lipid flip-flop, which causes protein leakage and cell death without a specific receptor [71–74]. It has variable degrees of activity among Gram-positive bacteria, which is also dependent on the accumulation of hydroxyl radicals [71]. Furthermore, lactocin Q shows selectivity in inhibition for Gram-positive bacteria but not for Gram-negative bacteria owing to physiochemical differences in the outer membrane [73]. Enterocin AS–48 interacts with the targeted membrane, most likely by utilising its ability to transit from water-soluble to a membrane-bound state that occurs upon the antimicrobial protein’s interaction with the bacterial membrane [75]. The proposed model is described in a recent review [76]. Carnocyclin A, a 60-amino acid circular bacteriocin from *Carnobacterium maltaromaticum* UAL307, is another lipid II-independent bacteriocin capable of direct interaction with the lipid bilayer, causing formation of ion-specific pores [77].

3.5. The folate biosynthesis pathway represents a mechanism of action not currently targeted by bacteriocins

There are multiple targets within the folate synthesis pathway, including dihydrofolate reductase (DHFR), a precursor shared between eukaryotic and prokaryotic organisms, as well as dihydropterote synthase (DHPS), an enzyme unique to prokaryotic organisms [78]. Presently known inhibitors of these two targets interact with enzymatic neighbours in the folate pathway and include sulphonamides [78]. Currently there are no characterised bacteriocins capable of inhibiting bacteria using either of these modes of actions. Given this, further research into bacteriocins capable of inhibiting this pathway with similar properties to DHFR or DHPS could provide a valuable avenue of research.

4. A novel target: septum formation utilised by garvicin A and lactococcin 972

Whilst it is significant that bacteriocins hit four of the classic drug targets, it is important to recognise that targets using novel systems may be a more effective method of treating infections. Bacteriocins aim at one such novel target in the inhibition of septum formation.

SeptA consists of an in-growth of the cytoplasmic membrane and the mucoprotein layer during the final stages of mitosis. Addition of antibiotics during cytophisis results in the development of a bulge and inhibition of the cell cycle [3]. Two bacteriocins, garvicin A and lactococcin 972, have thus far been shown to have this mechanism of action [79,80]. Garvicin A is specifically active against other *Lactococcus garvieae* strains, whilst lactococcin 972 inhibits only closely related *Lactococcus* spp. The mechanism of action in lactococcin 972 is through the blocking of septum invaginations, which results in cellular elongation and widening [80].

Although the target is similar to cell wall formation inhibition, lactococcin 927 uses different target machinery and mechanisms of action positioning it a separate group of particular interest. It is important that other proteins, such as the FtsZ, FtsA and ZipI rings, be considered as new and potential targets for these bacteriocins [81].

Table 1 lists the mentioned bacteriocins in this review along with appropriate references, their targets and the bacteria that produce them.

As of this review, there are no documented natural FtsZ-inhibiting antibiotics, however other small-molecule antimicrobials are being investigated for FtsZ inhibition [82]. These molecules exclusively inhibit FtsZ and subsequent formation of the bacterial cell wall.

Bacteriocins have been heavily evaluated as efficacious at four of the conventional antibiotic targets, usually in vitro systems. It is critical to note that bacteriocins are capable of addressing these routes through distinct mechanisms of action that differ from antibiotics. Septum formation and other associated distinctive targets of cell membrane formation are of particular interest [83]. Other internal targets, such as gene expression and protein production, are also of interest. Evaluation is necessary to identify the interactivity of the mechanisms of action of these peptides in combination as well as to engineer them in order to improve the spectrum of action and stability and to overcome other obstacles that decrease their efficacy in human systems.


One way to address novel targets and to improve the efficacy and stability of current bacteriocins is through their manipulation. Some bacteriocins that have been engineered include microcin B17, geobacilcin I, nisin, lactacin 3147, enterocin ES-52 and pediocin PA-1.

Microcin B17 acts as a DNA gyrase inhibitor. It was found that the polyglycine in the N-terminus is used as an anchor for microcin synthase, and the C-terminal Ser-His-Ile contribute to critical stabilisation interactions in the bacteriocin [17]. The derivative Mcc[Gly46-Ile69] showed similar cleavage activity and increased stability compared with the parental molecule.

A geobacilcin I engineered derivative carrying an AsnValAla linker as a replacement of the molecule’s Leu19 and serving as a bridge between the C and D rings of the molecule has an eight times higher minimum inhibitory concentration (MIC) than the parental molecule. It is further postulated that stability could be improved through mutation of DhaS5 based on the nisin analogue I4K/DhaS5/F6L, which had increased stability and antimicrobial activity [25,84]. The resulting MIC increase in the mentioned study is indicative that more research is necessary to investigate the region specificity of bacteriocins for improved efficiency.

The ‘popularity’ of nisin among investigators has led to the generation of numerous mutations in the FNDL box, the ring positions
<table>
<thead>
<tr>
<th>Bacteriocin ID</th>
<th>Target</th>
<th>Mechanism of action</th>
<th>Primary producer(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nisin A</td>
<td>Lipid II/pore formation</td>
<td>Inhibition of cell wall formation</td>
<td><em>Lactococcus lactis</em></td>
<td>[3,33,40]</td>
</tr>
<tr>
<td>Microcin B17</td>
<td>DNA gyrase</td>
<td>Prevents DNA decatenation</td>
<td><em>Escherichia coli</em></td>
<td>[17]</td>
</tr>
<tr>
<td>Colicin family</td>
<td>DNA and RNA</td>
<td>DNase and RNase</td>
<td><em>E. coli</em></td>
<td>[20,59,60]</td>
</tr>
<tr>
<td>Geobacillin I</td>
<td>Lipid II</td>
<td>Membrane disruption</td>
<td><em>Geobacillus thermotolerans</em></td>
<td>[25]</td>
</tr>
<tr>
<td>Nukacin ISK-1</td>
<td>Lipid II</td>
<td>Inhibition of cell wall formation</td>
<td><em>Staphylococcus warneri</em></td>
<td>[43–45]</td>
</tr>
<tr>
<td>NAI-107</td>
<td>Lipid II</td>
<td>Inhibition of cell wall formation</td>
<td><em>Microbacterium sp. strain</em></td>
<td>[46,47]</td>
</tr>
<tr>
<td>Bac-GM17</td>
<td>Lipid II</td>
<td>Membrane disruption</td>
<td><em>Bacillus clausii GM17</em></td>
<td>[50]</td>
</tr>
<tr>
<td>Carocin S2</td>
<td>RNA</td>
<td>RNase</td>
<td><em>Pectobacterium carotovorum</em></td>
<td>[61]</td>
</tr>
<tr>
<td>Pin E, F, J and K</td>
<td>Lipid II</td>
<td>Membrane disruption</td>
<td><em>Lactobacillus plantarum</em></td>
<td>[64]</td>
</tr>
<tr>
<td>Dysgalactacin</td>
<td>Manno- phosphotransferase system (man-PTS)</td>
<td>Membrane disruption</td>
<td><em>Streptococcus dysgalactiae subsp. equisimilis</em></td>
<td>[65]</td>
</tr>
<tr>
<td>Lactococcus A, B</td>
<td>man-PTS</td>
<td>Membrane disruption</td>
<td><em>L. lactis</em> subsp.</td>
<td>[66]</td>
</tr>
<tr>
<td>Pediocin-like bacteriocins</td>
<td>man-PTS</td>
<td>Membrane disruption</td>
<td>Unnamed producer(s)</td>
<td>[66]</td>
</tr>
<tr>
<td>Mesentericin Y105</td>
<td>man-PTS</td>
<td>Membrane disruption</td>
<td><em>Leuconostoc mesenteroides Y105</em></td>
<td>[67]</td>
</tr>
<tr>
<td>Lactocin Q</td>
<td>Cellular membrane</td>
<td>Toroidal pore formation, protein leakage</td>
<td><em>L. lactis</em> Q5</td>
<td>[71–74]</td>
</tr>
<tr>
<td>Garvicin A</td>
<td>Not determined</td>
<td>Inhibition of septum formation</td>
<td><em>Lactococcus garvieae</em></td>
<td>[78]</td>
</tr>
<tr>
<td>Lactocin 972</td>
<td>Not determined</td>
<td>Inhibition of septum formation</td>
<td><em>L. lactis</em></td>
<td>[79]</td>
</tr>
<tr>
<td>Subtilosin A</td>
<td>Cellular membrane</td>
<td>Pore formation</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>[91–93]</td>
</tr>
<tr>
<td>Lactocin 481</td>
<td>Lipid II</td>
<td>Inhibition of cell wall formation</td>
<td><em>L. lactis</em></td>
<td>[48,49]</td>
</tr>
<tr>
<td>Cloacin DF13</td>
<td>16S subunit</td>
<td>RNase</td>
<td><em>E. coli</em></td>
<td>[54–58]</td>
</tr>
<tr>
<td>Aureocin A53</td>
<td>Cellular membrane</td>
<td>Membrane destruction</td>
<td><em>Staphylococcus aureus</em></td>
<td>[68,69]</td>
</tr>
<tr>
<td>Aureocin A70</td>
<td>Cellular membrane</td>
<td>Membrane destruction</td>
<td><em>S. aureus</em></td>
<td>[68,70]</td>
</tr>
<tr>
<td>Pep5</td>
<td>Lantibiotic</td>
<td>Pore former</td>
<td><em>Staphylococcus epidermidis</em></td>
<td>[62]</td>
</tr>
<tr>
<td>Epidermin</td>
<td>Lantibiotic</td>
<td>Pore former</td>
<td><em>S. epidermidis</em> Tu3298</td>
<td>[62,63]</td>
</tr>
</tbody>
</table>

and the hinge regions. It has been found that mutations D-19A, F-18H, F-18M, L-16D, L-16K and L-16A enhance the production of nisin [85]. The N-terminal thioester ring positions have been randomised and removed in combinations [84]. It has been found that mutating ring A results in increased activity, removal of ring D and E results in the inability to make cell pores, and the opening of ring B eliminates antimicrobial activity while retaining autoinducer activity [80]. In hinge experiments, N20/M21P and M21P/K22 mutants were still capable of binding lipid II, however they were unable to form cellular pores [29]. In addition, the mutants retained potent antimicrobial activity against vegetative *Bacillus anthracis* cells but not spores [40]. Mutants with hinge regions of AAK, NAI and SLS have also been produced and have increased antimicrobial activity against *L. lactis, Streptococcus agalactiae, Mycobacterium smegmatis* MC2155 and *S. aureus* RF122 [86]. Another study evaluating nisin V, a Met21Val mutant of nisin A, demonstrated enhanced antilisterial activity in mouse models [87]. The nisin V mutant exhibited a 1.1 log reduction compared with nisin A in the treatment of a bioluminescent *L. monocytogenes* mutant [87].

Lactocin 3147 is a two-peptide lantibiotic with at least 14 derivatives that contain different combinations of specific subsets of genes [88]. Additional copies of genes that encode for the biosynthetic/production machinery and the regulator LtnR result in high-level overproduction, whilst additional copies of structural genes, such as *ltnAIA2*, result in reduced production of lactocin 3147.

Hybridisation of bacteriocins has also indicated increased efficacy in the case of eneterocin E50/pediocin PA-1 and pediocin PA-1/eneterocin E50-52 in that MICs were reduced by 32-fold and 64-fold, respectively, compared with their natural counterparts, as tested against various Gram-positive and Gram-negative bacteria [89].

These different strategies represent potential methods of manipulating bacteriocins to improve their overall efficacy and stability. Such strategies are being more readily applied and represent means of addressing current shortcomings in bacteriocin applications.

6. **Bacteriocin synergy in combination with other bacteriocins and conventional antibiotics**

Bacteriocins possess numerous potentials for therapeutic applications, not only as alternatives but also as synergists to antibiotics. There are several scenarios in which antibiotic–bacteriocin synergy could act to enhance current insufficient infection therapies [90]. The bacteriocin subtilosin A has been shown to be a potent synergist with clindamycin, metronidazole, lauramide arginine ethyl ester (LAE) and polycline against the bacterial vaginosis–associated pathogen *Gardnerella vaginalis* [91–93]. Furthermore, it has been shown that the two-peptide lactacin 3147 synergistically interacts with polymyxin to inhibit *Cronobacter* spp. and *E. coli* [28].
Bacteriocins have been identified as capable of synergy with other antibiotics. As previously stated, the four plantaricins (Pln E, F, J and K) have anti-\textit{Candida} activity [64]. The combinations of PlnJ/K and PlnEF were most effective at inhibiting \textit{Candida albicans} [64]. This increased efficacy positions these two combinations as potential alternatives to currently available antibiotic combinations. Similarly, it has been found that lactacin Q when combined with nisin could overcome certain hurdles, such as the inactivation of nisin at alkaline pH values, while improving overall efficacy since these two bacteriocins are in the same category [74].

Treatments that take advantage of synergistic relationships between conventional antibiotics and bacteriocins have shown a lot of promise. There are almost limitless combinations, so the field is still in its infancy. Combinatory treatments can be tailored to target specific sites or pathways to improve and/or broaden efficacy [94]. A combination of two lipid-binding agents, nisin and ramoplanin, displayed two-way synergy against 14 of 20 tested MRSA isolates [95]. Bacteriocins have also shown the ability to reduce MICs against initially non-sensitive pathogens when combined with the appropriate antibiotic [28]. In addition, disinfectants that are commonly used in oral care have shown strong synergy with a newly isolated bacteriocin, PsVP-10, against oral isolates [96]. However, bacteriocin–antibiotic interactions can be antagonistic and completely diminish the efficacy. For example, nisin and chloramphenicol antagonised each other and did not inhibit the growth of MRSA [95].

7. In vivo studies

Bacteriocin synergy and effectiveness has been measured in both in vitro and in vivo systems. Two recent studies of interest concern the use of nisin in conjunction with conventional antibiotics for membrane permeabilisation of multidrug-resistant \textit{Salmonella enterica} serovar Typhimurium. It has been indicated that the combination of nisin with ceftriaxone or with cefotaxime assists in permeating the outer membrane of \textit{Salmonella} spp. [83]. Membrane permeabilisation from a β-lactam antibiotic allows for the uptake of nisin. Such facilitation allows for the inhibition of DNA and subsequent protein synthesis. Inhibition is time- and concentration-dependent but has also indicated direct immunomodulatory activity when tested in \textit{Salmonella}-infected mice [97]. Such studies are extremely promising and represent a positive direction for further elucidating the mechanisms of action of bacteriocins in in vivo systems.

8. Limitations

Despite the remarkable potential for the incorporation of bacteriocins in synergistic applications, there are some pitfalls that have not been tested. Much like conventional antibiotics, the overuse of bacteriocins can lead to resistant pathogens [33]. Many would argue that the application of another antimicrobial might postpone the issue of resistance, rather than resolving it. Furthermore, synergistic therapies could create a dual-resistant pathogen epidemic.

Studies in LAB have shown that resistance carries a significant fitness cost, with resistant strains having a slower growth rate than their sensitive ancestor. Treatment with a combination of bacteriocins, for instance nisin and a class Ila bacteriocin, would theoretically reduce the incidence of resistance. There is currently conflicting evidence as to whether resistance to one class of LAB bacteriocin can result in cross-resistance to another class.

Another critical concern is delivery of bacteriocins. Current administration routes of conventional antibiotics include oral, intravenous, subcutaneous and intramuscular routes [90,98]. Peptides given orally that are larger than 3 kDa may not be easily absorbed due to their size, and those that are smaller than that may be denatured by digestive proteases. These issues, and others like them such as short plasma half-life, are some of the reasons for bacteriocin engineering particularly in vivo systems. Not only should these studies focus on improving overall efficacy but they should also aim to improve the stability of these peptides. It is critical that these studies be performed in vivo in order to accurately identify the behaviours of these peptides in realistic environments. The authors suggest the review by Arthur et al. [90] for a further discussion on recent research in bacteriocin delivery research.

9. Conclusion

The era of the most successful infection prevention and treatment therapies is coming to an end. For around a century, antibiotics have been a reliable and effective method for treating almost all documented infections. However, after continuous use and careless regulation, antibiotics have driven the evolution of resistant organisms. In nosocomial and healthcare settings, immunocompromised and susceptible individuals are commonly exposed to and infected with these strains. Once contracted, the antibiotic–resistant infections are significantly more difficult to treat with traditional antibiotics. Since the emergence of this dilemma, a variety of novel therapeutic treatments have been explored.

Bacteria have a number of tools to allow for improved and successful allelopathy. Bacteriocins have been of particular interest as they are known only in their food industry and have shown promise in the medical industry. Bacteriocins position themselves as fulfilling four of the five classic drug targets, using novel targets, being easily engineered to overcome shortcomings, and act as potent synergists to other bacteriocins as well as conventional antibiotics.

10. Recommendation for future research

Bacteriocins represent one of the most studied microbial defense systems. It is clear from both the abundance and variation that bacteriocins are the weapons of choice in the microbial world. Therefore, understanding the evolutionary relationships and ecological functions of these successful toxins could have a substantial impact. Although it is largely understood that bacteriocins play a role in mediating microbial dynamics and maintaining diversity, the mechanism of action is poorly defined. Again, the ramifications of further research into these and other mechanisms would greatly improve our understanding of microbial interactions. Applications of mechanism of action in synergistic relationships as well as improved efficacy and stability could prove extremely beneficial. Research into mechanisms of action is not entirely academic; bacteriocins are already enormously useful in food preservation and maintain the potential to be equally important as alternatives, synergists or part of a multiple hurdle approach with antibiotics.

Acknowledgment

The authors would like to thank Dane Jensen for his insight and expertise in the food industry.

\textbf{Funding:} None.

\textbf{Competing interests:} None declared.

\textbf{Ethical approval:} Not required.
I. Bacteriocin-encoding mobilome, antibiotic contribution


References


[62] Fontana MB, Freire De Bastos MDC, Brandelli A. Bacteriocins Pep5 and epi-


[64] Sharma A, Srivastava S. Anti-Candida activity of two-peptide bacteriocins, plan-
taricins (Pn UF and JX) and their mode of action. Fungal Biol 2014;118: 264–75.


[68] Neto DJA, de Freire Bastos DC, Sahi H-G. Mode of action of the antimicro-


[71] Li M, Yoneyama F, Toshimitsu N, Zendó T, Nakayama J, Sonomoto K. Lethal hydroxyl radical accumulation by a lactococcal bacteriocin, lactocin Q. Anti-


[77] Gong X, Martin-Vischer LA, Nahdney D, Vederas JC, Duszyk M. The circu-


[79] Maldonado-Barragán A, Cárdenas N, Martínez B, Ruiz-Barba JL, Fernández-


[90] Arthur TD, Kovač VL, Chikindas ML. On bacteriocin in delivery systems and poten-

[91] Kovač VL, Volski A, Chikindas ML. The natural antimicrobial subtilosin A syn-


[93] Noll KS, Piarchad MN, Khaykin A, Sinko PJ, Chikindas ML. The natural antimicro-

[94] Wolska KJ, Grzes K, Anna K. Synergy between novel antimicrobials and con-


