Genetically Engineered Bacteriocins and their Potential as the Next Generation of Antimicrobials

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Abstract: The discovery of penicillin by Fleming in 1928 was an historical milestone in the fight against infectious disease. Over the following fifty years, pharmaceutical companies discovered and developed over 100 antibiotics effective against a wide range of human pathogens. More recently, the dramatic rise in antibiotic-resistant pathogens has stimulated renewed efforts to identify, develop or redesign antibiotics active against these multi-resistant bacteria. This review focuses on such efforts directed at one large and highly diverse family of toxins, the bacteriocins, which hold great promise as the next generation of antimicrobials. The majority of bacteriocins differ from traditional antibiotics in one critical way: they have a relatively narrow killing spectrum and are, therefore, toxic only to bacteria closely related to the producing strain. Accordingly, they can be considered "designers drugs" that target specific bacterial pathogens. In this review we focus on recent attempts to generate custom designed bacteriocins using genetic engineering techniques. These efforts illustrate the potential of genetically-modified bacteriocins to solve some of the most challenging problems in disease control.

INTRODUCTION

Since the identification of penicillin in 1928 and its subsequent production on a massive scale, antibiotics have revolutionized approaches to human health [1]. The ability of antibiotics to cure individuals of otherwise debilitating, and sometimes fatal, infectious diseases has been regarded as nothing short of a medical miracle. For example, the use of antibiotic therapies, in combination with improved sanitation and vaccination, have reduced mortality rates from childhood pneumonia in the United States by 97% over the 58-year period of 1939 to 1996 [2]. With the availability of a powerful and effective arsenal of drugs, pharmaceutical companies abandoned their antimicrobial drug development programs, as there seemed to be little need for new compounds [3].

Bacterial resistance to antimicrobials was observed shortly after their initial wide-scale use [4]. Since then, the levels of resistance have continued to rise dramatically, to the point that by 2000 the World Health Organization cautioned that infectious diseases may become untreatable as a result of high levels of multiply resistant pathogens [5].

At first, antibiotic resistance was thought to be confined to hospital settings, where the use of antibiotics was most intensive; approximately one third of all hospitalized patients receive antibiotics with at least half of those prescriptions being either unnecessary, poorly chosen or incorrectly administered [6, 7]. Compounding the problem further, an almost exclusive reliance on broad-spectrum antibiotic agents has contributed to a rapid emergence of multi-resistant pathogens [8, 9]. The increasing threat of antibiotic resistance is also the result of antibiotic use in agricultural and food production settings. In the agricultural industry, the use of antibiotics for disease control, prophylactic agents and growth promotion, has contributed significantly to the emergence of resistant bacteria pathogenic to animals [10, 11] and plants [12]. Additionally, bacteria isolated from animals in environments unrelated to clinical or agricultural management settings have been shown to naturally acquire high levels of antibiotic resistance [13].

Ironically, it is likely that the extensive benefits of antibiotic use has contributed to the limited array of effective drugs available today for treating multi-resistant bacteria. Only recently has the alarming nature of this problem re-motivated research efforts to find alternatives to our increasingly limited antibiotic resources. Numerous antibacterial agents are now being considered, such as bacteriophage [14], probiotic bacteria [15, 16], antimicrobial peptides [17, 18], and bacteriocins [19, 20]. In order to optimally exploit the desired activities of these varied antimicrobial leads, researchers often employ chemical or genetic engineering methods [18, 21].

In this review we introduce a promising family of antimicrobial leads, the bacteriocins. These potent toxins have received increased attention due to their powerful but narrow killing activity, stability, and low toxicity to humans. Furthermore, we will describe attempts to genetically engineer bactetiocins for the purpose of making them more suitable for clinical and agricultural use.

Bacteriocins

The first bacteriocin was originally identified in 1925 by Gratia as an antimicrobial protein produced by *Escherichia coli* [23] and was named colicin in accordance to the producing species in which it was identified. Today it is known that bacteriocins comprise a large and functionally

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diverse family of toxins found in all major lineages of Bacteria and Archaea [24]. Yet, there are certain features that unite them as a family; they are all ribosomally synthesized proteinaceous compounds, and are active against bacteria closely related to the producing bacteria. In fact, two features distinguish the majority of bacteriocins from antibiotics: (i) antibiotics are not ribosomally synthesized and (ii) bacteriocins have a relatively narrow killing-spectrum [24]. Bacteriocin genes are either chromosomally or plasmid encoded with resulting toxins employing a variety of killing mechanisms, including cytoplasmic membrane pore formation, cell wall interference, and nuclease activity [25, 26].

Bacteriocins of Gram-Positive Bacteria

Bacteriocins produced by Gram-positive bacteria resemble many of the antimicrobial peptides produced by eukaryotes, such as defensins [18]; they are generally cationic, amphiphilic, membrane-permeabilizing peptides, approximately 2-6 kDa in size [27]. Typically, the biosynthesis of bacteriocins of Gram-positive bacteria is self-regulated with specifically dedicated transport mechanisms facilitating its release [24]. To date, bacteriocins produced by lactic acid bacteria (LAB), fermenting bacteria long used in the preservation of meat and milk, are best characterized. Four main groups of LAB antibiotics have been identified: Class I modified bacteriocins, known as lantibiotics, Class II, heat stable minimally modified bacteriocins, class III, larger heat labile bacteriocins and Class IV, complex bacteriocins carrying lipid or carbohydrate moieties [19, 28-32]. This review will mainly focus on the potential applications of Class I lantibiotics because the reported applications of class II, III and IV bacteriocins are limited.

Class I Bacteriocins - Lantibiotics

Lantibiotics are ribosomally synthesized bacteriocins that target a broad range of other Gram-positive bacteria and are characterized by their high content of uncommon amino acids, such as thioether bridges of lanthionine and 3-methyllanthionine or dehydroalanin and dehydrobutyrin [33]. Lantibiotic gene clusters are localized on the bacterial chromosome or on mobile elements such as plasmids or transposons. They are synthesized as precursor peptides with a characteristic N-terminal leader peptide and a C-terminal pro-peptide domain in which specific amino acid residues are post-translationally modified. The leader peptide may function either to keep the bacteriocin inactive until export, to facilitate interaction with the transporter protein or to promote interaction with the modification enzymes. The precursor peptide is encoded by a structural gene, which is part of a gene cluster with genes required for modification, proteolitic processing, transport, autoimmunity and regulation [19, 31, 32, 34]. Lantibiotic production is regulated by a quorum sensing strategy in which the antimicrobial peptide functions as a signal molecule for measuring the density of a population and triggering a regulatory system that induce its own expression [35].

The lantibiotics are subdivided into two groups: type A and B. Type A lantibiotics are small (2-5 kDa), elongated, screw shaped proteins that contain positively charged molecules, and kill via membrane polarization. Type B lantibiotics are smaller (about 2 kDa), globular in shape and kill by interfering with cellular enzymatic reactions such as cell wall synthesis [19, 31, 32].

Bacteriocins of Gram-Negative Bacteria

Most bacteriocins of Gram-negative bacteria are large in comparison to bacteriocins of Gram-positive bacteria, and range in size from less then 10 kDa to greater then 20 kDa. Bacteriocins of Gram-negative bacteria differ from bacteriocins of Gram-positive in two fundamental ways: (i) they are usually released through cell lysis and (ii) they are often dependent on host regulatory pathways, like SOS regulation [24].

Most enteric bacteria produce bacteriocins. Recent surveys of *Hafnia alvei*, *Citrobacter freundii*, *Klebsiella oxytoca*, *K. pneumonia*, and *Enterobacter cloacae* reveal levels of bacteriocin production ranging from 3 to 26 percent of environmental enteric isolates [36]. Fifteen to fifty percent of *E. coli* strains produce one or more colicins, the most extensively studied bacteriocin group [37]. Molecular investigations reveal that enteric bacteriocins employ similar killing mechanisms, although they often utilize novel receptor recognition and translocation functions, ensuring their narrow killing specificities [38, 39].

Colicins

Colicins are plasmid-encoded high molecular weight proteins (over 20kDa) that are active against *E. coli* strains and other closely-related bacteria, such as *Salmonella* [26]. The colicin family has been extensively studied and serves as a model for investigating the mechanisms of bacteriocin structure/function, genetic organization/regulation and evolution [25, 26, 40]. Over 30 types of colicins have been identified, based upon killing activity and immunity specificity [25, 41].

SOS regulation ensures that colicin production occurs principally during times of stress, for example when levels of nutrients or oxygen are depleted [25]. Immediately following induction, colicin molecules are released by lysis of the producer cell into the environment where they bind to specific cell surface receptors on susceptible cells. Having gained access into the cell, the colicins are translocated across the inner membrane [22] and kill the sensitive target by one of several mechanisms, including pore formation in the cytoplasmic membrane, nonspecific DNAse activity or inhibition of protein biosynthesis by cleaving 16S rRNA or tRNAs [25, 41].

Genes encoding colicin functions are found in clusters that include a toxin-encoding gene; an immunity gene, encoding a protein conferring self-specific protection to the cell against its own colicin; and, frequently, a lysis gene, encoding a protein involved in colicin release via lysis or pseudo-lysis of the producing cell [25, 26].

Microcins

Enteric bacteria produce an additional group of bacteriocins, the microcins. Only nine microcins have been identified so far [42, 43], and, unlike colicins, few have been characterized at the level of protein structure or mode of action. Microcins are smaller than colicins (less than 10 kDa)

and their synthesis is neither lethal to the producing strain nor SOS dependent [44]. They share some features with low molecular weight bacteriocins produced by Gram-positive bacteria: they are thermostable, resistant to certain proteases, relatively hydrophobic and resistant to extreme pH [42]. The killing spectrum of microcins is broad compared to that of colicins but is also primarily directed against genera of Enterobacteriaceae [44]. Microcins kill their target sensitive cells by forming pores or by disrupting the cells' membrane potential [42, 45, 46].

Pyocins

Another well-studied group of bacteriocins produced by Gram-negative bacteria are the pyocins of *Pseudomonas*. In contrast to the comparatively lower levels of enteric bacteriocin production [37], over 90% of *P. aeruginosa* strains produce at least one pyocin [47]. In contrast to the plasmid-encoded colicins, the genes encoding pyocins are found exclusively on the chromosome. The pyocin-encoding gene clusters comprise of tightly linked toxin and immunity-encoding genes and in some cases a lysis gene cassette [48]. As with colicins, pyocins expression is induced by DNA damaging agents, triggering the SOS response [49].

Three types of pyocins have been described R-, F-, and S-types. Both the R- and F-types have a rod-like structure resembling bacteriophage tail fibres. R-type pyocins appear as hollow cylinders, consisting of an extended sheath and a core, and kill by depolarizing the cytoplasmic membrane of sensitive cells [50]. The F-type pyocins are flexuous non-contractile rods with a square like structure at one end and a fiber-like structure at the other end [51, 52]. S-type pyocins are colicin-like, soluble, protease sensitive proteins, which kill by pore formation, RNase or DNase activity [53, 54]. Unlike colicins, S-type pyocin gene clusters lack a lysis gene, indicating that different mechanisms might be involved in their release [55]. The killing spectrum of S-type pyocins is limited to *P. aeruginosa*, while R- and F-type pyocins kill more broadly, including other Gram-negative bacteria such as *Neisseria* and *Haemophilus* [56, 57].

Bacteriocins Active Against Phytopathogens

Bacteriocins that are active against plant pathogens are not well described. In fact, little is known about their structure, killing activity, regulatory systems and killing spectra. However, described bacteriocins of *Erwinia carotovora* subsp. carotovora and *Serratia plymithicum*, known as carotovoricins and serracin P respectively, resemble phage tails in structure and are induced by DNA damaging agents [58, 59]. Glycinicin A, the best described baceriocin produced by *Xanthomonas campestris* pv. glycines [60], is a heterodimer of two polypeptides. Glycinicin A was found to be active against most tested *Xanthomonas* phytopathogenic bacteria strains [61].

Application of Bacteriocins in Human Health and Agricultural Settings

The application of bacteriocins in the food industry has received considerable attention, as has their potential for clinical and agricultural use (summarized in Table **1**). We will review the applications along with recent attempts to genetically engineer bactetiocins in order to make them more suitable for disease control in human and agricultural settings.

Lantibiotics

The potential use of lantibiotics in food, human and animal health applications has been well documented [19, 62-64], with several features making them particularly attractive for such applications; a relatively broad killing spectrum, an auto-regulation system, stability and cost-effective production processes [19, 27]. Moreover, several lantibiotics are produced by food-grade bacteria that have been safely consumed by humans for centuries.

The best-studied lantibiotic is undoubtedly nisin, produced by *Lactococcus lactis*. It is thus far the only bacteriocin that has been approved by the FDA as a food preservative, and it is being used for this purpose in more then 50 countries [65]. Nisin has been also considered for pharmaceutical applications. For example, it was suggested that nisin has potential in treating peptic ulcer disease by inhibiting *Helicobacter pylori* growth and colonization [66]. Nisin was additionally used to inhibit growth of multi-drug resistant pathogens such as *Staphylococcus* and *Streptococcus* spp. [67]. Bower and his colleagues (2002) treated catheters and tracheotomy tubes with nisin, which had a protective effect against infection by Gram-positive bacteria, albeit for only a short period (5-12 hr), and produced no systematic or adverse effects [68].

Pharmaceutical applications were found in other lantibiotics. Epidermin and gallidermin, produced by *Staphylococcus epidermidis* and *S. gallinarum* respectively, are being explored for treatment of juvenile acne due to their specific and potent activity against *Propionibacterium acnei* [27, 69, 70]. Lanthiopeptin produced by *Streptoverticillium cinnamoneum* had demonstrated antiviral activity against herpes simplex virus infection [71]. Mersacidin is produced by *Bacillus subtilis*, and inhibits methicillin-resistant *Staphylococcus* with a killing efficiency similar to vancomycin [72]. It was further suggested to use lantibiotics to treat food that must remain bacteria-free for immunocompromised patients [64].

The potential of class I type B lantibiotics in human health applications has been investigated as lantibiotic activity was found to inhibit eukaryotic cell functions. Ancovenin, a bacteriocin produced by *Streptomyces* spp., is a natural inhibitor of angiotensin I, which serves in regulating cardiac and vascular function [73]. Duramycin and cinnamycin, produced by *Streptoverticillium* and *Streptomyces* spp., show promise as anti-inflammatory and anti-allergy drugs due to their ability to inhibit phospholipase A2, which plays a role in the release of prostaglandins and leukotrienes, both potent promoters of inflammations and allergies [74].

Lantibiotics have also been investigated for use in addressing animal health concerns. The two-peptide lantibiotic, lacticin 3147, produced by *L. lactis* was found to be active against mastitis-causing bacteria *Streptococci* and *Staphylococci*. Mastitis is the most costly disease in dairy cattle. It can be effectively treated with antibiotics, but the antibiotic residues found in the milk of treated cows may contribute to the selection for antibiotic resistance in humans

Table 1. Bacteriocin as Antibiotic Agents: Examples f Suggested Applications

Bacteriocin	Producer strain	Potential use	Reference
Lantibiotics			
Ancovenin	*Streptomyces* spp.	Treating high blood pressure	[73]
Cinnamycin	*Streptoverticillium* and *Streptomyces* spp.	Treating inflammations and allergies	[74]
Duramycin	*Streptoverticillium* and *Streptomyces* spp.,	Treating inflammations and allergies	[74]
Epidermin	*Staphylococcus epidermidis*	Treating skin infections	[69]
Gallidermin	*Staphylococcus gallinarum*	Treating skin infections	[70]
Lacticin 3147	*Lactococcus lactis*	Treating mastitis infections	[75, 76]
Lanthiopeptin	*Streptoverticillium cinnamoneum*	Treating Herpes simplex virus	[71]
Mersacidin	Bacillus subtilis	Treating vancomycin resistant strains	[72]
Mutacin	*Streptococcus mutans*	Treating dental carries	[102]
Nisin	*Lactococcus lactis*	Treating peptic ulcer Antimicrobial inhibiting multi-drug resistant pathogens Antimicrobial barrier in implanted medical devices	[66] [67] [68]
Colicins			
Ia	*Escherichia coli*	Component in an engineered species specific antibiotic	[101]
E1, E4, E7, E8, K & S4	*Escherichia coli*	Treating hemorrhagic colitis and hemolytic uremic syndrome	[78]
Microcins			
24	*Escherichia coli*	Treating salmonelosis in chicken	[79]
B17	*Escherichia coli*	Antibacterial agent in cattle	[84]
E294	*Klebsiella pneumoniae*	Controlling cell proliferation	[89]
J25	*Escherichia coli*	Treating salmonelosis in chicken	[85]
L	*Escherichia coli*	Treating salmonelosis	[86]
Pyocins			
S-35	*Pseudomonas aeruginosa*	Treating pulmonary infections	[48]
	Pseudomonas syringae pv. ciccaronei	Treating olive knot disease	[93]
Gram-negative produced bacteriocins			
Serracin-P	Serratia plymithicium	Treating fire blight disease	[58]
Glycinicin A	*Xanthomonas campestris* pv. glycines	Treating black rot, citrus canker, bacterial spot, and leaf spot diseases	[61, 104]
Carotovoricin	*Erwinia carotovora* subsp. carotovora	Treating soft rot disease	[110]
	Xanthomonas campestris pv. oryzae	Treating blight infection	[94]
	Ralstonia solanacearum	Treating tobacco wilt infection	[93]

who drink that milk. Thus, bacteriocins such as lacticin 3147 show considerable potential in the prevention of infectious disease in agricultural settings [75, 76].

Colicins and Microcins

One drawback of the popular LAB bacteriocins is that they only inhibit the growth of Gram-positive bacteria. Gram-negative infectious agents such as *Aeromonas*, *Escherichia*, *Salmonella*, *Yersinia* and *Pseudomonas* are only rarely sensitive to LAB bacteriocins unless they are coupled with chelating agents. Consequently, the potential for bacteriocins active against Gram-negative pathogens has been investigated. Colicins E1, E4, E7, E8, K and S4 [77] together with microcin J25 [78] and microcin 24 [79] are the most effective growth inhibitors of *E. coli* O157:H7, a shiga

toxin-producing strain that is the leading cause of hemorrhagic colitis and hemolytic uremic syndrome in humans. The cattle rumen serves as the major reservoir for *E. coli* O157:H7, however, management strategies to control the pathogen using antibiotics has been problematic. Studies have shown that antibiotic therapy increases the amount of shiga toxin released, and therefore, induces higher levels of bacterial virulence [80]. Recently, several studies have reported that administration of colicin and microcin producing bacteria into the cow gut have reduced the level of enteric pathogens in the animal, possibly by preventing acquisition of new pathogenic strains [77, 81-83]. Colicin E and B are already being marketed by PBS Animal health® and Horse Health USA®, respectively, for prevention and control of pathogenic *E. coli* strains in newborn piglets and foals.

Microcins have been shown to be potential alternatives to the currently used antimicrobial agents of quinolones and coumarins, inhibitors of prokaryotic topoisomerase II (DNA gyrase), a central compound in DNA replication. These DNA gyrase antagonists are a rapidly expending class of agents with promising properties for the treatment of infectious diseases. Microcin B17, produced by *E. coli*, kills in a similar manner as the quinolones and the coumarins, thus defining a third class of DNA gyrase inhibitors [84]. Microcins J25 [85] and L [86], both produced by *E. coli*, exhibit strong antimicrobial activity against *Salmonella enterica* serovars typhimurium and enteritidis, which cause diarrheal illness in humans.

Like some lantibiotics [87], colicins and microcins exhibit the ability to inhibit the growth of eukaryotic cells by inducing apoptosis, or programmed cell death [88]: Microcin E294, produced by *Klebsiella pneumoniae*, induced biochemical and morphological changes typical of apoptosis in human cell lines [89], and therefore, may have potential as an anti-cancer drug. Colicin E9, a DNase produced by *E. coli*, shares a common features with proteins that regulate apoptosis through permeability of cells organelles [90].

Bacteriocins Active Against Phytopathogens

The potential of Gram-negative produced bacteriocins as a mean of biological control in fighting plant pathogens has been investigated, influenced by the prevalence of antibiotic-resistant phytopathogenic bacteria [91] and growing health concerns associated with chemical pesticides [92]. To date, several bacteriocins have shown promising results at curbing plant pathogens. Dipping plants in a suspension of a bacteriocin-producing avirulent strain of *Ralstonia solanacearum* prevented tobacco wilt infection [93]. The incidence and severity of bacterial blight infection that causes leaf streak in rice was reduced by treatment with a nonpathogenic bacteriocin-producing strain of *X. campestris* pv oryzae [94]. *S. plymithicium* produces a colicin-like bacteriocin, which is active against *Erwina amylovora*. This pathogen is the causative agent of fire blight, a costly disease to the apple and pear industry [58]. A pyocin produced by *Pseudomonas syringae* pv. ciccaronei inhibited the multiplication of *P. syringae* subsp. *savastanoi*, the causative agent of olive knot disease. This bacteriocin also effects the epiphytic survival of the pathogen on leaves and twigs of treated olive plants [95].

GENETICALLY ENGINEERING BACTERIOCINS TO CREATE NOVEL ANTIBIOTICS

Knowledge of the genetic organization and biosynthetic pathways of increasing numbers of bacteriocins has facilitated the analysis and modification of bacteriocins and their producing hosts to improve the potency of bacteriocins as antimicrobial agents [19, 96]. Successful production of engineered bacteriocins depends on many factors; cell growth, expression levels, location of the final recombinant product, post translational modification and regulation [97]. Additionally, bacteriocins must meet specific requirements for them to be effective drugs; they must be active against the intended target pathogen and stable in the proposed environment of use. Lastly, the choice of host cell and host-encoded genetic elements can be critical to the successful expression of the gene of interest since expression systems are a combination of both components [96]

Bacteriocins with novel characteristics can be generated by either mutating bacteriocin-encoding genes or by fusing genes from different bacterial species. Genetic modification of bacteriocins and their producing hosts can offer several advantages over using them in their native form. For example, it is useful to enlarge the killing spectrum of bacteriocins, as they often have a narrow killing range, and may not be effective against all strains of a targeted pathogen. Gene fusion is a useful tool in modifying and expanding the killing spectra of bacteriocins [96]. Such an approach was employed for addressing nosocomial infections caused by *P. aeruginosa*, the primary causative agent in pulmonary infections among patients with cystic fibrosis. Such infections are difficult, if not impossible, to treat with classical antibiotic approaches [98], and therefore, the use of bacteriocins was investigated. Pyocin S-35, isolated from a *P. aeruginosa* strain, cultured from the sputum of a patient with cystic fibrosis, was modified to enhance its killing range. The translocation and killing domains of the S-35 activity protein and its cognate immunity protein were fused to the receptor-binding domain of pyocin S1. The resulting construct was effective against a broader range of *P. aeruginosa* strains than either S1 or S35 pyocins alone [47].

Gene fusion can also be utilized to alter the killing spectra of bacteriocins in order to target pathogenic species not sensitive to the bacteriocin in its native form. For example, in order to design a species-specific antibiotic, the gene cluster encoding a channel-forming colicin (Ia) and its cognate immunity protein from *E. coli* was fused with a pheromone-encoding gene (*agrD*) from *S. aureus* [99-101]. The resulting pheromonicin had specific affinity for a *S. aureu*s membrane receptor and targeted the pore forming function of Ia at the membrane. Injections of pheromonicin were more effective than penicillin in eliminating pathogenic *S. aureus* from a mouse model [101].

In addition to being administrated as purified proteins, bacteriocins can be used as probiotics, with the host designed to produce the specifically required bacteriocin. If a bacteriocin is to be used as a probiotic, the producing strain must be able to competitively colonize the environment and cannot be pathogenic to the host [102]. In addressing this, bacteriocin genes or proteins of distantly related bacteria

have been fused together, generating active bacteriocins in non-native producing strains that have to date been considered safe for humans. The gene encoding microcin V, produced by *E. coli*, was inserted before an LAB class II bacteriocin signal peptide. The resulting construct was the first Gram-positive strain that successfully produced a bacteriocin of a Gram-negative bacterium [103]. This system may serve as a model for the heterologous expression of other small bacteriocins active against Gram-negative bacteria from LAB, bacterial species considered harmless to humans.

Gene fusions have been employed to improve bacteriocin stability in a wide range of environmental conditions. Glycinicin A, produced by *Xanthomonas campestris* pv. glycines, is active against a phytopathogenic *Xanthomonas* spp., including the causative agents of black rot, citrus canker, bacterial spot, and leaf spot diseases [61]. A fusion of two genes encoding the glycinicin subunits resulted in a chimeric protein that retained wild type activity and provided increased stability at both higher and lower pH and higher temperatures [104].

The bacteriocin expression systems have been explored for their use in novel vaccine production and types of vaccination. The controlled production of lactic acid bacteria (LAB) proteins is largely based on the well-characterized nisin controlled expression (NICE) system. It has been shown that the nisin promoter could be employed in a series of transcriptional and translational fusion vectors that were extremely useful in expressing a variety of genes [66, 105]. For example, the NICE system was used to display on a coprotein of human papillomavirus, a compound constitutively produced in cervical cancer, in an attempt to design a therapeutic vaccine. The oncoprotein was displayed on the cell wall of *L. lactis* bacteria and administrated intranasally to mice, inducing a specific immune response [106].

Genetic engineering efforts can also be employed to manipulate the producing strain. A bacteriocin active against a target pathogen can be cloned into an avirulent bacteria strain known to successfully colonize the intended host. Recent studies indicate that microcin 24, produced by *E. coli*, holds promise in the prevention of *Salmonella* contamination in chickens [79, 107]. Wooley and colleagues transformed plasmids containing microcin 24 gene fragments into a nonpathogenic avian *E. coli* strain. Addition of the recombinant strain to drinking water significantly reduced the chickens intestinal *Salmonella typhimurium* load [108].

Some bacteriocins active against targeted disease-causing bacteria may be produced by pathogenic strains themselves; clearly problematic when considering human, animal and plant health applications. One strategy to counteract this problem would be to modify the bacteriocin-producing strain such that it loses its pathogenicity. *Streptococcus mutans*, a causative agent of dental caries, has been reported to produce mutacins active against neighboring plaque-forming strains. A positive correlation exists between mutacin production and the ability of a strain to colonize the oral cavity, and consequently, a nonpathogenic mutacin-producing strain was constructed for replacement therapy of dental caries [102]. Cavity formation is associated with the pathogenic strain's ability to convert sugar into enamel-eroding lactic acid, a reaction catalyzed by lactate dehydrogenase [109, 110]. Therefore, the lactate dehydrogenase-encoding gene of a pathogenic *S. mutans* strain was deleted and, in order to counteract resulting metabolic imbalance, was replaced with an alcohol dehydrogenase-encoding gene [111]. The resulting strain did not generate lactic acid and produced similar quantities of mutacin when compared to the wild type strain. Animal studies showed that the genetically modified strain was significantly less pathogenic and can colonize the oral cavity, inhibiting the growth of pathogenic strains [112-114].

Two different approaches using mutagenesis, site directed and random, have been explored to generate more effective bacteriocins. The use of nisin in clinical settings is problematic as it has low solubility at physiological pH (pH 7). By using site-directed mutagenesis to introduce lysine residues into nisin Z, the solubility at pH 7 was enhanced and, more importantly, the novel bacteriocin had comparable antimicrobial activity to native nisin Z [115]. This may enable the use of the FDA approved nisin as a therapeutic agent.

Employing random mutagenesis, a bacteriocin-producing strain was selected to prevent soft rot disease in plants. Pathogenic *Erwinia carotovora* subsp carotovora, produces carotovoricin, a bacteriocin active against several strains of *E. carotovora* [59]. Bacteriocins producing strains were exposed to a chemical mutagen and were screened for loss of pathogenicity. The resulting strain prevented soft rot, black rot, and bacterial seedling blight of rice [116].

CONCLUSIONS

Recent studies reveal that most, if not all, major lineages of bacteria produce one or more bacteriocins, comprising a diverse and abundant family of potent antimicrobials. An increasing number of studies reveal the potential for these toxins to serve as the next generation of antibiotics for use in human health and agricultural settings. In many cases, relatively simple methods of genetic engineering allow the modification of both the bacteriocin protein and the producing host to meet the varied needs of health and agricultural applications.

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