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Functions and emerging applications of bacteriocins

Michael L Chikindas[1,2], Richard Weeks[1], Djamel Drider[3], Vladimir A Chistyakov[4] and Leon MT Dicks[5]

Bacteriocins, defined as ribosomally synthesized antimicrobial peptides, have traditionally been used as food preservatives, either added or produced by starter cultures during fermentation. In-depth studies of a select few bacteriocins opened exiting new research fields and broadened the application of these antimicrobial peptides. The possibility of developing bacteriocins into next generation antibiotics, accompanied with the rapid development in genetics and nanotechnology, paves the way to even more fascinating applications such as novel carrier molecules (delivery systems) and the treatment of cancer. Also, some bacteriocins are found to regulate quorum sensing which suggests novel applications for this group of substances. While there is some interesting translational research on bacteriocins from Gram-negative bacteria, the majority of application-oriented studies are focused on bacteriocins from Gram-positive microorganisms, mostly lactic acid bacteria. The applications of bacteriocins are expanding from food to human health.

Addresses
[1] Health Promoting Naturals Laboratory, School of Environmental and Biological Sciences, Rutgers State University, 65 Dudley Road, New Brunswick, NJ 08901, USA
[2] Center for Digestive Health, New Jersey Institute for Food, Nutrition and Health, 61 Dudley Road, New Brunswick, NJ 08901, USA
[3] Université de Lille, EA 7394 – ICV - Institut Charles Viollette, 59000 Lille, France
[4] D. I. Ivanovsky Academy of Biology and Biotechnology, Southern Federal University, Rostov-on-Don 344090, Prospekt Stachki 194/1, Russia
[5] Department of Microbiology, Stellenbosch University, 7600 Stellenbosch, South Africa

Corresponding author: .Chikindas, Michael L (tchikind@sebs.rutgers.edu)

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Introduction

All living organisms produce antimicrobial proteins (AMPs), many of which are referred to as antimicrobial peptides because of their relatively small size. Within the eukaryotic immune system, AMPs are believed to serve as first line of defense in protecting the host against hostile intruders [1]. Bacteria produce two types of AMPs: those that are ribosomally synthesized (also known as bacteriocins), and non-ribosomally synthesized AMPs, with no structural genes coding for these AMPs, e.g. ε-poly-L-lysine [2].

Definitions: current classifications can be confusing

Some investigators prefer separating 'true' bacteriocins such as colicins, first discovered by Gratia in 1925 [3], and colicin-like bacteriocins, from so-called bacteriocin-like inhibitory substances (BLIS) [4]. There is a continuing interest in bacteriocins from lactic acid bacteria (LAB), and some authors suggest a stand-alone classification for these AMPs [5•]. Moreover, the broad variety of bacteriocins, their origins, complexities of production, and mechanisms of actions, justify the need for stand-alone classifications for several other groups of bacteriocins. It is appropriate to conclude that bacteriocins in general should be defined as ribosomally-produced multi-functional substances of a proteinaceous nature, with pronounced antimicrobial activity at certain concentrations. In addition, there is no need to define bacteriocins as having a net positive charge and amphipathic nature, since there are anionic bacteriocins such as subtilosin A reported in the literature [6].

Bacteriocins: applications are determined by the functions

'Primary' and 'secondary' functions: signaling and protection of an ecological niche

Many, if not most of the presently known bacteriocins were first discovered and studied as antimicrobials. For more information, readers should be referred to various sources such as Fairweather's book reviewing the involvement of bacteriocins in the regulation of various processes within microbial communities [7], and to more recent reviews (Hegarty *et al.* [8]). Synthesis of the bacteriocin nisin in *Lactococcus lactis* is elegantly controlled by a cascade of events in which nisin itself serves as a pheromone-like primary 'trigger' for its own production [9]. In streptococci, a signaling-involved membrane protein (ComM) regulates the production of a bacteriocin transporter, and triggers the cell's immunity towards lytic enzymes [10]. Moreover, in *Streptococcus mutans*, an unmodified intracellularly-located bacteriocin, mutacin V, may work as a lytic agent against the producer cell when in the presence of a high concentration of a

competence-stimulating peptide. This may act as an evolutionary factor driving the exchange of genetic material with non-lysed competent cells [11]. There are also reports on bacteriocins inhibiting quorum sensing in various microorganisms at sub-MIC concentrations [12]. From an evolutional point of view, it is logical for microorganisms to utilize quorum sensing inhibition as a means of defense, rather than killing cells that invade their ecological niche. Instead of producing large amounts of bacteriocins, the bacteriocin-producers prevent intruders from settling down by arresting biofilm formation via inhibition of quorum sensing with low-level production on bacteriocins [13]. In summary, there are many examples of bacteriocins exhibiting a variety of regulatory functions, including their own production [14]. Undoubtedly, they also play an essential role in kin recognition [15•].

Natural levels of production: enough for killing, or just to repel?

Data on the production and concentration of bacteriocins in the producer's usual ecological environment is scarce. Nevertheless, studies mimicking the natural environment will shed light on our assumptions of the primary function of bacteriocins. When analyzing microbial inactivation curves as a reflection of the bacterial response to the environmental stressor nisin at sub-MIC concentrations, a step-like response by the foodborne pathogen *Listeria monocytogenes* was observed [16]. While the aim of this study was to evaluate the pathogen's survival in the food environment with gradually decreasing concentrations of nisin, this can also be seen as an extrapolation of the natural condition where intruder cells are facing relatively low concentrations of the bacteriocin diffused throughout the environment. Based on the available literature, we speculate that the primary functions of bacteriocins are signaling and repelling rather than killing. Qualitatively different behavior is observed at concentrations far exceeding those in the microbial 'free-range' life.

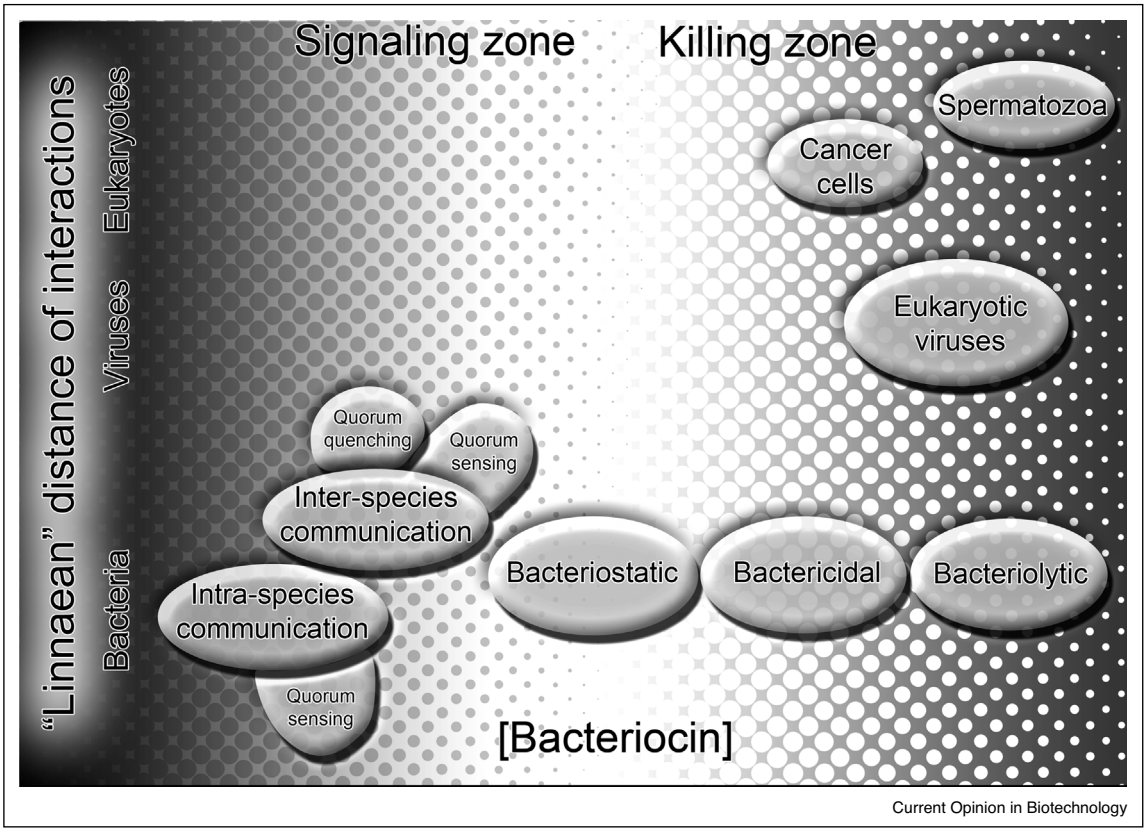
When used in concentrations higher than in the natural environment of the producer microorganisms, some bacteriocins reveal a variety of additional functions, of which their antimicrobial activity is the most studied [17]. At MBC (minimal bactericidal concentration), they often act as membrane perturbers or as pore-forming substances [18]. Some bacteriocins can kill sensitive cells by interfering with the cell division process. Lipid II serves as a recognition 'anchor' for various AMPs (including nisin). However, some bacteriocins have the ability to segregate lipid II, thus preventing the targeted cell from normal cycling [19]. Interference with cell division, as well as other biological functions, may occur at substantially different concentrations for different bacteriocins, acting against different target microorganisms. For instance, micromolar concentrations of salivaricin B (a bacteriocin first found in *Streptococcus salivarius*) are required to interfere with septum formation in *Micrococcus luteus* and *Streptococcus pyogenes*, as opposed to nanomolar concentrations of nisin A required for the same function [20]. Interference with bacterial septum formation may also result in bacteriostatic action for some bacteriocins [21]. Last but not least, there are numerous bacteriocins reported to cause lysis of sensitive cells [22].

Bacteriocins' functions expanded: human health applications

Some bacteriocins, such as subtilosin A from *Bacillus subtilis*, were reported as having anti-viral [23] and spermicidal [24] activities. Subtilosin's antiviral activity is likely due to interference with the late stages of virus replication. Concentrations of subtilosin A that immobilize and de-activate human spermatozoa are inactive against lactobacilli isolated from the human gastrointestinal tract. In contrast, spermicidal concentrations of nisin were shown to inhibit LAB [25]. Moreover, several attempts have been undertaken to systematize bacteriocins' function in relation to their structure, and some systemic approaches have been reported to improve the functioning of a number of bacteriocins [26•]. Taking a broad view of this and other critical reviews, we were able to map out an 'ecology of bacteriocins' based on presently available information (Figure 1).

While the primary application of bacteriocins has always been in food preservation [26•], antimicrobial resistance to conventional antibiotics presents new opportunities for the exploration of bacteriocins' application in a variety of healthcare products where undesired and potentially resistant microorganisms must be controlled [27•,28]. The National Institute of Health (NIH) recently encouraged a complementary approach in searching for novel drug formulations, where the activity of conventional antimicrobials can be enhanced when combined with novel and, often naturally derived, antimicrobials [29,30]. A multipronged approach utilizing synergistically acting antimicrobials with different targets in controlled bacteria has been studied for many years. Different bacteriocins were reported as acting synergistically with various food-grade substances [31] and with bacteriophages [32], although the latter study provided no FICs (fractional inhibitory concentrations) or isobolograms to validate the observed synergy. Potential areas of interest include oral and skin care, and respiratory, gastrointestinal, urogenital tract and other infections. In addition to anti-viral activity, bacteriocins could potentially be used in the post-surgical control of infectious bacteria [33]. Nisin, being the oldest discovered and most studied bacteriocin, is of particular interest in a variety of human health related applications due it its ability to control many Gram-positive pathogens and because of its enhanced range of action, including Gram-negative microorganisms, when used in combination with various

Figure 1



Bacteriocins: signaling and killing.

antimicrobials, in particular acting on the outer-membrane of Gram-negative bacteria [34,35]. One of the most intriguing new fields of investigation is the study of bacteriocins as potential anti-cancer agents [36]. Bacteriocins are able to selectively act against cancer cells, most likely due to the distinctive differences in the membranes.

Food applications: preservation and beyond

Most of the food applications involving bacteriocins can be divided into three categories: Partially purified bacteriocins (e.g., Nisaplin®, containing 2.5% nisin), dairy and other food-grade fermented products containing bacteriocins in the form of a crude fermentate (e.g., MicroGARD® series of bacteriocin-containing products [37]), and bacteriocin-producing protective cultures (Table 1, Figure 2, [38,39]). Control of foodborne pathogens in a variety of food products and by many bacteriocins has been studied by several groups over decades and was recently reviewed [40]. However, it appears thus far that nisin is the only commercially produced food-grade bacteriocin utilized as a preservative in a variety of food products, having FDA approved GRAS (generally recognized as safe) status for certain applications. Nevertheless, there are encouraging reports on the effective control of foodborne pathogens with multi-bacteriocin producing microorganisms, where nisin is utilized in combination with other bacteriocins [41]. Several groups are working on the improvement of bacteriocins' performance in food environments. The challenges being addressed include the engineering of derivatives with increased resistance to proteolytic enzymes (which does not always come with better activity) [42], enhanced activity, and inhibition spectrum [43•]. Some of the reported attempts followed the approach of rational design via modification of the bacteriocin's amino acid sequence [44], and were rather successful.

In addition to the improvement of bacteriocins' antimicrobial activity by sequence manipulation, their effectiveness in the food environment can be improved via intelligently controlled delivery. In a model study with nisin and *M. luteus*, one of the most sensitive reference microorganisms, it was shown that the most effective inhibition occurs when an initial phase of fast release is followed by a phase of slow release in smaller quantities, just enough to control the remaining population [45]. While appropriate food-grade delivery systems are still under investigation, they are available for a variety of pharmaceutical applications [46].

Table 1

Examples of commercially available bacteriocin-producing food-grade microorganisms [37,38]

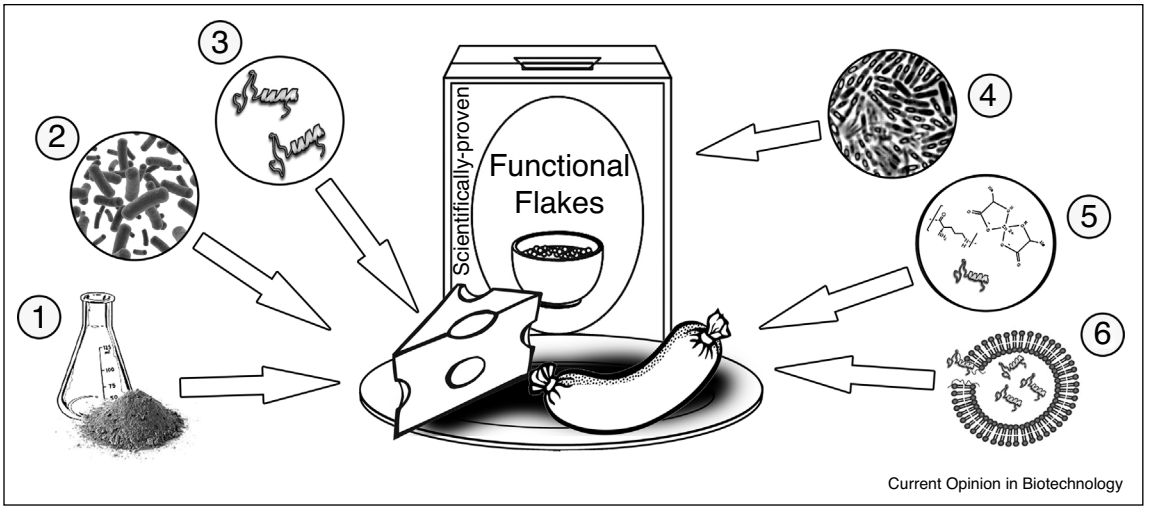
Product name	Microorganism	Bacteriocin	Manufacturer	Application
BioSafe™	*Lactococcus lactis* subsp. *lactis* BS-10	Nisin A	Chr. Hansen	Cottage, feta, and ripened cheeses, prevention of late blowing and off-flavors due to clostridia
HOLDBAC™ (formerly 'Bio Profit' by Valio, with same species but different strains)	*Propionibacterium freudenreichii* subsp. *shermanii* DSM 706 and *Lactobacillus rhamnosus* DSM 7061	Undefined bacteriocins – see US20150150298 A1, publication date: June 4, 2015	Dupont Nutrition Biosciences Aps	Inhibition of mold and psychrotrophes in cottage cheese
Bactoferm™ F-LC	*Pediococcus acidilactici*, *Lactobacillus curvatus* and *Staphylococcus xylos*	*L. curvatus* is producing sakacin A and *P. acidicactic*i is likely to produce pediocin PA-1/AcH	Chr. Hansen	Control of *Listeria monocytogenes* and as a meat starter
ALCMix1	*Lactobacillus plantarum* and *Staphylococcus carnosus*	Produce plantaricin and carnocin bacteriocins, respectively	Danisco DuPont	Anti-listerial cultures for fermented sausages and cooked ham
Bactoferm™ B-SF-43	*Leuconostoc carnosum*	Leucocin	Chr. Hansen	Control of listeria in vacuum and modified atmosphere stored meat products
Bactoferm™ B-2	*Lactobacillus sakei*	Sakacin	Chr. Hansen	Control of listeria in vacuum and modified atmosphere stored meat products
Bactoferm™ B-FM	*Staphylococcus xylosus* and *L. sakei*	Sakacin	Chr. Hansen	Control of listeria in vacuum and modified atmosphere fresh meat products

One new and emerging potential role for bacteriocins is likely to be aimed at functional foods where bacteriocin-producers will be consumed either with the food or as a health-promoting over-the-counter formulation with the goal of positive modulation of the gastrointestinal (GI) microbiota [47••]. In the first reported experiments, the bacteriocin-producing *Lactobacillus salivarius* UCC118 was able to modify the GI microbiota in diet-induced obese mice, while its bacteriocin-free derivative did not cause the described changes [48].

Conclusion

Bacteriocins are one of many natural defense mechanisms bacteria use to compete against microorganisms in the same environment. Since the first discovery of nisin, many bacteriocins with unique structures and different modes of

Figure 2



Bacteriocins: from simple use to sophisticated targeted applications. 1 — milk or other food-grade bacteriocin-containing fermentate; 2 — bacteriocin-producing protecting microbial culture; 3 — partially-purified food-grade bacteriocin; 4 — bacteriocin-producing active probiotic culture (possibly spore-former); 5 — bacteriocin with synergistically-acting natural-derived antimicrobials; 6 — implementation of controlled delivery systems for improved stability and effectiveness of bacteriocins.

activity have been described, and the genes coding for the production, secretion and immunity of most have been reported. During the last decade, many investigators shifted their focus on bacteriocins for food preservation to the treatment of infections and antibiotic-resistant disease-causing bacteria. This exciting new era of bacteriocin research will undoubtedly lead to new inventions and new applications. With the rapid rate at which genome sequences are becoming available, genome mining becomes easier, and with the latest techniques in gene synthesis and protein expression, we can look forward to novel bacteriocins with very dedicated applications.

Conflict of interest

None declared.

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