Trends in Microbiology

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REVIEWS

Review

Mobilization of Microbiota Commensals and Their Bacteriocins for Therapeutics

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With the specter of resurgence of pathogens due to the propagation of antibiotic-resistance genes, innovative antimicrobial strategies are needed. In this review, we summarize the beneficial aspects of bacteriocins, a set of miscellaneous peptide-based bacterium killers, compared with classical antibiotics, and emphasize their use in cocktails to curb the emergence of new resistance. We highlight that their prey spectrum, their molecular malleability, and their multiple modes of production might lead to specific and personalized treatments to prevent systemic disorders. Complementarily, we discuss how we might exploit prevailing bacterial commensals, such as *Streptococcus salivarius*, and deliberately mobilize their bacteriocin arsenal 'on site' to cure multiresistant infections or finely reshape the endogenous microbiota for prophylaxis purposes.

Highlights

The number of bacterial strains resistant to antibiotics has dramatically increased during the past decades so that some microbes are totally insensitive to current antibiotics.

Small antibacterial peptides known as bacteriocins are widely used for natural intraspecies and interspecies competition and could be exploited for human needs, in medicine or agriculture.

Beneficial features of bacteriocins could allow them to substitute antibiotics or foster their action and might reduce the emergence of resistant strains.

Friendly bacteria of the human microbiota might be mobilized to produce bacteriocins and prevent bacterial infection at the external surface of human epithelia.

The Resurgence of Pathogens

When he discovered penicillin in 1928, the first widely consumed antibiotic, the Nobel Prize laureate Alexander Fleming already espied the threat of multidrug-resistant microorganisms[i]. However, in the late 1960s, many brilliant scientists and doctors boasted that humankind was about to eradicate most types of infectious diseases thanks to antibiotics [1]. The net result of more than 60 years of misuse in animal feed or within the food industry, and clinical overprescription, is the rise of nosocomial infections and the re-emergence of forgotten or contained diseases [2]. The problem is so critical that the World Health Organization (WHO) recently sounded the alarm and drew up the list of high-priority pathogens problematic for their antibiotic resistance[ii]; these pathogens include the Gram-positive bacteria *Staphylococcus aureus* (MRSA and VRSA for methicillin- and vancomycin-resistant *S. aureus*, respectively), enterococci (VRE for vancomycin-resistant enterococci), and *Streptococcus pneumoniae*. In this perspective, the WHO called for massive investments in research to dodge the current scenario that places infection diseases as the first mortality cause in 2050, beyond cardiovascular disorders and cancers [3].

Contrasting with the need for new drugs, the number of novel antibiotics in clinical trials is constantly declining [4]. Following the 'Golden Age' of antibiotics (1950–1960), biotech and pharma companies neglected to fund new projects, mainly for reasons of patent protection lost and high development costs, and steered their research toward bankable products dedicated to chronic therapy [5]. This void leaves a market niche for small start-up companies to undertake novel approaches and identify a new generation of antimicrobial compounds.

The goal of this review is to showcase bacteriocins as a credible alternative to antibiotics, drawing the list of their comparative effects and benefits. We also discuss how we could mobilize the bacteriocin-producers of our endogenous **microbiota** (see Glossary) to restrain bacterial infections.

Bacteriocins as a Backup Plan for Traditional Antibiotic Treatments

In intricate and overcrowded environments, microorganisms doggedly compete with each other for territories and nutrients and therefore have developed a plethora of defense mechanisms [6].

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Among them, bacteriocins are currently considered as the most widely distributed mechansim [7]. By definition, bacteriocins are secreted, ribosomally synthesized peptides, of prokaryotic origin, which have antibacterial properties. Usually, they are associated with immunity protein (s) encoded in the genome. They gather disparate proteinaceous toxins with various modes of action, typically membrane permeability and cell wall damaging, but also metabolic pathway interference. Due to low sequence homology, and depending on the considered features (e.g., function, sequence, or producer host), bacteriocins are controversially categorized into four classes [8,9].

Class I bacteriocins include proteolysis- and heat-resistant small peptides substantially modified by specific enzymes at the post-transcriptional level; they include **lantibiotics**, sactipeptides, glycocins, and lasso peptides. According to their structure and function, this class is subdivided into type A and type B.

Class II bacteriocins are subdivided into (at least) four subtypes (pediocin-like, two-peptides, circular, and nonpediocin-like linear); they comprise temperature- and pH-resistant small peptides with no or minor post-transcriptional processing such as disulfide bonds.

Class III bacteriocins – bacteriolysins – incorporate the large heat-labile proteins (>10 kDa).

Class IV bacteriocins consist of lipid- or carbohydrate-conjugated complex proteins.

Although their discovery by André Gratia preludes penicillin [10], bacteriocins were marginalized for human needs and mainly confined to biopreservative applications [11]. One of the rare widely used molecules in this category is nisin, a broad-spectrum lantibiotic that is produced at the industrial level for food preservation under the E234 European denomination since the 1970s [12]. Initially isolated from lactic acid bacteria, its biosynthesis and mode of action are extensively documented [13]. Nisin targets the lipid-anchored precursor of peptidoglycan (lipid II), resulting in a loosened peptidoglycan meshwork, and ultimately in the leakage of cytosolic components by the formation of pores. Currently, nisin is of interest for clinical applications, specifically for local and external treatments. Several reports highlight the *in vitro* effects of nisin on multiresistant priority enemies [*S. aureus* (MRSA), enterococci, mycobacteria, and streptococci] and secondary pathogens such as *Cutibacterium acnes*, *Mycobacterium smegmatis*, and species of *Bacillus* and *Clostridium* (even against resistant forms such as spores) [12,14], as well as the *in vivo* modulation of animal microbiota when nisin is orally administered [15,16]. Beyond its antibacterial properties, nisin affects fungal populations (e.g., *Candida albicans*) and reduces tumorigenesis in cell line and mouse models [12].

Given their anti-**persister** [17], bactericidal, or bacteriostatic effects, bacteriocins might be (re) considered to incrementally furnish our arsenal against bacterial pathogens, especially those that are omni-resistant, with no last-resort curative drugs. Moreover, the surface bacterial infections usually rely on a biofilm *modus operandi* that better resists aggressive treatments. The strong antibiofilm properties of several bacteriocins might be used to dismantle the biofilm structure or prevent adhesion and biofilm formation [18]. Alternatively, they might potentiate antibiotics if used in combination, as shown for nisin [19–21]. The benefits of bacteriocins rely on six pillars: spectrum, stability, bioengineering, diversity, production, and safety (Table 1).

Prey Spectrum

The broad host range inherent in most antibiotics usually provokes drastic collateral effects on the whole microbiota (skin, digestive, urogenitory, and respiratory tracts), generating an imbalance in microbial populations. In extreme cases, they create a persisting **dysbiosis**, opening the door for diabetes, obesity, and cardiovascular, inflammatory, atopic, and immune disorders [22]. Several

Glossary

Commensal: resident microorganisms of the microbiota, harmless for the host's health.
Competence: refers to a transient physiological state of bacterial cells that allows foreign DNA acquisition.
Cytotoxicity: deleterious effect on eukaryotic cells provoked by biological or chemical compounds.
Deep sequencing: next-generation sequencing technology to collect billions of nucleotide reads at a high-throughput scale.
Dysbiosis: deleterious imbalance in microbiota populations that can be provoked by broad-spectrum antimicrobials, resulting in severe systemic disorders.
Immunity: refers to a bacterial protein or structural component that prevents or dampens the toxic effect of a bacteriocin.
Lantibiotic: post-translationally modified bacteriocin characterized by the presence of a high proportion of unusual and dehydrated amino acids.
Metagenomics: collection of genes or genomes sequenced from environmental samples. These approaches study the communities of microorganisms as a whole.
Microbiota: community of microorganisms naturally dwelling on host epithelia. They colonize surfaces to prevent the establishment of harmful microbes, challenge the immune system, and process some nonmetabolizable molecules for the host.
Persister (cell): dormant form of an antibiotic-sensitive bacterium that is recalcitrant to antibiotic treatment.
Pheromone: molecule secreted by a living organism to inform surrounding kin or friend cells about a specific concern.
(Pan-)predatiome: set of antimicrobial compounds actively secreted by a cell (or the cells of a microbial community) to hinder growth of surrounding specific organisms.
Predation: refers to a defensive/attacking behavioral strategy of a bacterium to kill competitors.
Probiotic: a live microbial strain provoking a beneficial effect on health.

Table 1. Properties of Bacteriocins vs Antibiotics

Characteristic	Bacteriocin	Antibiotic
Stability (e.g., T° and pH)	High for class I and II	Low
Environmental resilience	Low	High
Molecular diversity	>800	~150
Cytotoxicity	Low	Low to high
Bioflexibility (engineering)	High	Low
Production:		
Fermentation	Possible	Possible
Chemical synthesis	Easy for class II	Difficult
In vitro/ex vivo	Easy for class II	Difficult
In vivo mobilization	Possible	Difficult
Prey spectrum	Broad and narrow	Usually broad

bacteriocins also exhibit a broad (and even transphylum) spectrum (e.g., subtilosin A, amyloliquefacin RX7, lacticin 3147, pentocin MQ1, salivaricin B, garvicin ML, or nisin, mentioned above). However, a significant proportion of the bacteriocins have a narrow spectrum of activity that is related to their phylogeny or their ecology, such as thuricidin CD, sakacin A, plantaricin, and lactococcin A [9,23,24]. As a metaphor, the broad-range compounds act as nuclear bombs, leaving a 'half-naked ground' exposed to colonization by infectious bacteria. In contrast, a narrow-spectrum antimicrobial drug is similar to a surgical strike that specifically targets the menacing pathogenic populations and limits the distortion of the ecosystem homeostasis. Superimposed, this kind of specialized treatment places less selective pressure on autochthonous and peaceful bacteria, minimizing the global enrichment of resistant strains/clones and thence the propagation of resistance genes to pathogens. In the **deep sequencing** and **metagenomics** era that affords high-speed and low-price analyses at the quantitative and qualitative level, 'sniper' bacteriocins might be exploited for personalized and protective medicine/solutions. Dynamically accessing the microbiota composition and tracking real-time lineage rebalancing could be done in the future using these tools.

Stability

At the physicochemical level, bacteriocins are typically less labile than antibiotics and can support high temperatures and extreme pH [25]. In this regard, the stability is directly related to the diverse structure of bacteriocins and to the level of post-translational modifications (cyclization, disulfide bridges, and nonconventional amino acids). On the other hand, due to their peptide backbone, bacteriocins could be sensitized to proteases compared with chemically-based antibiotics. Thus, they would display a lower biological half-life in nature and organic environments. This is considered as an asset to dampen the emergence of resistance, since low remnant and sublethal concentrations of toxins are mutagenic and favor the progressive acclimation of an initial clonal population [26].

Molecular Amenability

Rational modulation or conception of *de novo* chemical compounds to expand our antibacterial arsenal is a costly and long-lasting route with an unpredictable chance of success. The genetic basis of bacteriocins might alleviate the barriers of the low-throughput chemical approaches. With successive randomized mutational screens, bacteriocin sets are likely to be optimized without any preconception. Therefore, peptide variants could be biocrafted regarding their efficacy, stability, **cytotoxicity**, or spectrum for specific medical or biotechnological applications (Figure 1). For instance, besides the natural nisin variants (A, Z, F, Q, H, U, U2, and P) that harbor

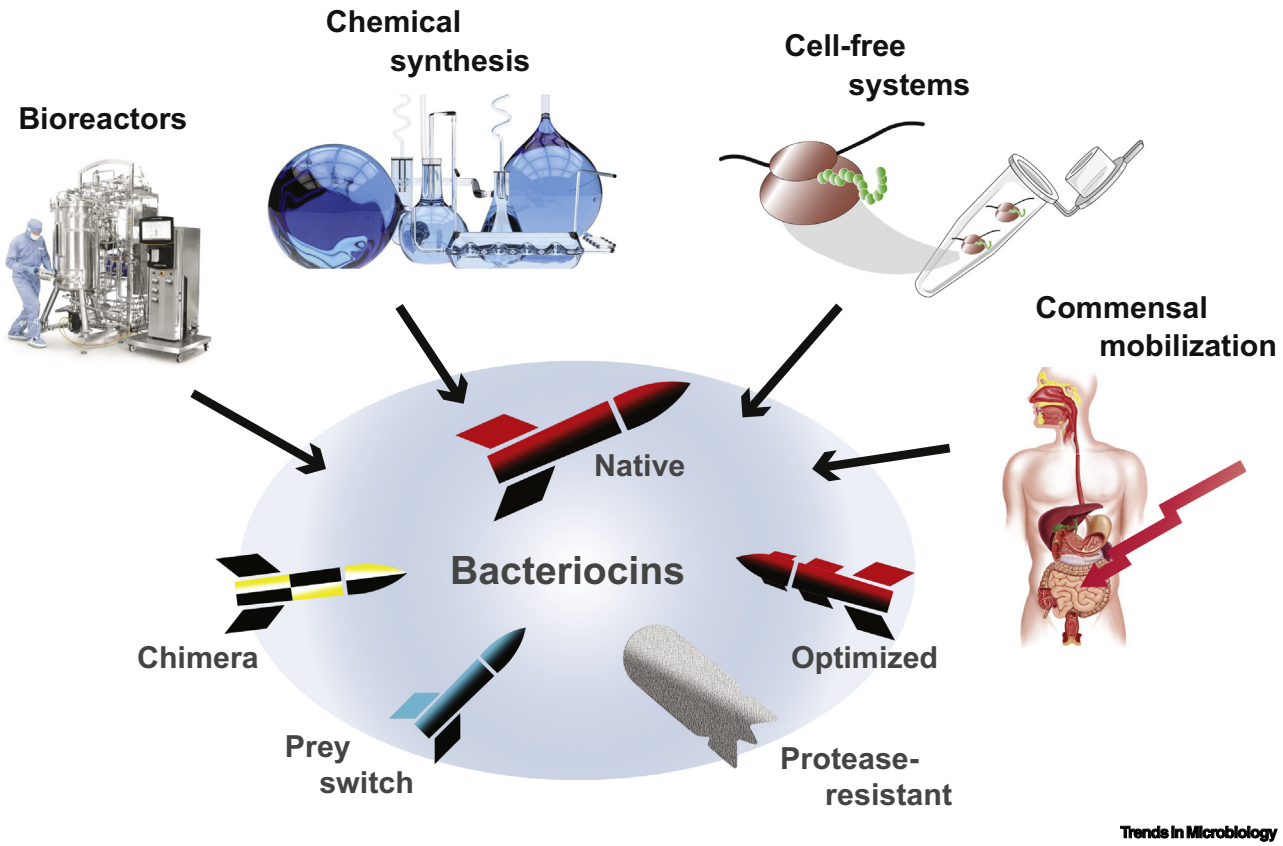

Figure 1. Bio- and Chemically-based Production of Tractable Bacteriocins. Current and past advances in biomolecule production substantially expanded the way to synthesize active bacteriocins (missile cartoons). The ancestral (and potentially obsolete) purification/extraction methods from fermentation processes (bioreactors) is now in competition with *in vitro* (chemical synthesis), *ex vivo* (cell-free systems), and *in vivo* mobilization (commensal bacteria) strategies. This panoply of available techniques, combined with the high molecular plasticity of bacteriocins, will lead to high-throughput bioengineering. Ultimately, bacteriocins will be remodeled to manufacture variants that are more robust (stability or efficacy), with different prey-spectrum, bifunctional, and/or protease-insensitive characteristics. The red arrow depicts the 'on site' induction of bacteriocin secretion from endogenous populations of producers. The cell-free systems are schematized by an mRNA (black line) trapped in a ribosome machinery producing a nascent peptide.

slightly different stability, diffusion, and solubility, several studies have reported bioengineered mutants of nisins A and Z with modulated pharmacokinetic properties [12] or that dodge bactericidal resistance [27]. Such approaches will allow exploration of a range of peptide sequences that would be counterselected *in vivo* due to intrinsic (production and secretion by the host) or extrinsic (e.g., external pH, temperature, and protease environment) constraints. Finally, the modular nature of bacteriocins has been used to design chimeric bifid molecules and generate dual bacterial killers [28].

Diversity

Up to now, the medical and veterinary fields have at their disposal less than 150 different antibiotic molecules[iii]. According to the BAGEL database that collects data-mining and experimental data, the number of indexed bacteriocins has increased above 800 units [29]. However, it does not take into account all natural variants of individual bacteriocins, while novel stand-alone bacteriocin genes are difficult to detect, especially if they are not in close vicinity of genes coding for **immunity**, transporter, or modification enzyme. For instance, the bacteriocin databases only pinpointed a fifth of effective class II bacteriocins in *S. salivarius* [30]. Therefore, we can reasonably predict that this number is a large underestimation of the genuine bacteriocin panoply.

Low Cytotoxicity

Several antibiotics exhibit damaging collateral effects on human health. Conversely, bacteriocins are assumed to have low or no cytotoxicity [31], mainly because tested bacteriocins originate from lactic acid bacteria that have long been used in fermentation and dairy products as biopreservatives, and the healthy human digestive tract is hypercolonized by bacteriocin-proficient **commensal** strains. For instance, class II bacteriocins, nisin, and other lantipeptides, have been proved to be cytoneutral toward various eukaryotic cell lines [32], even at doses 100-fold higher than the saturated killing concentration [33]. Some studies reported cytotoxic effects for bacteriocins such as cytolysins [34], microcin E492 [35], or the antimicrobial peptide P40 [36]. Therefore, we will need a case-by-case survey of cytotoxicity for each single bacteriocin (or in combination) that will be administered into or onto the human body. However, from a different angle, cytotoxic bacteriocins might be exploited as an antitumor strategy since they preferentially insert into negatively charged membranes of cancer cells [37,38]. Moreover, bacteriocins toxic to eukaryotic cells are worthy of interest considering that peptide-based antimicrobials can be engineered to minimize their cytotoxicity while maintaining their bactericidal effects [39].

Production

So far, the canonical way to manufacture commercial bacteriocins is by batch fermentation from a natural producer strain (Figure 1). Such a one-at-a-time method needs to be specifically optimized for each produced molecule, while the inherent toxicity of bacteriocins curbs *in vivo* production, even in immunity-proficient bacteria. In the case of nisin, the yield strongly relies on physicochemical properties of the medium, and numerous residual components of the initial medium impinge on efficient purification [40].

The current advances in chemical synthesis [41] have cleared the path to a high level of production of bacteriocin-based antimicrobial compounds (Figure 1) in agreement with clinical safety requirements (Good Manufacturing Practice). Although they cannot be considered as bacteriocins from a rigorous definition point of view, as they are not ribosomally produced, a C terminal to N terminal *in vitro* assembly is able to produce bioactive molecules. Indubitably, the main advantage is the design of peptidomimetics with side-chain modifications (e.g., crosslinking agent) or encompassing exotic or D-form amino acids to optimize stability and/or proteolysis insensitivity. Again, the small nonmodified bacteriocins (class II) are particularly appropriate in this context. Several glycosylated or circular (head-to-tail or disulfide-bridged) bacteriocins can be synthesized at low yield [41]. Concerning class I bacteriocins, an option might be to synthesize the peptide precursor followed by an incubation with purified modification enzyme(s).

Beside the heavily processed bacteriocins, the second current pitfall of chemical synthesis is the long and/or hyperhydrophobic peptide motif that can self-aggregate, hindering the elongation steps. To circumvent this, cell-free protein synthesis, developed 60 years ago, is a tantalizing production alterative [42] (Figure 1). Again, class I bacteriocins appear cumbersome with such an *ex machina* method, except if the peptide precursor and processing enzyme(s) are coproduced in reconstituted transcription–translation systems (*in vitro*) or if the peptide precursor is incubated in a producer strain cell lysate (*ex vivo*).

Finally, bacteriocin secretion has been demonstrated as an essential factor for digestive tract colonization through intraspecies and interspecies competition [43,44]. A strategy would be to stimulate bacteriocin production in indigenous populations of the microbiota via activating signal molecules (Figure 1). Alternatively, **probiotic** blends of bacteriocin-producing strains might be orally administered [45]. In both scenarios, the rational is to promote the development of commensal subpopulations and protect the digestive tract from invasive strains, ultimately re-

equilibrating the microbiota with beneficial bacterial populations, while preventing dysbiosis generated by broad-host-range antibiotics [22].

S. salivarius and Microbiota as a Solution against Emerging Pathogens

Efficient probiotics must feature four *sine qua non* characteristics: a substantial antipathogen activity, a noncytotoxic effect, a high prevalence in the human population, and a mild-to-strong persistence in/on the human body. *S. salivarius* appears to be a promising species for probiotic use. First of all, several studies identified bacteriocinogen strains of this species with high potency against infectious pathogens [30,46,47]. Their prey spectra are diverse and presumably reflect their bacteriocin gene content. For example, the strains HSISS4, YU10, or NU10 inhibit bacteria closely related to priority pathogens, such as *S. aureus* or enterococci, and etiological agents provoking important or common health issues (e.g., *Streptococcus pyogenes*, *Streptococcus mutans*, *Listeria monocytogenes*, and *Micrococcus luteus*) [30,47]. Perhaps the BLIS (bacteriocin-like inhibitory substances) cocktail released by the commercialized strain K12™ is the most effective poison produced by *S. salivarius*. Besides the targets mentioned above [47], BLIS hinders the *in vitro* growth of bacteria responsible for pneumonia (*S. pneumoniae*; mid-priority target) [48], vaginal colonization (*Streptococcus agalactiae*) [49], otorhinolaryngeal infection (*Moraxella catarrhalis*) [50], and diphtheria (*Corynebacterium diphtheriae*) [50]. It was also proposed that *S. salivarius* could reduce acne by inhibiting *C. acnes* [51].

The *S. salivarius* population is dominant and is genetically diverse in the human digestive tract [52,53]. Initially considered as restricted to the upper part of the tract, this commensal also dwells in the lumen of the small intestine and, to a minor extent, in the colon [54,55]. Shortly after delivery, *S. salivarius* is acquired by infants from breast feeding and pioneers in the mouth will serve as a reservoir for the gut [56]. Widespread in the healthy human population [57–59], an appropriate *S. salivarius* population balance in the microbiota is assumed to contribute to nutritional health [60], and to biomark disorders since some childhood obesities are associated with a reduction in *S. salivarius* abundance [61]. In the competitive mouth ecosystem, *S. salivarius* incorporates into the incipient pellicle that covers tooth enamel and coaggregates with other periodontal microbes at each step of colonization: with the primosettlers *Veillonella* and *Prevotella* spp., the intermediate settlers *Fusobacterium nucleatum* and *Candida albicans*, and the late settlers *Tannerella forsythia* and *Porphyromonas gingivalis* [62,63].

Up to now, there has not been a report about a potential toxic effect of *S. salivarius* on human cells. On the contrary, superimposed on its specific antibacterial properties, *S. salivarius* stimulates multiple beneficial aspects for human health. The strains K12™ and M18™ have been administered for prophylactic or probiotic treatments for reasons that go beyond mere bacteriocin production [45,48]. In clinical trials, they reduced dental plague [64] and protected against recurrent episodes of streptococcal pharyngitis, tonsillitis, or otitis, with the suggestion that effective antibiotic absorption doses might be reduced [65,66]. By adhering to various human epithelial cells [67], *S. salivarius* colonizes and persists on oral or vagina mucosa, promoting re-epithelization [68] and excludes settlement of virulent bacteria such as *S. agalactiae* or *S. pneumoniae* [48,49]. Moreover, residence of strain K12™ on bronchial epithelial cells is also suspected to dampen the proinflammatory response. Indeed, this strain downregulates the NF-κB pathway, the secretion of the proinflammatory cytokine interleukin (IL)-8, and the expression of host genes that promote bacterial adhesion [69]. Finally, halitosis (malodor) has large social and economic implications and typically correlates with periodontitis (organic pathology). The strain K12™ is active against bacteria involved in this inconvenience, decreases the level of volatile compounds from breath and therefore improves oral hygiene [70]. Altogether, these concrete examples shine a light on the positive multifactorial implications of *S. salivarius* on host health.

The *S. salivarius* Pan-predatiome

Canonically, living organisms maximize their rate of prosperity by releasing a heterogeneous set of molecules bioactive against bellicose non-self entities. This notion of a **predatiome** includes fratricide behaviors that prevent surrounding kin microbes from consuming all vital resources. *S. salivarius* uses the same strategy by secreting a cocktail of bacteriocins, each endowed with specific chemical and biological properties. However, the genomic content of bacteriocin genes is highly variable from one strain to another. As several *S. salivarius* strains are naturally transformable, and therefore able to perform intraspecies exchanges of genomic DNA [30,71], a large repertoire of antimicrobial compounds scattered in different organisms (**pan-predatiome**) may ensure a reshuffling of antibacterial weapons in hostile environments.

Salivaricin A (SalA)

Categorized into six subtypes (SalA–SalA5), salivaricin A is a class I lantibiotic that kills *S. pyogenes*, *S. pneumoniae*, *Corynebacterium* spp., and *M. luteus* strains, potentially by binding to lipid II [72]. Akin to nisin, it is an autoinducing signal molecule that upregulates its own production [73]. Even if the *salA* locus is (partially) defective with no expression of killing molecules, salivaricin A is probably the most widespread bacteriocin in *S. salivarius* species. A reason might be that the immunity protein SalY (membrane-spanning subunit of a putative efflux pump) could cross-protect against eukaryotic cationic peptides [74].

Salivaricin B (SboA)

Salivaricin B is a broad-spectrum polycyclic lantibiotic, usually encoded on a megaplasmid in the close neighborhood of the salivaricin A2 gene [75]. Due to a high plasmid instability in bacteriocin repression conditions, both salivaricins are supposed to positively drive the maintenance of megaplasmid-bearing strains in the biotope [46]. Surprisingly, salivaricin B has no associated immunity gene and does not dissipate the membrane potential but rather interferes with cell wall biogenesis by aberrant accumulation of precursors [76].

Salivaricin D (SlvD)

As a highly potent lantibiotic, salivaricin D is resistant to proteases such as trypsin and has a broad spectrum of sensitive strains, including *S. pyogenes*, *S. pneumoniae*, *Clostridium* spp., and *M. luteus* [77]. Beside sensory, modification, and transport proteins, the *slvD* locus (12 genes split into two operons) encodes four immunity proteins and a second nisin-like precursor (SlvN) that is potentially active.

Salivaricin E (SrnA)

SrnA is a lantibiotic encoded on a megaplasmid with two other highly similar variants (SrnA′ and SrnA″). Akin to salivaricin D, it is associated with dedicated sensory, modification, and transport proteins. Salivaricin E is active against *S. mutans* [78].

Salivaricin G32 (SlnA)

This nisin-like lantibiotic is close to the bacteriocin SA-FF22 produced by *S. pyogenes* and is active against *Listeria* spp., *Enterococcus* spp., *Leuconostoc* spp., *M. luteus*, and *S. pyogenes* [79].

Salivaricin 9 (SivA)

The *sivA* gene is part of an eight-unit operon (including genes for sensor and transport systems, the precursor peptide, and the modification enzyme) present on a megaplasmid. Probably autoinducible, salivaricin 9 is a class I lantibiotic that mainly permeabilizes membranes of *S. pyogenes* and some other enterococci [80,81].

Streptin (SrtA)

Streptin is a broad-host-range lantibiotic [82].

Blp Cocktails (BlpK, SlvV-Z)

While lantibiotics are usually borne on (mega)plasmids, *S. salivarius* typically harbors a main chromosomal *blp* (bacteriocin-like peptide) locus completed by several secondary loci that code for

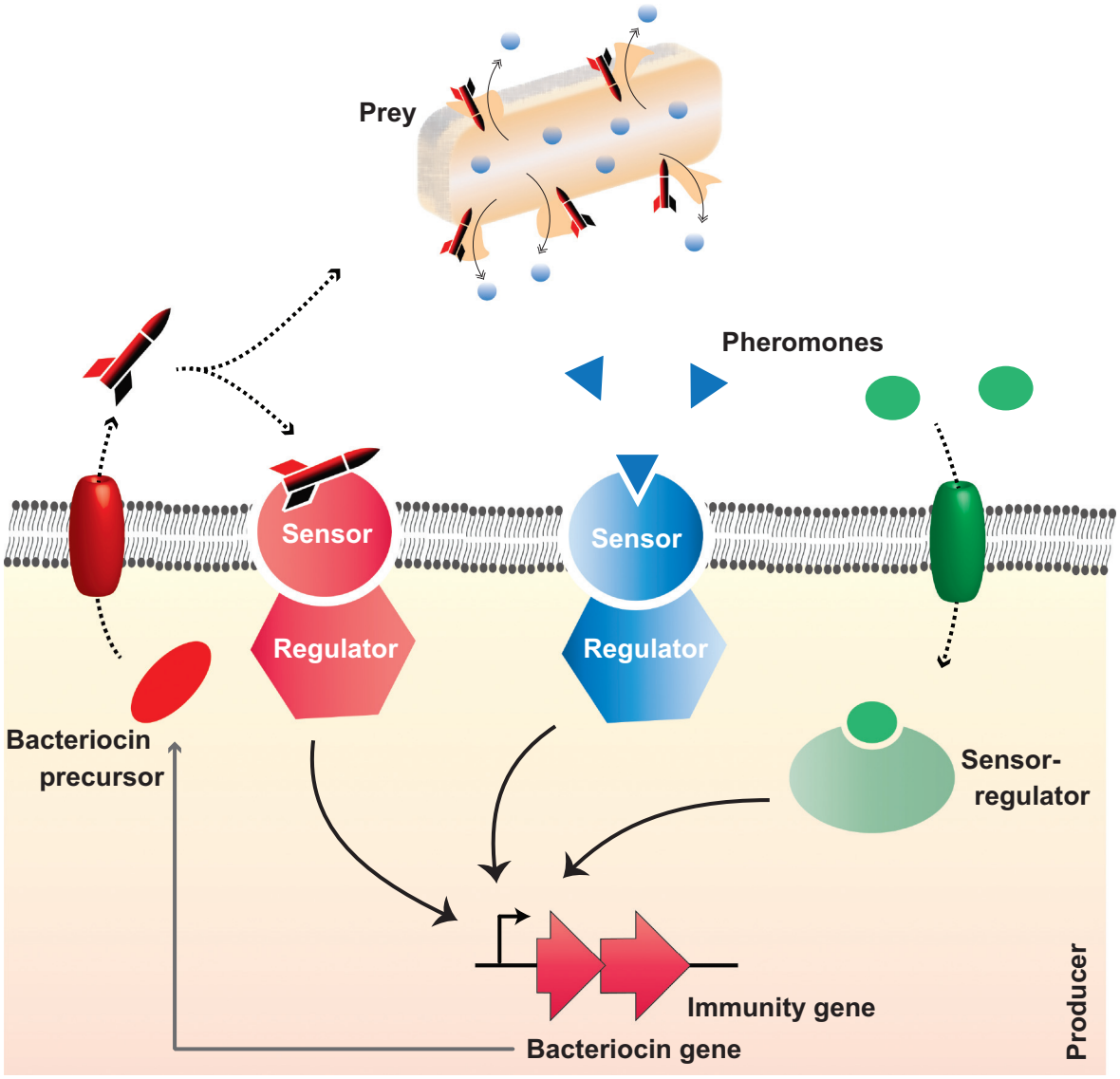

Figure 2. Pheromone-based Mobilization of Bacteriocins. Generic regulatory network (unbroken arrows) for bacteriocin secretion. Bacteriocin precursors (red ovoid shape) are synthesized intracellularly in the producer cell. Next, a specific complex translocates them through the plasma membrane and finalizes their processing/maturation into effective bacterial killers (missile cartoons). Canonically, the toxic effect is associated with pore formation in the cell envelop of surrounding prey bacteria and leakage of cellular constituents (blue balls). Active bacteriocins can auto-regulate their own production (red pathway). They bind and activate a membrane histidine kinase (sensor; red circle) of a two-component system that conveys the information to a response regulator (red hexagon). Alternatively, bacteriocin production could be under the control of quorum-sensing mechanisms. In this context, a signaling pheromone either activates a membrane-bound two-component system (blue pathway) or is internalized and interacts with an intracellular regulator of the ComR/RRNPP family (green pathway). All turned-on regulators directly occupy bacteriocin gene promoters to positively drive gene transcription.

class II salivaricins with a double-glycine maturation site. In strain HSISS4, these *S. aureus*-killer bacteriocins are coinduced, and each individual bacteriocin takes part in the poisoning effect, even though BlpK and SlvX make the major contribution [30].

Predation Cues in *S. salivarius*

At the ecosystem level, it is assumed that it is counterproductive to be hyperaggressive [83], and extruding bacteriocin is costly and partly autotoxic from the producer standpoint. As a result, bacteriocin production is usually finely regulated (Figure 2). *S. salivarius* lantibiotics are co-encoded with sensory two-component systems that often autoregulate their own abundance, arguing for a nonconstitutive accumulation strategy of attacking molecules. However, the inducing conditions are species-specific and are mostly poorly understood, meaning that bacteriocin production in administered probiotic strains remains erratic and dependent on environmental cues. In contrast, Blp cocktail secretion is synchronized through a well-documented quorum-sensing mechanism [30,84]. *S. salivarius* and its close cousin *Streptococcus vestibularis* aside, the rest of the *Streptococcus* genus coordinates *blp* expression via a membrane sensor system composed of the two-component system BlpRH and the activating **pheromone** BlpC [84]. Notably, the BlpRH activity is directly or indirectly modulated by committing regulators of **competence** (a transient physiological state that allows acquisition of exogenous DNA) in order to connect **predation** to genetic evolution. Surprisingly, in *S. salivarius*, the BlpRHC module is decayed or missing. As a substitute, the cytoplasmic cell–cell communication sensor ComR exerts the direct Blp control [30]. Given that ComR complexes with the short pheromone ComS [85], and directly activates the master regulator of competence (ComX) at the transcriptional level [86], this genetic circuitry intricately couples competence and predation. Presumably, the concomitant or preliminary release of bacteriotoxins ensures the provision of DNA originating from killed cells in the close environment of bacteria capable of internalizing it.

With such a communication mode, we might envision that the pheromone receptors could be hijacked and bacteriocin mobilized *in vivo* on demand in dominant commensals of the microbiota, in a similar way to how it was performed *in vitro* [30]. Providing formulated preparations, supplemented with an activating molecule, might clean oral and/or intestine epithelia from specific destabilizing virulent microbes, while the cleared landscape would be subject to recolonization by the bacteriocin producer. This strategy is particularly interesting for short nonmodified peptide signaling molecules resembling ComS that are supposed to resist most digestive proteases and the stomach barrier. As a complement, indigenous commensal populations could be bolstered with probiotic strains or fecal transplants responsive to analogous easy-to-handle pheromones.

Concluding Remarks

Facing the urgency of finding solutions to the decline in antibiotic efficacy, the underexploited potential of bacteriocins might be raised as a sustainable solution, either to replace antibiotic molecules or to restore their past potency (see Outstanding Questions). Their particular properties, and the flexibility in their production methods (Figure 1), are a tremendous asset for use in medical, agricultural, and biotechnological applications (Figure 3). A comprehensive vision of spectra, and the use of narrow-range bacteriocins that specifically target undesired microbes, will lead to efficient personalized medicine and tend to minimalize collateral systemic disturbances provoked by nonnatural treatments.

At the human health-care level, a precise evaluation of the efficacy, the stability, and the incorporation kinetics in/on the human body is absolutely needed to contemplate bacteriocins as serious therapeutics, whereas cytotoxic or immunogenic bacteriocins should be cautiously discarded. Currently, there is a very limited number of bacteriocins (or bacteriocin-based probiotics) selected

Outstanding Questions

Why are bacteriocins underexploited for human needs while antibiotic use is widespread?

What will be the impact of bacteriocin treatment on the microbiota equilibrium?

How will the microbiota be shaped when bacteriocin-producing strains are artificially mobilized?

How genetically flexible are bacteriocins to generate more stable, more efficient, and inescapable killing variants?

Will novel production modes (chemical synthesis, cell-free extracts) allow economical and sustainable development of bacteriocins?

What is the relationship between prey spectrum and ecological niche?

Is it possible to design mutant or chimeric bacteriocins to modulate the prey spectrum?

Will the use of bacteriocins substantially sap the emergence of multidrug-resistant bacteria?

What will be the resilience and diffusion of bacteriocins in/on the human body or treated environments?

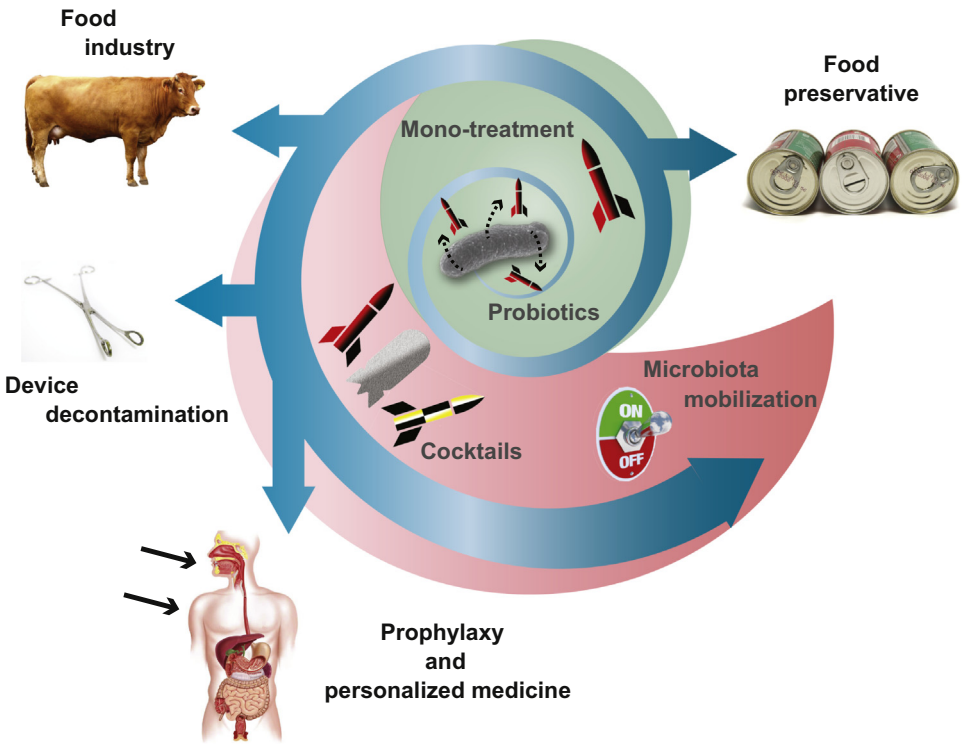

Figure 3. Timeline of Bacteriocin Utilization for Human Needs. Spiral-shape scheme of bacteriocin-based treatments applied for human food and health. For 70 years, living bacteriocin producers (probiotics) and single bacteriocins (mono-treatments) have been used for food preservation or to counteract the misbalance of microbial populations in the human digestive tract. In the future, bacteriocins alone or in rational combinations (cocktails) might be exploited to externally and locally cure multidrug-resistant infections or microbiota disorders. Additionally, they could also be used in the veterinary field, in the food industry, or to sterilize contaminated medical devices. Ideally, we might mobilize 'on demand' the endogenous populations of the microbiota via inducing molecules to finely re-equilibrate the microbial ratio and prevent systemic dysbiosis. The green circle pinpoints the achieved developments, while the red shade heralds future prospects.

for tests in (pre)clinical trials [87]. Pursuing and intensifying the clinical studies on promising bacteriocins will enhance the success rate for medical applications.

In addition to the benefit from the chemically bioactive nature of bacteriocins, we might requisition the poisoning skills of local populations that are pre-established or provided on biological surfaces (e.g., skin, vagina, oro-gastrointestinal tract) to maintain or reinstate the microbiota homeostasis. Moreover, stable and economically relevant pheromones would be a molecular on/off remote control that robustly activates bacteriocin production. However, identification of signal molecules will be required to desynchronize predation from competence, as both phenomena are usually entwined. Indeed, a simultaneous activation of gene-capture processes could propagate virulent or antibiotic-resistance traits in the producer populations.

To conclude, the emergence of resistance to toxins is inherent in evolving organisms, and it would be naive to reckon that bacteriocins are the exception to the rule. Nonetheless, the intrinsic characteristics of bacteriocins (e.g., narrow spectrum or low environmental remanence), combined with rational consumption by humans and livestock, is likely to delay the emergence of resistance.

The number of exploitable bacteriocins will increase over time and favor the design of cocktails that could attack the prey from diverse molecular angles to drastically reduce the probability of resistant clone outbreaks. This integrative strategy with next-generation antimicrobials might ensure an optimal bacterial containment in our war against pathogens.

Acknowledgments

Funding support was from FNRS (Belgium) and IUAP (Belspo Belgium) grants to P.H. The Walloon Region (Belgium) subsidized Syngulon through a RPR (Responsable Projet de Recherche) grant to hire J.M. P.H. is Senior Research Associate at FNRS (Belgium).

Disclaimer Statement

J.M. and P.G. are currently employed by and cofounder of the Syngulon company, respectively. The authors declare that they have no other conflict of interests.

Resources

[i]www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-lecture.html

[ii]www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

[iii]https://en.wikipedia.org/wiki/List_of_antibiotics

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