


REVIEW



Gut microbiota as a source of novel antimicrobials

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ABSTRACT

Bacteria, Archaea, Eukarya and viruses coexist in the human gut, and this coexistence is functionally balanced by symbiotic or antagonistic relationships. Antagonism is often characterized by the production of antimicrobials against other organisms occupying the same environmental niche. Indeed, close co-evolution in the gut has led to the development of specialized antimicrobials, which is attracting increased attention as these may serve as novel alternatives to antibiotics and thereby help to address the global problem of antimicrobial resistance. The gastrointestinal (GI) tract is especially suitable for finding novel antimicrobials due to the vast array of microbes that inhabit it, and a considerable number of antimicrobial producers of both wide and narrow spectrum have been described. In this review, we summarize some of the antimicrobial compounds that are produced by bacteria isolated from the gut environment, with a special focus on bacteriocins. We also evaluate the potential therapeutic application of these compounds to maintain homeostasis in the gut and the biocontrol of pathogenic bacteria.

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1. Introduction

Antimicrobials are compounds that kill or inhibit the growth of microorganisms. The availability of antimicrobials, and antibiotics in particular, has improved the quality of life and increased life expectancy. However, antibiotic resistance has become a major threat. Indeed, since the golden era of antibiotic discovery (1940–1960), when many compounds were discovered, especially from soil-derived actinomycetes, few new classes of antibiotics have been discovered, and the majority of new compounds have resulted from chemical modifications of existing ones. In an effort to address this challenge, different approaches have been proposed, like the use of discovery platforms,¹ but there has been insufficient investment globally in developing novel procedures/techniques to identify new antimicrobials, and the characterization of these compounds thereafter.

Traditionally, the production of antimicrobials has been considered as an expression of competition and antagonism in bacteria, both inter- and intra-species, whose niches overlap.² However, it should also be noted that some antimicrobials, and antimicrobial peptides in particular, might have a social purpose, serving to

facilitate signaling between individual strains, although not necessarily an indication of cooperation.³ Antimicrobial activity has been proposed to promote biodiversity, as reflected by the differences in metabolic input of antimicrobial producers, sensitive and resistant species and in the ecological fitness that these bacteria show in a shared environment.^{4,5} More specifically, production of antimicrobials might confer an advantage to the producer against sensitive bacteria, but this production is the result of a metabolic effort, as is the maintenance of immunity among resistant bacteria, which requires less metabolic input than antimicrobial production, but more than that of sensitive bacteria. This has been referred to as the rock-scissors-paper (RSP) dynamic game that ultimately leads to a balanced co-existence in microbial communities.^{4,5} Different studies, both theoretical and experimental, have been carried out to understand the dynamics of the microbial communities and the role that antibiotics play in this context. These considerations further support the premise that natural sources containing a wide diversity of microorganisms continue to be an underexploited source of new antimicrobials that can be identified and developed at an enhanced rate with the aid of new ‘omics’ technologies,

computational advances and development of new analytical tools.⁶

The human microbiome represents an excellent example of such an environmental niche and contains trillions of microorganisms that have co-evolved with the human host, leading to the view that humans can be considered to be super-organisms, constituted by a vast number of symbiotic relationships. Arguably, the most important microbial communities in humans can be found in the GI tract (the colon is estimated to contain over 70% of all the microbes in the human body⁷), in the mouth, skin, and urogenitalia. Microbial communities can be also found in, for example, lungs, breast milk and eyes.⁸ These communities are dynamic and change over time, due to characteristic processes of ecological succession, where microbial communities are substituted by new ones and can be affected by changes of environmental conditions. In the GI tract, the first microbial species that are established in infants are succeeded by new species to form a more mature and complex community and the balance can be altered by the use of antibiotics, dietary changes or introduction of exogenous pathogens.^{9,10}

As the human microbiome is such a dense and diverse compilation of different microbial ecosystems that has not yet been fully explored, it is not difficult to regard it as a promising source of novel metabolites. In 2015, Donia and Fischbach reviewed the chemical spectrum of metabolites produced by the human microbiota and concluded that its diversity was as broad as any other microbial ecosystem, but that these metabolites were highly specific for interacting within the human host.¹¹ More recently, Mousa et al¹² (2017) extensively reviewed the same topic, highlighting the variety of specialized metabolic compounds produced by this human microbiota and the effects within the human host. These metabolites include lipids and glycolipids, oligosaccharides, terpenoids, polyketides, amino acids, non-ribosomal peptides and ribosomally synthesized post-translationally modified peptides (RiPPs). This chemical variety also reflects a variety of functions: immunomodulatory, cytotoxic, antioxidant and, of course, antimicrobial, although the function of many of these compounds is not yet identified. New compounds and functions continue to be discovered and characterized. Among the vast number of relevant papers are two recent examples that have shown that a human nasal commensal produces a novel peptide antibiotic that is able to limit *Staphylococcus aureus*

colonization and maintain homeostasis¹³ and that an antimicrobial produced by skin commensal bacteria has potential for the treatment of atopic dermatitis.¹⁴ In the latter case, the observations point to dysbiosis of the skin microbiome as the origin of the problem and a rebalancing of the situation as a potential solution.

Given the aforementioned observations, the variety of species that are coexisting in the human microbiome, most densely in the GI tract, and the number of antimicrobials with a human microbiome origin that have already been described, we can assume that the human gut can continue to be a promising source of new antimicrobials that can help to address specific aetiologies and complex systemic conditions such as metabolic syndrome, inflammatory bowel disease, colorectal cancer and diabetes, that have been started to be associated with an imbalance in gut microbiota communities.¹⁵

Here we review the variety of antimicrobials produced by bacteria present in the human GI tract and, in the process, highlight the importance of a targeted approach to discover new effective antimicrobials that can help to maintain good health in humans and to address the problem of antimicrobial resistance.

2. Gut microbiota composition and distribution

Gut microbiota composition is heterogeneous across the GI tract as a result of variations in a broad variety of parameters including fluctuations in temperature, pH, water activity and gas composition as well as differences in physiology throughout the gut. Nutrients are a very important factor because they are the main source of energy for gut bacteria. Their chemical and physical nature determines flow rates in the gut and, therefore, the process of adhesion and washing out of microorganisms. Surface tension can also be affected, having an effect on the physiology of bacteria. Further information on gut conditions and how they influence composition has been extensively reviewed previously.^{10,16}

It is important to consider that the gut microbiota composition is also not static. As noted earlier, alteration of the microbiome structure starts from the moments after birth and varies significantly initially.^{17,18} However, it stabilizes after the first⁷ or third¹⁰ year of life, maintaining a relatively consistent composition that tends to recover even after acute changes, such as a single exposure to antibiotics.^{16,17}

More sustained changes, like dietary alterations over an extended period can, however, induce longer lasting structural changes.⁷

Observations of variation among individuals, throughout their intestinal tracts and across their life span, has made it clear that it is challenging to describe what constitutes a healthy gut microbiota.⁸ However, it has been argued that a functional approach to categorize the gut microbiota may more accurately reflect its 'health'. This is based on the fact that the microbiota is capable of carrying out a variety of metabolic and molecular functions. This functionality is somewhat redundant and thus remains relatively even in situations where changes in taxonomy are evident.¹⁰

There have been considerable advances in approaches to determine microbial composition and the identification of their functional attributes. High throughput new generation DNA sequencing platforms in particular have helped to identify, and partially characterize microbes, most of which cannot be cultured. Care needs to be taken however as the vast majority of investigations relating to the human gut microbiota have been performed using stool samples and, as pointed out by Bajaj et al¹⁹ and others, fecal communities do not reflect the gut mucosal microbiota or microbial communities at locations in the upper sections of the GI tract.¹⁶

In general, the dominant bacterial phyla in the gut are Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria and Verrucomicrobia.¹⁶ Differences in physiological conditions result in a heterogeneous distribution of bacteria across the GI tract. The presence of bile acids, lower pH, higher oxygen levels and antimicrobial compounds (of both host and bacterial origin) favors colonization of the small intestine by groups of facultative anaerobes, such as Firmicutes (Lactobacillales) and Proteobacteria (Enterobacteriales). In the proximal part of the small intestine, the extreme environmental conditions result in it having a lower microbial density. In the distal part of the small intestine, near the ileum, these conditions become less limiting. Microbial density is highest in the colon where the transit time is slower, and the absence of easily digestible nutrients facilitates the proliferation of fermentative organisms that are able to degrade more complex compounds, including dietary fiber. These organisms include, for example, representatives of the Bacteroidetes (Bacteroidaceae, Prevotellaceae and Rikenellaceae) in the lumen and Firmicutes (Lachnospiraceae and Ruminococcaceae) in the inter-fold regions.¹⁶

Despite the fact that bacteria have been most extensively studied, there are other groups of microbes in the human GI tract that have not been as well researched but have an important role in the ecological dynamics, like archaea, viruses and eukaryotes.^{10,20} Archaea is represented by Euryarcheota and Crenarchaeota. *Methanobrevibacter smithii* has been identified as the dominant human methanogen where it is estimated to be present in 50% of the adult population²¹ and the concept of archaeobiotics has been proposed, as probiotics of archaeal origin.²² Eukarya presence includes amoebae and fungi. The concept 'mycobiome' has been proposed to cover the diversity and dynamics of fungi in the human body.²³ It has been suggested that mycobiome and microbiome are intimately regulated, influencing each other in the colonization of the gut niche and having a crucial effect on the overall health status of the host. Recent reviews describe the human mycobiome and its implications in health and disease.^{24,25} "Virome" is another recent concept to define the community of viruses that can be found within the human host.²⁶ There are also eukaryotic viruses, plant derived viruses that have a dietary origin, giant viruses and bacteriophages.²⁶ Antimicrobials produced by representatives of these groups in the gut have not been identified and characterized yet, to the best of our knowledge. However, Archaea, Eukarya and viruses are already known for their antimicrobial capabilities. Fungi are an especially important source of antibiotics²⁷ and Archaea have also been reported to show antimicrobial activity.^{28,29} Some bacteriophages produce lytic enzymes that can kill bacteria,³⁰ and the use of the phages derived from the human gut has been proposed as a potential therapeutic to modulate gut composition (phage therapy).^{26,31} Further examination of these groups might reveal novel antimicrobials and modulation strategies.

3. Non-peptide antimicrobial activity in the gut

Bacteria develop different antagonism strategies to gain ecological advantages over other bacteria in the gut. Some of them are direct strategies, like competitive removal of essential substrates, accumulation of D-amino acids, lowering the oxidation-reduction potential and co-aggregation.³² Another strategy involves the production of substances of metabolic origin that have the ability to limit the growth of surrounding bacteria. Such activity can be specific or non-specific, where the regulatory agent does not address a specific target, hydrogen

peroxide (H_2O_2) being a good example of a chemical with such activity. H_2O_2 is well known for its antimicrobial properties that are mediated through oxidizing effects on bacterial cells and their molecular structures.³³ It has been suggested that H_2O_2 contributes to maintain a healthy microbiota.³⁴ H_2O_2 can function synergistically with other compounds produced by bacteria, such as lactic acid,³⁵ but its presence has also been linked to chronic inflammatory lesions in colon.³⁶ Lactic acid and other organic acids are believed to function by virtue of their undissociated form crossing the lipid membrane and its subsequent dissociation in the neutral pH once inside, producing ions that cause stress in the cell. Lactic acid is produced by the carbohydrate metabolism of the so called lactic acid bacteria (LAB), while the important group of short chain fatty acids (SCFA), such as formate, acetate, propionate and butyrate, are produced by bacteria fermenting starches, dietary fiber, sugars, proteins and amino acids³⁷ and their antimicrobial activity is due to acidification of the environment.³⁸ There are other compounds of bacterial origin, like diacetyl (2,3-butanedione),^{39,40} ethanol, CO_2 , ammonia or phenolic compounds, which have also been associated with antimicrobial activity,¹¹ but, as with the previous examples, their non-specific activity and other side effects,⁴¹ make them unsuitable for clinical applications and they will not be discussed further in this review.

4. Peptidic antimicrobial activity in the gut

Bacteria are also capable of producing antimicrobial peptide compounds that are considered to be target-specific. Peptides with antimicrobial properties are classified based on their biosynthesis, which can be ribosomal or non-ribosomal. In general, antimicrobial peptides consist of 10–50 amino acids and their ability to kill bacteria depends on their interaction with bacterial membranes and cell walls. Selectivity depends on membrane or cell wall composition.⁴² The levels of production and accumulation of these compounds in the gut depend on the producer strains, their chemical structure, bioavailability and physical conditions within the surrounding environment, making the identification and direct isolation of those compounds difficult. Recently, a bioinformatics tool, ClusterFinder, has been used to identify thousands of biosynthetic gene clusters from human metagenomic samples, encoding among other compounds, antimicrobial peptides, highlighting the potentially underexploited therapeutics derived from the human microbiome.^{43,44}

4.1. Non-ribosomal peptides (NRPs)

NRPs are secondary metabolite peptides synthesized by multienzyme complexes of multifunctional peptide synthetases.⁴⁵ NRPs constitute a large class of bacterial products in several environments and their study has provided many of the antibacterial compounds currently in use, such as penicillins, vancomycin or polymyxin that are considered as peptide derived.⁴⁵ However, there are very few NRPs characterized from the human microbiota.¹¹ Although analysis of the Antimicrobial Peptide Database (APD3) did not show any NRPs of human gut origin,²⁹ Donia and Fischbach listed some NRPs that had been characterized from bacterial producers isolated from the human microbiome.¹¹ The peptides cereulide, zwittermicin and tilivalline were isolated from pathogens from the human gut although they are not normally present in human gut microbiome.¹¹ Their activity is described as cytotoxic, alongside with colibactin activity, produced by *Escherichia coli*.⁴⁶ Zwittermicin activity has been reported as antimicrobial⁴⁷ but resistance genes for it have also been identified.⁴⁸ Therefore, the use of such NRPs as therapeutics would be limited.

4.2. Ribosomally synthesized peptides (Bacteriocins)

Bacteriocins are ribosomally synthesized peptides that exhibit antimicrobial activity. The first bacteriocin was discovered by Gratia in 1925⁴⁹ and named “colicin”. Since then, many more bacteriocins have been isolated and characterized, mostly based on their bioactivity, in a wide variety of environments and from many different bacterial species. Bacteriocins have been proposed as mediators in population dynamics and their ecological role in this regard has been studied in recent decades.^{5, 50, 51} A considerable number of these peptides have originated from bacterial isolates from foods, though many important bacteriocins are produced by bacteria that inhabit the GI tract of humans and animals. Indeed, given the density and diversity of microbial populations present in the gut, it may represent a source of bacteriocin producers that is, as yet, relatively underexplored. Bacteriocins address some of the problems described for traditional antibiotics and that is why their range of applications is expanding towards human and veterinary therapeutics.^{52,53} They have low toxicity, making them suitable for use as food and beverage preservatives, since traditional antibiotics are prohibited for

use in foods. Low toxic effects have been proven for many of the bacteriocins in different studies,^{54,55} although a small percentage have shown some cytotoxic activity.⁵⁶ Bioinformatics is also helping to identify putative anti-tumoral activity of bacteriocins, as has been described recently.^{57,58} Antiviral and antifungal properties of bacteriocins represent other potential application of these peptides.^{59,60}

Some bacteriocins can exhibit broad-spectrum while others exhibit narrow-spectrum antimicrobial activity. The former have the possibility of being used as a traditional therapeutical antibiotic, while the narrow spectrum antimicrobials are more suitable for targeting specific harmful microorganisms without altering the natural populations,⁶¹ contributing to maintaining a balance in microbial populations. As a general rule, bacteriocins produced by Gram-positive bacteria show better activity against Gram-positive pathogens and Gram-negative bacteriocins work better on Gram-negative pathogens, but with purified bacteriocins and bioengineered derivatives it has been possible to cross this barrier.⁶² Furthermore, their activity can be enhanced by the presence of other substances, such as lactic acid, that allows the permeabilization of the bacterial membrane. One of their most attractive features is that resistance is not easily developed against them. In order to do that, target cells would have to alter membrane composition⁶³ or the receptors.^{64,65} Transporters have also been pointed as a mechanism to develop resistance,⁶⁶ and although they are not very common, expression of resistance proteins have also been reported.^{67,68} Bacteriocin combination or using strains that produce more than one, in order to maximize efficacy is another possibility,⁶⁹ and they can also be combined with other compounds to act synergistically.^{70,71} In addition, bioactivity is in a nano- to micromolar range, while an absence of taste, color or odor, makes them suitable for industrial and clinical purposes.⁶³

Another asset of such peptides is that they offer the possibility of being bioengineered, either by gene manipulation or partial or complete chemical synthesis, to improve properties such as specific activity, stability and host range. Rational design might be the key to obtain more potent bacteriocins, based on the structure-function relationship;⁷² and bacteriocins could be modified to have broader activity against new pathogen targets,⁶² or narrowed activity to target specific pathogens, to treat biofilms and resistant bacteria,^{73,74} or increase protease resistance against chymotrypsin or trypsin, which can

aid their survival in the gut. Key residues have been identified, along with the mechanisms that control activity and regulation that have been studied using natural variants and the generation of peptide derivatives.⁷⁵ Delivery matrices have been examined to maintain bacteriocin stability and in this regard, nanotechnology has been used as one of the most important approaches, with the use of nanoliposomes or nanoparticles of different composition.⁷⁶ Improvement of delivery strategies might offer new therapeutic possibilities for bacteriocins, like new targets or controlled delivery.^{77,78}

4.2.1. Identification of bacteriocins

Identification of new bacteriocins originally relied on methods to identify antimicrobial or antagonistic activity of a bacterial strain on a target bacterium. Bioassays for detecting inhibitory activity can present variations for adapting to specific needs.⁷⁹ One technique is to inoculate media with the specific indicator strain and add a spot of the bacteriocin producing strain or its cell free supernatant (spot test). A common variation is the growth on agar of the producer strain and subsequent overlay with indicator-inoculated soft agar (overlay assay). In both cases the activity is estimated by the inhibition of growth of the indicator strain (Fig. 1). Well diffusion assays and disc assays both involve placement of the cell free supernatant of the producer strain in a well or on a paper disc in indicator inoculated agar followed by incubation, with the bacteriocin producing a zone of inhibition which can be used to measure specific activity (Fig. 1).⁷⁹ The indicator strain can also be co-cultured with the producer strain or its supernatant and growth assessed by measurement of optical density.⁸⁰ Despite the extended use of these techniques and their variations, this approach has a limitation, as just a small percentage of bacteria are able to grow in culture and *in vitro* growth may not provide the appropriate conditions to induce the biosynthesis of bacteriocins.⁸¹ Some of the bacteriocin-like compounds identified required the presence of a trigger. For example, the presence of trypsin has proven to be effective as an antimicrobial inducer in human gut isolates,⁸² and both cell density and carbon source were necessary to induce bacteriocin production in *Streptococcus pneumoniae*,⁸³ reinforcing the idea that the environment plays an important role in the production of these compounds. Understanding all the factors and interactions that take place in the environment of the potential producing bacteria and the development of

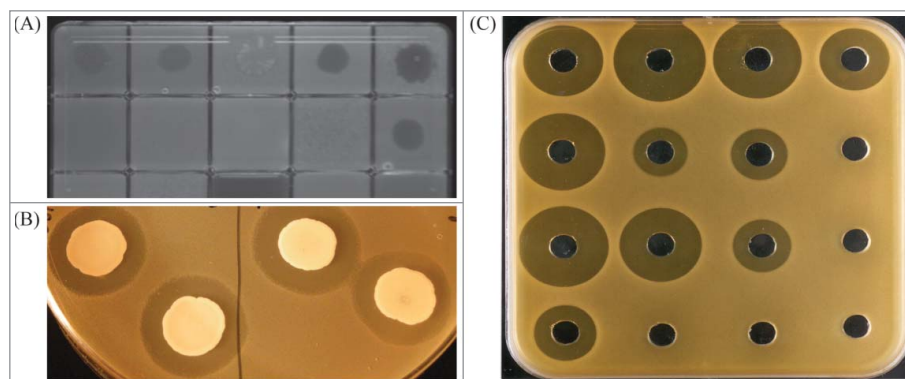


Figure 1. Examples of assays performed to identify antimicrobial activity. (a) spot test; (b) overlay; (c) well diffusion.

strategies to mimic these interactions can be a gargantuan task. A recently developed peptidogenomics strategy attempts to establish a mass spectral molecular network of microbes grown in a plate and integrate this information with genomic and phenotypic information.⁸⁴ This could be a good first step towards a holistic approach to microbial interactions that could lead to the identification of novel compounds produced from these simultaneous and multilateral interactions in a more realistic and complex environment such as the GI tract.

The development of molecular tools removes the requirement for functionality influenced by culture conditions and induction. This has allowed the identification of putative bacteriocin clusters in genomes, both in culturable and unculturable species. There are public software and databases to identify putative bacteriocin clusters in sequenced genomes and also offer access to bacteriocin peptide sequences. BAGEL with 482 sequences⁸⁵ and BACTIBASE with 345 sequences⁸⁶ are the most commonly used. Broader databases are available to screen for potential new bacteriocin clusters in the gut, like the Human Microbiome Project's reference genome database.^{87,88} An analysis of this database by Walsh et al identified 74 putative bacteriocin-encoding gene clusters in different phyla.⁸⁷ A more accurate analysis of the distribution and frequency of specific bacteriocin types can be addressed by the use of Hidden Markov Model, as performed for LanB homologues, allowing the identification of seven new lantibiotic producers.⁸⁸ Drissi et al developed an extensive database for bacteriocins, BUR ("Bacteriocins of the URMITE database"), where they combined BAGEL, BACTIBASE and NCBI databases for bacteriocin published sequences and also retrieved 641 available genomes from the GI tract that they analyzed in order to incorporate as much bacteriocin data

as possible.⁸⁹ The study of this database provided some interesting observations. First, it showed differences between bacteriocins produced by gut bacteria and bacteria from other environments. These differences were found in the amino acid composition and peptide length, with those for gut bacteria generally being shorter and having less positively charged and less hydrophobic residues, lower percentages of aspartic acid, leucine, arginine and glutamic acid, and higher percentages of lysine and methionine.⁸⁹ The most predominant class was class I, with 44% of bacteriocins produced in the gut, while class III was less represented (17.3%). This is also a significant difference with bacteriocins from other environments, where class III represents 59.2% and class I only 13%. Different bacterial groups generate different classes of bacteriocins: Actinobacteria (58%), Proteobacteria (94%) and Bacteroidetes (exclusively) were identified mainly as producers of class III bacteriocins, while Firmicutes showed production of bacteriocins of all classes more evenly (20% class I, 45% class II and 35% class III).⁸⁹

In Tables 1, 2 and 3 we have compiled the known bacteriocins isolated from the human gut based on the analysis of BAGEL3, BACTIBASE and APD3 databases.

4.2.2. Classification of bacteriocins

Due to the heterogeneous nature of the bacteriocins, there have been different criteria to classify them (e.g. by primary structure, molecular weight, mode of action, heat stability, genetic properties). Currently the most accepted is that based on their structure. This review will follow the classification scheme proposed by Cotter et al,⁵⁶ to highlight the diversity of bacteriocins produced by members of the gut microbiota. Examples of bacteriocin structures and gene operon organization are shown in Figs 2 and 3.

Table 1. Class I bacteriocins produced by bacteria originally isolated from the human gut.

Class I (modified)					
Group	Distinctive feature	Bacteriocin from human gut isolate	Gut Producer first isolated from human	Activity against	Reference
Microcins		Microcin L	<i>E. coli</i> LR05	<i>Shigella sonnei</i> <i>E. coli</i> <i>Pseudomonas aeruginosa</i>	150
		Microcin M	<i>E. coli</i> Nissle 1917	<i>E. coli</i> , <i>Salmonella</i>	151
		Microcin V (ColicinV)	<i>E. coli</i>	<i>E. coli</i>	49
		Microcin H47 (MccH47)	<i>E. coli</i> H47	<i>E. coli</i> , <i>Salmonella</i> , <i>Enterobacter</i> , <i>Shigella</i> , <i>Klebsiella</i> , <i>Proteus</i> spp.	152
		Microcin E492	<i>Klebsiella pneumoniae</i> RYC492	<i>Escherichia</i> , <i>Klebsiella</i> , <i>Salmonella</i> , <i>Citrobacter</i> , <i>Enterobacter</i>	153
Lasso peptide Sactibiotics	Lasso knot structure Sulphur- α -carbon linkages	Microcin J25	<i>E. coli</i> AY25	<i>E. coli</i> , <i>Salmonella</i> sp, <i>Shigella flexneri</i>	96
		Thuricin CD	<i>Bacillus thuringiensis</i> DPC 6431	<i>Clostridium difficile</i> , <i>Bacillus cereus</i> , <i>Bifidobacterium firmus</i> , <i>Listeria monocytogenes</i>	154
Lantibiotics	Lanthionine bridges	Ruminococcin A	<i>Ruminococcus gnavus</i>	<i>Bacteroides</i> sp, <i>Clostridium</i> sp, <i>Bifidobacterium</i> sp, <i>B. cereus</i>	116
		Nisin O	<i>Blautia obeum</i> A2-162	<i>Clostridium perfringens</i> , <i>C. difficile</i> , <i>Lactococcus lactis</i>	118
		Cytolysin ClyLI and ClyLs	<i>Enterococcus faecalis</i>	Gram-positive bacteria	114
		BLD 1648	<i>Bifidobacterium longum</i> (strain DJO10A)		117
Linear azole-or azoline-containing peptides	Heterocycles but no other modifications	Microcin B17	<i>E. coli</i> Nissle 1917	<i>E. coli</i> , <i>Salmonella</i> , <i>Shigella</i>	151

Examples of Class I (Linaridins, Proteusins, Patellamide-like cyanobactins, Anacyclamide-like cyanobactins, Thiopeptides, Bottrromycins and Glycocins) have not yet been described from human gut isolates. Classification of bacteriocin is based on Cotter et al, 2013.

Table 2. Class II bacteriocins produced by bacteria originally isolated from the human gut.

Class II (unmodified or cyclic)					
Group	Distinctive feature	Bacteriocin from human gut isolate	Gut Producer first isolated from human	Activity against	Reference
IIa peptides (pediocin PA-1-like bacteriocins)	Conserved YGNV motif (N represents any amino acid)	Bac43	<i>Enterococcus faecium</i> VRE82	<i>E. faecalis</i> , <i>E. faecium</i> , <i>Enterococcus hirae</i> , <i>Enterococcus durans</i> , and <i>L. monocytogenes</i>	122
		Bacteriocin RC714	<i>E. faecium</i> strain RC714	<i>E. faecalis</i> , <i>E. faecium</i> , <i>L. monocytogenes</i> , <i>Listeria innocua</i> , <i>Listeria murrayi</i> , <i>Listeria grayi</i> , <i>Lactobacillus paracasei</i> , <i>Lactobacillus plantarum</i> , <i>Leuconostoc</i> sp., and <i>Pediococcus pentosaceus</i>	124
		Bacteriocin 31	<i>E. faecalis</i> YI717	<i>E. hirae</i> 9790, <i>E. faecium</i> , and <i>L. monocytogenes</i>	155
IIb peptides	Two unmodified peptides are required for activity	ABP-118	<i>Lactobacillus salivarius</i> UCC-118	<i>L. monocytogenes</i>	127
		Acidocin J1132	<i>Lactobacillus acidophilus</i> JCM1132	<i>Lactobacillus</i> sp	126
		Gasserin T	<i>Lactobacillus gasseri</i> SBT 2055	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	130
IIc peptides	Cyclic peptides	Lactacin F	<i>Lactobacillus johnsonii</i>	<i>Lactobacillus</i> sp, <i>E. faecalis</i>	156
		Gasserin A	<i>L. gasseri</i> LA39	<i>Bacillus</i> , <i>Clostridium</i> , <i>Lactobacillus</i> spp, <i>Lactococcus lactis</i> , <i>Leuconostoc mesenteroides</i> , <i>Listeria</i> spp, <i>Pediococcus cerevisiae</i> , <i>S. aureus</i> , <i>Streptococcus agalactiae</i>	157
		Reuterin 6	<i>Lactobacillus reuteri</i> LA 6	<i>L. acidophilus</i> , <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> and <i>L. delbrueckii</i> subsp. <i>lactis</i>	158
IId peptides	Unmodified, linear, non-pediocin-like, single-peptide bacteriocins	AS-48	<i>E. faecalis</i> AS-48	Gram-positive and Gram-negative	159
		Microcin S	<i>E. coli</i> G3/10	<i>E. coli</i> G3/10	136
		Rhamnosin A	<i>Lactobacillus rhamnosus</i> strain 68	<i>Micrococcus lysodeikticus</i> ATCC 4698	138
		Bac32	Vancomycin-resistant <i>E. faecium</i> (VRE) 200	<i>E. faecium</i> , <i>E. hirae</i> , and <i>E. durans</i>	139

Examples of Class II (IIe) have not yet been described from human gut isolates. Classification of bacteriocins is based on Cotter et al, 2013.

Table 3. Class III bacteriocins produced by bacteria originally isolated from the human gut.

Class III					
Group	Distinctive feature	Bacteriocin from human gut isolate	Gut Producer first isolated from human	Activity against	Reference
Bacteriolysins		Colicins Bacteriocin 28b	<i>E. coli</i> <i>Serratia marcescens</i>	Enterobacteria <i>E. coli</i>	49 141
Non-lytic bacteriocins		Bacteriocin helveticin J	<i>L. acidophilus</i> NCFM		142

4.2.2.1. Class I bacteriocins. This class contains different subgroups, which are all small heat-stable peptides of less than 10 kDa and share the process of enzymatic modification during their biosynthesis. The use of characteristic amino acids and structures is distinctive and will have an influence on their future function. The most studied group is lantibiotics, but the development and implementation of more accurate and advanced techniques is broadening our understanding of other modifications discovered more recently. Here we discuss the subgroups of this peptide class whose producers were isolated initially from the human gut microbiota.

4.2.2.1.1. Microcins. Microcins are peptides produced by Enterobacteriaceae⁹⁰ (Fig. 2). They are differentiated from colicins, also produced by Enterobacteriaceae, by their smaller molecular mass (microcins are smaller than 10 kDa, while colicins are larger than 20 kDa). Microcin operons are constituted by genes encoding a precursor, secretion proteins and elements for self-immunity (Fig. 3). Microcins are subdivided in two groups, based on molecular masses and presence of disulphide bonds and post-translational modifications.⁹⁰ Thus, class I microcins are encoded on plasmids, smaller than 5 kDa and have extensive post-translational modifications. Class II microcins (between 5–10kDa) can be further classified into class IIa, encoded by plasmids, with one, two or no disulphide bonds and no post-translational modifications, and class IIb, encoded on chromosomes and a non-mandatory post-translational modification.⁹⁰ They are considered to be hydrophobic and have heat, pH and protease stability.⁹¹ Microcins are known for having a precursor peptide that needs to be cleaved during their transport from the producer cells.⁹² Currently there are at least 16 microcins identified, but only eight of them have been characterized structurally.⁹² The mode of action of microcins is described as a “Trojan horse”,⁹⁰ mimicking essential compounds for nutrition, for example an iron-siderophore complex, that the target cell will detect using outer-membrane receptors and will incorporate. They can also be secreted as a non-active compound and be processed to the

bioactive form by the target cell. Once inside the cell, microcins bind enzymes or interact with the inner membrane.^{90,93}

4.2.2.1.2. Lasso peptides. These were first described in 1991 by the discovery of anantin.⁹⁴ They are RiPPs of bacterial origin characterized by their distinctive structure as a knot, including a macrolactam ring crossed through by the C-terminal tail. This structure is sustained by steric interactions and disulphide bridges and it is very stable. There are three classes of lasso peptides described so far, and the criterion followed is the number of disulphide bridges: class I has two disulphide bonds, class II no bonds and class III just one.⁹⁴ They have been reported to have inhibitory activity against enzymes, receptor antagonistic behavior and a few of them display antimicrobial activity.⁹⁵ Although during the first years their discovery was activity-based, increased application of genome mining has resulted in a rapid increase of discovery of lasso peptide biosynthetic gene clusters. In a recent review by Heggeman et al⁹⁵ a total of 38 known lasso peptides were identified. Currently almost all lasso peptides have been isolated from soil, sludge and water bacteria,⁹⁵ with the exception of zucnodin, isolated from an erythroleukemia cell line, and microcin J25, isolated from baby feces.⁹⁶ They have also attracted attention because they have been proposed to be used as new chemical scaffolds, due to their stability.

Microcin J25 is the paradigm of lasso peptides and has been studied as a typical example of its class. It has 21 amino acids and is produced by *E. coli* AY25 that was isolated from newborn feces. Typically, it forms a ring by a lactam linkage between the first glycine and the glutamate at position 8 (Fig. 2B). The remaining thirteen residues form the tail, with the phenylalanine in position 19 and tyrosine at position 20 acting as plugs.⁹⁷ The biosynthetic cluster has four genes: a precursor (*mcjA*), cleavager of leader peptide (*mcjB*), a macrolactam ring formation gene (*mcjC*) and a gene that encodes the ATP-binding cassette transporter (*mcjD*) (Fig. 3). Despite their genomic

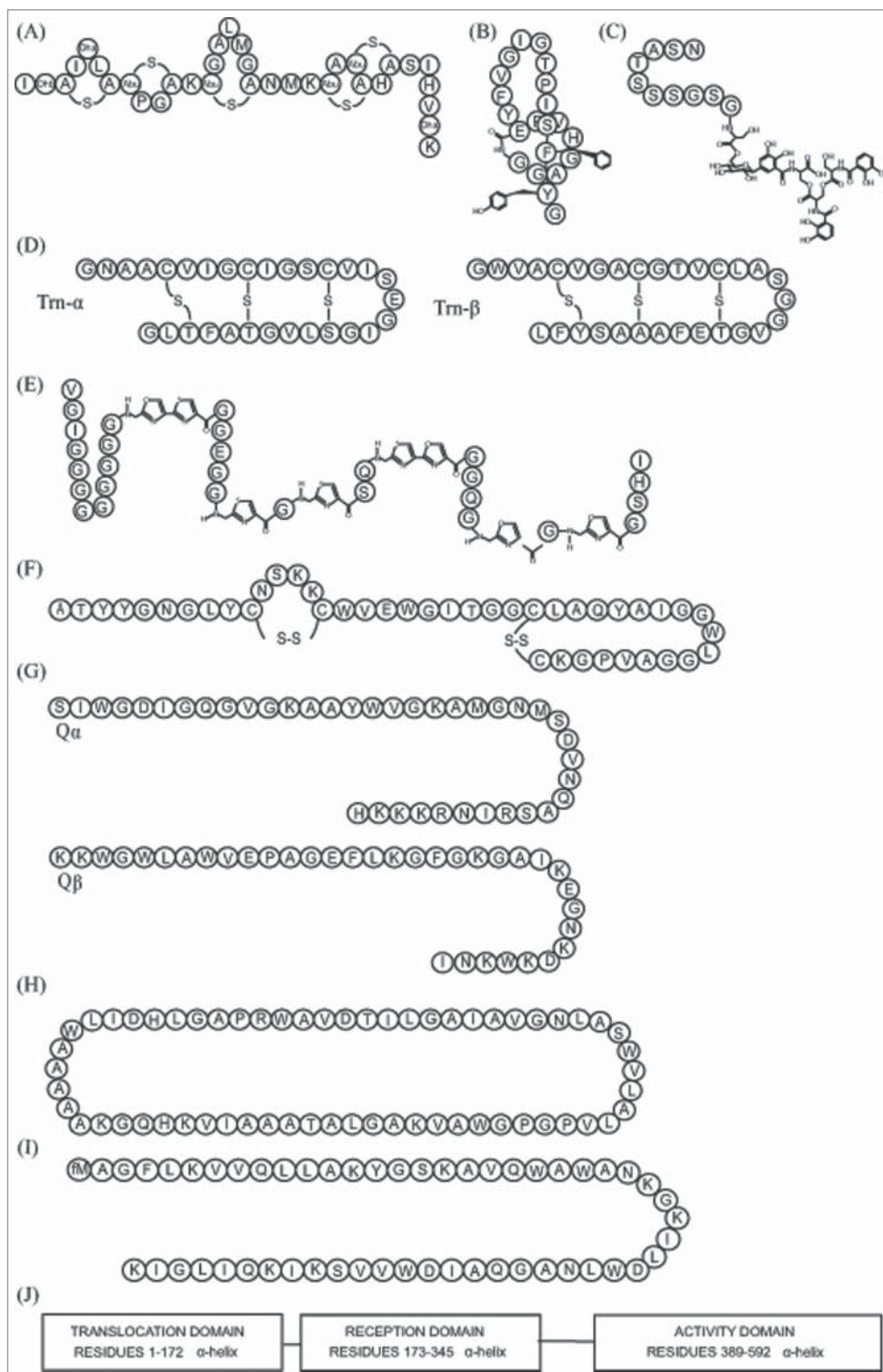


Figure 2. Examples of bacteriocin structures. (a) nisin A¹, lantibiotic¹⁶⁰; (b) microcin J25, lasso peptide¹⁶¹; (c) microcin E-492, microcin¹⁶²; (d) thuricin CD subunits, sactipeptide¹⁵⁴; (e) microcin B17, linear azole or azoline containing peptides¹⁶³; (f) enterocin NKR-5-3C¹, class IIa¹⁶⁴; (g) lactococcin Q¹, class IIb¹⁶⁴; (h) lactocyclin Q¹, class IIc¹⁶⁴; (i) lactacin Q¹, class II d¹⁶⁴; (j) colicin A¹, class III.⁴⁹ ¹Note that these bacteriocins have not been described as of human gut origin.

identification, biosynthesis of lasso peptides has not been fully elucidated.⁹⁸ MccJ25 antimicrobial activity is based on inhibiting the RNA polymerase of Gram-

negative bacteria but another activity has been described, depolarizing cell membranes in *Salmonella* and *E. coli* species that overexpress *fhuA*.⁹⁵

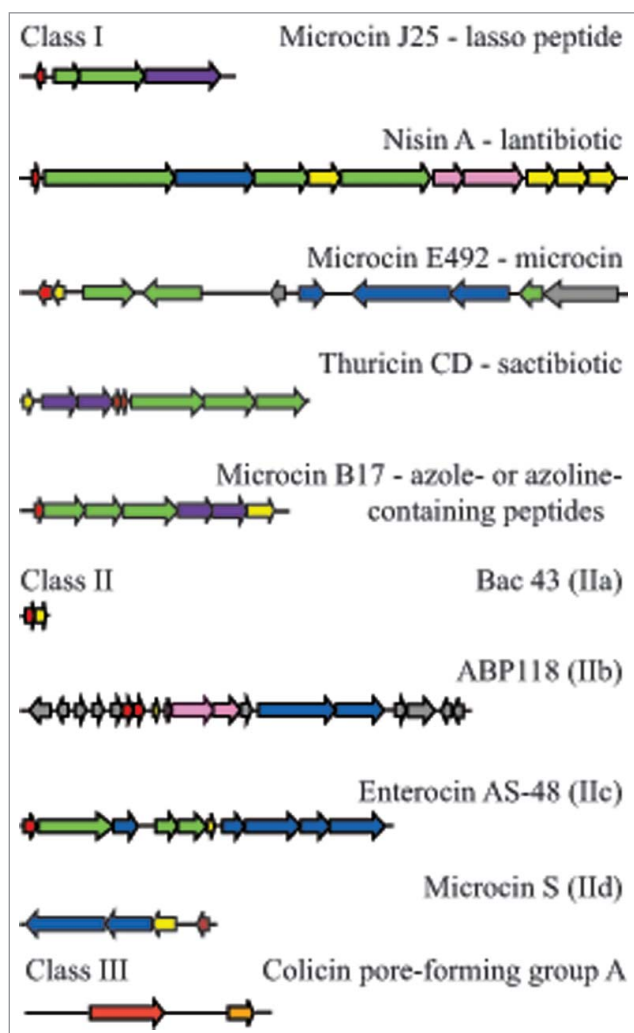


Figure 3. Examples of bacteriocin cluster organization among the different bacteriocin classes. Genes are colored according to the function of their products: red, precursor peptides; green, post-translational modifications; blue, export; yellow, immunity; pink, regulation; purple, export and immunity; orange, lysis; grey, unknown function. Class I: microcin J25⁹¹; nisin A¹⁰⁹; microcin E-492⁹¹; thuricin CD¹⁰⁰; microcin B17⁹¹; class IIa bacteriocin 43¹²²; class IIb ABP-118¹²⁷; class IIc AS-48¹⁶⁵; class II d microcin S¹³⁷; class III colicin.⁴⁹

4.2.2.1.3. Sactibiotics. Sactipeptides are a general group of peptides characterized by having an intramolecular bridge between a cysteine sulphur and an α -carbon and do not always show inhibitory activity; the term sactibiotic is used to describe sactipeptides with antimicrobial activity. Many of them have been identified by genome mining⁹⁹ and currently there are four sactibiotics described: subtilisin A, propionicin F, thuricin H and thuricin CD.¹⁰⁰ Among them, only thuricin CD has been isolated from a bacterium from the human gut. Produced by *Bacillus thuringiensis* DPC6431, it is particularly important because it has been shown to have very

specific activity against *Clostridium difficile*, *Bacillus cereus*, *Bifidobacterium firmus* and *Listeria monocytogenes*.¹⁰¹ It is composed of two subunits, Trn α and Trn β (Fig. 2D). Its gene cluster contains structural genes, transport, immunity and post translational modification genes (Fig. 3). The presence of genes encoding members of the radical S-adenosylmethionine superfamily of proteins is also a specific characteristic. Thuricin CD's mode of action has just started to be elucidated. Mathur et al showed that both subunits act on the cell membrane, irreversibly collapsing the membrane potential and suggested that the peptides are inserted in the membrane of the cell, forming a pore that leaks ions and ultimately leading to cell lysis.¹⁰² The effect of thuricin CD was similar to vancomycin and metronidazole, both routinely used to treat *C. difficile* infections.

4.2.2.1.4. Linear azole- or azoline-containing peptides.

These peptides, also called LAPs, are characterized by presenting different combinations of heterocyclic rings of thi-azole and (methyl)oxazole. These are produced by a cyclodehydration of cysteine and serine or threonine, and a flavin mononucleotide dependent dehydrogenation reaction.¹⁰³ Their mode of action is not yet fully understood. Microcin B17 is a LAP produced by *E. coli* isolated from baby feces¹⁰⁴ (Fig. 2E). Its cellular target is the DNA gyrase and it has been extensively studied to discover the link between its structure and function, in order to obtain information to design new antimicrobials.¹⁰⁵ There is an increasing interest in studying the inhibition of topoisomerases and gyrases as targets for designing antimicrobials, and the study of microcin B17 can offer further insights into the structure and functional analysis of this antimicrobial target.¹⁰⁶ *In vivo* studies have already demonstrated their ability to control infections in infants¹⁰⁷ and cattle.¹⁰⁸

4.2.2.1.5. Lantibiotics. The term “lantibiotic” comes from “lan-thionine containing antibiotic”. Similar to sactibiotics, lantibiotics are embedded in a bigger group, lantipeptides, not all of which show antimicrobial activity. This subgroup is constituted by small peptides (<5 kDa) with presence of modified amino acids such as dehydroalanine (Dha) and dehydrobutyryne (Dhb), among others.¹⁰⁹ Dha and Dhb are derived from the dehydration of Ser and Thr residues respectively, the process followed in order to obtain 2,3-didehydroalanine and (Z)-2,3-didehydrobutyryne; an additional modification is the stereospecific intramolecular addition of a cysteine residue to form

lanthionine (Lan) or methylanthionine (MeLan) bridges. In total up to 17 uncommon amino acids have been described.¹¹⁰ The function of these modified amino acids is not known, but it has been suggested that they interact with free sulfhydryl groups on the cell envelopes of target organisms.¹⁰⁹

Genes encoding lantibiotics are organized in clusters which are often highly conserved and have similar functions. A basic lantibiotic cluster contains genes for a precursor peptide, known as LanA, modification enzymes (LanB and LanC or LanM), usually a protease to remove the leader peptide (LanP); an ABC transporter (LanT) that translocates the peptide and sometimes also encodes the protease activity; a two-component regulatory system (LanR and LanK), and proteins responsible for self-immunity (LanI and LanFEG) (Fig. 3). Defined steps in the production and maturation of lantibiotics involve synthesis of the prelantibiotic, dehydration and linkage reactions, removal of the leader sequence, and secretion. Depending on the enzymes present during the maturation process four groups have been differentiated: class I, LanBC-modified (involves LanB, with dehydratase function and LanC, a cyclase); class II, LanM-modified (LanM encodes both dehydratase and cyclase functions); class III uses LanKC and class IV uses LanL, but only class I and II show antimicrobial activity.¹¹¹

The most common mechanism of action of lantibiotics is through lipid II binding. Lipid II plays an important role in the formation of cell wall and interfering with its function is fatal for the cell. The two ways of disrupting the cell wall are by inhibiting its synthesis or by pore formation.¹¹¹ Nisin, the most studied lantibiotic (Fig. 2A), has both functions, but not all lantibiotics have the same mode of action. This activity on the cell wall is the reason that lantibiotics are not very effective on Gram negative bacteria, due to their outer membranes that present a barrier for entry to the bacterial cell wall. It has been a common belief that lantibiotics are only produced by Gram-positive bacteria, but the recent identification of pinensins, lantibiotic type bacteriocins produced by the Gram-negative bacteria *Chitinophaga pinensis*, that also show antifungal activity,¹¹² is an example of how broad metabolic diversity is and that there are functions still to be discovered. At the moment, the front line in lantibiotic research is focused on the transference of the *in vitro* efficacy to *in vivo* tests, and the use

of bioengineering to improve mode of action and efficiency of delivery systems.¹¹³ There are many lantibiotics that are being tested *in vivo* for a variety of health applications, especially targeting bacteria of clinical relevance, and some are now in pre-clinical development.¹¹³

Some of the lantibiotics that can be found in the human gut are synthesized by gut pathogens, like the two-component cytolyisin, produced by *Enterococcus faecalis*.¹¹⁴ This cytolyisin is one factor contributing to its virulence and facilitates antibiotic resistant infections,¹¹⁵ and it is highly efficient against other Gram-positive bacteria. Cytolyisin is composed of two subunits, large (L) and small (S); the gene cluster also encodes proteins for post translational modification, export, and a protease for activation and immunity. Its activity is regulated by a two-component system that functions via quorum-sensing autoinduction. The products of two genes, *cylR1* and *cylR2*, repress transcription of the bacteriocin components so that production is low. When cell density increases, the small subunit of cytolyisin reaches levels high enough to overcome the repression and induce transcription of the bacteriocin components.¹¹⁴ Other lantibiotics are produced by commensal gut microbes. Ruminococcin A, produced by *Ruminococcus gnavus*, was the first characterized bacteriocin from a strict anaerobe isolated from a human fecal sample and requires trypsin for antimicrobial activity,¹¹⁶ while genomic analysis of *B. longum* DJO10A identified the presence of a novel lantibiotic gene cluster with activity against a related strain.¹¹⁷ Recently, a new variant of nisin (nisin O), has been identified from the human gut bacterium *B. obeum* A2-162.¹¹⁸ This showed activity against important gut pathogens *C. difficile* and *C. perfringens*.¹¹⁸

4.2.2.2. Class II bacteriocins. Class II constitutes the most extensive group of bacteriocins. They are characterized by being small heat stable peptides (<10 kDa) that do not undergo post translational modifications. According to their specific relationships between structure and function, they can be classified into four subgroups. There have been different criteria to the establishment of these subgroups. Here we will use the one proposed by Cotter et al.⁵⁶

4.2.2.2.1. Class IIa. Also known as pediocin-like bacteriocins, these contain an N-terminal consensus sequence YGNGVXaaCXaaK/NXaaXaaC which is

highly conserved (Fig. 2F). This includes the “pediocin box” (YGNGV). They are characterized also by having two cysteines in the conserved region which form a disulfide bond required for antimicrobial activity.¹¹⁹ The C-terminus is responsible for specificity and their mode of action is via permeabilization of the bacterial cell wall. The sugar transporter mannose phosphotransferase system (Man-PTS) is the target receptor, present in Gram-positive and Gram-negative bacteria,¹²⁰ and they have particularly strong activity against *Listeria* spp.¹¹⁹ Biosynthesis of the peptide commonly requires at least four genes: a structural gene encoding a precursor, and the genes encoding an immunity protein, an ATP-binding cassette transporter and a protein for extracellular translocation. They are regulated by quorum sensing, and the regulatory system is composed of three genes, encoding an inducer peptide, a membrane associated histidine protein kinase, and a cytoplasmic response regulator.¹²¹ Bacteriocin 43 was isolated and characterized from a vancomycin-resistant clinical isolate *Enterococcus faecium* (VRE). Its sequence showed high homology with bacteriocin 31 from *E. faecalis* YI717 and bacteriocin RC714 from *E. faecium* strain RC714,¹²² both clinical isolates too.^{123,124} It is unusual in requiring only 2 genes, one structural and one for immunity¹²² (Fig. 3).

4.2.2.2.2. Class I Ib. This class is represented by bacteriocins that need two different peptides to act synergistically in order to exert their antimicrobial activity¹²⁵ (Fig. 2G). Normally their genes are located on the same operon and they are expressed simultaneously (Fig. 3). The killing mechanism involves membrane permeabilization. The peptides that constitute the bacteriocin have similar characteristics to one peptide bacteriocins, being usually cationic and hydrophobic or amphiphilic, with a length of 30 to 50 amino acids. Their synthesis includes a double-glycine leader type sequence in the N terminus that it is cleaved off during secretion of the peptide to the outside of the cell.

A range of bacteriocins of this class originating from bacteria of human gut origin are identified in BAGEL3, BACTIBASE and APD3 databases. Acidocin J1132 was produced by from *L. acidophilus* JMC 1132, isolated from human feces;¹²⁶ it was found that its production relied on the pH of the medium not being above 7, with maximum production at pH 5. The mode of action against other taxonomically close strains showed a bactericidal activity.¹²⁶ ABP-118 was the first bacteriocin to be isolated and characterized from a bacterium

that was already considered a probiotic.¹²⁷ *L. salivarius* UCC118, isolated from the ileal-caecal region of the human GI tract was proven to produce ABP-118 *in vivo* and it has been tested in order to assess its potential activity, resulting in the control of *L. monocytogenes* infection in mice.¹²⁸ Its activity has also been tested as a gut microbiota regulator, leading to population shifts by increasing Bacteroidetes and Proteobacteria and decreasing Actinobacteria.¹²⁹

Gassericin T is another representative of this class. Produced by *L. gasseri* SBT 2055 isolated from human faeces, it is part of the lactacin F family, sharing a 60% similarity with lactacin F.¹³⁰ Lactacin F, another class IIb bacteriocin produced by a gut bacterium, requires two hydrophobic peptides LafA and LafX for its antimicrobial activity and it has been shown to be effective in combination with nisin.¹³¹

4.2.2.2.3. Class I Ic. These are cyclic peptides that contain a covalent bond between the C and N termini (Fig. 2H). They are generally cationic and have some degree of hydrophobicity. Initially their mode of action was identified as disruption of the cell membrane allowing permeabilization of the bacterial cell membrane to small molecules, leading to cell death. However, inhibition of nucleic acid, protein, cell wall synthesis and enzyme activity have also been reported as additional mechanisms of inhibition.¹³² Their structure is believed to confer a 3-dimensional stability and makes them more resistant to proteolysis.

Some bacteriocins of human gut origin could be found in the databases. Among them, the paradigm of a circular bacteriocin is considered to be AS-48, an α -helical and cationic peptide first isolated from *E. faecalis*. It is extensively distributed among different *Enterococcus* species from clinical to food isolates, and a number of different derivatives, both engineered and natural are available (e.g., AS-48J, isolated from goat cheese¹³³). It maintains stability and solubility through a broad range of pH and temperature, and the most attractive feature is its broad spectrum of activity, affecting both Gram-positive and Gram-negative bacteria. Its applications as a food preservative is well documented, but new functions arise from the implementation of new strategies, like liposome-encapsulation.¹³⁴ The successful treatment against *S. aureus* from bovine mastitis opens the possibility of veterinary applications. Other applications being investigated include its use as a leishmanicidal agent¹³⁵ or against bacterial biofilms.¹³⁴

Gassericin A is produced by an isolate from the human gut *L. gasseri* LA39. It has the same primary amino acid sequence as reuterin 6 produced by *L. reuteri* LA6, both isolated from feces of the same baby,¹³² but differs in one D-alanine residue, which confers on gassericin A a broader spectrum of activity. In fact, gassericin A can inhibit the growth of *L. reuteri* LA6, but not the other way around. The activity of reuterin 6 is also influenced by the presence of substances on the surface of the target cells and low pH values, highlighting once more the importance of the secondary and tertiary structures of bacteriocins and the close relationship between this and the mode of action and efficacy.

4.2.2.2.4. Class IId. These are unmodified, linear, non-pediocin like, single peptides (Fig. 2I). This group was proposed as a miscellaneous collection to group different bacteriocins of class II that did not fit elsewhere. There were a few representatives of this class identified from human gut in databases. Microcin S is a bacteriocin produced by *E. coli* G3/10 that is part of the probiotic marketed as Symbioflor, constituted by six different *E. coli* genotypes, and used for gastrointestinal disorder treatments, especially intestinal bowel syndrome (IBS).¹³⁶ Microcin S inhibits the adhesion of the enteropathogenic *E. coli* E238/6 to intestinal epithelial cells.¹³⁷ Rhamnosin A¹³⁸ and Bac 32 were also assigned to this subclass of bacteriocins.¹³⁹ Bac 32 is encoded by a plasmid and has been identified as present in a wide number of clinical isolates of *E. faecium*, having a narrow spectrum of action that only includes other Enterococci.¹³⁹

4.2.2.3. Bacteriocins class III. These are large (> 30 kDa), heat-labile proteins, usually with different domains (Fig. 2J). Bacteriocins of this group are not very well characterized. They are classified into two groups depending on their mode of action: class IIIa or bacteriolysins, which lyse the cells, and class IIIb or non-lytic bacteriocins, which disrupt the membrane potential. Colicins are the most important type of bacteriocin representing class IIIa.⁴⁹ The first colicin (colicin V) was originally isolated from *E. coli*, and is now classified as a microcin, but many colicins have been isolated from other enteric bacteria. They have a narrow spectrum of action and they act by binding to specific receptors on the surface of the target cells. They have different modes of action: inhibiting macromolecular synthesis, causing DNA breakdown and stopping protein synthesis. Colicins can be further

divided into two subgroups based on their encoding plasmids: group A are on small plasmids and excreted and group B are encoded by large plasmids and are not excreted, although some of them have mixed characteristics. Colicin operons are formed, as a general trend, by a structural gene, an immunity gene and a lysis protein gene. Bacteriocin 28b, isolated from many biotypes of *Serratia marcescens* also belongs to class III. Its structural analysis showed similarities to colicins and it is active against *E. coli*.¹⁴⁰ The different biotypes of *S. marcescens* that produce bacteriocin 28b were clinical isolates, driven by a study of nosocomial infections caused by this bacterium.¹⁴¹ A helveticin J bacteriocin has also been identified from the genome of *L. acidophilus* NCFM, a probiotic strain that is widely commercialized.¹⁴²

5. Applications of gut produced antimicrobials

The use of broad spectrum antibiotics has associated problems of increasing antibiotic resistance and concomitant disturbance of the beneficial commensal microbiota. Natural antimicrobials produced by bacteria from the human GI tract are attracting interest in their potential to offer specific solutions to health problems. Bacteriocins can be considered not only as antimicrobials but also, when produced in a mixed microbial community, as regulatory elements to balance the ratio between desirable and undesirable bacteria, offering competitive advantage to beneficial bacteria against bacteria that cause dysbiosis. In an effort to establish the microbiota alterations associated with certain pathologies, correlations between proportions and presence or absence of certain bacterial groups and functionality have been studied. This has revealed dysbiotic relationships between gut microbiota and host pathologies. The disruption of the balance of the microbiota in the gut has been associated with metabolic disease, type 2 diabetes, colorectal cancer or IBD.⁵⁰ Rational application of bacteriocins, either directly or produced by bacteria *in situ* as probiotics, is being studied as a method to modulate the structure and function of the gut microbiota and to control these acute and chronic pathologies. Guinane and Cotter (2013) summarized the microbial associations with chronic intestinal diseases.¹⁵ Others focus on the probiotic use of bacteriocins and their use to modulate the gut microbiota.¹⁴³ A strategy of production of many bacteriocins with low activity and a few

specific highly active antimicrobials points towards a functionality focused on maintaining homeostasis and providing defensive strategies against elements that could cause dysbiosis. There is an increasing amount of research in this area, but the complexity of GI tract microbial ecology requires further understanding. For example, recent evidence in a tilapia model suggests that abrupt interruption of a probiotic administration can cause gut dysbiosis by increasing host susceptibility to pathogens and other alterations in the intestinal epithelium and metabolites.¹⁴⁴ It needs to be taken into consideration whether these results can be extended to humans when designing probiotic treatments.

Bacteriocins can control and protect from specific infections. Microcins have recently proved their ability to act as narrow-spectrum antimicrobials to limit the proliferation of Enterobacteriaceae during intestinal inflammation by giving *E. coli* Nissle 1917, a probiotic originally isolated from the human gut, a competitive advantage.¹⁴⁵ This is the first evidence of the ecological role that microcins might play in the gut and opens a door to further therapeutical applications.

An example of the regulatory potential of bacteriocins in gut microbial populations is bactofencin A. It is produced from a *L. salivarius* isolated from porcine intestine and, when tested in a distal colon model, although it did not have a direct inhibitory activity against *Clostridium*, *Bacteroides*, *Bifidobacterium* and *Fusobacterium*, their populations were altered in a positive way.¹⁴⁶ More recently, in an experiment where water administered to mice was supplemented with different bacteriocin producer strains, initial 16S rDNA analysis at phylum level suggested that gut microbiota populations were not affected. But a deeper analysis at lower taxonomic levels showed that potential problematic bacteria, like *Clostridium* spp. or *Staphylococcus* spp., were inhibited.¹⁴⁷ The consensus is that we do not see large changes in *in vivo* experiments at the level of higher taxonomy level, but there are certainly beneficial and subtle changes that are favorable for the host without major disturbances in the gut, which is a desirable feature. Also, these studies teach us the importance of the scale of analysis to understand effects on the gut microbiome.

One of the main challenges for bacteriocin production *in situ* is that the producer, in order to be effective, needs to survive and colonize the human gut and the antimicrobial to be active in the gut environment. Thuricin CD

illustrates this assertion. Despite its efficacy against *C. difficile*, when delivering *B. thuringiensis* DPC6431 directly through the GI tract of mice, results showed that this approach was not a good delivery option.¹⁴⁸ However, alternative routes were found to be effective, like rectal administration.¹⁴⁸ Also, other possibilities can be explored, like synbiotics and delivery systems to help protect the producer strain or the compound from harsh acidic and enzymatic environments of the GI tract. Tablets of nisin with a pectin / HPMC polymer mixture are an example of this delivery strategy.¹⁴⁹

6. Concluding remarks

The search for new antimicrobials has become one of the most urgent tasks to address the challenges of antibiotic resistance. Understanding the environment where problematic bacteria grow and searching for the strategies used by their natural enemies to control them has proven successful. Translating this idea into practical solutions against acute infections and systemic syndromes will be supported by the identification of different antimicrobials of human microbiota origin. Bacteriocins are highly promising, due to the variety of advantages that they present. Current limitations in the identification and isolation of these compounds can be addressed by incorporating new techniques like genomic and bioinformatics tools, genome mining and metagenomics, but there is the need to translate the genomic information into the laboratory, and ultimately to achieve efficacy *in vivo*. In addition, further understanding of the microbial ecology of the system under study may lead to the development of more effective treatments, both for targeted intervention and to maintain gut homeostasis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors contributions

EG-G, MM, PC, and AN designed the manuscript; EG-G wrote the manuscript; MJM, PC and AN critically revised the manuscript and approved the final version.

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