Chapter 4
Bacteriocins from Gram-Negative Bacteria: A Classification?

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Abstract Bacteria produce an arsenal of toxic peptides and proteins, which are termed bacteriocins and play a role in mediating the dynamics of microbial populations and communities. Bacteriocins from Gram-negative bacteria arise mainly from Enterobacteriaceae. They assemble into two main families: high molecular mass modular proteins (30–80 kDa) termed colicins and low molecular mass peptides (between 1 and 10 kDa) termed microcins. The production of colicins is mediated by the SOS response regulon, which plays a role in the response of many bacteria to DNA damages. Microcins are highly stable hydrophobic peptides that are produced under stress conditions, particularly nutrient depletion. Colicins and microcins are found essentially in *Escherichia coli*, but several other Gram-negative species also produce bacteriocin-like substances. This chapter presents the basis of a classification of colicins and microcins.

Introduction

To understanding the diversity and complexity of nature and its components at the different levels of organization, scientists need classification. Since the ancient times, Greeks and Latins tried to classify plants. Since then, classification of organisms has been for many centuries one of the main concerns for scientists. A classification is an orderly arrangement of organisms or objects in a hierarchical series. In this way, biologists reconstruct the pattern of events that have led to the distribution and diversity of life in the course of evolution. The basis of the contemporary phylogenetic classification of organisms results from the double influence of Carl von Linné (1707–1778) and Charles Darwin (1809–1882). It was further modified, according to

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the revolutionary concepts introduced in 1950, by the entomologist Willy Hennig, who founded the *phylogenetic systematics* or *cladistics*. Phylogenetic classifications are often so hardly complex that they are subject to controversies and thus to changes, as fast as novel species are discovered. But apart *natural classification* of species, plants, animals and microorganisms, which is constructed in connection with evolutionary relationships, deciphering the Tree of Life, *artificial classifications* may be structured for convenience, using readily identifiable characters that are not necessarily indicative of phylogenetic relationships. Classifications rely on comparative analyses of characters, functions, properties, molecules and so on that allow bringing out similarities or differences at the organism, cellular or molecular level. Adding more parameters generally allows improving classifications. Indeed, molecules are also classified: they gather according generally to their molecular masses, functionalities, chemical or biological properties as well.

The production of molecules able to inhibit the growth of microorganisms is probably the most widespread defence strategy developed in nature. From mammals to bacteria, antimicrobial peptides contribute to the strategies that allow organisms to fight against pathogens (innate immunity) or to develop themselves at the detriment of others for the conquest of a given niche (competitions). The term bacteriocin was introduced at first in the 1950s by François Jacob (Jacob et al. 1953). It was further used by Tagg et al. (1976) and Klaenhammer (1988), successively to define proteinaceous compounds of bacterial origin being lethal to bacteria that were different from the producing strain. The sensible bacterial species are generally closely related to the producer organism or occupy the same ecological niche. It is worth noting that nowadays, in the literature, the term bacteriocin is very often restricted to the ribosomally synthesized antibacterial peptides of Gram-positive bacteria, principally from lactic acid bacteria. However, the first bacteriocin to be described had been identified in 1925 from the Gram-negative bacterium *Escherichia coli* (Gratia 1925). It inhibited the growth of another *E. coli* strain. This pioneering work has inaugurated the very numerous studies that were further conducted on these antimicrobial (poly)peptides or proteins.

Actually, bacteriocins can be considered today as antimicrobial (poly)peptides or proteins that are ribosomally synthesized by many bacteria, have generally a narrow spectrum of antibacterial activity, and against which the producing strains are protected by a dedicated immunity system. They may undergo or not posttranslational modifications that endow them with very diverse structures and mechanisms of action. It has been estimated that 30–99% of bacteria and archaea very probably are able to produce at least one bacteriocin (Klaenhammer 1988; Riley 1998). Moreover, many strains have been shown to produce two or more bacteriocins (Gordon and O'Brien 2006). Indeed, bacteriocins have been largely found in *E. coli* and some other enterobacteria, as well as in lactic acid bacteria. Therefore, for both historical and economical reasons, colicins from enterobacteria and bacteriocins from lactic acid bacteria have been more extensively studied than other bacteriocins, leading to the very impressive literature on these antimicrobial peptides and proteins. Parallel to colicins, which are large proteins, lower molecular mass bacteriocins have been identified in Enterobacteriaceae (Asensio et al. 1976;

Baquero and Moreno 1984). The name microcin was coined by Asensio to differentiate these lower molecular mass and protease-resistant bacteriocins produced by enteric bacteria from colicins (Asensio et al. 1976). This was the starting point for numerous studies leading to the identification of 14 microcins, and the analysis and characterization of the mechanisms involved in the production and antibacterial activity of seven of them (for reviews refer to Destoumieux-Garzón et al. 2002; Duquesne et al. 2007a; Severinov et al. 2007). It is striking to note that the first bacteriocin to be described, colicin V (ColV) (Gratia 1925), is produced by *E. coli* and is indeed a microcin, based on several parameters including its low molecular mass, noninducible production and dedicated export system (Fredericq et al. 1949; Duquesne et al. 2007a).

Although colicins and microcins are the most studied bacterial antimicrobial (poly)peptides/proteins, *E. coli* is not the single species to produce bacteriocins for killing neighbouring bacteria. A limited number of bacteriocins have been characterized in other Gram-negative organisms. Some of them are related to colicins, showing a similar domain organization, such as pyocins and lumicins in *Pseudomonas* species (Jacob 1954) and *Photorhabdus luminescens* (Sharma et al. 2002), respectively, pesticins in *Yersinia pestis* (Ferber and Brubaker 1979) or klebicins in *Klebsiella pneumoniae* (Chibber and Vadehra 1986) (see below). It is worth noting that *K. pneumoniae* is also the producer of a microcin (de Lorenzo 1984), namely microcin E492. Others, sharing part of the characteristics of lower molecular mass bacteriocins, are frequently termed bacteriocin-like inhibitory substances (BLIS), according to the recommendations of Ray and colleagues (Jack et al. 1995). Such BLIS have been also characterized in archaea. Archaea possess a remarkable variety of cell envelope structure and chemical, which make them having heterogeneous response to Gram staining. However, they are rather considered as being closer to Gram-positive than Gram-negative bacteria (Gupta 1998). For this reason, bacteriocins from archaea will not be considered here.

Identified Gram-negative bacteria producers of BLIS include mainly *Vibrio* (Farkas-Himsley and Seyfried 1962), *Myxococcus* (Munoz et al. 1984) and *Aeromonas* (Messi et al. 2003). A few bacteriocins produced by the genus *Vibrio* have been identified, namely vibriocins isolated from *V. cholerae* (Wahaba 1965) and *V. harveyi* (Mc Call and Sizemore 1979) and the bacteriocins from *V.* sp strain NM10 (Sugita et al. 1997), *V. vulnificus* (Shehane and Sizemore 2002) and *V. mediterranei* (Carraturo et al. 2006). Among these BLIS, a very restricted number has been well identified. In majority, they have been only partly purified and/or characterized. Therefore, BLIS are not prone to classification.

But what criterions have to be considered to elaborate a classification for bacteriocins, given the heterogeneity of bacterial sources, of peptide structures and of mechanisms of action? In the case of Gram-positive bacteria, the high number of bacteriocins isolated, purified and fully characterized makes the answer easier, even if elaborating the classification remains complex. The classification into groups and subgroups relies essentially (1) on the primary structures, including the size of the (poly)peptide and the presence or absence of consensus sequences and of posttranslational modifications, and (2) on the mechanisms of action and bacterial target

specificity (for more details on this classification, the reader is referred to Chap. 3 by Paul Cotter). Contrary to antimicrobial peptides from eucaryotes, where the three-dimensional structures acquired by the peptides are one of the important criterions considered for their classification, this aspect is not taken into account for bacteriocins, presumably as a lower number of three-dimensional structures has been determined. In Gram-negative bacteria, except for colicins where the classification is clear enough, a classification based on similar criterions is made more difficult, given the great structural heterogeneity inside the very restricted group of bacteriocins formed by microcins. Finally, it is worth noting that, similar to all classifications, the classification of bacteriocins requires constant revision, as it continuously evolves according to the knowledge that accumulates rapidly, as soon as novel bacteriocins or mechanisms of action are brought to light.

This introductory chapter to bacteriocins from Gram-negative bacteria presents their classification, dealing with colicins, colicin-like bacteriocins and microcins, successively. The less-studied bacteriocins, which are described in this chapter, will not be spoken of later in the book, while for a more extensive description of colicins and microcins, readers are referred to the corresponding chapters, which provide comprehensive overviews of these classes of bacteriocins.

The Colicin Classification

Colicins are the most studied bacteriocins from Gram-negative bacteria (for reviews see Konisky 1982; Braun et al. 1994; Braun et al. 2002; Duché et al. 1995; Cascales et al. 2007). They are high-molecular mass (30–80 kDa) bactericidal proteins (Table 4.1 and Fig. 4.1) produced by many *E. coli* strains in periods of stress. Colicins are produced by *E. coli* strains harbouring one colicinogenic plasmid. The production of colicins is lethal for the producing cells, as a consequence of the concomitant production of a lysis protein, coexpressed with the colicin. The typical colicin operon contains one to three genes: a gene encoding the colicin (*cxa* for colicin X activity), a gene often located downstream from the structural gene that encodes the immunity protein (*cxi* for colicin X immunity or *imX*) and a gene encoding the lysis protein (or bacteriocin release protein, BRP), whose product permits the release of the colicin into the external medium (for reviews, the reader is referred to Van der Wal et al. 1995; Braun et al. 2002). Regulation of colicin gene clusters is mediated by the SOS response regulon, which plays a role in the response of bacteria to DNA damages (Walker 1995). Since 1963, it was demonstrated that the various identified colicins had different modes of action (Nomura 1963). The classification elaborated for colicins relies on both their killing mechanisms and the uptake machineries they use (for reviews, the reader is referred to Braun 1995, Cascales et al. 2007). Indeed, colicins kill target cells through three main mechanisms (Cascales et al. 2007; refer to Chap. 14 by Miklos de Zamarockzy) (Table 4.1) either (1) by making voltage-dependent channels in the inner membrane of the target bacteria, (2) by a nuclease action in the cytoplasm or (3) by degrading

Table 4.1 Main characteristics of group A and B colicins and colicin-like bacteriocins[a]

Name of the bacteriocin Producer	Active bacteriocin Number of residues, gene, 3D structure (PDB)	Receptor	Translocation system	Killing mechanism	Immunity protein Number of residues, gene, 3D structure (PDB)
Group A					
Colicin A Citrobacter freundii Escherichia coli	592, *caa*, 1COL[b]	BtuB	OmpF TolA, B, Q, R	Pore-forming	178, *cai*
Colicin K E. coli	548, *cka*	Tsx	OmpF, OmpA TolA, B, Q, R	Pore-forming	196, *cki*
Colicin N E. coli	387, *cna*, 1A87	OmpF	OmpF TolA, Q, R	Pore-forming	131, *cni*
Colicin U E. coli	618, *cua*	OmpA	OmpF, LPS TolA, B, Q, R	Pore-forming	174, *cui*
Colicin S4 E. coli	499, *csa*	OmpW	OmpF, TolA, B, Q, R	Pore-forming	179, *csi*
Colicin E1 E. coli	522, *cea*	BtuB	TolC TolA, Q, R	Pore-forming	113, *ceiA*
Colicin E2 E. coli	581, *ceaB*	BtuB	OmpF, TolA, B, Q, R	DNase	86, *ceiB*
Colicin E3 E. coli	551, *ceaC*, 1JCH[c]	BtuB	OmpF, TolA, B, Q, R	rRNase	84, *ceiC*, 1JCH[c]
Colicin E4 E. coli	177, *cea4*	BtuB	OmpF, TolA, B, Q, R	rRNase	85, *cei4*
Colicin E5 E. coli	unknown, *cea5*	BtuB	OmpF, TolA, B, Q, R	tRNase	83, *cei5*
Colicin E6 E. coli	551, *cea6*	BtuB	OmpF, TolA, B, Q, R	rRNase	85, *cei6*
Colicin E7 E. coli	576, *cea7*, 1M08[d]	BtuB	OmpF, TolA, B, Q, R	DNase	87, *cei7*, 1AYI

(continued)

Table 4.1 (continued)

Name of the bacteriocin / Producer	Active bacteriocin / Number of residues, gene, 3D structure (PDB)	Receptor	Translocation system	Killing mechanism	Immunity protein / Number of residues, gene, 3D structure (PDB)
Colicin E8 / *E. coli*	unknown, *cea8*	BtuB	OmpF, TolA, B, Q, R	DNase	85, *cei8*
Colicin E9 / *E. coli*	582, *cea9*, 1FSJ	BtuB	OmpF, TolA, B, Q, R	DNase	86, *cei9*, 1IMQ
Cloacin DF13 / *Enterobacter cloacae*	561, *ccl*	IutA	TolA, Q, R	rRNase	85, *cim*
Pyocin AP41 / *Pseudomonas aeruginosa*	776	(?)	TolA, B, Q, R	DNase	–
Alveicins A / *Hafnia alvei*	408, *aat*	(?)	TolA, B, Q, R	Pore-forming	*aai*
Alveicin B / *H. alvei*	358, *abt*	(?)	TolA, B, Q, R	Pore-forming	*abi*
Marcescin 28b / *Serratia marcescens*	–	Omp4^e/OmpA^f	OmpF, TolA, B, Q, R	Pore-forming	–
Group B					
Colicin B / *E. coli*	510, *cba*, 1RH1	FepA	FepA (?), TonB, ExbB, D	Pore-forming	175, *cbi*
Colicin 1a / *E. coli*	626, *cia*, 1CH	Cir	Cir, TonB, ExbB, D	Pore-forming	111, *imm*
Colicin 1b / *E. coli*	626, *ciaB*	Cir	Cir (?), TonB, ExbB, D	Pore-forming	115
Colicin 5 / *E. coli*	490, *cfa*	Tsx	TolC, TonB, ExbB, D	Pore-forming	43, *cfl*
Colicin 10 / *E. coli*	490, *cta*	Tsx	TolC, TonB, ExbB, D	Pore-forming	43, *ckl*
Colicin D / *E. coli*	697, *cda*	FepA	FepA (?), TonB, ExbB, D	tRNase (Arg tRNA)	87, *cdi*

Colicin M *E. coli*	271, *cma*	FhuA	FhuA (?) TonB, ExbB, D	Degradation of peptidoglycan Muramidase	117, *cmi*
Pesticin *E. coli*	357, *pst*	FyuA	FyuA (?) TonB, ExbB, D	Muramidase	141, *pim*
Pyocin S1 *Pseudomonas aeruginosa*	618, *pyoS1A*	ferripyoverdine receptor	(?) TonB, ExbB, D	DNase	87, *pyoS1I*
Pyocin S2 *P. aeruginosa*	690, *pyoS2A*	ferripyoverdine receptor	(?) TonB, ExbB, D	DNase	87, *pyoS2I*
Pyocin S3 *P. aerugoinosa*	768, *pyoS3A*	ferripyoverdine receptor	TonB, ExbB, D	DNase	154, *pyoS3I*
Undetermined group					
Klebicin C *K. pneumoniae* *K. oxytoca*	619, *kca*	(?)	non-TonB	rRNase	84, *kci*
Klebicin D *K. pneumoniae*	716, *kda*	(?)	(?)	tRNase	88, *kdi*
Pyocin S4[g] *P. aeruginosa*	764	(?)	(?)	tRNase	–
Pyocin S5[g] *P. aeruginosa*	498	(?)	(?)	Pore-forming	–
Carocin S1 *Herwinia carotovora*	361, *caroS1K*	(?)	(?)	DNase (?)	129, *caroS1I*

[a] References are given in the main text
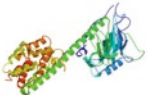[b] C-terminal killing domain
[c] Colicin E3 + immunity protein
[d] Nuclease domain
[e] In *S. marsescens*
[f] In *E. coli*
[g] Predicted by genome sequence in silico analysis

Name of the bacteriocin	Three-dimensional structure
Colicin A Group A (PDB: 1COL)	
Colicin N Group A (PDB: 1A87)	
Colicin B Group B (PDB: 1RH1)	
Colicin Ia Group B (PDB: 1CH)	

Fig. 4.1 Three-dimensional structures of pore-forming colicins of groups A and B

the peptidoglycan (Barreteau et al. 2010). The activity of colicins requires a recognition step that uses a number of receptors normally involved in the uptake of essential nutrients, such as vitamin B12 (cobalamin), siderophore-bound iron or nucleosides, which have been parasitized by colicins, helping them entering more efficiently in target bacteria. The receptors that have been hijacked by colicins are principally the siderophore receptors FhuA (hydroxamate siderophores), FepA, Cir and Fiu (catecholate siderophores) (Ferguson and Deisenhofer 2002), the cobalamine receptor BtuB (Taylor et al. 1998) and the nucleoside receptor Tsx (Bremer et al. 1990). Porins, the membrane proteins that form aqueous channels in the outer membrane and control the passive diffusion of specific small metabolites (sugars, phosphates, amino acids and so on) into the bacterial cells (Nikaido and Vaara 1985; Nikaido 2003), are also used by some colicins (Table 4.1). In many cases, the colicin translocation step remains poorly understood since it is questionable if the receptor is used also as a translocator, or the translocation step does need a second protein partner. For example colicin Ia uses Cir for both recognition and translocation (Jakes and Finkelstein 2009), while colicin A uses BtuB for the recognition step and OmpF for translocation (Lazzaroni et al. 2002) (for a detailed description of this aspect, the reader is referred to Chap. 14 by Miklos de Zamarockzy). The receptors used for the recognition step are each coupled to one of two protein machineries, namely the Ton (Krewulak and Vogel 2008) and Tol membrane systems (Lloubès et al. 2001). These two machineries are anchored at the inner membrane and provide energy to the receptors, using the proton-motive force. The Ton system is composed of three inner membrane proteins TonB, ExbB and ExbD, while the Tol system contains several proteins of similar topology TolA, TolQ,

TolR, and a periplasmic protein TolB and an outer membrane-anchored lipoprotein Pal, which is very probably not required for colicin import (Cascales et al. 2007). Colicins of group A require the Tol proteins or a subset of these proteins (e.g. TolA/TolQ for colicin E1 and TolA/TolQ/TolR for colicin N), while all colicins of group B require TonB/ExbB/ExbD (Table 4.1). Moreover, colicins of group A are encoded by small plasmids and are released into the culture medium, while colicins of group B are encoded by large plasmids and are not released into the medium. Group A comprises colicins A, E1 to E9, K, N, U (Cascales et al. 2007), and S4 (Pilsl et al. 1999; Arnold et al. 2009), which are translocated by the Tol machinery; group B comprises colicins B, D, Ia, M, 5 and 10, which use the Ton system (Cascales et al. 2007) (Table 4.1). In addition, colicin-like bacteriocins, which have been characterized in non-*E. coli* strains, have been shown (or are supposed) to split also into the two groups A and B: cloacin DF13 (Oudega and de Graaf 1976; Thomas and Valvano 1993), klebicins (Chavan et al. 2005), pyocin AP41 (Michel-Briand and Baysse 2002), marcescin 28b (Guasch et al. 1995a, b; Enfedaque et al. 1996) and alveicins (Wertz and Riley 2004) fit with group A and pesticins (Rakin et al. 1996) and pyocins S1 to S5 (Michel-Briand and Baysse 2002) with group B (Table 4.1).

Most colicins and colicin-like bacteriocins are organized into three protein functional domains, each corresponding to one step of the mode of action: (1) the central domain is involved in binding to the receptor (R-domain for Receptor), and thus recognizes and adheres to specific regions on the surface of target cells; (2) the N-terminal domain is responsible for translocation (T-domain for Translocation) and enables entry of the bacteriocin into the target cells and (3) the C-terminal domain, which is the active region, is the killing domain (C-domain, for Cytotoxicity) (Braun et al. 1994; Cascales et al. 2007). Inside each of the two groups, A and B, previously defined, colicins and colicin-like bacteriocins may be assembled according to their killing mechanisms, pore-forming, nuclease or peptidoglycan degradation activities.

The Microcin Classification

Microcins are the lower molecular mass bacteriocins produced by Gram-negative bacteria, mostly *E. coli*, in stress conditions (Baquero and Moreno 1984; Duquesne et al. 2007a), and particularly, in poor nutrient conditions. They are peptides ranging from 1 to 10 kDa that are generally resistant to proteases, extreme pHs and temperatures. They are encoded by gene clusters carried by plasmids or in some cases by the chromosome. The gene clusters involved in the production of microcins include a variable number of genes, but show a conserved organization: open reading frames encode the precursor of the microcin, secretion proteins and self immunity factors and, in some cases, posttranslational modification enzymes. In contrast with the bacteriocins from Gram-positive bacteria, where identification of a high number of representatives sharing common structural features and mechanisms of action allows identifying classes and subclasses, and with colicins that share

characteristics related to their uptake and mode of cell killing that have been exploited for their classification, classifying microcins appears as a difficult task. This is related to the restricted number of representatives identified until now and the high diversity in structures and mechanisms of action they exhibit (Table 4.2). They also arise from various biosynthetic pathways, including or not the acquisition of different complex posttranslational modifications, and display various mechanisms of uptake. In the 2000s, Pons and colleagues proposed the first microcin classification into two classes, depending on the presence or absence of a posttranslational modification (Gaillard-Gendron et al. 2000; Pons et al. 2002a). However in 2004, while microcin E492 was described as unmodified until then (Pons et al. 2002b), the finding of a modified form of microcin E492 carrying a siderophore at the C terminus (Thomas et al. 2004) prompted our group to propose a novel classification (Duquesne et al. 2007a). This classification takes into account the three following criteria: (1) the presence, nature and localization of posttranslational modifications, (2) the gene cluster organization and (3) the sequences of the leader peptides, therefore assembling microcins into two classes (Table 4.2 and Fig. 4.2).

Class I gathers peptides with a molecular mass below 5 kDa, which have supported extensive backbone posttranslational modifications, namely microcins B17, C7-C51 and J25 (Duquesne et al. 2007b; Severinov et al. 2007). Microcin B17 contains four thiazole and four oxazole rings that result from an unusual posttranslational modification of six glycines, four serines and four cysteines in the 39–66 region of the 69-amino acid precursor of microcin B17. Microcin C7-C51 is an *N*-formylated heptapeptide carrying a C-terminal modified nucleotide: a C-terminal aspartic acid is covalently linked to a phosphoramidate group, itself substituted with both an adenosine moiety and a propylamine chain (Fig. 4.2). Microcin J25 is a lasso peptides, which means that it adopts the typical compact lasso structure consisting of an N-terminal ring formed by a lactam bond between the N-terminal extremity (Gly/Cys) and the side chain of an acidic residue (Asp/Glu) in position 8 or 9, where the C-terminal tail is irreversibly threaded (Rebuffat et al. 2004) (Fig. 4.2). Class II includes higher molecular mass peptides (in the 5–10 kDa range) and is itself further subdivided into two subclasses, IIa and IIb. Class IIa contains plasmid-encoded peptides without posttranslational modification and forming possibly disulfide bonds (MccL, MccV and Mcc24) (Fig. 4.2). Class IIb gathers those chromosome-encoded linear microcins that carry a C-terminal siderophore posttranslational modification (microcins E492, M, H47 and presumably I47 and G47 that have been hypothesized through genome analyses), (Poey et al. 2006; Vassiliadis et al. 2010), (Fig. 4.2). It is to note that microcin 24 does not fit perfectly with the criteria previously defined for any of the classes. This microcin has neither been isolated nor biochemically characterized, but based on its precursor sequence predicted from the gene, it contains neither disulfide bond nor posttranslational modification. Nevertheless, it was incorporated into class IIb to which it was more tightly related when considering its gene cluster organization (Duquesne et al. 2007a). For a more detailed description of the microcins from classes I and II, the reader is referred to Chaps. 15 and 16 by Konstantin Severinov et al. and Vassiliadis et al. 2010, respectively.

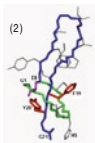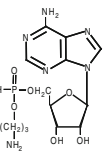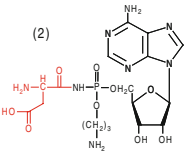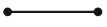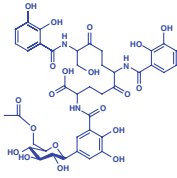**Table 4.2** Main characteristics of class I and II microcins[a]

Name of the microcin	Active microcin Number of residues of the precursor of the mature peptide, gene, microcin 3D structure (PDB)	Post-translational modification Name of the genes involved	Receptor	Translocation system	Killing mechanism
Class I					
B17	69, 43, *mcbA*	Thiazole and oxazole rings *mcbB, mcbC, mcbD*	OmpF	OmpF (?) SbmA	DNA gyrase inhibition
C7-C51	7, 7, *cea*	Modified nucleotide *mccB, mccC, mccE*	OmpF	OmpF (?) YejA, B, E, F	Cleavage in the target cell into a modified aspartyl adenylate that inhibits Asp-tRNA synthetase
J25	58, 21, *mcjA*, 1Q71	Lasso structure *mcjB, mcjC*	FhuA	Fhua (?) TonB, ExbB, D, SbmA	RNA polymerase inhibition Mitochondrial proteins and lipids damages
Class II					
Class IIa					
L	105, 90, *mclC*	2 disulfide bonds	Cir	Cir TonB, ExbB, D SdaC	Membrane permeability modification (?)
V	103, 88, *cvaC*	1 disulfide bond	Cir	Cir (?) TonB, ExbB, D	Membrane permeability modification
24	90, 73, *mfS*	no disulfide bond	(?)	(?)	Mannose permease (ManYZ) targeting (?)
Class IIb					
E492	103, 84, *mceA*	Siderophore anchored at the C terminus *mceC, mceD, mceI, mceJ*	FepA, Cir, Fiu	FepA, Cir, Fiu (?) TonB, ExbB, D	Inner membrane channels ManYZ targeting (mannose permease)

(continued)

Table 4.2 (continued)

Name of the microcin	Active microcin Number of residues of the precursor of the mature peptide, gene, microcin 3D structure (PDB)	Post-translational modification Name of the genes involved	Receptor	Translocation system	Killing mechanism
M	92, 77, *mcmA*	Siderophore anchored at the C-terminus *mcmL, mcmK*	FepA, Cir, Fiu	FepA, Cir, Fiu (?) TonB, ExbB, D	(?)
H47	75, 60, *mchB*	Siderophore anchored at the C-terminus *mchA, mchS1, mchD mchC*	FepA, Cir, Fiu	FepA, Cir, Fiu (?) TonB, ExbB, D	F_0F_1 ATP synthetase
I47[b]	77, 62, *mchS2*	Siderophore anchored at the C-terminus	FepA, Cir, Fiu	FepA, Cir, Fiu (?) TonB, ExbB, D	(?)
G492[b]	89, 74, *mceL*	Siderophore anchored at the C-terminus	FepA, Cir, Fiu (?)	FepA, Cir, Fiu (?) TonB, ExbB, D	(?)

[a] References are given in the main text
[b] Predicted by genome sequence in silico analysis

Name of the bacteriocin	Structure
Microcin J25 Class I (1) primary structure; (2) three-dimensional structure (PDB: 1Q71)	(1) G G A G H V P E Y F V G I G T P I S F Y G (2)
Microcin C7-C51 Class I (1) native microcin; (2) toxic entity generated in target cells	
Microcin V Class IIa	ASGRDIAMAI GTLSGQFVAG GIGAAAGGVA GGAIYDYAST HKPNPAMSPS GLGGTIKQKP EGIPSEAWNY AAGRLCNWSP NNLSDVCL
Microcin E492 Class IIb	GETDPNTQLLNDLGNNMAWGAALGAPG GLGSAALGAAGGALQTVGQGLIDHGPV NVPIPVLIGPSWNGSGSGYNSATSSSGSGS

Fig. 4.2 Structures of microcins of classes I and II

Similar to colicins, microcins have receptor-mediated mechanisms of action. This is reflected in their minimal inhibitory concentrations, which are in the nanomolar range, while they are in the micromolar range for antimicrobial peptides of eukaryotic origin, which act through a direct interaction with phospholipid membrane bilayers. To improve their intake into sensible bacteria, microcins hijack receptors involved in the uptake of essential nutrients, such as the iron siderophore receptors (FhuA, FepA, Cir and Fiu) or the porin OmpF (Duquesne et al. 2007a) (Table 4.2). These receptors, which are also exploited by bacteriophages, antibiotics and bacterial toxins, are thus critical for bacteria for which they represent an Achille's hill. In general, translocation of microcins requires the TonB–ExbB–ExbD complex (Duquesne et al. 2007a), which uses the proton-motive force from the cytoplasmic membrane for energy transduction to the outer membrane and its receptors. However, microcin B17 uses the outer-membrane porin OmpF and the protein SbmA, and microcin C7–C51 requires OmpF and the inner-membrane ABC-transporter YeJ to be actively transported through the inner membrane (refer to Chap. 15 by Konstantin Severinov et al.). Microcins exhibit very heterogeneous killing mechanisms (Table 4.2). Class I microcins inhibit vital bacterial enzymes. Microcins B17 and J25 inhibit DNA gyrase

(Heddle et al. 2001) and RNA polymerase (Mukhopadhyay et al. 2004, Adelman et al. 2004), respectively. Microcin C7–C51 needs a preliminary cleavage inside the target bacteria to generate the toxic entity, which is a mime of aspartyl adenylate that inhibits aspartyl tRNA synthetase, thus blocking protein synthesis at the translation step (Metlitskaya et al. 2006). More complex and subtle mechanisms appear to be developed in certain cases, as microcin J25 has been shown to target mitochondria and the respiratory chain (Niklison-Chirou et al. 2010) in addition to its inhibitory effect of RNA polymerase. Class II microcins rather target the inner membrane or their components. Microcin H47 targets the F_0 proton channel of ATP synthetase (Rodríguez and Laviña 2003). Microcins E492 (Lagos et al. 1993; Bieler et al. 2006) and V (Yang and Konisky 1984) both permeabilize the inner membrane. However, microcin E492 not only forms channels in the inner membrane (Lagos et al. 1993) but also requires the ManYZ inner-membrane components of the mannose permease, which is involved in the active uptake of mannose and related hexoses, to exert its bactericidal activity (Bieler et al. 2006). Microcins can also require proteins at the inner membrane for activity, such as SdaC, which is involved in serine uptake, for microcin L (Gérard et al. 2005) or SbmA for microcins B17 (Yorgey et al. 1994) and J25 (De Cristobal et al. 2006). The exact role of these proteins is presumably helping microcins passing through the inner membrane.

Conclusions

In contrary to the classification of bacteriocins from Gram-positive bacteria, which mainly relies on the structural features, the classification of colicins is based on functional criteria that are the recognition machineries and the killing mechanisms. In the case of microcins, due to the high heterogeneity of this restricted class of bacteriocins as regards the structural characteristics, the complexity of the genetic systems and the killing mechanisms developed, the classification has to take into account these different criteria to assemble microcins sharing common characters. For bacteriocins produced by Gram-positive and Gram-negative bacteria, from both commensal and environmental origin, a classification helps pointing more easily similarities or differences in the strategies they developed to make bacteriocinogenic strains better adapted to the natural conditions in a given niche. No doubt that genomic advance will contribute to the rapid identification of novel bacteriocins (refer to Chap. 5 by Oscar Kuipers and colleagues), which will allow describing novel mechanisms at different levels in bacteriocin production and activity, i.e. synthesis of the bacteriocins, immunity of the producing bacteria, penetrating capacity inside target bacteria and killing mechanisms. This will possibly change the classification and also modify and improve our use of bacteriocins as models for deciphering novel, clever and sophisticated strategies of antibacterial activity and of resistance to antimicrobials, thus providing tools for various applications in the environment and for the development of novel antibiotics.

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