

Lantibiotics produced by *Actinobacteria* and their potential applications (a review)

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Abstract

The phylum *Actinobacteria*, which comprises a great variety of Gram-positive bacteria with a high G+C content in their genomes, is known for its large production of bioactive compounds, including those with antimicrobial activity. Among the antimicrobials, bacteriocins, ribosomally synthesized peptides, represent an important arsenal of potential new drugs to face the increasing prevalence of resistance to antibiotics among microbial pathogens. The actinobacterial bacteriocins form a heterogeneous group of substances that is difficult to adapt to most proposed classification schemes. However, recent updates have accommodated efficiently the diversity of bacteriocins produced by this phylum. Among the bacteriocins, the lantibiotics represent a source of new antimicrobials to control infections caused mainly by Gram-positive bacteria and with a low propensity for resistance development. Moreover, some of these compounds have additional biological properties, exhibiting activity against viruses and tumour cells and having also potential to be used in blood pressure or inflammation control and in pain relief. Thus, lantibiotics already described in *Actinobacteria* exhibit potential practical applications in medical settings, food industry and agriculture, with examples at different stages of pre-clinical and clinical trials.

INTRODUCTION

The phylum *Actinobacteria* is one of the major phyla of the domain Bacteria and contains Gram-positive filamentous bacteria with a high G+C DNA content and different morphological, physiological and metabolic characteristics [1–3]. Most bacteria belonging to this phylum are free-living micro-organisms that are ubiquitously found in both aquatic and terrestrial ecosystems and include the following: (i) several pathogens, such as species of *Corynebacterium*, *Mycobacterium*, *Nocardia*, *Propionibacterium* and *Tropheryma*; (ii) soil inhabitants, such as *Micromonospora* spp. and *Streptomyces* spp.; (iii) plant commensals, such as *Frankia* spp.; and (iv) gastrointestinal commensals, such as *Bifidobacterium* spp. [1]. Their diversity ensures one of the main features of this group: versatility in the production of biologically active compounds, including antimicrobial substances [4]. Until 2010, about 34 000 microbial bioactive compounds had already been reported and close to 40 % of them are produced by *Actinobacteria*, especially *Streptomyces* spp., which produce nearly 80 % of the actinobacterial compounds. Approximately 10 000 actinobacterial substances exhibit antimicrobial and/or antitumour activity [5]. Moreover, most of the antimicrobials already used in human and veterinary medicine or agriculture are either

natural or semisynthetic derivatives of actinobacterial and fungal products [6]. These data highlight the importance and the potential practical application of the actinobacterial antimicrobial substances, notably to control pathogenic micro-organisms.

The actinobacterial antimicrobials belong to many different groups, including conventional antibiotics and bacteriocins [1]. In this review, we will focus on lantibiotics produced by these micro-organisms as members of this bacteriocin class represent new alternatives to fight against emerging multi-drug-resistant pathogens. We will also update the classification scheme of the bacteriocins already described in this phylum, based on the proposal of Alvarez-Sieiro and coworkers [7], and discuss the best characterized actinobacterial lantibiotics, highlighting their potential biotechnological applications.

BACTERIOCINS PRODUCED BY *ACTINOBACTERIA*

Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins produced by prokaryotes [8]. They are usually small peptides (<10 kDa) which are cationic and amphipathic [9–12]. Actinobacterial bacteriocins inhibit the

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Abbreviations: CDI, *Clostridium difficile* infections; Dha, dihydroalanine; Dhb, dihydrobutyrine; HIV, human immunodeficiency virus; HSV, herpes simplex virus; Lan, lanthionine; Lab, labionin; MeLan, β -methyl-lanthionine; PLA₂, phospholipase A₂; DPH, 3,3-dimethylamino-1-propylamide hydrochloride; PE, phosphatidylethanolamine.

growth of species related to the producer, such as michiganin A [13], although many of them possess a broad spectrum of inhibitory activity that includes non-related bacteria (microbisporicin), protozoans (thiostrepton), yeasts and fungi (cinamycin) and viruses (labyrinthopeptin A1 and duramycin) [14–18]. In addition to antimicrobial activity, some actinobacterial bacteriocins and their semisynthetic derivatives also exhibit anti-inflammatory [19–21], anti-allergic [20], antitumour [22–24] and antinociceptive activities [25, 26], and may also act in blood pressure regulation [19, 27]. Some of these peptides have already found clinical applications, such as thiostrepton, a thiopeptide used in dermatologic ointment for veterinary use [28], and others are in stages of pre-clinical and clinical trials, such as NAI-107, NAI-112 [29] and lancovutide/duramycin (<https://clinicaltrials.gov/ct2/show/study/NCT00671736?term=Lancovutide&rank=1>; accessed September 17, 2016), which reinforces the great potential of practical application of bacteriocins produced by *Actinobacteria*.

The increasing number of new bacteriocins with distinctive features being continuously described makes difficult the establishment of a classification system for these compounds. There are several publications that discuss and update the bacteriocin classification [8, 9, 11, 30–33]. Alvarez-Sieiro and coworkers [7] proposed a broader bacteriocin classification scheme, which will be adopted in this review since it allows us to best accommodate the diversity of actinobacterial bacteriocins. This classification scheme divides the bacteriocins into only three classes. Class I includes peptides smaller than 10 kDa that undergo enzymatic post-translational modifications of some amino acid residues, known as ribosomally synthesized and post-translationally modified peptides. Class II bacteriocins are unmodified peptides smaller than 10 kDa and class III is formed by unmodified bacteriocins larger than 10 kDa. *Actinobacteria* produce bacteriocins belonging to all three classes and most subclasses, except for subclasses IIb (whose antimicrobial activity depends on two unmodified peptides), IIc (which comprises single-peptide, unmodified bacteriocins that are synthesized without leader peptide sequences) and IId (which comprises single-peptide and unmodified bacteriocins that are not pediocin-like bacteriocins). Table 1 summarizes the bacteriocin classification scheme adopted in this review, describing the main features of each class and subclass and giving examples of actinobacterial bacteriocins. For class I, only the ribosomally synthesized and post-translationally modified peptide subclasses already described in *Actinobacteria* are listed, such as lanthipeptides, thiopeptides, lasso peptides, linaridins, bottromycins and linear azole-containing peptides.

ACTINOBACTERIAL LANTIBIOTICS AND THEIR POTENTIAL BIOTECHNOLOGICAL APPLICATIONS

Lanthipeptides are peptides that undergo post-translational modifications which give rise to rare amino acids, such as

lanthionine (Lan), β -methyl-lanthionine (MeLan), didehydroalanine (Dha) and didehydrobutyrine (Dhb), among others [30]. Lan and MeLan possess internal rings formed by thioether bridges formed between Dha or Dhb, respectively, and a cysteine (Cys) residue [8, 10, 34]. Therefore, lanthipeptide modifications depend on the occurrence of two main reactions: (i) dehydration of serine (Ser) or threonine (Thr) residues to form Dha or Dhb, respectively, and (ii) cyclization for formation of Lan or MeLan, respectively. The lanthipeptides that exhibit antimicrobial activity are called lantibiotics and their antibacterial activity is related to the inhibition of cell wall biosynthesis by binding to lipid II (to its pyrophosphate-sugar moiety), thus preventing the incorporation of the cell wall precursor units into the nascent peptidoglycan, and/or to pore formation, interfering with cellular membrane functions [8, 34, 35]. Of medical importance is the fact that binding of lantibiotics to lipid II is not antagonized by vancomycin, a drug commonly used in the control of infections caused by Gram-positive pathogens [36]. Since their mechanisms of action are different from those of other antimicrobials already used in clinical settings, lantibiotics have been considered important alternatives for treatment of infections caused by resistant bacteria carrying prevailing resistance mechanisms [8, 10, 34]. Actinobacterial lantibiotics generally target lipid II. As lipid II is not the product of a single gene but derived from multiple enzyme-catalysed reactions, such lantibiotics have a reduced propensity for resistance development [37]. Therefore, their antimicrobial activity combined with their low tendency to generate resistance makes these lantibiotics highly attractive for medical applications [38].

Lantibiotics may exhibit either linear or globular structures and are divided into three types or subclasses based on their biosynthetic machinery [8]. The type I lantibiotics are modified by the dehydratase LanB and the cyclase LanC; the type II is modified by a bifunctional enzyme, LanM, with both activities, and the type III is modified by a trifunctional enzyme, LanKC (with lyase, kinase and cyclase activities) (Table 2).

Lantibiotics are generally synthesized in the form of inactive precursor peptides. Their production depends on the presence of a gene cluster, organized in one or more operons, located either on mobile genetic elements, such as plasmids, or on the bacterial chromosome of the producer strain [10, 11, 34]. The number of genes found in these clusters varies (generally from 6 to 15) depending on the lantibiotic produced. Besides the lantibiotic structural gene, these genes encode at least functions related to post-translational modifications of amino acids, proteolytic processing of the precursor peptide for production of the mature and bioactive peptide, bacteriocin transport and immunity. Some clusters also carry genes involved in regulation of lantibiotic production. These gene clusters will not be described in the present review as detailed information about them can be found in the numerous reviews on lantibiotics that can be found in the literature [10, 11, 34, 35, 39, 40].

Table 1. Classification scheme for bacteriocins produced by *Actinobacteria*

	Distinctive features	Examples	Activity	References
Class I (heat-stable, <10 kDa, modified peptides)				
Lanthipeptides	Contain sulfur- β -carbon linkages	Planosporicin Actagardine Labyrinthopeptins	Antibacterial (G+ and G–) Antibacterial (G+) Antibacterial (G+), antiviral, antinociceptive	[45] [57] [93]
Thiopeptides	Contain a central pyridine, a dihydropyridine or piperidine ring as well as heterocycles	Thiostrepton	Antibacterial (G+), antiprotozoal, anticancer	[14, 96, 97]
Lasso peptides	Possess a lasso structure	GE2270 A Lariatins	Antibacterial (G+ and G–) Antibacterial (<i>Mycobacterium</i>)	[98] [99]
Linear azole-containing peptide	Possess heterocyclic rings of thiazole and (methyl)oxazole	Siamycin Goadsporin	Antibacterial (G+ and G–) and antiviral Antibacterial (<i>Streptomyces</i>)	[100, 101] [102]
Botromycins	Contain a macrocyclic amidine, a decarboxylated carboxy-terminal thiazole and carbon-methylated amino acids	Botromycin A2	Antibacterial (G+ and <i>Mycoplasma</i>)	[103, 104]
Linaridins	Possess a linear structure with dehydrated amino acids	Cypemycin	Antibacterial (<i>Micrococcus luteus</i>), anticancer	[23]
Class II (heat-stable, <10 kDa, unmodified peptides)				
Ila	Pediocin-like peptides	Bifidocin B	Antibacterial (G+)	[105]
IIf	Two-peptide	ND		
IIfc	Leaderless peptides	ND		
IId	Non-pediocin-like, single-peptide	ND		
Class III (thermo-labile, >10 kDa proteins)				
IIIa	Bacteriolysins	Ipomycin	Antibacterial (<i>Streptomyces ipomoeae</i>)	[106]
IIIb	Non-lytic	AFP-1	Antifungal	[107]

ND, Not detected in *Actinobacteria*; G+, Gram-positive bacteria; G–, Gram-negative bacteria.

Table 2. Classification scheme of lantibiotics produced by *Actinobacteria*

Type	Distinctive features	Example	Producer <i>Actinobacteria</i>	Reference
I	Processed by LanB and LanC	Microbisporicin A1 and A2	<i>Microbispora corallina</i>	[15]
		Planosporicin	<i>Planomonospora alba</i>	[45]
II	Processed by a bifunctional LanM	Variacin	<i>Kocuria varians</i>	[52]
		Actagardine	<i>Actinoplanes garbadinensis</i> and <i>Actinoplanes liguriae</i>	[54]
		Deoxyactagardine B	<i>Actinop. liguriae</i>	[68]
		Michiganin A	<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i>	[13]
		Cinnamycin	<i>Streptomyces cinnamoneus</i>	[17]
		Duramycin	<i>Strep. cinnamoneus</i>	[81]
		Ancovenin	<i>Streptomyces</i> spp.	[27]
III	Contain labionin; processed by LanKC	Labyrinthopeptins A1, A2 and A3	<i>Actinomadura namibiensis</i>	[93]
		NAI-112	<i>Actinoplanes</i> spp.	[25]

Although lantibiotics are mainly produced by species of *Firmicutes*, potent lantibiotics have been detected in species of *Actinobacteria* [41], as described below. However, only the best characterized lanthipeptides that exhibit antimicrobial activity will be covered by this review.

TYPE I LANTIBIOTICS

Microbisporicin or NAI-107

Microbisporicin or NAI-107 (Fig. 1a) is produced as a complex of two structurally related lantibiotics containing 24 amino acids (aa), five thioether bridges (three Lan, one MeLan and one AviCys; the latter amino acid results from the oxidative decarboxylation of a terminal Cys residue) and amino acid modifications that have never been reported in the literature, such as a 5-chloro-tryptophan (Trp) and a proline (Pro) hydroxylation [15, 42]. Microbisporicin/NAI-107 has been detected in strains of *Microbispora corallina* [15, 42, 43] and *Actinoallomurus* spp. [43, 44]. This lantibiotic was first isolated in a project that aimed to identify new compounds that act as cell wall synthesis inhibitors from screening a collection of about 40 000 rare actinomycetes [15, 45]. The most abundant congeners of microbisporicin/NAI-107 are named A1 (2246 Da) and A2 (2230 Da), and they differ only in position 14, with a 3,4-dihydroxy-proline in microbisporicin A1 and a 4-hydroxy-proline in variant A2. Both congeners exhibit the same mechanism of action and similar inhibition spectrum, proving to be potent lantibiotics that act by blocking cell wall synthesis [15]. This blocking occurs by the microbisporicin/NAI-107 binding to lipid II as well as to other bactoprenol-bound precursors, impairing membrane functions and resulting in a rapid bactericidal activity [46]. Other minor congeners have also been described in strains of *Microbispora* spp., differing by the presence of two, one or zero hydroxyl groups at Pro-14, by the presence of a chlorine at Trp-4 and/or by the presence of a sulfoxide on the thioether linkage of the first Lan. However, these minor congeners showed some variation in antimicrobial activity, being less effective than A1 and A2, particularly against staphylococci and enterococci [42].

Microbisporicin/NAI-107 is considered one of the most potent lantibiotics, exhibiting a strong activity against aerobic

and anaerobic Gram-positive pathogens. Its spectrum of activity includes drug-resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* and *Streptococcus pneumoniae* resistant to penicillin, presenting efficacy comparable and sometimes superior to those observed for vancomycin and teicoplanin. This lantibiotic also exhibits activity against *Clostridium* spp. and *Propionibacterium* spp., and some Gram-negative bacteria as well, such as *Neisseria* spp., *Haemophilus influenzae* and *Moraxella catarrhalis* [15]. Microbisporicin proved to be effective in treating different types of infections (sepsis, endocarditis and granuloma pouch) caused by Gram-positive multiple drug-resistant pathogens *in vivo* [47]. Pharmacokinetic and pharmacodynamic studies using a neutropenic murine thigh infection model showed a dose-dependent bactericidal effect of microbisporicin/NAI-107 and a prolonged suppression of bacterial growth (>35 h). In addition, the *in vivo* NAI-107 effectiveness was similar to or even better than that observed for drugs already used to treat methicillin-resistant *Staph. aureus*, such as ceftaroline, daptomycin, oxazolidinones and vancomycin [48]. Sustained concentrations of microbisporicin/NAI-107 were observed in plasma, which may explain the compound efficacy in different animal models of infection, such as the acute lethal infection model in mice, the granuloma pouch model in rats, rat endocarditis [47] and the neutropenic murine thigh infection model [48]. Microbisporicin/NAI-107 is at the final stages of pre-clinical development [29] proving to be a potent lantibiotic with promising chances of being used to treat infections caused by a variety of Gram-positive bacteria of medical importance.

A brominated variant of microbisporicin/NAI-107, known as NAI-108, carrying a 5-Br-tryptophan, was detected in cultures of *Microbispora* spp. and *Actinoallomurus* spp. when potassium bromide (KBr; at 8.6 mM) was added to the growth medium. NAI-108 was produced by substitution of chlorine found in NAI-107 structure (in Trp-4) with bromine and it represents the first example of a bromine-containing lantibiotic. This variation was responsible for the generation of a more potent lantibiotic, with an inhibitory spectrum similar to that of microbisporicin/NAI-107 but effective at lower concentrations than those required for inhibition by the parental peptide. NAI-108 turned out

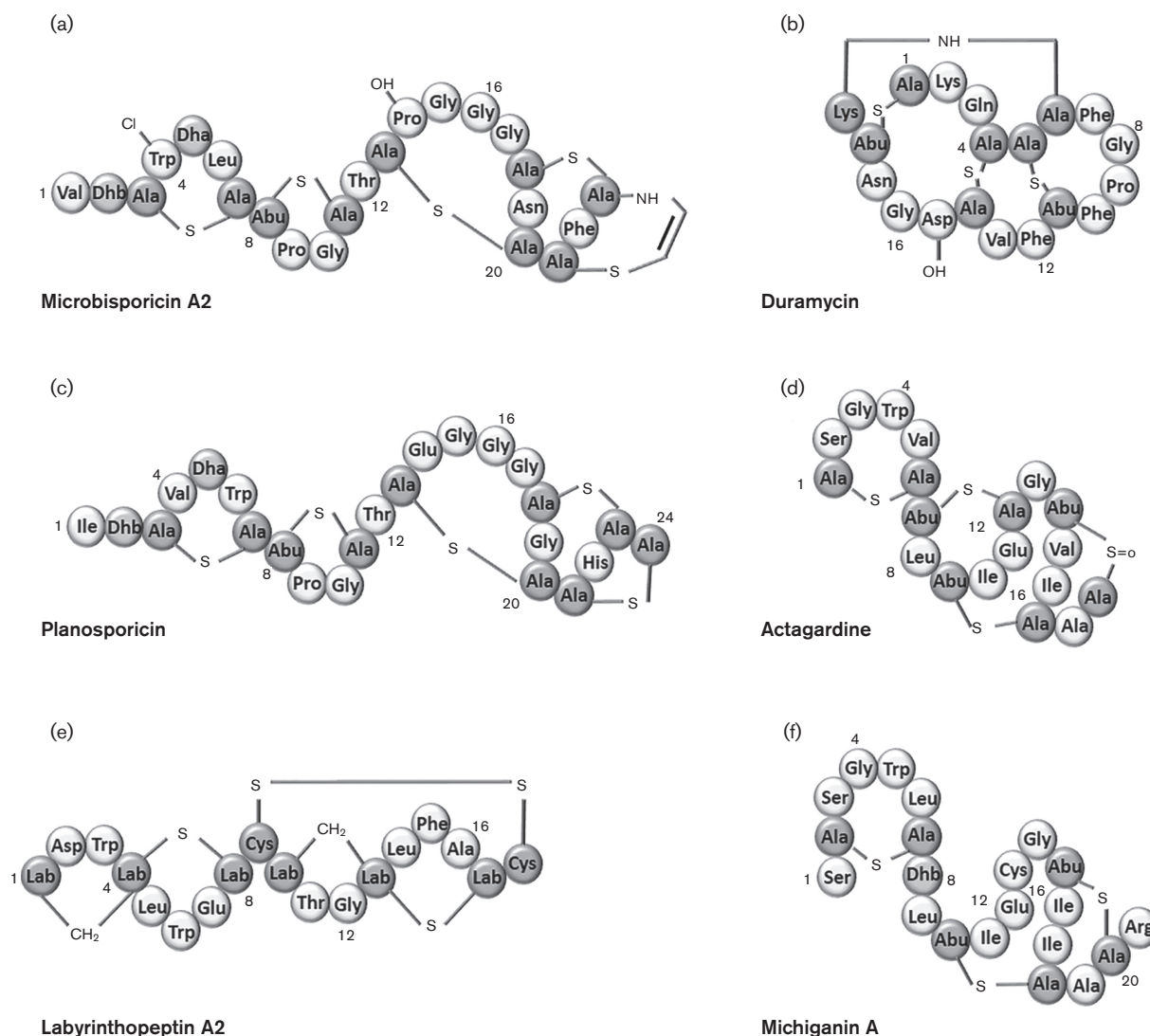


Fig. 1. Representative scheme of the main actinobacterial lantibiotics.

to be about twice as active as microbisporicin/NAI-107 against important pathogens such as *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp. At $0.25 \mu\text{g ml}^{-1}$, it also rapidly killed the target strain *Staph. aureus* ATCC 29213, leading to a 5- $\log_{10}$ reduction in viable cells within 30 min. A lower inhibition was observed with microbisporicin/NAI-107 that caused only a 3- $\log_{10}$ reduction in viable cells, when used at the same concentration. For both, microbisporicin/NAI-107 and NAI-108, the target strain growth rate in the presence of the antimicrobial was comparable to that observed in the control with no antimicrobial added. Therefore, NAI-108 was consistently more active than microbisporicin/NAI-107. Although NAI-108 is still at an early study phase, it is another attractive option for treatment of infections caused by multidrug-resistant Gram-positive bacteria [43].

Planosporicin or lantibiotic 97518

Planosporicin (Fig. 1c), a cell wall biosynthesis inhibitor of 24 aa (2194 Da), is produced by *Planomonospora* sp. DSM 14920 and it was detected in the same project that described microbisporicin [45]. Planosporicin and microbisporicin/NAI-117 exhibit a similar primary structure, with over 70 % sequence similarity (differing in only six amino acids, occupying the positions 1, 4, 6, 14, 19 and 22), and five thioether linkages. Planosporicin possesses four Lan residues and one MeLan residue as this lantibiotic presents a Lan residue instead of an AviCys residue at the C-terminal region [49, 50]. Planosporicin production, which is encoded by a cluster of 15 genes, is confined to the stationary phase of growth in broth and to the onset of morphological differentiation when *Planomonospora* spp. are grown on solid medium [51].

Despite sharing remarkable structural and antibacterial spectrum similarities, inhibiting important Gram-positive pathogens, planosporicin exhibited a less potent antimicrobial activity than microbisporicin/NAI-117 against all tested micro-organisms, including staphylococci, streptococci and enterococci, among others [15]. The difference in the effectiveness of these two lantibiotics seems to be related to subtle structural differences in the region between amino acids 12 and 18. In this region, microbisporicin is more flexible. Moreover, planosporicin presents a negative charge due to the presence of a glutamic acid residue (Glu-14) in this region, not found in microbisporicin. These two features seem to influence the interaction of both lantibiotics with the membrane and, therefore, their antibacterial activity [50].

TYPE II LANTIBIOTICS

Variacin

Variacin was detected in two strains of *Kocuria varians* (formerly referred to as *Micrococcus varians*) isolated from Italian-type raw salami as part of the natural meat microbiota. The mature lantibiotic is composed of 25 aa (2658 Da), containing one Lan and one MeLan rings, and exhibits a significant similarity (92 %) to lactacin 481, a type II lantibiotic produced by *Lactococcus lactis*. Variacin inhibits a wide range of Gram-positive food spoilage bacteria; important food-borne pathogens, such as *Enterococcus* spp., *Listeria monocytogenes* and *Staph. aureus*; and vegetative forms and spores of *Clostridium* spp. and *Bacillus* spp. In addition to its broad inhibitory spectrum, which suggests a potential of this lantibiotic as a food biopreservative, variacin is also thermostable and resistant to a wide pH range (pH 2 to 10), desirable features for this application [52]. When variacin was added in the form of a spray-dried fermented ingredient (at 1 or 3 % of total weight) in chilled dairy products, it was effective in controlling a cocktail of psychotrophic strains of the spore-forming food-borne pathogen *Bacillus cereus*, even under conditions of temperature abuse. At 8 °C,

variacin at 1 % of fermented ingredient inhibited the growth of *B. cereus* in all products and models to which it was applied. At 12 °C, the addition of 3 % fermented ingredient was required to inhibit outgrowth of the spores [53]. However, further studies, such as (i) tests of the variacin cytotoxicity to eukaryotic cells at the concentrations used in the study of O'Mahony and coworkers, (ii) the effectiveness of the bacteriocin in other food matrices and (iii) other bacteriocin properties (such as shelf-life and sensitivity to gastrointestinal conditions), should be encouraged so the feasibility of using this lantibiotic as a natural antimicrobial to control undesirable micro-organisms in food products is confirmed.

Actagardine and derivatives

Actagardine (Figs 1d and 2) was first described in 1976 under the name gardimycin. This lantibiotic is produced by *Actinoplanes garbadinensis* and *Actinoplanes liguriae* [54–56]. It is composed of 19 aa (1890 Da) and four intramolecular rings, one Lan and three MeLan, with the C-terminal MeLan oxidized to sulfoxide [57–59]. This sulfoxide bond is not common among lantibiotics. The oxidation of the thioether linkage between residues 14 and 19 of the mature peptide for formation of the sulfoxide bond is catalysed by the product of the *garO* gene, which encodes a luciferase-like monooxygenase. Deletion of *garO* resulted in production of deoxyactagardine, with a slightly lower activity than its cognate, suggesting a certain influence of the sulfoxide group on antimicrobial activity [60]. This result, however, contrasts with a study performed with nisin, a lantibiotic produced by *Lc. lactis*, in which the oxidation of its Lan residues led to nisin inactivation [61].

Actagardine acts by inhibiting cell wall synthesis by blocking the transglycosylation reaction [36, 62, 63]. This lantibiotic exhibits a potent activity against Gram-positive pathogens, particularly *Streptococcus* spp. and *Clostridium* spp. [54–56]. *In vivo* tests using mice experimentally infected with *Strep. pneumoniae* or *Streptococcus haemolyticus* (formerly referred to as *Diplococcus haemolyticus*)



Fig. 2. Peptide sequences of actagardine and its derivatives. The thioether bonds that are shared by all these peptides are shown as black lines on the actagardine sequence. Amino acids that differ from actagardine sequence are marked in red.

showed that actagardine has an antimicrobial activity comparable to those of ampicillin and cephaloridine [54].

Upon detection of actagardine, several natural and semisynthetic derivatives have been described (Fig. 2). These lantibiotics share in their structures at least three thioether bridges and 14 of the 19 aa of their chains [64]. The D metabolite, accidentally generated during actagardine purification, differs from actagardine by only the absence of a MeLan residue, and *in vitro* and *in vivo* tests showed significantly better antimicrobial activity of this compound against *Streptococcus* spp. and *Staph. aureus* [65]. Several basic carboxamides of actagardine have already been synthesized. The monocarboxamides were shown to be twice to fourfold more effective than actagardine in a murine model of *Streptococcus pyogenes* septicaemia. Among the monocarboxamides, the 3,3-dimethylamino-1-propylamide hydrochloride (DPH) was chosen for further analyses and, like actagardine, displayed a bactericidal activity, although at lower concentrations. The actagardine and DPH killed at least 99.9 % of the *Strep. pyogenes* viable cells exposed to a concentration of 10 times the MIC, the MIC_{DPH} being equal to 0.4 µg ml⁻¹ and the MIC_{Actagardine} equal to 1.6 µg ml⁻¹ [66].

The Ala(0)-actagardine (1961 Da; Fig. 2), produced by *Actinop. liguriae* ATCC 31048, differs from actagardine by only an additional alanine (Ala) residue at its N-terminus (Fig. 2), and it displays a slightly higher activity than actagardine, especially against strains of *Staphylococcus* spp. However, regarding streptococci and enterococci, Ala(0)-actagardine exhibited an inhibitory activity similar to that of actagardine. The semisynthetic derivatives Lys(0)-actagardine and Ile(0)-actagardine displayed an activity either better (from twofold to fourfold lower MICs) or similar to that of Ala(0)-actagardine against the strains tested [67].

Actinop. liguriae NCIMB41362 produces another lantibiotic related to actagardine, the deoxyactagardine B (Fig. 2). This peptide has also 19 aa and differs from actagardine in residues 15 and 16 [actagardine contains valine (Val) and isoleucine (Ile) and deoxyactagardine, leucine (Leu) and Val, respectively] and by the absence of the sulfoxide bond in the MeLan bridge between residues 14 and 19 [68]. Semisynthetic derivatives of deoxyactagardine B, NVB302 and NVB333, display antimicrobial activity against important bacterial pathogens and are already in clinical trials.

NVB302 (aminoheptylamido-deoxyactagardine; Fig. 2) completed part of the phase I of clinical trials for the treatment of *Clostridium difficile* infections (CDI) (www.evaluationgroup.com/Universal/View.aspx?type=Story&id=344702; accessed 17 September 2016). *Clost. difficile* is an important pathogenic micro-organism implicated in 20–30 % of cases of antibiotic-associated diarrhoea and 90 % of cases of pseudomembranous colitis [69]. One advantage of using NVB302 in the treatment of CDI is the fact that this antimicrobial exhibits selective *in vitro* activity against *Clost. difficile* compared with other intestinal bacteria [70]. As vancomycin is used to treat severe cases of colitis and

diarrhoea caused by *Clost. difficile* [71, 72], NVB302 effectiveness was compared to that of vancomycin. *In vitro* tests were then performed using a human gut model inoculated with a faecal emulsion prepared from *Clost. difficile*-negative faeces of health volunteers. On days 14 and 21, spores (~10⁷ c.f.u.) of a virulent *Clost. difficile* strain were inoculated into the system, followed by instillation of either NVB302 (125 mg l⁻¹) or vancomycin (125 mg l⁻¹) on day 30. Each antimicrobial was instilled four times daily for 7 days. From day 37 until day 50, gut microbiota populations and the *Clost. difficile* cytotoxin titres were monitored. NVB302 exhibited an activity comparable to that of vancomycin as both antimicrobial instillations effectively eliminated vegetative *Clost. difficile* from the gut model, leading also to cytotoxin reduction to undetectable levels 5–7 days after drug instillation. Moreover, NVB302 exhibited a reduced effect on the intestinal microbiota. However, neither NVB302 nor vancomycin displayed activity against *Clost. difficile* spores. Taken together, these results showed that NVB302 succeeded in treating simulated CDI in a human gut model [70]. Animal model tests have also shown that NVB302 is effective in treating CDI, reaching high concentration levels in the colon and without significant toxic effects. NVB302 was also resistant to pH and enzymes present in the gastric fluid and it was not absorbed systemically, which minimizes the appearance of collateral effects [73].

NVB333 (Fig. 2) was synthesized from binding 3,5-dichlorobenzylamine to the C-terminal region of deoxyactagardine B. This semisynthetic lantibiotic presents activity against clinically relevant Gram-positive pathogens such as *Staph. aureus* resistant to meticillin, vancomycin, linezolid and daptomycin; vancomycin-resistant *Enterococcus*; and penicillin-resistant *Strep. pneumoniae*. Although the NVB333 MICs against most strains tested were either twice or fourfold higher than those determined for vancomycin, this peptide showed excellent results in *in vivo* tests using disseminated infection, thigh infection and lung infection models, with results comparable to and even better than those observed for vancomycin. Additionally, NVB333 was well tolerated at the dose levels used and there were no signs of any drug-related adverse effects. These results coupled with a low resistance rate to NVB333 make this compound a novel promising candidate for the treatment of systemic infections caused by Gram-positive pathogens [37].

More recently, two other actagardine-related lantibiotics have been described, NAI-802 (2188 Da; Fig. 2), produced by *Actinoplanes* sp. ID104802 and ID104771, and a semisynthetic derivative, obtained following amidation of NAI-802 with benzylamine, which resulted in the diamidated derivative 3. NAI-802 has two additional amino acids, an N-terminal Ala and a C-terminal arginine (Arg), when compared to actagardine, these additions being the only differences found between both lantibiotics. The NAI-802 producer strains also produce minor amounts of its Ala(0)-congener 2 (Fig. 2). The amino acid additions found in NAI-802 seem to have improved its activity against *Staphylococcus* spp. and

Streptococcus spp., with MICs ranging from 0.5 to 32 $\mu\text{g ml}^{-1}$ for NAI-802, whereas the MICs for actagardine ranged from 2 to 64 $\mu\text{g ml}^{-1}$. In relation to enterococci, the MICs observed for NAI-802 ($\geq 128 \mu\text{g ml}^{-1}$) were similar to those for actagardine [64]. The improved activity of NAI-802 is probably related to the additional positive charge due to the presence of a C-terminal Arg. Positively charged residues are believed to play an important role in the interaction of lantibiotics with the negatively charged bacterial membranes [74]. Such importance is confirmed by the antimicrobial activity of the diamidated derivative 3, which carries one additional positive charge through conversion of the C-terminal carboxylate into the corresponding basic amide. The diamidated derivative 3 exhibited an improved antimicrobial activity when compared to NAI-802, exhibiting from a fourfold to an eightfold reduction in MIC values, even against enterococci. NAI-802 and derivative 3 showed also a significant activity against Gram-positive anaerobic bacteria, such as *Clostr. difficile*, *Clostridium butyricum*, *Clostridium perfringens* and *Peptostreptococcus asaccharolyticus* with MIC ranges of 0.25–2 $\mu\text{g ml}^{-1}$ and <0.125 –8 $\mu\text{g ml}^{-1}$, for NAI-802 and derivative 3, respectively. These MIC values were comparable to those determined for vancomycin (0.25–1 $\mu\text{g ml}^{-1}$). Hence, NAI-802 and its diamidated derivative 3 are potential novel antimicrobial agents for the treatment of infections caused by Gram-positive pathogens, including resistant strains [64].

Michiganin A

Clavibacter michiganensis subsp. *michiganensis* is known not only as the agent of bacterial wilt and canker of tomato but also for producing many antimicrobial substances [75, 76], including michiganin A [13]. Michiganin A is a 21 aa heat-stable lantibiotic (2145 Da; Fig. 1f) related to actagardine and, like other actagardine-related bacteriocins, presents two rings of MeLan and one of Lan. Michiganin A differs from actagardine by the absence of the sulfoxide bond and by only four residues, the amino acids added to the N- and C-termini and the residues 5 and 15, which are Val and Val in actagardine, and Leu and Ile in michiganin A, respectively [13, 60]. Holtsmark and coworkers [13] suggested that michiganin A acts by inhibiting cell wall synthesis due to its high similarity to mersacidin and actagardine, including the presence of a conserved Glu residue, known to be important for interaction with lipid II. However, michiganin A has a rather narrow spectrum of target organisms. This lantibiotic inhibits, at nanomolar concentrations (in the 10–100 nM range), the growth of the closely related bacterium *Clavibacter michiganensis* subsp. *sepedonicus* [13, 77], an important phytopathogen that causes potato ring rot, responsible for major economic losses worldwide [78]. Despite the requirement of more studies, michiganin A is a potential new antimicrobial for plant disease control. Although its spectrum of activity is quite narrow, such a feature offers an advantage, contributing to cover agriculture's need for more sustainable and effective strategies for control of specific phytopathogens, without affecting plant-beneficial micro-organisms [77].

Cinnamycin group

Cinnamycin (2042 Da), duramycin (2014 Da; Fig. 1b) and ancovenin (1959 Da) form a group of natural variants that present other biological activities in addition to a weak antimicrobial action. Lantibiotics belonging to the cinnamycin group exhibit a similar structure as globular 19 aa peptides with one Lan, two MeLan and an unusual lysinoalanine bridge between Lys-19 and Ser-6 (Fig. 3). They also exhibit a modification in position 15, an aspartate hydroxylation yielding the *erythro*-3-hydroxy-aspartic acid. Among the cinnamycin group, ancovenin is the most different variant, neither presenting the aspartate 15 modification nor the lysine–alanine bridge (Fig. 3) [20].

Cinnamycin is produced exclusively by *Streptomyces* spp. [79]. Despite this lantibiotic having also been described in *Streptoverticillium* spp., this genus was unified with *Streptomyces* in 1990 [80]. This lantibiotic inhibits the growth of *Bacillus subtilis*, anaerobic bacteria, fungi and yeasts (although less intensely). Additionally, it also inhibits the proliferation of herpes simplex virus (HSV) type 1 [17]. Duramycin was isolated from *Streptomyces cinnamoneus* and initially studied because of its inhibitory action against *Rhodococcus fascians* [81], a phytopathogen that infects a wide range of plants causing a leafy gall formation [82]. Some natural variants of duramycin, duramycins B (1951 Da) and C (2008 Da), have already been described [34].

Ancovenin and cinnamycin inhibit the activity of the angiotensin-converting enzyme, presenting a potential application in blood pressure regulation [19, 27]. Cinnamycin and duramycin act as phospholipase A₂ (PLA₂) inhibitors [20, 21]. In fact, it is an indirect inhibition because the lantibiotics bind at a 1:1 ratio and with high affinity and exclusive specificity to the substrate of PLA₂, the phosphatidylethanolamine (PE) [21, 83]. This binding alters the operation of ion channels [84–86], a feature that is exploited by the pharmaceutical industry for cystic fibrosis treatment, as discussed below [87, 88]. Furthermore, as PLA₂ provides arachidonic acid, by releasing this fatty acid from phospholipids in the cell membranes to the enzymes responsible for the synthesis of eicosanoids, which are associated with inflammation, such lantibiotics can also be used in regulation of inflammatory processes [19, 89]. The inhibition of PLA₂ has also been explored in treatment of diseases such as atherosclerosis, diabetes and cancer [89].

In vitro studies using airway epithelium showed that duramycin enhances the chlorine secretion (via alternative chloride channel activation) due to the intracellular calcium increase [85]. Because of this activity, duramycin, also called Moli1901 or lancovutide, is in phase II of clinical trials to be used in the treatment of cystic fibrosis. Cystic fibrosis is a genetic disorder that causes an exocrine organ dysfunction. The lung disease is characterized by inhibition of chlorine secretion into airway surface liquid, creating an ion imbalance which results in a thick mucus layer which in turn hinders the ciliary functions and airway clearance, forming a favourable environment for multiplication of micro-organisms [90]. Then duramycin acts

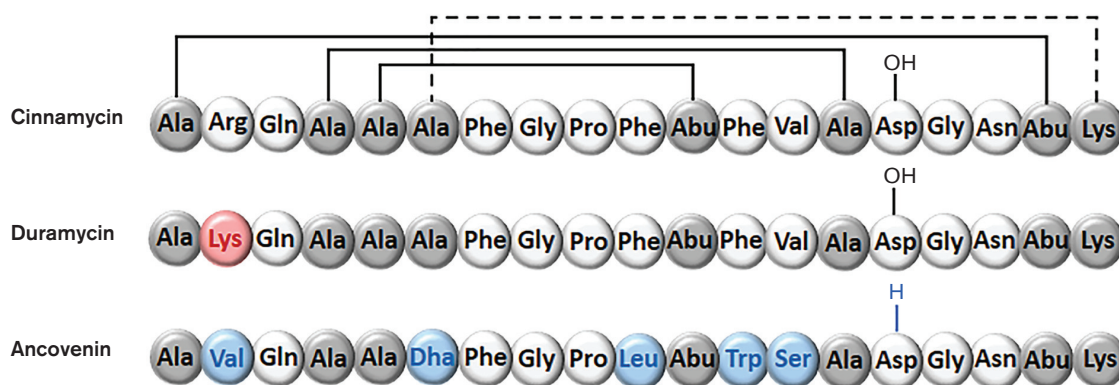


Fig. 3. Comparison of the sequences of the peptides belonging to the cinnamycin group. The thioether bonds that are shared by all peptides are shown as black lines on the actagardine sequence. The dashed line represents the lysine–alanine bridge, which is not present in ancovenin.

indirectly, rehydrating airway surface liquid of patients with cystic fibrosis. The finished phase IIb of clinical trials approved intrapulmonary administration by inhalation of nebulized duramycin in the treatment of cystic fibrosis patients, proving to be safe and with improvement in lung function [87, 88].

Recent studies have also shown inhibitory activity of duramycin on the multiplication of viruses and tumour cells. Both pancreas tumour cells and viruses of the *Flaviviridae* and *Filoviridae* families have PE on their surfaces. In tumour cells, duramycin binds to PE, increasing apoptosis and necrosis and reducing cell proliferation in a dose-dependent manner (between 0.125 and 12.5 $\mu\text{mol l}^{-1}$) [91]. For the West Nile, dengue and ebola viruses, duramycin inhibited the viral entry processes. Therefore, duramycin, as other lantibiotics that bind to PE, has potential to inhibit the activity of tumour cells and viruses [18, 91].

TYPE III LANTIBIOTICS

Labyrinthopeptins

The labyrinthopeptins are lanthipeptides that do not possess Lan and MeLan residues. Instead, these lantibiotics contain a Lan variant known as labionin (Lab), which is a carbacyclic ring which requires two Dha and one Cys for its formation. Lab is thus formed by a Lan residue covalently bound to Dha by a methylene bridge [26, 92]. Labyrinthopeptins were initially detected in *Actinomadura namibiensis* DSM 6313 culture and this bacterium produces three types of labyrinthopeptins called LabA1, LabA2 and LabA3. The labyrinthopeptins are composed of 18–21 aa and, in addition to Lab, they also possess a disulfide bond between two Cys residues. The variants LabA1 and LabA3 differ only by addition of an aspartic acid (Asp) in the N-terminal region of LabA3, and LabA2 (Fig. 1e) is the smallest variant with 18 aa [26, 93].

LabA1 exhibits a very effective antiviral activity, acting against both human immunodeficiency virus (HIV) and HSV,

preventing cell entry of HIV and HSV, and cell-to-cell viral transmission of HIV. Its anti-HSV activity was comparable to those shown by antiviral compounds (acyclovir and cidofovir) and this lantibiotic kept its anti-herpetic activity even against drug-resistant strains. Additive or synergistic effects were also demonstrated between LabA1 and antiviral drugs already used in the clinic (tenofovir, acyclovir, saquinavir, raltegravir and enfuvirtide). Therefore, LabA1 presents favourable properties that qualify its application in prevention and control of transmission of sexual diseases caused by HIV and HSV [16]. On the other hand, although LabA2 showed only a moderate anti-HSV activity and no anti-HIV activity [16, 26], it presents a potent antipain activity [26], as also observed for NAI-112.

NAI-112

NAI-112 was another lantibiotic that was detected during a screening program that aimed to search for new cell wall inhibitors. NAI-112 is a Lab-containing lantibiotic produced by *Actinoplanes* sp. DSM 24059. It is a 22 aa neutrally charged peptide presenting, besides Lab residues, a methyl-labionin (MeLab) in its C-terminal region. In addition, NAI-112 carries a 6-deoxyhexose moiety N-linked to Trp-13. Thus, NAI-112 was the first lantibiotic in which the presence of MeLab and N-glycosylation was reported. Despite its modest antimicrobial activity observed against *Staphylococcus* spp. and *Streptococcus* spp., which contrasts with the properties of most lantibiotics, NAI-112 displayed antinociceptive activity in animal models, reducing pain symptoms in mice in both the formalin and the chronic constriction injury tests, without toxicity signs. Therefore, like LabA2, NAI-112 has potential to be used for pain relief applications. However, the mechanism of action of NAI-112 as antinociceptive agent has not been established yet [25].

CONCLUDING REMARKS

The phylum *Actinobacteria* comprises important human pathogens, such as *Mycobacterium tuberculosis*, *Mycobacterium leprae* and *Corynebacterium diphtheriae*, among

others, and animal and plant pathogens as well. However, as shown in this review, its members may also be potential rich sources of new compounds with a variety of biological properties. Regarding lantibiotics, which were the focus of this review, these properties include the following: (i) antimicrobial drugs to be added to the arsenal of novel compounds required to control multidrug resistance pathogens involved in human, animal or plant infections; drugs for (ii) blood pressure or (iii) inflammation control; and (iv) drugs with an antinociceptive activity. Regarding their antimicrobial activity, not only bacterial strains but also viruses and tumour cells can be targeted by actinobacterial lantibiotics. Many of them have unusual structural features, when compared to most lantibiotics, including the presence of a lysinoalanine bridge and/or residues of erythro-3-hydroxy-aspartic acid, MeLab or Lab, representing compounds with interesting and additional biological properties that deserve further investigation.

Taking into account the numerous species that belong to this phylum, the diversity of their habitats and the number of bacteriocins described in *Actinobacteria*, this phylum can be considered still unexplored regarding novel biologically active compounds. The diversity of biological properties exhibited by the substances already under study certainly encourages the search for new compounds produced by *Actinobacteria*. In this respect, the increasing number of genome sequences provides a new valuable tool for identifying bacteriocins from this group of micro-organisms, following a genome-mining approach using antimicrobial substance databases, such as BACTIBASE [94] and BAGEL3 [95] for bacteriocins, and antiSMASH (<http://antismash.secondarymetabolites.org/>) for antibiotics and secondary metabolites.

Additionally, the study of natural variants of lantibiotics is also important and has contributed to the knowledge about modifications that affect the activity of these compounds. This knowledge can be used to guide the design of new semisynthetic derivatives with improved characteristics. Furthermore, since the information for the synthesis of lantibiotics is genetically encoded on the bacterial DNA, it also allows genetic engineering modifications of the lantibiotic structural gene for production of a greater variety of substances with potential applications.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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