fermentation

MDPI

Review

Unraveling the Biosynthetic Logic Behind the Production of Ramoplanin and Related Lipodepsipeptide Antibiotics

Oleksandr Yushchuk [1,2], Kseniia Zhukrovska [2], Elisa Binda [1] and Flavia Marinelli [1,*]

[1] Department of Biotechnology and Life Sciences, University of Insubria, 21100 Varese, Italy; oleksandr.yushchuk@uninsubria.it (O.Y.); elisa.binda@uninsubria.it (E.B.)
[2] Department of Genetics and Biotechnology, Ivan Franko National University of Lviv, 79005 Lviv, Ukraine; kseniya-oksana.zhukrovska@lnu.edu.ua
* Correspondence: flavia.marinelli@uninsubria.it; Tel.: +39-0332-42-1546

Abstract: This review focuses on the genetic and biotechnological aspects of the biosynthesis of ramoplanin (Rmp), enduracidin (End), and other related lipodepsipeptide antibiotics, herein named collectively ramoplanin and ramoplanin-related lipodepsipeptide (RRLDPs). These compounds exhibit a promising antimicrobial activity against Gram-positive bacterial pathogens, showing no cross-resistance with vancomycin. Rmp is in clinical development for human treatment and End has been used as animal growth promoter for decades. Other RRLDPs as ramoplanose and janiemycin had been poorly investigated in the past, whereas new molecules as chersinamycin have been recently discovered, attracting a renewed interest in this class of antibiotics. Nowadays, sequence and annotation of the biosynthetic gene clusters (BGCs) of Rmp, End, and several other RRLDPs are available, and researchers are focused on understanding the biosynthetic logic behind the production of these compounds. Interestingly, producers of Rmp and chersinamycin belong to the so-called "non-common" actinomycetes from the family *Micromonosporaceae*, whereas End is produced by different members of the genus *Streptomyces*. To the best of our knowledge, no reviews summarize and systematize the current information on the biosynthesis of RRLDPs. Therefore, in this review, we aim to fill this gap. We first describe and compare the BGCs for known RRLDPs, giving an insight on how they were discovered and developed. Next, we review the biosynthetic pathways of these antibiotics, as well as the regulation of their biosynthesis. Then, we focus on the production processes of RRLDPs, demonstrating how cultivation and nutritional factors influence their production. Finally, we provide a short outline of future directions in studying RRLDPs.

Keywords: ramoplanin; enduracidin; chersinamycin; lipodepsipeptides binding to lipid-II; biosynthetic gene cluster; genes; antibiotics

Citation: Yushchuk, O.; Zhukrovska, K.; Binda, E.; Marinelli, F. Unraveling the Biosynthetic Logic Behind the Production of Ramoplanin and Related Lipodepsipeptide Antibiotics. *Fermentation* **2024**, *10*, 544. https://doi.org/10.3390/fermentation10110544

Received: 30 September 2024
Revised: 18 October 2024
Accepted: 21 October 2024
Published: 24 October 2024

1. Introduction

In the past century, a multitude of polypeptide antibiotics of bacterial origin has been isolated and investigated [1,2]. These can generally be divided into two superclasses based on their principal biosynthetic mechanisms: ribosomally synthesized and post-translationally modified peptides (RiPPs) [3], and non-ribosomally synthesized peptides (NRPs) (i.e., those produced involving non-ribosomal peptide synthetases [4]). Producers of the former and latter compounds have been discovered across various Gram-positive and Gram-negative bacterial lineages, although the members of the phylum *Actinomycetota* (traditionally named actinomycetes) represent the largest source of bacterial polypeptide antibiotics. Apart from actinomycetes, RiPPs have been predominantly found in Gram-positive bacteria from the phylum *Bacillota*, including *Bacillus* spp. (e.g., subtilin [5]), *Lactococcus* spp. (e.g., nisin [6]), *Staphylococcus* spp. (e.g., epidermin [7]), *Streptococcus* spp. (e.g., salivaricin [8]), *Enterococcus* spp. (e.g., cytolysin [9]), *Carnobacterium* spp. (carnocin [10]), or in Gram-negative myxobacteria (e.g., myxarylin in *Pyxidicoccus fallax* [11]). Producers

of non-ribosomally synthesized peptide antibiotics have been identified within a much broader variety of bacterial phyla, including *Bacillota* (e.g., gramicidin and polymyxin from *Bacillus brevis* and *Paenibacillus polymyxa*, respectively [12,13]), *Pseudomonadota* (e.g., teixobactin from *Eleftheria terrae* and ajudazols from *Chondromyces crocatus* [14,15]), or *Cyanobacteriota* (e.g., anabaenopeptins from *Planktothrix* spp. [16]).

Among RiPPs and non-ribosomal peptide antibiotics, numerous compounds target different steps of peptidoglycan (PG) biosynthesis [17]. PG biosynthesis, intensively discussed elsewhere (e.g., [18]), is a vital process in eubacteria absent in eukaryotes, and consequently it represents a good target for antibiotics. Among the PG-targeting antibiotics, lipid II binders have been the most exploited [17]. Although lipid II molecules constitute a small fraction of cell-membrane phospholipids [19], their role is essential for the PG biosynthesis and turnover [20]. Such lipid II-binding NRPs as glycopeptide antibiotics (GPAs), vancomycin, and teicoplanin represent the "last defense line" antibiotics to treat infections caused by multidrug-resistant Gram-positive pathogens [21,22], while nisin (an RiPP) is widely used as a food preserving agent [23].

Among lipid II binders, large cyclic non-ribosomally synthesized lipodepsipeptides such as ramoplanin (Rmp) and enduracidin (End) (as well as some other related compounds, more recently discovered, hereafter referred to as RRLDPs—ramoplanin and related lipodepsipeptides), occupy a special niche [24]. These antibiotics interact with lipid II as dimers, forming stable salt bridges with the pyrophosphate portion and enclosing the polar head of lipid II [25]. RRLDPs exhibit a promising antimicrobial activity spectrum against Gram-positive bacterial pathogens, being more active against *Staphylococcus* spp. (including the methicillin-resistant mutants) than GPAs and showing no cross-resistance with vancomycin towards vancomycin-resistant enterococci (VRA) [26–28]. End has been used as an animal growth promoter for decades in various farm animals [29–35], and notably no cases of End-resistant isolates have been reported yet, implying that resistance is not easily acquired [32]. Rmp has long been investigated for therapeutic applications in humans, but in vivo animal studies demonstrated that it is not orally absorbed, it is instable in plasma, and poorly tolerated if injected, posing significant hurdles to its clinical development [36]. Orally administered Rmp has been recently reconsidered for the treatment of gastrointestinal infections and has progressed into phase III clinical trials, but there is no recent information about its status [37].

In the last decade, novel RRLDPs have been described, enriching this class of promising potential antibiotics and re-attracting the general interest for their peculiar mode of action in overcoming resistant barriers to the current antibiotics in clinics [38–40]. Notably, as in the case of Rmp, most of them are produced by relatively uncommon actinomycetes genera, which are difficult to manipulate genetically and present intriguing challenges in handling and fermentation [38,39]. Sequencing of the corresponding biosynthetic gene clusters (BGCs) might enable comparing the different producing pathways, opening the way for an integrated view of RRLDP biosynthesis. Indeed, numerous aspects of RRLDP biosynthesis remain still unexplored and warrant additional investigations. There is a notable absence of contemporary review articles focusing specifically on RRLDP biosynthesis that summarize all these recent findings. In this review, we aim to update the landscape of RRLDP discovery among the producing actinomycetes, with a special focus on reconstructing and discussing the biosynthetic logics behind RRLDP production, on the basis of BGC comparison of old and novel antibiotics belonging to this class. Herein, we also report the gene-engineering and biotechnological strategies to improve the producing strains and processes, which have been optimized mostly during Rmp and End discovery and development. Although the advances in the chemical synthesis of RRLDPs are not covered by this review, it has to be mentioned that Rmp and its analogues can be produced by synthetic chemistry as an alternative to biological production, e.g., [41]. To this purpose, a recently developed solid-phase peptide synthesis and macrocyclization applied to RRLDPs [42,43] represents a promising and innovative method to efficiently explore their structural activity relationship (SAR). Our hope is that a thorough understanding of these aspects among the

different RRLDPs might contribute in the future to the re-purposing of this antibiotic class, re-engineering, and re-designing a new generation of molecules able to tackle the diffusion of resistance in Gram-positive pathogens.

2. Ramoplanin and Related Lipodepsipeptides: Producer Strains and History of Their Discovery

Rmp and End are the best studied RRLDPs that were isolated as a result of extensive bioactivity-guided screening programs in the previous century. End (also known as enramycin [44]) was the first RRLDP antibiotic to be isolated in 1968 [45] by the Takeda Chemical Industries (Japan). The producing microorganism was an actinomycete strain obtained from a soil sample collected in Nishinomiya (Japan), and subsequently classified as *Streptomyces fungicidicus* B-5477 (ATCC 21013) [28]. Shortly after (in 1970), another RRLDP, named janiemycin, was isolated from *Streptomyces macrosporeus* ATCC 21388 [46]. Although the initial structural analysis indicated that janiemycin was chemically related to End [46], neither the chemical structure of the compound nor its producer were further investigated. More than a decade later, in 1983, Rmp, initially referred to as A-16686 (also known as MDL 62198), was discovered. Rmp was obtained from an actinomycete strain isolated from a soil sample collected in Vaghalbod (India) by Gruppo Lepetit (Italy) [47]. The producer of Rmp was initially identified as *Actinoplanes* sp. ATCC 33076 [47] and has received a valid classification as *Actinoplanes ramoplaninifer* ATCC 33076 only recently [48]. Later, an unknown *Actinoplanes* sp. of undisclosed origin was shown to produce the so-called ramoplanose (UK-71,903), an RRLDP structurally almost identical to Rmp [49]. Similar to janiemycin, the producer of ramoplanose was not further investigated at that time. Of note, several steps in Rmp biosynthesis (discussed below) were investigated in *Actinoplanes* sp. SIPI-A.2006 (e.g., [50]), which likely is a synonym of ATCC 33076.

Recent findings demonstrated that End production is not limited to *S. fungicidicus* ATCC 21013. *S. fungicidicus* TXX3120, L-69, and SG-01 were additionally reported as End producers, although their origins and relation to ATCC 21013 remain unclear [51–53]. *Streptomyces* sp. NJWGY3665 (isolated from soil samples collected in Nanjing, China) [54], *Streptomyces atrovirens* MGR140 (CGMCC No. 3365) (isolated from mangroves of Jiulong river, China) [40,55], and *Streptomyces* sp. MC079 (a deep-sea Pacific Ocean sediment isolate) [56] were also identified as active producers of End. The approaches used to identify *Streptomyces* sp. NJWGY3665 and *Streptomyces* sp. MC079 as End producers remain unclear. Instead, a targeted screening guided by the End halogenase gene detection was employed to identify *S. atrovirens* MGR140 [40].

A recent study conducted a systematic screening of actinomycetes from the soil samples collected worldwide for potential RRLDP producers [39]. After cultivation in complex liquid media, cultural broths of the isolates were extracted with acetone, and the obtained crude extracts were tested against *Bacillus subtilis* HB0950. The latter served as a reporter strain, as it contains the *lacZ* gene (coding for β-galactosidase) fused to the *liaI* promoter (*PliaI*), which activates the expression of *lacZ* in response to the cell-wall stress induced by lipid II binders [57,58]. Consequently, extracts containing lipid II binders induced chromogenic conversion of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and were subjected to LC-MS to identify mass peaks resembling Rmp [39]. Forty-nine isolates were thus found to produce RRLDPs, either exactly Rmp or, more often, related molecules. These isolates were identified as follows: 1 *Actinoplanes* sp., 34 *Micromonospora* spp., 3 *Nocardia* spp., 1 *Amycolatopsis* sp., 1 *Lechevalieria* sp., 1 *Actinomadura* sp., 3 *Streptomyces* spp., and 5 taxonomically unidentified isolates [39]. The majority of these isolates were subspecies of *Micromonospora chersina* (10 isolates) and *Micromonospora chaiyaphumensis* (10 isolates) [39]. Consequently, subsequent research aimed to determine whether *M. chersina* DSM 44151, already known as the producer of the enedyine antitumor antibiotic dynemicin [59], could also produce RRLDPs [38]. As a result, *M. chersina* DSM 44151 was found to produce a novel RRLDP, named chersinamycin [38].

Thus, to-date, RRLDPs have been shown to be produced by different actinomycetes genera, belonging at least to the orders *Micromonosporales*, *Nocardiales*, *Pseudonocardiales*, *Streptosporangiales*, and *Kitasatosporales*. Further exploration of uncommon taxa of actinomycetes holds great promise for the discovery of novel RRLDPs.

3. Structural Features of Rmp and Related Lipodepsipeptides

The chemical structures of five RRLDPs were investigated so far to a different extent [25,38,46,60,61]. These are Rmp, End, and chersinamycin, produced by *A. ramoplaninifer* ATCC 33076 [62], *S. fungicidicus* ATCC 21013 [63], and *M. chersina* DSM 44151 [38], respectively, as well as janiemycin (*S. macrosporeus* ATCC 21388) [46] and ramoplanose (*Actinoplanes* sp.) [49]. All of these compounds feature a non-ribosomally synthesized oligopeptide core, consisting of 17 proteinogenic and non-proteinogenic amino acids. In the process of biosynthesis, the linear oligopeptide is cyclized through an ester linkage between the 3-OH group of amino acid in position 2 (AA2) and the terminal carboxyl group of AA17 [64,65]. An aliphatic side chain is attached to the terminal amino group of AA1. Consequently, RRLDPs can be characterized as 49-membered lipidated macrocyclic depsipeptides. The primary structural differences among RRLDPs arise from variations in the amino acid composition of the oligopeptide core, differences in halogenation and glycosylation patterns, and variations in the structure and branching of aliphatic side chains.

3.1. Rmp Complex, Ramoplanose, and Chersinamycin

Rmp is produced in *A. ramoplaninifer* ATCC 33076 as a complex of structurally related congeners [47,66]. The complex consists of factors A1 (6–12%), A2 (72–86%), and A3 (8–14%) in standard cultivation conditions [62,67]. Production of additional factors A'1, A'2, and A'3 was also observed, although only under certain production conditions, accounting for ca. 15% of the total production. All of these compounds share a non-ribosomally synthesized 17 amino acid macrocyclic core, which comprises 5 proteinogenic (asparagine, Asn; phenylalanine, Phe; glycine, Gly; leucine, Leu; and alanine, Ala) and 12 non-proteinogenic amino acids (Figure 1a) [47,62,68]. The non-proteinogenic amino acids include 3-hydroxyasparagine (3-OH-Asn), 4-hydroxyphenylglycine (Hpg, 6 residues), ornithine (Orn, 2 residues), and *allo*-threonine (aThr, 3 residues) [62] (Figure 1a). In all the congeners of Rmp, AA17 is monochlorinated (resulting in 3-chloro-Hpg). A 2,3-4,5-unsaturated aliphatic side chain is attached to the terminal amino group of AA17. The differences among Rmp congeners are determined by the length and branching of this side chain, which is identical in factors A1 and A'1, A2 and A'2, and A3 and A'3 (Figure 1a) [62,66]. Finally, a D-mannosyl-D-mannose disaccharide is attached to AA11 (Hpg) in factors A1, A2, and A3 via an *O*-glycosidic bond (Figure 1a) [62]. Alternatively, in factors A'1, A'2, and A'3, this disaccharide is replaced by a single D-mannose (Figure 1a) [66].

Ramoplanose, produced by an unidentified *Actinoplanes* sp., shares structural identity with Rmp factor A1 but carries an additional D-mannose residue on the D-mannosyl-D-mannose disaccharide attached to AA11 (Figure 1a) [49].

In contrast to the Rmp complex, chersinamycin is reported to be produced in *M. chersina* DSM 44151 as a single compound [38]. Structurally, chersinamycin closely resembles Rmp (Figure 1b). Rmp and chersinamycin share 15 out of the 17 amino acids forming the aglycone, but chersinamycin has 3,5-dihydroxyphenylglycine (Dpg) and valine (Val) in positions AA13 and AA15, respectively, which differ from Hpg and Leu found in the corresponding positions of Rmp [38]. Additionally, chersinamycin carries a saturated C10 aliphatic side chain (Figure 1b). The chlorination and glycosylation patterns of chersinamycin match those of Rmp factors A1-3 (Figure 1b) [38].

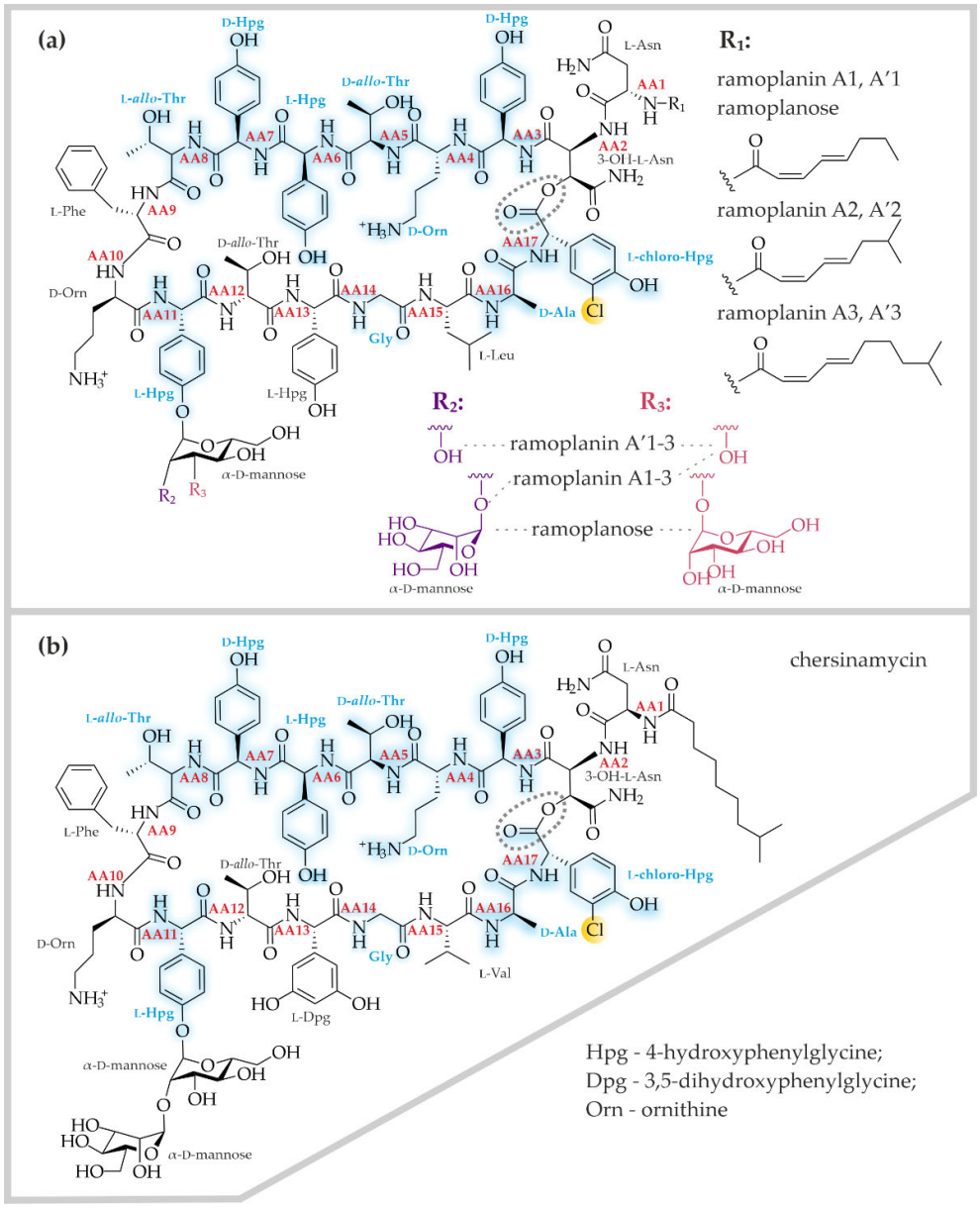


Figure 1. Structures of Rmp complex and ramoplanose (**a**), and chersinamycin (**b**). Amino acids that are identical in Rmp, chersinamycin, and End are highlighted in blue; the chlorination site is highlighted in yellow, while the ester linkage closing the cyclic structure between the 3-OH group of amino acid in position AA2 and the terminal carboxyl group of AA17 is marked with a dashed line.

3.2. End and Janiemycin

End is produced in *S. fungicidicus* ATCC 21013 as two congeners, End A (I) and B (II), differing only in the length of the aliphatic side chain (Figure 2); trace amounts of the monochlorinated versions of A and B, indicated as C and D, respectively, were also detected [63,69]. Amino acid composition of the End peptide core differs from Rmp and chersinamycin [45,70], featuring 5 proteinogenic (aspartic acid, Asp; Thr; serine, Ser; Gly; and Ala) and 12 non-proteinogenic amino acids. These include aThr (2 residues), Hpg (6 residues), Orn (1 residue), citrulline (Cit, 1 residue), and enduracididine (Ecd, 2 residues) (Figure 2). The last is a rare non-proteinogenic amino acid containing an unusual five-membered cyclic guanidine moiety [71] (Figure 2). Ecd was initially described as a component of End (as it comes from its eponymous name) [71], but it was later found also in other peptide antibiotics originating from actinomycetes, such as minosamyno-

mycin (*Streptomyces* sp. MA514-A1 [72]), mannopeptimycins (*Streptomyces hygroscopicus* LL-AC98 [73]), and in other natural products [74].

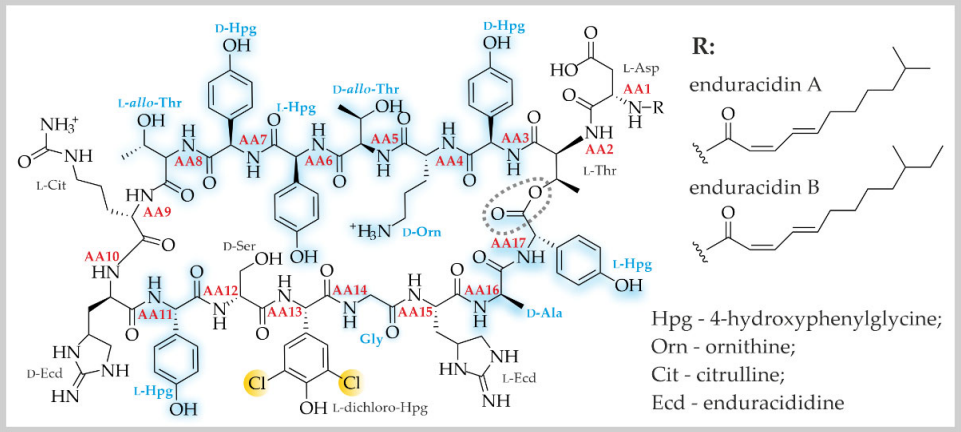


Figure 2. Structures of End complex. Amino acids that are identical in Rmp, chersinamycin, and End are highlighted in blue; chlorination sites are highlighted in yellow; the ester linkage between the 3-OH group of amino acid in position AA2 and the terminal carboxyl group of AA17 is marked with a dashed line.

The peptide core of End carries two chlorine atoms attached to AA13 Hpg (yielding 3,5-dichloro-Hpg) (Figure 2). Notably, as indicated by its initial structural analysis, janiemycin is a non-chlorinated analogue of End [46]. Unlike Rmp or chersinamycin, End is not glycosylated [45]. Finally, End A and B feature 2,3-4,5-unsaturated C12 and C13 aliphatic side chains, respectively, [63] (Figure 2).

4. Biosynthesis of Ramoplanin and Related Lipodepsipeptides: From Genes to Pathways and Compounds

Today, thousands of high-quality bacterial genomes are available for analysis, unraveling the unprecedented genetic variability of BGCs for different antibiotic families [75]. Currently, sequences of RRLDP BGCs are available from eight actinomycetes belonging to the orders *Micromonosporales*, *Kitasatosporales*, and *Pseudonocardiales*, namely *A. ramoplaninifer* ATCC 33076 (BGC named *ramo*) [65], *M. chersina* DSM 44151 (*chers*) [38], *S. fungicidicus* ATCC 21013 (*end*) [28,64], *Streptomyces* sp. SLBN-134 [38], *Streptomyces* sp. TLI_053 [38], *Amycolatopsis balhimycina* DSM 44591 [38], *Amycolatopsis orientalis* B-37 [38], and *Amycolatopsis orientalis* DSM 40040 [38] (Figure 3). Only in the first three strains, BGCs are known to be actively expressed, leading to the production of structurally elucidated RRLDPs, i.e., Rmp, End, and chersinamycin (see Section 3). Interestingly, some of the annotated RRLDP BGCs were found in the genomes of well-known GPA-producing strains such as *Am. balhimycina* DSM 44591 producing balhimycin [76], *Am. orientalis* DSM 40040 (KCTC 9412) synthetizing vancomycin [77], and in the norvancomycin-producer *Am. orientalis* B-37 [78]. Since no RRLDPs were ever detected from these strains, although they were extensively cultivated for GPA production, it might be assumed that corresponding RRLDP BGCs are silent [38]. As reported in Section 2, End production was also observed in *S. atrovirens* MGR140 [40], and the authors reported the identification of an *end*-like BGC; however, the sequence of this BGC has not been deposited yet in public databases. RRLDP BGCs from the strains belonging to *Streptosporangiales* and *Nocardiales*, reported above (Section 2) to produce RRLDPs [39], are also not available so far.

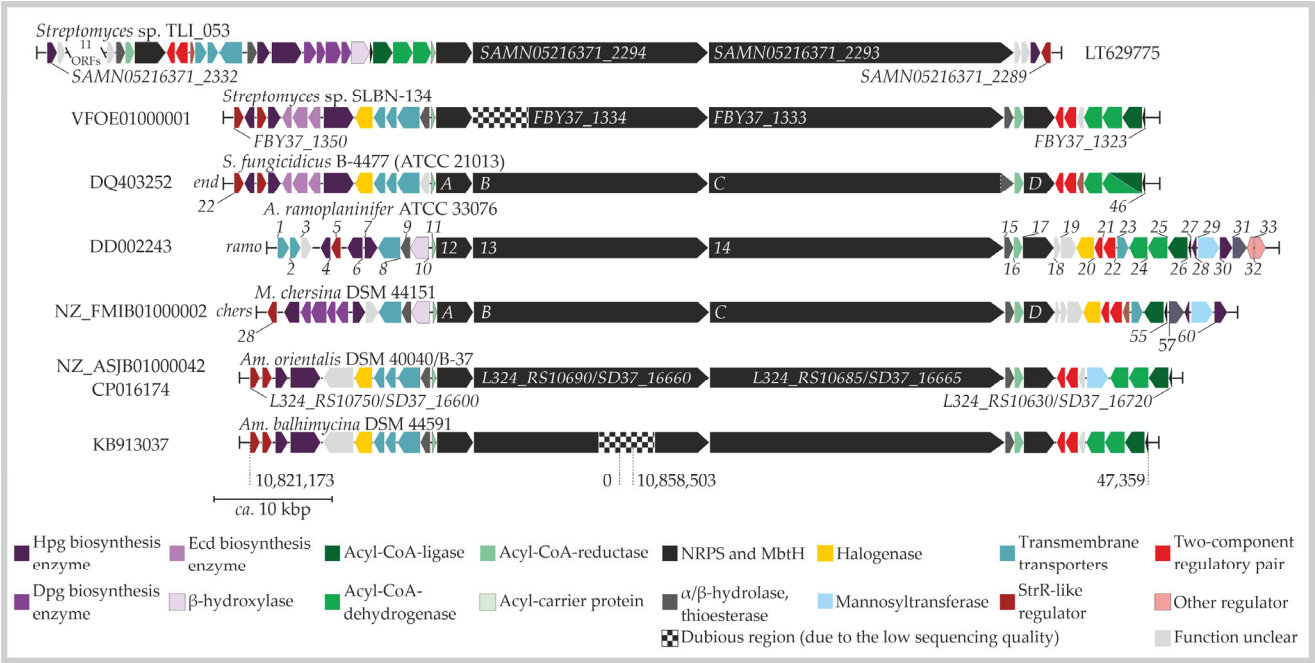


Figure 3. Genetic organization of the eight available RRLDP BGC sequences, including the ones leading to the production of known products (*end*, *ramo*, and *chers*), as well as the putative ones. The legend below the figure explains the color-coding; details are discussed in the main text.

Due to the 17 modular NRPS encoded within the retrieved RRLDP BGCs, these clusters are large (in range of ca. 80–100 kbp), likely implying a complex transcriptional machine involving several polycistronic transcriptional units. Their genetic organization is quite variable, although in the majority of them amino acid biosynthesis and transporter genes are located upstream from the NRPS genes, while the genes responsible for the processing of precursor fatty acids are downstream [38,64,79,80] (Figure 3). The BGC from *Streptomyces* sp. TLI_053 represents an exception, where the majority of biosynthetic and other genes are situated upstream from the NRPS genes [38] (Figure 3).

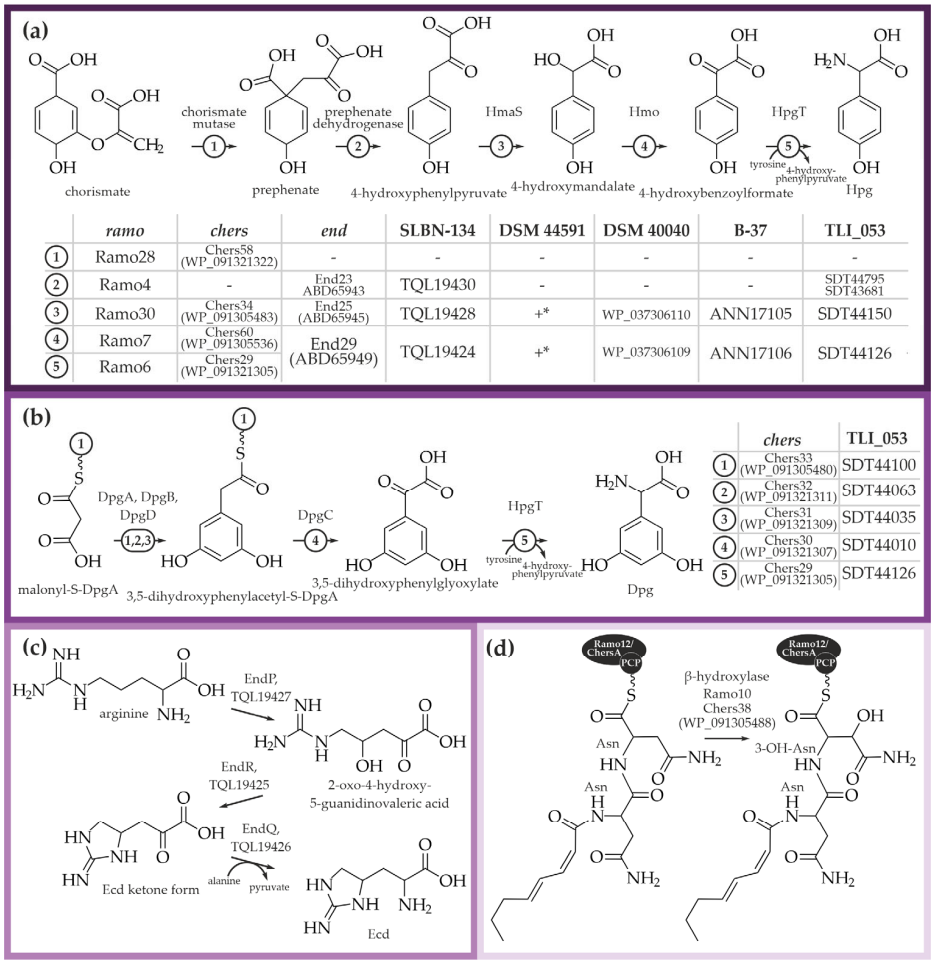
Similar to the biosynthesis of GPAs [81], the biosynthesis of RRLDPs could be divided into consequential stages. However, there is an essential difference. In those GPAs carrying a lipid chain (lipo-GPAs as teicoplanin), the attachment of the aliphatic side chain occurs as a tailoring reaction of the already cross-linked and glycosylated heptapeptide core [82,83]. Conversely, in RRLDPs, the fatty acid moiety (loaded on a standalone acyl-carrier protein domain of the NRPS) serves as starter unit for the non-ribosomal synthesis. This resembles the biosynthesis of multiple other cyclic lipopeptides from actinomycetes [84], or even of the antimycotic echinocandins from the ascomycete *Aspergillus nidulans* NRRL 11440 [85]. Hence, it is reasonable to delineate the following stages of RRLDP biosynthesis: (i) biosynthesis of non-proteinogenic amino acids and supply of the precursor amino acids; (ii) processing and activation of the starter fatty acid moiety; (iii) non-ribosomal biosynthesis and macrocyclization of the lipopeptide core, and its halogenation; and (iv) glycosylation of the halogenated lipopeptide core.

4.1. Biosynthesis of Non-Proteinogenic Amino Acids of RRLDPs

As mentioned above, the RRLDP peptide cores are formed in large proportion by non-proteinogenic amino acids. Some of them are obtained directly from the primary metabolism, e.g., Orn and Cit deriving from arginine (Arg) and glutamate (Glu) metabolism [86]. In the cases of Hpg, Dpg, or Ecd, the enzymes deputed to their biosynthesis are encoded within the RRLDP BGCs.

Biosynthesis of Hpg. Hpg residues are present in all the structurally elucidated RRLDPs (Rmp, End, and chersinamycin [38,62,63]) and their occurrence is predicted for the still-

putative RRLDPs in *Streptomyces* sp. SLBN-134, *Streptomyces* sp. TLI_053, *Am. balhimycina* DSM 44591, *Am. orientalis* B-37, and *Am. orientalis* DSM 40040 [38]. Consequently, genes encoding enzymes for Hpg biosynthesis are ubiquitously present in RRLDP BGCs. These enzymes closely resemble those found in GPA biosynthetic pathways, so their functions have been assigned on the basis of their homology with the Hpg biosynthesis enzymes experimentally investigated in the chloroeremomycin producer *Kibdelosporangium aridum* A82846 [87,88]. In this GPA producer, three enzymes are involved in Hpg biosynthesis, namely HpgT (4-hydroxyphenylglycine transaminase), Hmo (4-hydroxymandelate oxidase), and HmaS (4-hydroxymandelate synthase). HmaS utilizes 4-hydroxyphenylpyruvate as a substrate, producing 4-hydroxymandelate, which is subsequently oxidized into 4-hydroxybenzoylformate by Hmo. HpgT transaminates 4-hydroxybenzoylformate using tyrosine as an amino donor, yielding Hpg and 4-hydroxyphenylpyruvate [87,88] (Figure 4a). In *ramo* and *chers*, Hpg biosynthesis enzymes are encoded by *ramo6/chers29* (HpgT), *ramo7/chers60* (Hmo), and *ramo30/chers34* (HmaS) (Figure 3). Interestingly, in all the other RRLDP BGCs, Hpg biosynthesis enzymes are encoded by two genes, with one coding for an HpgT-Hmo fusion protein. These genes are orthologous to *end29* (coding for HpgT-Hmo fusion protein) and *end25* (HmaS) (Figure 4a). It is noteworthy that genes encoding HpgT-Hmo fusion proteins were also observed in BGCs for type V GPAs [89] (or glycopeptide-related peptides, GRPs [90]), such as corbomycin [91], and for the linear peptide feglymycin [92].



	ramo	*chers*	*end*	SLBN-134	DSM 44591	DSM 40040	B-37	TLI_053
1	Ramo28	Chers58 (WP_091321322)	-	-	-	-	-	-
2	Ramo4	-	End23 ABD65943	TQL19430	-	-	-	SDT44795 SDT43681
3	Ramo30	Chers34 (WP_091305483)	End25 (ABD65945)	TQL19428	+*	WP_037306110	ANN17105	SDT44150
4	Ramo7	Chers60 (WP_091305536)	End29 (ABD65949)	TQL19424	+*	WP_037306109	ANN17106	SDT44126
5	Ramo6	Chers29 (WP_091321305)						

	chers	TLI_053
1	Chers33 (WP_091305480)	SDT44100
2	Chers32 (WP_091321311)	SDT44063
3	Chers31 (WP_091321309)	SDT44035
4	Chers30 (WP_091321307)	SDT44010
5	Chers29 (WP_091321305)	SDT44126

+* - the corresponding coding ORF has been identified in the BGC sequence available but it has not been annotated yet.

Figure 4. Schematic representation of the biosynthetic pathways catalyzed by RRLDP BGC-encoded enzymes, leading to the production of non-proteinogenic amino acids found in RRLDP lipopeptide cores: 4-hydroxyphenylglycine (Hpg) (**a**); 3,5-dihydroxyphenylglycine (Dpg) (**b**); enduracididine (End) (**c**); and 3-hydroxyasparagine (3-OH-Asn) (**d**).

The initial substrate for HmaS is 4-hydroxyphenylpyruvate (Figure 4a), produced from prephenate involving a prephenate dehydrogenase [93]. In turn, prephenate is obtained from chorismate by a chorismate mutase (Figure 4a) [94]. To meet the high demand of 4-hydroxyphenylpyruvate in Hpg biosynthesis, *ramo*, *end*, and BGCs from *Streptomyces* sp. SLBN-134 and TLI_053 carry a gene for prephenate dehydrogenase (another prephenate dehydrogenase gene could be found upstream from the TLI_053 BGC). The absence of prephenate dehydrogenase genes in RRLDP BGCs of *Am. balhimycina* DSM 44591, *Am. orientalis* B-37, and DSM 40040 is likely due to the fact that these strains are GPA producers, and the corresponding GPA BGCs already carry genes for prephenate dehydrogenases [74–76,95,96]. Finally, *ramo* and *chers* also carry genes for type II chorismate mutase (*ramo28* and *chers58*, respectively), which share a remarkable similarity (54 and 64% of amino acid sequence identity, respectively) with Tei26*, a chorismate mutase coded within the *tei* BGC for the production of the GPA teicoplanin in *Actinoplanes teichomyceticus* NRRL B-16726 [97].

Biosynthesis of Dpg. A single Dpg residue is found as AA13 in chersinamycin peptide core, while two residues are predicted to occupy AA13 and AA17 positions in the putative RRLDP from *Streptomyces* sp. TLI_053 [38]. Both the corresponding BGCs contain four genes coding for Dpg biosynthesis (Figure 3). Again, these genes are remarkably similar to the Dpg biosynthetic genes found in GPA BGCs. Production of Dpg for GPA biosynthesis was investigated in chloroeremomycin and balhimycin producers [98,99], and involves DpgA (type III polyketide synthase), DpgB (enoyl-CoA hydratase), DpgC (3,5-dihydroxyphenylacetyl-CoA 1,2-dioxygenase), DpgD (enoyl-CoA hydratase), and HpgT. These enzymes act consequentially (Figure 4b), producing Dpg from four malonyl-CoA units. Thus, *chers* carries *chers33-30*, which code for DpgA-D enzymes; their orthologues are also present in the TLI_053 BGC (Figure 4b).

Biosynthesis of Ecd. Radioactive labeling experiments conducted in *S. fungicidicus* ATCC 21013 have revealed that Arg serves as a precursor of Ecd [100]. This is intriguing as Orn and Cit are also components of the End core. It is hypothesized that *endPQR* genes, which encode two pyridoxal phosphate (PLP)-dependent aminotransferases and an acetoacetate decarboxylase, respectively, are responsible for the Ecd biosynthesis [64] (Figure 4c). *end* shares this subset of genes with the mannopeptimycin BGC, where the orthologous *mppPQR* appear to be responsible for Ecd biosynthesis. In the mannopeptimycin pathway, Ecd is subsequently transformed into 3-OH-Ecd by MppO, which codes for a clavaminic acid synthetase-like oxygenase [101]. The amino acid sequence identities between MppP/EndP, MppQ/EndQ, and MppR/EndR are 81%, 68%, and 74%, respectively. The functions of MppP, MppQ, and MppR (derived from different mannopeptimycin producers) were investigated in a series of in vitro experiments [102,103], allowing the assignment of their functions in Ecd biosynthesis. Thus, MppP catalyzes the first reaction, synthesizing 2-oxo-4-hydroxy-5-guanidinovaleric acid directly from Arg [102]. The latter is further transformed by MppR into a ketone version of Ecd [103], further aminated by MppQ likely using alanine as an aminodonor [103]. Ecd biosynthesis genes seem to be rare in RRLDP BGCs, being found only in *end* and *end*-like BGC from *Streptomyces* sp. SLBN-134 (Figures 3 and 4c).

Biosynthesis of 3-OH-asparagine. 3-OH-asparagine residue is the AA2 of Rmp and chersinamycin peptide cores, where the 3-OH group plays a crucial role in the macrocyclization of the linear peptide precursor, participating in the formation of an ester linkage with the terminal carboxyl group of AA17 (Figure 1). End carries a threonine residue as AA2, which also provides a 3-OH group for macrocyclization. A thorough inspection of *ramo* and *chers* allowed us to identify genes (*ramo10* and *chers38*, respectively) coding for a diiron non-heme β-hydroxylase. These predicted enzymes share high similarity (64% of amino acid sequence identity) with Tei12*, a tyrosine β-hydroxylase involved in teicoplanin biosynthesis [97]. The function of the Tei12* orthologue from A40926 GPA biosynthesis in *Nonomuraea gerenzanensis* ATCC 39727—Dbv28—was investigated experimentally [104]. It was demonstrated that Dbv28 acts on the tyrosine residue in the NRPS-bound GPA aglycone; the deletion of the corresponding gene led to the production of an A40926 analogue

carrying tyrosine instead of 3-OH-tyrosine [104]. It is thus highly likely that the products of *ramo10* and *chers38* are responsible for the 3-hydroxylation of asparagine in Rmp and chersinamycin biosynthesis (Figure 4d), respectively. Notably, the deletion of *ramo10/chers38* might likely have a dramatic effect on antibiotic production, leading to the biosynthesis of a linear peptide. Interestingly, a Ramo10 orthologue (WP_091305488) is encoded also within the TLI_053 BGC. The putatively encoded compound is an RRLDP-carrying aspartate as AA2. Considering the similarities of asparagine and aspartate, it is reasonable to speculate that WP_091305488 is involved in the 3-hydroxylation of AA2, yielding 3-OH-aspartate, which consequently provides the 3-OH group needed for the formation of the ester linkage.

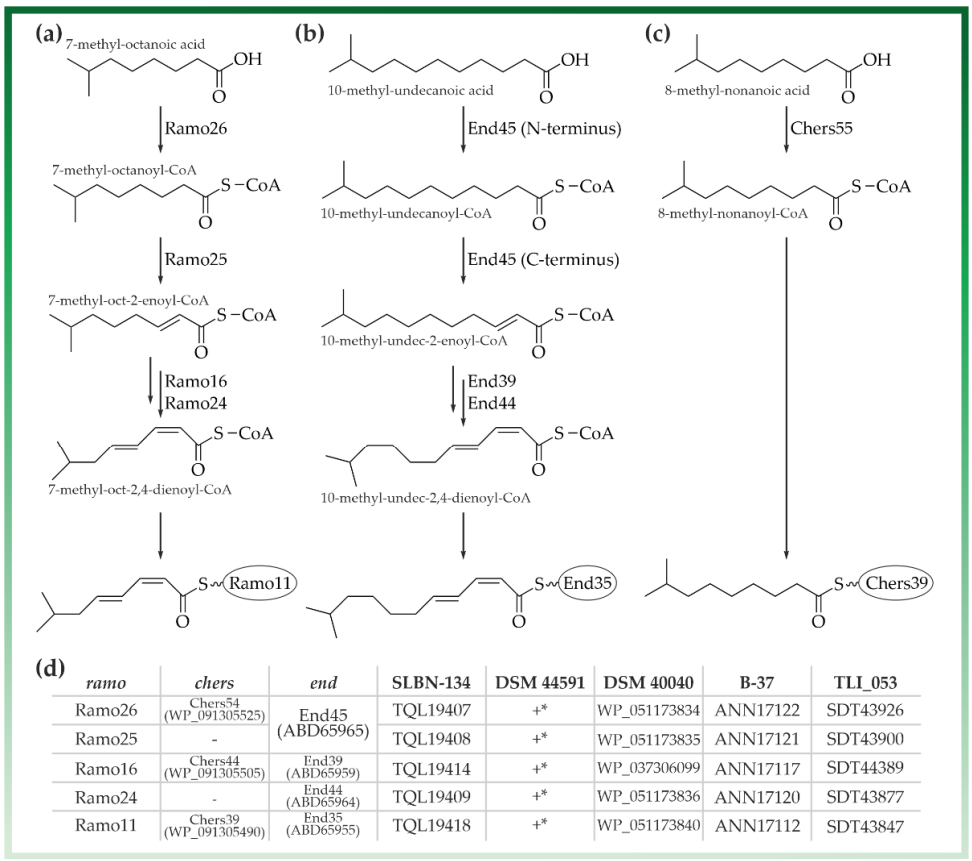
4.2. Modification and Activation of the Fatty Acid Starter Moieties

The fatty acid moiety is pivotal for RRLDP biosynthesis, serving as the starter unit for the non-ribosomal assembly. Rmp and End carry unsaturated fatty acid moieties, while in chersinamycin this lipid chain is fully saturated (Figures 1 and 2). In the biosynthesis of Rmp, End, and chersinamycin, fatty acid moieties (likely obtained from the pool of cell membrane lipids) are initially activated by acyl-CoA ligases encoded by *ramo26*, *end45*, and *chers55* (Figure 5) [35,38,64,105]. According to the initial annotation of *end*, *end45* codes for a fusion protein, where the N-terminal part is an acyl-CoA ligase, while the C-terminal is an FAD-dependent acyl-CoA-dehydrogenase (involved in further desaturation of the fatty acid moiety, see below) (Figure 5) [64]. In the case of Rmp biosynthesis, the fatty acids involved are octanoic acid (A1, A'1), 7-methyl-octanoic acid (A2, A'2), and 9-methyl-decanoic acid (A3, A'3). 10-methyl-undecanoic and 10-methyl-dodecanoic acids enter the biosynthesis of End to yield factors A and B, respectively. 8-methyl-nonanoic acid is instead activated in the biosynthesis of chersinamycin. The functions of *ramo26* were investigated in vivo, demonstrating that a Δ*ramo26* mutant is completely unable to produce Rmp [105]. Complementation of the mutant by expressing the N-terminal fragment of End45 restored the production of antibiotic, leading to Rmp congeners with longer aliphatic chains [105].

Activated fatty acid moieties in Rmp and End biosynthesis further undergo desaturation, achieved by an orthologous set of enzymes encoded in *ramo* and *end*. These are the FAD-dependent dehydrogenases Ramo25/End45 (C-terminus) and Ramo24/End44, which introduce double bonds, and the NAD-dependent reductases Ramo16/End39 that perform double-bond isomerization (Figure 5a,b) [64,105]. Interestingly, an orthologue of *end44* was recently deleted in End-producing *S. fungicidicus* TXX3120 [51]. The mutant strain retained its antimicrobial activity, as determined by the Kirby–Bauer *B. subtilis* growth inhibition assay, leading to the erroneous assumption that End44 does not participate in End biosynthesis. Unfortunately, the chemical structure of End congeners produced by the *end44* mutant was not analyzed, not providing more insight into the role of End44 in desaturating the aliphatic side chain of End [51]. Although the aliphatic chain of chersinamycin is saturated and not isomerized, its BGC carries a gene for a Ramo16 orthologue, Chers44 (Figure 5d), which might indicate that *chers* initially possessed *ramo24-25* orthologues, subsequently lost during BGC evolution.

It is noteworthy that *end45*, which appears as a fusion of two ORFs for acyl-CoA ligase and acyl-CoA dehydrogenase, might be an artifact of sequencing. This is suggested by the fact that the *end*-like BGC from *Streptomyces* sp. SLBN-134 contains two separate ORFs encoding these two proteins (as illustrated in Figures 3 and 5). This observation is also supported by the sequence of *end* obtained from *S. fungicidicus* TXX3120, a randomly mutated End-high producer, which also possesses two separate ORFs in place of *end45* [51].

Finally, the activated (and in the case of Rmp and End, desaturated) fatty acid moiety is attached to the standalone acyl-carrier protein (ACP), coded by *ramo11*, *end35*, or *chers39* in the different BGCs. The function of Ramo11 was verified in vitro [80]. The scenario, where the fatty acid moiety undergoes desaturation and isomerization while already tethered to the ACP, cannot be excluded as well [35,80].



(d)

ramo	*chers*	*end*	SLBN-134	DSM 44591	DSM 40040	B-37	TLI_053
Ramo26	Chers54 (WP_091305525)	End45 (ABD65965)	TQL19407	+*	WP_051173834	ANN17122	SDT43926
Ramo25	-		TQL19408	+*	WP_051173835	ANN17121	SDT43900
Ramo16	Chers44 (WP_091305505)	End39 (ABD65959)	TQL19414	+*	WP_037306099	ANN17117	SDT44389
Ramo24	-	End44 (ABD65964)	TQL19409	+*	WP_051173836	ANN17120	SDT43877
Ramo11	Chers39 (WP_091305490)	End35 (ABD65955)	TQL19418	+*	WP_051173840	ANN17112	SDT43847

+* - the corresponding coding ORF has been identified in the BGC sequence available but it has not been annotated yet.

Figure 5. Enzymes and reactions involved in fatty acid activation and modification in the initial phase of Rmp biosynthesis (exemplified for the A2/A'2 factors of the Rmp complex) (**a**), End (factor A) (**b**), and chersinamycin (**c**), and accession numbers for the orthologous sets of proteins coded within the BGCs for the putative RRLDPs (**d**).

Interestingly, orthologues of Ramo26/End45 (N-terminus)/Chers55, Ramo25/End45 (C-terminus), Ramo24/End44, Ramo16/End39/Chers44, and Ramo11/End35/Chers39 have been found in all the RRLDP BGCs so far known (Figures 3 and 5d), suggesting that these putative molecules also carry unsaturated aliphatic chains. However, the lengths of these chains may vary, given the unpredictability of Ramo26 orthologues specificities. In the *Streptomyces* sp. SLBN-134 *end*-like BGC, orthologues of the C- and N-terminal parts of End45 are coded by two separate ORFs [38].

4.3. Genes and Proteins Associated with the Non-Ribosomal Assembly Line of RRLDPs

RRLDP BGCs encode large NRPSs responsible for the biosynthesis of 17 aa peptide cores. NRPS genes are accompanied by genes encoding MbtH-like proteins, which are known to assist in the proper folding and assembly of NRPSs (Figures 3 and 6) [106]. A distinctive feature of all the RRLDP NRPSs is that their assembly line, encoded within three consecutive NRPS ORFs (*ramo12-13-14*, *chersA-B-C*, *endA-B-C*, and their homologues) [38,64,65], carries a condensation (C)-domain and a peptidyl-carrier protein (PCP)-domain instead of a complete module (M) for the incorporation of AA8 (Figure 6). This is complemented with an adenylation (A)-domain and an additional PCP-domain, coded within a standalone NRPS gene—*ramo17*, *chersD*, *endD*, and their orthologues in other RRLPD BGCs (Figures 3 and 6). Thus, the A-domain of M8 acts *in trans* (Figure 6) [80]. The fine details on how it functions remain unknown, although it was impossible to complement Δ*ramo17* with *endD*, but complementation was achieved using a hybrid version of EndD carrying the N-terminal region of Ramo17 [50].

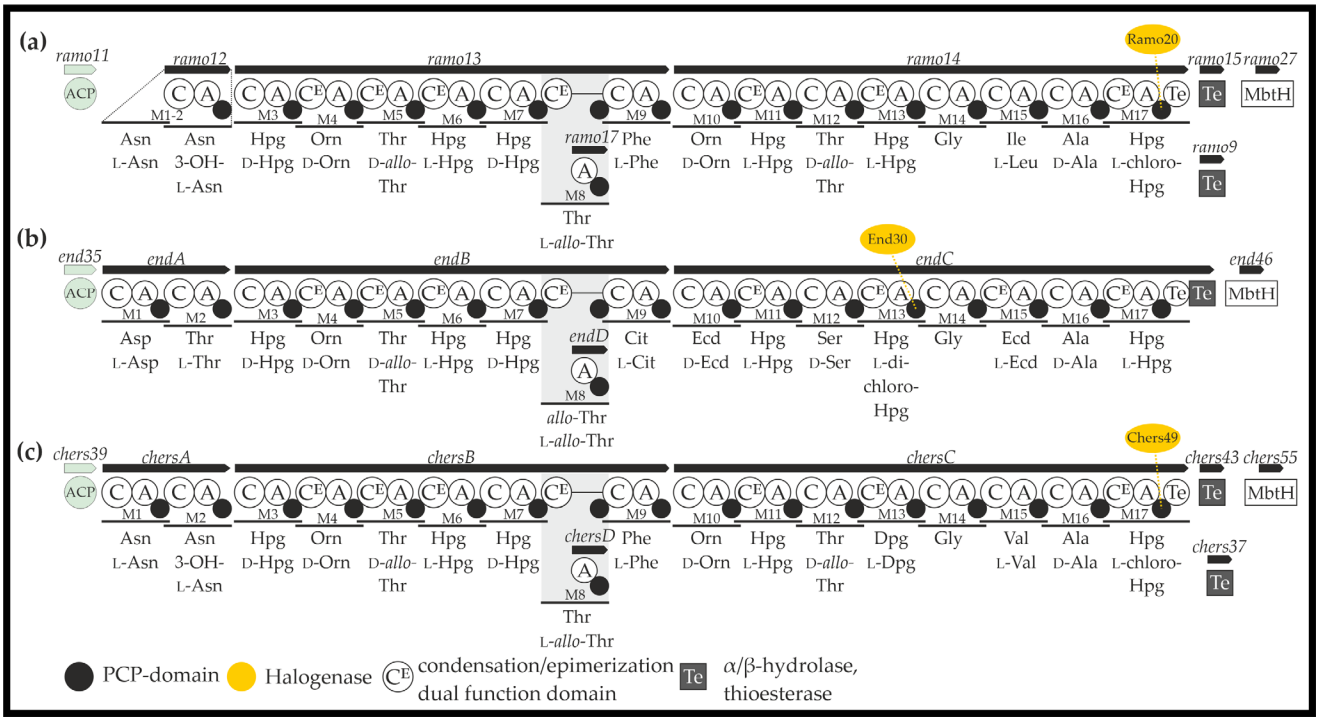


Figure 6. Module-domain organization of NRPS assembly lines in Rmp (**a**), End (**b**), and chersinamycin (**c**) biosynthetic pathways. Hydrolases/thioesterases, coded by separate genes, which might be involved in the detachment of the linear peptide from the NRPS and macrocyclization or/and proofreading, as well as halogenases, are also shown on the scheme.

Another unusual feature of RRLDP NRPSs is the complete absence of separate epimerization (E)-domains [38,64], although D-amino acids (derived from proteinogenic L-aminoacids) and non-proteinogenic L-amino acids (obtained from D-precursors) are abundant in the peptide cores. This is explained by the presence of dual function condensation (C)/E-domains (Figure 6) [38,64]. In addition, Rmp biosynthesis NRPS has a notable difference from all the other RRLDP NRPSs, having the first NRPS gene coding for only one module, which is believed to function iteratively, incorporating two Asn residues [65] (Figure 6a).

Biosynthesis of the peptide cores of RRLDPs is initiated with the fatty acid moiety tethered to a standalone ACP (as mentioned above). Consequently, all M1s of RRLDP NRPSs start with a C-domain (Figure 6). The non-ribosomal synthesis is terminated by a thioesterase (Te)-domain, found in every M17 of the known RRLDP NRPSs (Figure 6). This domain is believed to perform the detachment and macrocyclization of the lipopeptide. Additional genes encoding α/β-hydrolases/thioesterases are found following the NRPS modules in RRLDP BGCs. These include orthologues of Ramo15, encoded within every RRLDP BGC, although the corresponding gene in *end* seems to be fused with *endC*. The exact function of these enzymes is unclear, but they may assist in the lipopeptide detachment and macrocyclization or play a role in the biosynthetic proofreading mechanism [107,108]. The fusion of *endC* with the sequence coding for Ramo15 orthologue in the *end* might be again a sequencing artifact. This is suggested by the fact that in the *end* from *S. fungicidicus* TXX3120 and the *end*-like BGC from *Streptomyces* sp. SLBN-134, the corresponding sequences are identified as separate ORFs (Figure 3) [48].

Drawing from GPA biosynthesis, it can be inferred that halogenation of RRLDPs is associated with the non-ribosomal assembly [109,110]. A gene for a halogenase is present in each RRLDP BGC, except for the one found in *Streptomyces* sp. TLI_053 (Figures 3 and 6). The function of the halogenase gene in End biosynthesis was investigated in vivo by knocking out the corresponding gene (*end30*) [111]. The resulting Δ*end30* mutant pro-

duced dideschloro-End, and complementation with the native allele partially restored End production, yielding a mixture of End and monodes-/dideschloro congeners [111]. Complementation of Δ*end30* with the Rmp biosynthesis halogenase gene (*ramo20*) resulted in the production of monochlorinated End congeners, although Hpg13 was chlorinated despite the expectations that Ramo20 would chlorinate Hpg17 (the natural substrate in Rmp biosynthesis). A more surprising outcome was observed when *ramo20* was expressed in the wild-type *S. fungicidicus* ATCC 21013: trichlorinated End analogs were produced in the recombinant strain, and Hpg11 was found to be the third chlorination site [111]. The function of Ramo20 was also verified in vivo, with the knockout of *ramo20* leading to the production of deschloro-Rmp [112,113]. Complementation of Δ*ramo20* with End30 led to the production of Rmp (chlorinated at Hpg17) [113].

4.4. Glycosylation of RRLDPs

Genes for GT39-family [114] glycosyltransferases can be found in *ramo* (*ramo29*) and *chers* (*chers59*), and in the BGCs for the putative RRLDPs from *Am. orientalis* DSM 40040 and B-37 (*L324_RS10650* and *SD37_16700*, respectively) [38,65]. Respectively, Rmp and chersinamycin carry an α-1,2-dimannosyl at Hpg11 (Figure 1), while putative RRLDPs from *Am. orientalis* DSM 40040 and B-37 should be mannosylated as well (if produced at all) [38].

GT39-family glycosyltransferases are membrane-bound mannosyltransferases; Ramo29 and orthologues from the other RRLDP BGCs share similarity to the mannosyltransferases involved in GPA biosynthetic pathways (e.g., A40926, A50926, and teicoplanin), known to install a single α-D-mannose moiety at Dpg residue [82,83,115–117]. It is unclear how the second mannose residue is installed in the biosynthesis of Rmp and chersinamycin. Deletion of *ramo29* led to the production of non-glycosylated Rmp congeners [112,118], but this does not clarify whether Ramo29 acts iteratively (which is unlikely, considering the substrate specificities of GT39-family glycosyltransferases) or a second glycosyltransferase (perhaps coded outside the BGC) is required for the second mannosylation. Heterologous expression of *ramo29* in *S. fungicidicus* ATCC 21013 corroborates the latter assumption, as the recombinant strain was shown to produce an End derivative carrying a single α-D-mannose moiety at Hpg11 [119]. The additional putative mannosyltransferase might be membrane-associated and have an extracellular acting domain, since Rmp A'1-3 are bioconverted into Rmp A1-3 upon incubation with the mycelium of the producer strain [66].

Some other glycosyltransferases might be discovered in the still-unsequenced RRLDP BGCs in future: for instance, those involved in the glycosylation of ramoplanose carrying a mannose trisaccharide [49].

4.5. Transporters Coded Within RRLDP BGCs

RRLDP BGCs encode various combinations of membrane transporters, none of which have been investigated experimentally yet. Notably, each BGC encodes an orthologue of Ramo8—an MdlB-like ABC-type multidrug transport system, combining ATPase and permease domains within a single protein. Ramo8 and its orthologues are phylogenetically close to the ABC-transporters encoded within the BGCs for Type V GPAs (>7 aa) [120]. More broadly, they are related to transporters encoded within Type I-IV GPA BGCs, including the experimentally investigated balhimycin exporter, Tba [120,121]. Therefore, it is reasonable to assume that Ramo8 and its orthologues function as the primary exporters of RRLDPs.

4.6. Transcriptional Regulation of RRLDP Biosynthesis

As expected, RRLDP BGCs contain cluster-situated regulatory genes (CSRGs). These genes code for StrR-like pathway-specific transcriptional regulators (PSRs) and two-component regulatory pairs (Figure 4). *end* (as well as the BGCs from *Streptomyces* sp. SLBN-134, *Am. balhimycina* DSM 44591, *Am. orientalis* DSM 40040 and B-37) carries two CSRGs encoding two StrR-like PSRs [122]. These genes do not appear to be a product of duplication, as they occupy different positions in the phylogenetic tree of StrR-like regulators from antibiotic

BGCs [122]. On the contrary, a single StrR-like PSR is encoded within *ramo*, *chers*, and in the BGC from *Streptomyces* sp. TLI_053. Indeed, genes for two-component regulatory pairs are found in any RRLDP BGC [122]. Additionally, at the 3′-edge, *ramo* carries two genes that might have regulatory functions: *ramo32* (coding for a putative RNA-binding protein) and *ramo33* (coding for a LacI-family transcriptional regulator) [65]. However, orthologues of these genes are not present in any of the other sequenced RRLDP BGCs, and thus it cannot be ruled out that *ramo32* and *ramo33* are not actual components of the BCG.

So far, pathway-specific transcriptional regulation of RRLDP biosynthesis has been experimentally investigated only using the *end* BGC as a model. One of the CSRGs for StrR-like PSRs—*end22*—was knocked out and overexpressed in *S. fungicidicus* ATCC 31731, an industrial overproducer derived from ATCC 21013 [123]. Knockout of *end22* resulted in the complete cessation of End production, while overexpression led to a several-fold increase in the antibiotic production levels (as discussed in Section 6) [123]. The regulon of End22 was not identified, leaving the precise details of its regulatory mechanism unknown. Unfortunately, the role of the second CSRG (*end24*) for StrR-like PSR has not been investigated yet (Figure 7).

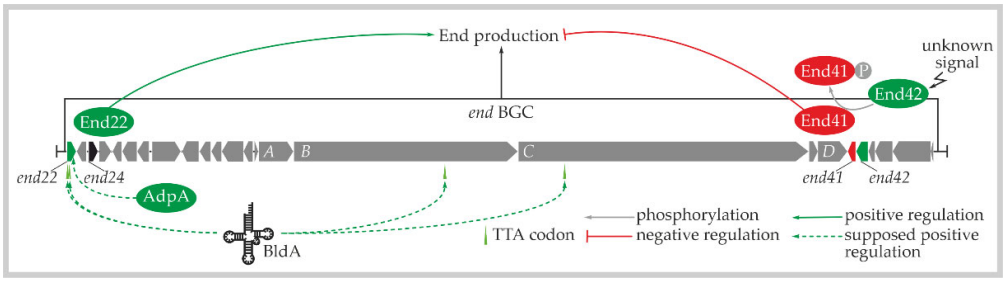


Figure 7. Schematic representation of experimentally investigated and hypothetical regulatory factors governing End biosynthesis. Please refer to the main text for more details.

Genes *end41-42*, coding for the two-component regulatory pair, were investigated in a similar fashion. Knockout of *end42* (coding for a sensor histidine kinase) decreased End production, while the knockout of *end41* (coding for a transcriptional response regulator) had no impact [123]. Overexpression of *end42* increased End production, whereas overexpression of *end41* had the opposite effect [123]. The specific details of End41-42-mediated regulation remain unclear, as is the ligand for End42. However, End41 likely acts as a transcriptional repressor in its non-phosphorylated form, with phosphorylation by End42 alleviating this repressive effect [123]. No further information has been reported yet on transcriptional organization of the RRLDP BGCs.

The expression of the RRLDP BGC is likely subjected to global transcriptional regulation. Four rare leucyl TTA codons could be found in *end* (Figure 7). $tRNA^{Leu}_{UAA}$ (coded by *bldA* gene [124]) is the only tRNA in *Streptomyces* spp. capable of decoding the TTA codon. The expression of *bldA* is part of a complex global regulatory network that intertwines antibiotic production with morphogenesis in *Streptomyces* spp. and is centered on the global transcriptional regulator of AraC/XylS family—AdpA [125]. Two TTA codons are present in *end22* (the gene for the key activator of End biosynthesis, see above), while the NRPS genes *endB* and *endC* each have one TTA codon. The presence of TTA codons in *end* suggests that End production is likely controlled by AdpA, a situation observed in numerous other *Streptomyces* spp. [124,126–129]. Any assumptions about the global regulation of RRLDP production in non-*Streptomyces* actinomycetes cannot be made, although AraC/XylS-family regulators might also be involved in the regulation of antibiotic production of non-*Streptomyces* actinobacteria [130,131].

5. Tools for the Genetic and Genomic Engineering of RRLDP Producers

A set of general gene-engineering tools has been employed to manipulate the producers of Rmp and End (Table 1). The expression of genes of interest was achieved in the

Rmp-producing strain *Actinoplanes* sp. SIPI-A.2006 (likely a synonym of ATCC 33076, as mentioned above), employing the φC31-based integrative vector pSET152 [132] (Table 1), where gene expression was driven by the *Saccharopolyspora erythraea* erythromycin resistance gene promoter (*ermEp*). The strength of different promoters for gene expression was not experimentally investigated in Rmp-producing *Actinoplanes* spp.; however, by extrapolating the data obtained for other *Actinoplanes* spp., the *ermEp* might not be the optimal choice to achieve a strong constitutive gene expression [133,134] and further investigation would be needed. In End-producing strains of *S. fungicidicus*, genes were expressed using either integrative pSET152 derivatives (Table 1) or replicative pIJ101 derivatives. In *S. fungicidicus* strains, *ermEp* was the most commonly used promoter for gene expression, although the usage of the tetracycline-inducible promoter *tcp830* was also reported (Table 1).

Table 1. List of plasmids used for gene expression and knockouts in Rmp and End producers.

Plasmid:	Used for:	Properties:	Reference:
pSET152	Gene expression in *Actinoplanes* sp. SIPI-A.2006 (=ATCC 33076)	φC31-based integrative vector	[50,112,113,118]
pXY152a	Gene expression in *S. fungicidicus* ATCC 21013	pSET152 [129] derivative, φC31-based integrative expression vector, gene expression achieved via *ermEp* promoter	[111,135]
pSET152*ermE*	Gene expression in *S. fungicidicus* ATCC 31731 (ATCC 21013 derivative)	pSET152 [129] derivative, φC31-based integrative expression vector, gene expression achieved via *ermEp* promoter	[123]
pIJ86	Gene expression in *S. fungicidicus* ATCC 21013	Replicative expression vector, pIJ101 [136] derivative, gene expression achieved via *ermEp* promoter	[119,137]
pMS17	Gene expression in *S. fungicidicus* ATCC 21013	φC31-based integrative expression vector, gene expression achieved via tetracycline-inducible promoter *tcp830*	[119,138]
pXY300	Gene knockouts in *S. fungicidicus* ATCC 21013 via homologous recombination	pGM160 [139] derivative carrying *oriT* from pOJ446 [132], thermosensitive pSG5 replicon-based replicative vector	[111,140]
pKC1139	Gene knockouts in *Actinoplanes* sp. SIPI-A.2006 (=ATCC 21013), *A. ramoplaninifer* ATCC 21013, and *S. fungicidicus* TXX3120 (industrial End producer) via homologous recombination	Thermosensitive pSG5 replicon-based replicative vector	[50,112,118,132]
pHZ1358	Gene knockouts in *S. atrovirens* MGR140 via homologous recombination	Replicative *cos*-site-containing pIJ101 [136] derivative, unstable and prone to chromosomal integration via homologous recombination	[40,141]
pCIMt005	Gene knockouts in *S. fungicidicus* ATCC 31731 (ATCC 21013 derivative) via homologous recombination	Thermosensitive pSG5 replicon-based replicative vector, derivative of pBlueScript II KS (+), carrying indigoidine synthetase cassette (*Streptomyces lavendulae* CGMCC 4.1386 gene *idgS* co-expressed with *Bacillus subtilis* phosphopantetheinyl transferase gene *sfp* using *ermEp*) [142] and pUWL201PW [143] *oriT*; as the presence of indigoidine synthetase cassette leads to accumulation of blue indigoidine, application of pCIMt005 simplifies the screening of mutants (where the cassette is lost)	[123,142]

Gene knockouts in ramoplanin-producing *Actinoplanes* spp. were achieved using an approach based on homologous recombination-mediated double crossing-over events [144].

For this purpose, thermosensitive pSG5 replicon-based vectors, replication of which is restricted at 37 °C, allowing homologous recombination into the chromosome [145], were utilized (Table 1). Similarly, pSG5 replicon-based vectors were used for gene knockouts in *S. fungicidicus* ATCC 21013, ATCC 31731, and TXX3120 (Table 1), while a pIJ101 derivative was employed for knocking out genes in End-producing *S. atrovirens* MGR140. Notably, CRISPR-Cas9-mediated gene knockouts have recently been performed in *S. fungicidicus* ATCC 31731 [123].

Intergeneric conjugation, utilizing *Escherichia coli* ET12567 strains as donors, was employed to deliver plasmid DNA to Rmp- and End-producing strains (see references in Table 1). In the case of End-producing *Streptomyces* spp., spores were used as recipients. However, the method of conjugal transfer in Rmp-producers remains unclear, as sporangia formation in *A. ramoplaninifer* ATCC 21013 occurs extremely rarely [48].

6. Ways and Approaches to Improve RRLDP Production in Actinomycetes

Significant effort has been put into optimization and improvement of Rmp and End production during the different phases of their discovery and development. In the case of Rmp, different reports described medium optimization, while for End strategies such as random mutagenesis and rational gene-engineering were published, in combination with medium optimization (Table 2). Although several media previously used for Rmp and End production were tested in *M. chersina* DSM 44151, chersinamycin production was only detected in H881 medium, which is typically used for dynemicin production [38,59,146] (Table 2). Chersinamycin production was reported only after a prolonged cultivation period (288 h). Although the exact productivity is not clear, ca. 1–3 mg/L of chersinamycin was obtained after purification [38].

Improvement of Rmp production. Initial reports described Rmp production on a fermenter scale, achieving over 60 mg/L of the complex after 60 h of cultivation (Table 2) [47]. Medium optimization led to the formulation of PB-medium described in [147] (Table 2). It was possible to achieve more than 100 mg/L of Rmp (factors A1-3 combined) in PB after 96 h of cultivation. Furthermore, the addition of 5 g/L of L-leucine significantly increased Rmp production to over 400 mg/L. This specifically enhanced the biosynthesis of the A2 factor, which then contributed to more than 96% of the total complex. Such a result was achievable because L-leucine is the precursor of the 7-methylocta-2,4-dienoyl aliphatic side chain of Rmp A2 [147]. Furthermore, addition of 5 g/L of L-valine induced Rmp A3 production to become dominant in the complex, as this amino acid serves as a precursor of the 9-methyldeca-2,4-dienoyl chain. However, in this scenario, the total Rmp production was similar to that in the non-modified medium [147]. A more recent report demonstrated that Rmp production could also be improved by substituting soybean meal in PB with meat-bone meal (>200 mg/L at 168 h of cultivation), poultry meal (>350 mg/L, 168 h), and fish meal (>400 mg/L, 164 h) [148].

Table 2. Media used for the fermentative production of Rmp and End and maximum antibiotic production achieved.

Antibiotic:	Producer Strain:	Vegetative Medium Composition (g/L):	Production Medium Composition (g/L):	Maximal Yield Achieved:	Reference:
Rmp	*A. ramoplaninifer* ATCC 33076	Meat extract—3; Yeast extract—5; Tryptone—5; Soluble starch—24; Glucose—1; $CaCO_3$—4	Soybean meal—30; Glucose—40; $CaCO_3$—1	>60 h, >60 mg/L	[47]
	A. ramoplaninifer ATCC 33076	Glucose—12; Soluble starch—13; Soybean meal—13; $CaCO_3$—4	**PB medium** Glucose—4; Maltose—20; Starch—4; Glycerol—20; Sucrose—20; Soybean meal—30; $CaCO_3$—6	96 h, >100 mg/L	[147]

Table 2. *Cont.*

Antibiotic:	Producer Strain:	Vegetative Medium Composition (g/L):	Production Medium Composition (g/L):	Maximal Yield Achieved:	Reference:
Chersinamycin	*M. chersina* DSM 44151	Fish meal—10, dextrin—30; lactose—10; $CaSO_4$—6; $CaCO_3$—5	**H881** Starch—10; Pharmamedia—5; $CaCO_3$—1; $CuSO_4$—0.05; NaI—0.5	288 h, 1–3 mg/L after the purification	[38]
End	*S. fungicidicus* B-5477	Not reported	Corn steep liquor—30; Glucose—20; Soluble starch—30; NaCl—5; $CaCO_3$—10	120 h, 170 mg/L	[28,111]
	S. fungicidicus B-5477	Corn steep liquor—35; Corn flour—25; Corn gluten meal—5; $CaCO_3$—30; Actocol antifoam—0.5	**Medium A** Corn flour—80; Corn gluten meal—30; Corn steep liquor—5; NaCl—1; $(NH_4)_2SO_4$—3; $ZnCl_2$—0.1; Lactose—10; Chicken oil—14; Potassium lactate—5	192 h, 550 mg/L	[149]
	S. fungicidicus B-5477	Corn steep liquor—35; Corn flour—25; Corn gluten meal—5; $CaCO_3$—30; Actocol antifoam—0.5	**Medium B** Corn flour—50; Corn gluten meal—40; Corn steep liquor—5; $Na_2H_2PO_4$—26; NaCl—5; $(NH_4)_2SO_4$—3; $ZnCl_2$—0.1; Lactose—10; Chicken oil—14; Potassium lactate—1	192 h, 530 mg/L	[149]
End	*S. fungicidicus* SG-01	Glucose—30, Corn steep liquor—30, Yeast extract—5; Ca_2CO_3—20; NaCl—8	Glucose—40; Soluble starch—40; Corn steep liquor—20; Corn gluten meal—30; NaCl—15; NH_4Cl—5; Ca_2CO_3—15	240 h, ca. 300 mg/L	[52]
End	*S. fungicidicus* ATCC 31731	Corn steep liquor—35; Corn starch—30; Glucose—20; $CaCO_3$—20	Sucrose—100; Glucose—10; Casamino acids—0.1; Yeast extract—5; MOPS—21; K_2SO_4—0.25; $MgCl_2$—10; $MnSO_4$—1 $\times$ 10^{-3}; $CuSO_4$—0.5 $\times$ 10^{-3}; $ZnSO_4$—1 $\times$ 10^{-3}; $CoCl_2$—1	192 h, 740 mg/L	[123]
End	*S. fungicidicus* TXX3120	Corn flour—35; Corn steep liquor—28; Cottonseed meal—5; $(NH_4)_2SO_4$—5; $FeSO_4$—0.36; KH_2PO_4 1.25; Light calcium carbonate—5; Olive oil—0.5 mL/L	Corn flour—80; Soybean flour—28; $(NH_4)_2SO_4$—4; Corn steep liquor—20; Glucose monohydrate—6; $FeSO_4$—0.1; L-lactic acid—2; Light $CoCO_3$—5; Thermostable α-Amylase—1500 IU/L; Soybean oil—0.5 mL/L	144 h, ca. 4000 U/mL	[51]
End	*S. atrovirens* MGR140	Not reported	Soluble starch—30; Soybean meal—10; Glucose—20; Yeast powder—10; $CaCO_3$—10; NaCl—5	Not reported, ca. 40 mg/L	[55]
End	*Streptomyces* sp. MC079	Glucose—20; Yeast extract—3; Peptone—2; $FeSO_4$—0.1; K_2HPO_4—2; NaCl—10	Glucose—40; Peptone—5; $FeSO_4$—0.1; K_2HPO_4—2; NaCl—10	160 h, 35 mg/L	[56]

Improvement of End production. The very first report in 1968 explored the dependence of End production on nitrogen and carbon sources in a basal medium [28]. The utilization of optimal nitrogen (corn steep liquor) and carbon (soluble starch combined with glucose) sources (Table 2) led to an End production of 170 mg/L in *S. fungicidicus* B-5477. Consequently, two other optimized media (A and B, Table 2), designed for End production in mutants (see below), also supported higher production in B-5477, yielding ca. 500 mg/L of the antibiotic [149]. UV-mutagenesis, selection of 3-fluoro-D,L-tyrosine-resistant mutants, and the combination of both methods were then applied to B-5477. Mutant obtained through UV-mutagenesis (named GAB-453) produced up to 1.730 g/L End in an optimized medium A [149]. Additionally, 2.56 g/L of End were produced by 3-fluoro-D,L-tyrosine-resistant mutant (Emt 36-3) in the optimized medium B. Finally, GAB-453 itself was used as a starting point to generate 3-fluoro-D,L-tyrosine-resistant mutant (Emt 2-140), which was able to produce a striking 4.34 g/L End in medium B [149].

A combined approach was used later to enhance End production in *S. fungicidicus* L-69 [53]. Although there is no information about the origin of L-69, its unusually high reported End-production level (ca. 1 g/L) suggests that L-69 might already be a product of an undisclosed mutagenesis process. In the first stage of mutagenesis, L-69 spores were treated with atmospheric and room temperature plasma (ARTP) to obtain streptomycin-resistant mutants (using a selection concentration of 20 mg/L streptomycin). The best-producing mutant from this stage yielded ca. 1.37 g/L of End and was subjected to a second mutagenesis step [53]. At this stage, spores of the previous mutant were treated with Cesium-137 γ-radiation and plated on a medium with an increased streptomycin concentration (50 mg/L). The top-producing mutant from this second stage exhibited an End production at the level of ca. 1.58 g/L [53]. Unfortunately, the composition of the production medium used here is unclear. Later, carbon ion radiation-induced mutagenesis was applied to another *S. fungicidicus* SG-01 [52], whose origin is unclear. Its reported End-production level (ca. 300 mg/L after 240 h of cultivation) hints that SG-01 might be also a wild-type strain, possibly a synonym of ATCC 21013. This mutagenesis approach yielded several mutants with significantly improved End production, reaching up to ca. 600 mg/L [49]. Notably, the addition of 10 g/L of L-arginine improved End production in an SG-01-derived mutant, though the effect was not tested in the initial strain [52]. End-overproducers obtained through random mutagenesis, as discussed above, were shown to gradually lose their elevated production levels [52,53].

A recombinant approach using the tools described in the previous section was used for *S. fungicidicus* ATCC 31731 (End high-producer derived from ATCC 21013 through random mutagenesis) [123]. In this case, genes coding for positive regulators of End biosynthesis (discussed in Section 4.6)—an StrR-like PSR (*end22*) and a sensor-histidine kinase (*end42*)—were overexpressed in ATCC 31731 using a pSET152 derivative with *ermEp* (see above) [123]. Although ATCC 31731 already produced a significant amount of End (ca. 0.8 g/L) in a variation of R5 medium [123,144], *end22* overexpression increased the production to ca. 3 g/L, while the overexpression of *end42* led to a production of ca. 1.7 g/L.

Another approach combining gene-engineering and random mutagenesis was applied to another industrial End-producer, *S. fungicidicus* TXX3120 [51]. Here, the expression levels of *end* genes were first assessed in TXX3120, identifying the *endC* NRPS gene as the least expressed [51]. It was thus hypothesized that *endC* expression could be a bottleneck for enduracidin production. The 5′-region of *endC* was substituted with *tsr* thiostrepton resistance gene. The recombinant strain then underwent ARTP mutagenesis to select for mutants with increased resistance to thiostrepton, suggesting that such mutants might carry beneficial mutations (e.g., in regulatory genes, promoter of the putative *end15-A-B-C-16-D* operon, etc.) that enhance *tsr* expression [51]. Subsequently, in several mutants with increased thiostrepton resistance, *tsr* was reverse-substituted with the 5′-terminal region of *endC*, restoring End production. As a result, these strains showed increased *endC* expression, and enduracidin production was enhanced by approximately two-fold,

although the exact quantity is difficult to ascertain since the production was reported in U/mL [51].

Very low levels of End production were detected in the environmental strains recently reported [40,55,56]. In *S. atrovirens* MGR140, End was initially detected in a non-optimized TSB medium, but production rates under these conditions were not reported [40]. An optimized medium was used in subsequent experiments (Table 2), where End production ranged from 30 to 50 mg/L [55]. Finally, spontaneous streptomycin-resistance mutants of *S. atrovirens* MGR140 with increased End production levels were obtained [55]. Lastly, another combination of media was employed for End production in *Streptomyces* sp. MC079, yielding ca. 35 mg/L of antibiotic after 160 h of cultivation [56]. Continuous control of pH during cultivation increased production to 40 mg/L (pH 5.5) and 46 mg/L (pH 5.8). A two-stage pH shift (pH 5.5 changed to pH 5.8 after 112 h of cultivation) further increased End production to ca. 53 mg/L [56].

7. Conclusions and Outline

Different aspects of the biology and chemistry of RRLDPs have been updated in recent decades. For instance, the mode of action of Rmp and End as lipid II binders has been clarified and reviewed after being the subject of intensive debate for a long time [24,25,150,151]. Additionally, the same reviews exhaustively covered the reports on chemical synthesis of RRLDPs [24,35], e.g., a total synthesis of Rmp A2 and ramoplanose aglycon [41], and a recent publication has described the advances in peptide synthesis using solid phase to prepare arylglycine-containing peptides including Rmp analogs [42], providing a new insight into SAR of RRLDPs. However, none of these reports investigated the growing body of evidence concerning the genetic background of RRLDP biosynthesis, the mechanisms involved in their biosynthesis, or the production conditions in various strains in vivo.

In this review, we focused on these topics, integrating the biosynthetic picture for different RRLDPs, comparing the available BGCs, and reviewing where the roles of different genes and enzymes have been experimentally confirmed or postulated by homology. The aim was to gain insight into the complex biosynthetic machinery behind the production of these NRP antibiotics and to offer an overview of the different tools (including genetic engineering approaches and cultivation media) used to study and enhance their biological production.

Most of the information available today has been derived from studies on Rmp and End due to their historical significance as a clinical antibiotic candidate and as an animal growth promoter, respectively. Nevertheless, recent experimental screening [39] and genome mining efforts [38] have demonstrated that many other RRLDPs likely exist in nature beyond Rmp and End, with the first newly discovered compound being chersinamycin [38]. Additionally, the growing diversity of RRLDP BGC sequences opens the door to combining different pathways to generate novel RRLDPs. Taken together, these opportunities renew interest in this class of antibiotics and establish the way for their further development.

Another intriguing aspect highlighted in this review, as well as in other recent works [97,121,152], is that RRLDP BGCs share phylogenetic relationships with GPA BGCs. GPAs also belong to the class of NRP antibiotics that bind lipid II. For example, recent work on the heterologous expression of the CSRG regulator from *M. chersina* DSM 44151—*chers28*, a StrR-like PSR—enhanced the production of the valuable GPAs teicoplanin and A40926 in *A. teichomyceticus* NRRL B-16726 and *N. gerenzanensis* ATCC 39727, respectively. In contrast, overexpression of *ramo5* (coding for the *chers28* orthologue) had no such effect [122].

The application of either native CSRGs (as demonstrated for End production in *S. fungicidicus* ATCC 31731 [123]) or heterologous CSRGs (yet to be tested) appears to be one of the most promising strategies to improve RRLDP production. For example, *A. ramoplaninifer* ATCC 33076, a producer of Rmp, would certainly benefit from this type of

investigation. Alternatively, these regulators could be used to activate seemingly silent RRLDP BGCs, following the approaches applied in GPAs [117,153].

By combining these various approaches, it may be possible to elevate RRLDPs into a clinically successful class of NRP antibiotics, which could potentially help in overcoming the spread of multidrug-resistant Gram-positive pathogens in the future. We hope that this review might ignite such efforts.

Author Contributions: K.Z. and E.B. performed the literature search; O.Y. and E.B. analyzed the literature; O.Y. prepared the figures; and O.Y. and F.M. wrote the original draft. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by an FEMS Ukraine Support Grant to K.Z. (2022), by Fondo Ateneo di Ricerca 2021/2022 to F.M., by Humboldt Research Fellowship for Postdocs from Alexander von Humboldt Foundation to O.Y., and through the EURIZON project, which is funded by the European Union under grant agreement No. 871072 to O.Y.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kleinkauf, H.; von Döhren, H. Peptide antibiotics, beta-lactams, and related compounds. *Crit. Rev. Biotechnol.* **1988**, *8*, 1–32. [CrossRef] [PubMed]
2. Hancock, R.E.; Chapple, D.S. Peptide antibiotics. *Antimicrob. Agents Chemother.* **1999**, *43*, 1317–1323. [CrossRef] [PubMed]
3. Arnison, P.G.; Bibb, M.J.; Bierbaum, G.; Bowers, A.A.; Bugni, T.S.; Bulaj, G.; Camarero, J.A.; Campopiano, D.J.; Challis, G.L.; Clardy, J.; et al. Ribosomally synthesized and post-translationally modified peptide natural products: Overview and recommendations for a universal nomenclature. *Nat. Prod. Rep.* **2013**, *30*, 108–160. [CrossRef] [PubMed]
4. Finking, R.; Marahiel, M.A. Biosynthesis of nonribosomal peptides. *Annu. Rev. Microbiol.* **2004**, *58*, 453–488. [CrossRef]
5. Gross, E.; Kiltz, H.H. The number and nature of α,β-unsaturated amino acids in subtilin. *Biochem. Biophys. Res. Commun.* **1973**, *50*, 559–565. [CrossRef]
6. Gross, E.; Morell, J.L. The structure of nisin. *J. Am. Chem. Soc.* **1971**, *93*, 4634–4635. [CrossRef]
7. Allgaier, H.; Jung, G.; Werner, R.G.; Schneider, U.; Zähner, H. Epidermin: Sequencing of a heterodetic tetracyclic 21-peptide amide antibiotic. *Eur. J. Biochem.* **1986**, *160*, 9–22. [CrossRef]
8. Ross, K.F.; Ronson, C.W.; Tagg, J.R. Isolation and characterization of the lantibiotic salivaricin A and its structural gene *salA* from *Streptococcus salivarius* 20P3. *Appl. Environ. Microbiol.* **1993**, *59*, 2014–2021. [CrossRef]
9. Gilmore, M.S.; Segarra, R.A.; Booth, M.C.; Bogie, C.P.; Hall, L.R.; Clewell, D.B. Genetic structure of the *Enterococcus faecalis* plasmid pAD1-encoded cytolytic toxin system and its relationship to lantibiotic determinants. *J. Bacteriol.* **1994**, *176*, 7335–7344. [CrossRef]
10. Stoffels, G.; Nissen-Meyer, J.; Gudmundsdottir, A.; Sletten, K.; Holo, H.; Nes, I.F. Purification and characterization of a new bacteriocin isolated from a *Carnobacterium* sp. *Appl. Environ. Microbiol.* **1992**, *58*, 1417–1422. [CrossRef]
11. Hug, J.J.; Frank, N.A.; Walt, C.; Šenica, P.; Panter, F.; Müller, R. Genome-guided discovery of the first myxobacterial biarylitide myxarylin reveals distinct C-N biaryl crosslinking in RiPP biosynthesis. *Molecules* **2021**, *26*, 1–17. [CrossRef] [PubMed]
12. Gause, G.F.; Brazhnikova, M.G. Gramicidin S and its use in the treatment of infected wounds. *Nature* **1944**, *154*, 703. [CrossRef]
13. Stansly, P.G.; Shepherd, R.G.; White, H.J. Polymyxin: A new chemotherapeutic agent. *Bull. Johns Hopkins Hosp.* **1947**, *81*, 43–54. [PubMed]
14. Kunze, B.; Jansen, R.; Höfle, G.; Reichenbach, H. Ajudazols, new inhibitors of the mitochondrial electron transport from *Chondromyces crocatus*. Production, antimicrobial activity and mechanism of action. *J. Antibiot.* **2004**, *57*, 151–155. [CrossRef] [PubMed]
15. Ling, L.L.; Schneider, T.; Peoples, A.J.; Spoering, A.L.; Engels, I.; Conlon, B.P.; Mueller, A.; Schäberle, T.F.; Hughes, D.E.; Epstein, S.; et al. A new antibiotic kills pathogens without detectable resistance. *Nature* **2015**, *517*, 455–459. [CrossRef]
16. Christiansen, G.; Philmus, B.; Hemscheidt, T.; Kurmayer, R. Genetic variation of adenylation domains of the anabaenopeptin synthesis operon and evolution of substrate promiscuity. *J. Bacteriol.* **2011**, *193*, 3822–3831. [CrossRef]
17. Müller, A.; Klöckner, A.; Schneider, T. Targeting a cell wall biosynthesis hot spot. *Nat. Prod. Rep.* **2017**, *34*, 909–932. [CrossRef]
18. Pavelka, M.S.; Mahapatra, S.; Crick, D.C. Genetics of peptidoglycan biosynthesis. In *Molecular Genetics of Mycobacteria*; Wiley: Hoboken, NJ, USA, 2015; pp. 511–533. [CrossRef]
19. de Kruijff, B.; van Dam, V.; Breukink, E. Lipid II: A central component in bacterial cell wall synthesis and a target for antibiotics. *Prostaglandins Leukot. Essent. Fat. Acids* **2008**, *79*, 117–121. [CrossRef]

20. Manat, G.; Roure, S.; Auger, R.; Bouhss, A.; Barreteau, H.; Mengin-Lecreulx, D.; Touzé, T. Deciphering the metabolism of undecaprenyl-phosphate: The bacterial cell-wall unit carrier at the membrane frontier. *Microb. Drug. Resist.* **2014**, *20*, 199–214. [CrossRef]
21. Marcone, G.L.; Binda, E.; Berini, F.; Marinelli, F. Old and new glycopeptide antibiotics: From product to gene and back in the post-genomic era. *Biotechnol. Adv.* **2018**, *36*, 534–554. [CrossRef]
22. Binda, E.; Marinelli, F.; Marcone, G.L. Old and new glycopeptide antibiotics: Action and resistance. *Antibiotics* **2014**, *3*, 572–594. [CrossRef] [PubMed]
23. And, H.C.; Hoover, D.G. Bacteriocins and their food applications. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2*, 82–100. [CrossRef] [PubMed]
24. Walker, S.; Chen, L.; Hu, Y.; Rew, Y.; Shin, D.; Boger, D.L. Chemistry and biology of ramoplanin: A lipoglycodepsipeptide with potent antibiotic activity. *Chem. Rev.* **2005**, *105*, 449–475. [CrossRef] [PubMed]
25. Hamburger, J.B.; Hoertz, A.J.; Lee, A.; Senturia, R.J.; McCafferty, D.G.; Loll, P.J. A crystal structure of a dimer of the antibiotic ramoplanin illustrates membrane positioning and a potential Lipid II docking interface. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 13759–13764. [CrossRef]
26. Somner, E.A.; Reynolds, P.E. Inhibition of peptidoglycan biosynthesis by ramoplanin. *Antimicrob. Agents Chemother.* **1990**, *34*, 413–419. [CrossRef]
27. O'hare, M.D.; Ghosh, G.; Felmingham, D.; Grüeberg, R.N. In-vitro studies with ramoplanin (MDL 62,198): A novel lipoglycopeptide antimicrobial. *J. Antimicrob. Chemother.* **1990**, *25*, 217–220. [CrossRef]
28. Higashide, E.; Hatano, K.; Shibata, M.; Nakazawa, K. Enduracidin, a new antibiotic. I *Streptomyces fungicidicus* no. B 5477, an enduracidin producing organism. *J. Antibiot.* **1968**, *21*, 126–137. [CrossRef]
29. Pedroso, A.A.; Menten, J.F.M.; Lambais, M.R.; Racanicci, A.M.C.; Longo, F.A.; Sorbara, J.O.B. Intestinal bacterial community and growth performance of chickens fed diets containing antibiotics. *Poult. Sci.* **2006**, *85*, 747–752. [CrossRef]
30. Wang, H.L.; Shi, M.; Xu, X.; Pan, L.; Zhao, P.F.; Ma, X.K.; Tian, Q.Y.; Piao, X.S. Effects of flavomycin, *Bacillus licheniformis* and enramycin on performance, nutrient digestibility, gut morphology and the intestinal microflora of broilers. *J. Poult. Sci.* **2016**, *53*, 128–135. [CrossRef]
31. Chen, Y.C.; Yu, Y.H. *Bacillus licheniformis*–fermented products and enramycin differentially modulate microbiota and antibiotic resistome in the cecal digesta of broilers. *Poult. Sci.* **2022**, *101*, 102010. [CrossRef]
32. Xuan, H.; Yao, X.; Pan, R.; Gao, Y.; Wei, J.; Shao, D.; Liu, K.; Li, Z.; Qiu, Y.; Ma, Z.; et al. Antimicrobial resistance in *Enterococcus faecium* and *Enterococcus faecalis* isolates of swine origin from eighteen provinces in China. *J. Vet. Med. Sci.* **2021**, *83*, 1952–1958. [CrossRef] [PubMed]
33. Li, H.; Liang, T.; Chu, Q.; Xu, F.; Li, Y.; Fu, L.; Zhou, B. Effects of several in-feed antibiotic combinations on the abundance and diversity of fecal microbes in weaned pigs. *Can. J. Microbiol.* **2017**, *63*, 402–410. [CrossRef] [PubMed]
34. Costa, M.C.; Bessegatto, J.A.; Alfieri, A.A.; Weese, J.S.; Filho, J.A.B.; Oba, A. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. *PLoS ONE* **2017**, *12*, 1–13. [CrossRef] [PubMed]
35. McCafferty, D.G.; Cudic, P.; Frankel, B.A.; Barkallah, S.; Kruger, R.G.; Li, W. Chemistry and biology of the ramoplanin family of peptide antibiotics. *Biopolym. Pept. Sci. Sect.* **2002**, *66*, 261–284. [CrossRef]
36. Farver, D.K.; Hedge, D.D.; Lee, S.C. Ramoplanin: A lipoglycodepsipeptide antibiotic. *Ann. Pharmacother.* **2005**, *39*, 863–868. [CrossRef]
37. Basśres, E.; Endres, B.T.; Dotson, K.M.; Jahangir Alam, M.; Garey, K.W. Novel antibiotics in development to treat *Clostridium difficile* infection. *Curr. Opin. Gastroenterol.* **2017**, *33*, 1–7. [CrossRef]
38. Morgan, K.T.; Zheng, J.; McCafferty, D.G. Discovery of six ramoplanin family gene clusters and the lipoglycodepsipeptide chersinamycin. *ChemBioChem* **2021**, *22*, 176–185. [CrossRef]
39. de la Cruz, M.; González, I.; Parish, C.A.; Onishi, R.; Tormo, J.R.; Martín, J.; Peláez, F.; Zink, D.; El Aouad, N.; Reyes, F.; et al. Production of ramoplanin and ramoplanin analogs by actinomycetes. *Front. Microbiol.* **2017**, *8*, 1–12. [CrossRef]
40. Li, X.G.; Tang, X.M.; Xiao, J.; Ma, G.H.; Xu, L.; Xie, S.J.; Xu, M.J.; Xiao, X.; Xu, J. Harnessing the potential of halogenated natural product biosynthesis by mangrove-derived actinomycetes. *Mar. Drugs* **2013**, *11*, 3875–3890. [CrossRef]
41. Jiang, W.; Wanner, J.; Lee, R.J.; Bounaud, P.Y.; Boger, D.L. Total synthesis of the ramoplanin A2 and ramoplanose aglycon. *J. Am. Chem. Soc.* **2002**, *124*, 5288–5290. [CrossRef]
42. Marschall, E.; Cass, R.W.; Prasad, K.M.; Swarbrick, J.D.; McKay, A.I.; Payne, J.A.E.; Cryle, M.J.; Tailhades, J. Synthetic ramoplanin analogues are accessible by effective incorporation of arylglycines in solid-phase peptide synthesis. *Chem. Sci.* **2023**, *15*, 195–203. [CrossRef] [PubMed]
43. Paquette, A.R.; Boddy, C.N. Macrocyclization strategies for the total synthesis of cyclic depsipeptides. *Org. Biomol. Chem.* **2023**, *21*, 8043–8053. [CrossRef] [PubMed]
44. Kawakami, M.; Nagai, Y.; Fujii, T.; Mitsuhashi, S. Anti-microbial activities of enduracidin (enramycin) in vitro and in vivo. *J. Antibiotics* **1971**, *24*, 583–586. [CrossRef] [PubMed]
45. Asai, M.; Muroi, M.; Sugita, N.; Kawashima, H.; Mizuno, K.; Miyake, A. Enduracidin, a new antibiotic. II Isolation and characterization. *J. Antibiot.* **1968**, *21*, 138–146. [CrossRef]
46. Meyers, E.; Weisenborn, F.L.; Pansy, F.E.; Slusarchyk, D.S.; Von Saltza, M.H.; Rathnum, M.L.; Parker, W.L. Janiemycin, a new peptide antibiotic. *J. Antibiot.* **1970**, *23*, 502–507. [CrossRef]

47. Cavalleri, B.; Pagani, H.; Volpe, G.; Selva, E.; Parenti, F. A-16686, a new antibiotic from *Actinoplanes*: I. Fermentation, isolation and preliminary physico-chemical characteristics. *J. Antibiot.* **1984**, *37*, 309–317. [CrossRef]
48. Marcone, G.L.; Binda, E.; Reguzzoni, M.; Gastaldo, L.; Dalmastri, C.; Marinelli, F. Classification of *Actinoplanes* sp. ATCC 33076, an actinomycete that produces the glycolipodepsipeptide antibiotic ramoplanin, as *Actinoplanes ramoplaninifer* sp. nov. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 4181–4188. [CrossRef]
49. Skelton, N.J.; Harding, M.M.; Mortishire-Smith, R.J.; Rahman, S.K.; Williams, D.H.; Rance, M.J.; Ruddock, J.C. Structure elucidation and solution conformation of the glycopeptide antibiotic ramoplanose (UK-71,903): A cyclic depsipeptide containing an antiparallel β-sheet and a β-bulge. *J. Am. Chem. Soc.* **1991**, *113*, 7522–7530. [CrossRef]
50. Pan, H.X.; Li, J.A.; Shao, L.; Zhu, C.B.; Chen, J.S.; Tang, G.L.; Chen, D.J. Genetic manipulation revealing an unusual N-terminal region in a stand-alone non-ribosomal peptide synthetase involved in the biosynthesis of ramoplanins. *Biotechnol. Lett.* **2013**, *35*, 107–114. [CrossRef]
51. Zhang, J.; He, Z.; Xu, J.T.; Song, S.; Zhu, Q.; Wu, G.; Guan, Y.; Wu, X.; Yue, R.; Wang, Y.; et al. Semi-rational mutagenesis of an industrial *Streptomyces fungicidicus* strain for improved enduracidin productivity. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 3459–3471. [CrossRef]
52. Liu, L.; Hu, W.; Li, W.-J.; Wang, S.-Y.; Lu, D.; Tian, X.-J.; Mao, Y.-Q.; Liu, J.; Chen, J.-H. Heavy-ion mutagenesis significantly enhances enduracidin production by *Streptomyces fungicidicus*. *Eng. Life Sci.* **2019**, *19*, 112–120. [CrossRef] [PubMed]
53. Zhang, D.; Wang, Q.; Liang, X. Breeding high producers of enduracidin from Streptomyces fungicidicus by combination of various mutation treatments. In *Proceedings of the Lecture Notes in Electrical Engineering*; Zhang, T.-C., Nakajima, M., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; Volume 332, pp. 133–142.
54. Hu, Y.; Yang, W.; Wan, W.; Shen, F.; Lei, Z.; Wang, D. Investigation on ultrasound-assisted extraction and separation of enduracidin from *Streptomyces* sp. NJWGY3665. *Appl. Biochem. Biotechnol.* **2012**, *166*, 830–838. [CrossRef] [PubMed]
55. Zhong, T.H.; Zeng, X.M.; Zhang, Y.H.; Chan, Z.H.; Luo, Z.H.; Yang, X.W.; Xu, W. Discovery, gene modification, and optimization of fermentation of an enduracidin-producing strain. *J. Asian Nat. Prod. Res.* **2018**, *20*, 633–648. [CrossRef]
56. Chan, Z.; Zhong, T.; Yi, Z.; Xiao, J.; Zeng, R. A pH shift feeding strategy for increased enduracidin production during fed–batch fermentation by a deep–sea bacterium *Streptomyces* sp. MC079. *Biotechnol. Bioprocess Eng.* **2015**, *20*, 908–914. [CrossRef]
57. Mascher, T.; Margulis, N.G.; Wang, T.; Ye, R.W.; Helmann, J.D. Cell wall stress responses in *Bacillus subtilis*: The regulatory network of the bacitracin stimulon. *Mol. Microbiol.* **2003**, *50*, 1591–1604. [CrossRef]
58. Mascher, T.; Zimmer, S.L.; Smith, T.A.; Helmann, J.D. Antibiotic-inducible promoter regulated by the cell envelope stress-sensing two-component system LiaRS of *Bacillus subtilis*. *Antimicrob. Agents Chemother.* **2004**, *48*, 2888–2896. [CrossRef]
59. Gao, Q.; Thorson, J.S. The biosynthetic genes encoding for the production of the dynemicin enediyne core in *Micromonospora chersina* ATCC 53710. *FEMS Microbiol. Lett.* **2008**, *282*, 105–114. [CrossRef]
60. Castiglione, F.; Marazzi, A.; Meli, M.; Colombo, G. Structure elucidation and 3D solution conformation of the antibiotic enduracidin determined by NMR spectroscopy and molecular dynamics. *Magn. Reson. Chem.* **2005**, *43*, 603–610. [CrossRef]
61. Kurz, M.; Guba, W. 3D structure of ramoplanin: A potent inhibitor of bacterial cell wall synthesis. *Biochemistry* **1996**, *35*, 12570–12575. [CrossRef]
62. Ciabatti, R.; Kettenring, J.K.; Winters, G.; Tuan, G.; Zerilli, L.; Cavalleri, B. Ramoplanin (A-16686), a new glycolipodepsipeptide antibiotic. III. Structure elucidation. *J. Antibiot.* **1989**, *42*, 254–267. [CrossRef]
63. Iwasaki, H.; Horii, S.; Asai, M.; Mizuno, K.; Miyake, A.; Ueyanagi, J. Enduracidin, a new antibiotic: VIII: Structures of enduracidins A and B. *Chem. Pharm. Bull.* **1973**, *21*, 1184–1191. [CrossRef]
64. Yin, X.; Zabriskie, T.M. The enduracidin biosynthetic gene cluster from *Streptomyces fungicidicus*. *Microbiology* **2006**, *152*, 2969–2983. [CrossRef] [PubMed]
65. Farnet, C.M.; Zazopoulos, E.; Staffa, A. Gene Cluster for Ramoplanin Biosynthesis. EP 1326983B1, 24 June 2001.
66. Gastaldo, L.; Ciabatti, R.; Assi, F.; Restelli, E.; Kettenring, J.K.; Zerilli, L.F.; Romanò, G.; Denaro, M.; Cavalleri, B. Isolation, structure determination and biological activity of A-16686 factors A′ 1, A′ 2 and A′ 3 glycolipodepsipeptide antibiotics. *J. Ind. Microbiol.* **1992**, *11*, 13–18. [CrossRef] [PubMed]
67. Mobarakai, N.; Quale, J.M.; Landman, D. Bactericidal activities of peptide antibiotics against multidrug-resistant *Enterococcus faecium*. *Antimicrob. Agents Chemother.* **1994**, *38*, 385–387. [CrossRef]
68. Kettenring, J.K.; Ciabatti, R.; Winters, G.; Tamborini, G.; Cavalleri, B. Ramoplanin (A-16686), a new glycolipodepsipeptide antibiotic. IV. Complete sequence determination by homonuclear 2D NMR spectroscopy. *J. Antibiot.* **1989**, *42*, 268–275. [CrossRef]
69. Hori, M.; Sugita, N.; Miyazak, M. Enduracidin, a new antibiotic: VI: Separation and determination of enduracidins A and B by column chromatography. *Chem. Pharm. Bull.* **1973**, *21*, 1171–1174. [CrossRef]
70. Hori, M.; Iwasaki, H.; Horii, S.; Yoshida, I.; Hongo, T. Enduracidin, a new antibiotic: VII: Primary structure of the peptide moiety. *Chem. Pharm. Bull.* **1973**, *21*, 1175–1183. [CrossRef]
71. Horii, S.; Kameda, Y. Enduracidin, a new antibiotic. V: Structures of new basic amino acids, enduracididine and allonduracididine. *J. Antibiot.* **1968**, *21*, 665–667. [CrossRef]
72. Hamada, M.; Kondo, S.; Yokoyama, T.; Miura, K.; Iinuma, K.; Yamamoto, H.; Maeda, K.; Takeuchi, T.; Umezawa, H. Minosaminomycin, A new antibiotic containing myo-inosamine. *J. Antibiot.* **1974**, *27*, 81–83. [CrossRef]
73. He, H.; Williamson, R.T.; Shen, B.; Graziani, E.I.; Yang, H.Y.; Sakya, S.M.; Petersen, P.J.; Carter, G.T. Mannopeptimycins, novel antibacterial glycopeptides from *Streptomyces hygroscopicus*, LL-AC98. *J. Am. Chem. Soc.* **2002**, *124*, 9729–9736. [CrossRef]

74. Atkinson, D.J.; Naysmith, B.J.; Furkert, D.P.; Brimble, M.A. Enduracididine, a rare amino acid component of peptide antibiotics: Natural products and synthesis. *Beilstein J. Org. Chem.* **2016**, *12*, 2325–2342. [CrossRef] [PubMed]
75. Medema, M.H.; Kottmann, R.; Yilmaz, P.; Cummings, M.; Biggins, J.B.; Blin, K.; De Bruijn, I.; Chooi, Y.H.; Claesen, J.; Coates, R.C.; et al. Minimum information about a biosynthetic gene cluster. *Nat. Chem. Biol.* **2015**, *11*, 625–631. [CrossRef] [PubMed]
76. Shawky, R.M.; Puk, O.; Wietzorrek, A.; Pelzer, S.; Takano, E.; Wohlleben, W.; Stegmann, E. The border sequence of the balhimycin biosynthesis gene cluster from *Amycolatopsis balhimycina* contains *bbr*, encoding a StrR-like pathway-specific regulator. *J. Mol. Microbiol. Biotechnol.* **2007**, *13*, 76–88. [CrossRef] [PubMed]
77. Jeong, H.; Sim, Y.M.; Kim, H.J.; Lee, D.W.; Lim, S.K.; Lee, S.J. Genome sequence of the vancomycin-producing *Amycolatopsis orientalis* subsp. orientalis strain KCTC 9412T. *Genome Announc.* **2013**, *1*, e00408-13. [CrossRef]
78. Lei, X.; Yuan, F.; Shi, Y.; Li, X.; Wang, L.; Hong, B. Draft genome sequence of norvancomycin-producing strain *Amycolatopsis orientalis* CPCC200066. *Genome Announc.* **2015**, *3*, e00296-15. [CrossRef]
79. Dörr, T.; Delgado, F.; Umans, B.D.; Gerding, M.A.; Davis, B.M.; Waldor, M.K. A transposon screen identifies genetic determinants of *Vibrio cholerae* resistance to high-molecular-weight antibiotics. *Antimicrob. Agents Chemother.* **2016**, *60*, 4757–4763. [CrossRef]
80. Hoertz, A.J.; Hamburger, J.B.; Gooden, D.M.; Bednar, M.M.; McCafferty, D.G. Studies on the biosynthesis of the lipodepsipeptide antibiotic ramoplanin A2. *Bioorganic Med. Chem.* **2012**, *20*, 859–865. [CrossRef]
81. Yushchuk, O.; Ostash, B. Glycopeptide antibiotics: Genetics, chemistry, and new screening approaches. In *Natural Products from Actinomycetes: Diversity, Ecology and Drug Discovery*; Springer: Singapore, 2022; pp. 411–444. [CrossRef]
82. Li, T.L.; Huang, F.; Haydock, S.F.; Mironenko, T.; Leadlay, P.F.; Spencer, J.B. Biosynthetic gene cluster of the glycopeptide antibiotic teicoplanin: Characterization of two glycosyltransferases and the key acyltransferase. *Chem. Biol.* **2004**, *11*, 107–119. [CrossRef]
83. Yushchuk, O.; Ostash, B.; Pham, T.H.; Luzhetskyy, A.; Fedorenko, V.; Truman, A.W.; Horbal, L. Characterization of the post-assembly line tailoring processes in teicoplanin biosynthesis. *ACS Chem. Biol.* **2016**, *11*, 2254–2264. [CrossRef]
84. Zhang, S.; Chen, Y.; Zhu, J.; Lu, Q.; Cryle, M.J.; Zhang, Y.; Yan, F. Structural diversity, biosynthesis, and biological functions of lipopeptides from *Streptomyces*. *Nat. Prod. Rep.* **2022**, *40*, 557–594. [CrossRef]
85. Cacho, R.A.; Jiang, W.; Chooi, Y.H.; Walsh, C.T.; Tang, Y. Identification and characterization of the echinocandin B biosynthetic gene cluster from *Emericella rugulosa* NRRL 11440. *J. Am. Chem. Soc.* **2012**, *134*, 16781–16790. [CrossRef] [PubMed]
86. Warneke, R.; Garbers, T.B.; Herzberg, C.; Aschenbrandt, G.; Ficner, R.; Stülke, J. Ornithine is the central intermediate in the arginine degradative pathway and its regulation in *Bacillus subtilis*. *J. Biol. Chem.* **2023**, *299*, 104944. [CrossRef] [PubMed]
87. Hubbard, B.K.; Thomas, M.G.; Walsh, C.T. Biosynthesis of L-*p*-hydroxyphenylglycine, a non-proteinogenic amino acid constituent of peptide antibiotics. *Chem. Biol.* **2000**, *7*, 931–942. [CrossRef]
88. Choroba, O.W.; Williams, D.H.; Spencer, J.B. Biosynthesis of the vancomycin group of antibiotics: Involvement of an unusual dioxygenase in the pathway to (S)-4-hydroxyphenylglycine. *J. Am. Chem. Soc.* **2000**, *122*, 5389–5390. [CrossRef]
89. Nicolaou, K.C.; Boddy, C.N.C.; Bräse, S.; Winssinger, N. Chemistry, biology, and medicine of the glycopeptide antibiotics. *Angew. Chem. Int. Ed.* **1999**, *38*, 2096–2152. [CrossRef]
90. Gavriilidou, A.; Adamek, M.; Rodler, J.-P.; Kubach, N.; Kramer, S.; Huson, D.H.; Cryle, M.J.; Stegmann, E.; Ziemert, N. Phylogenetic distance and structural diversity directing a reclassification of glycopeptide antibiotics. *bioRxiv* **2023**, 2023.02.10.526856. [CrossRef]
91. Culp, E.J.; Waglechner, N.; Wang, W.; Fiebig-Comyn, A.A.; Hsu, Y.P.; Koteva, K.; Sychantha, D.; Coombes, B.K.; van Nieuwenhze, M.S.; Brun, Y.V.; et al. Evolution-guided discovery of antibiotics that inhibit peptidoglycan remodelling. *Nature* **2020**, *578*, 582–587. [CrossRef]
92. Gonsior, M.; Mühlenweg, A.; Tietzmann, M.; Rausch, S.; Poch, A.; Süssmuth, R.D. Biosynthesis of the peptide antibiotic feglymycin by a linear nonribosomal peptide synthetase mechanism. *ChemBioChem* **2015**, *16*, 2610–2614. [CrossRef]
93. Xu, S.; Yang, Y.; Jin, R.; Zhang, M.; Wang, H. Purification and characterization of a functionally active *Mycobacterium tuberculosis* prephenate dehydrogenase. *Protein Expr. Purif.* **2006**, *49*, 151–158. [CrossRef]
94. Kloosterman, H.; Hessels, G.I.; Vrijbloed, J.W.; Euverink, G.J.; Dijkhuizen, L. (De)regulation of key enzyme steps in the shikimate pathway and phenylalanine-specific pathway of the actinomycete *Amycolatopsis methanolica*. *Microbiology* **2003**, *149*, 3321–3330. [CrossRef]
95. Thykaer, J.; Nielsen, J.; Wohlleben, W.; Weber, T.; Gutknecht, M.; Lantz, A.E.; Stegmann, E. Increased glycopeptide production after overexpression of shikimate pathway genes being part of the balhimycin biosynthetic gene cluster. *Metab. Eng.* **2010**, *12*, 455–461. [CrossRef]
96. Goldfinger, V.; Spohn, M.; Rodler, J.P.; Sigle, M.; Kulik, A.; Cryle, M.J.; Rapp, J.; Link, H.; Wohlleben, W.; Stegmann, E. Metabolic engineering of the shikimate pathway in *Amycolatopsis* strains for optimized glycopeptide antibiotic production. *Metab. Eng.* **2023**, *78*, 84–92. [CrossRef] [PubMed]
97. Yushchuk, O.; Ostash, B.; Truman, A.W.; Marinelli, F.; Fedorenko, V. Teicoplanin biosynthesis: Unraveling the interplay of structural, regulatory, and resistance genes. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 3279–3291. [CrossRef] [PubMed]
98. Chen, H.; Tseng, C.C.; Hubbard, B.K.; Walsh, C.T. Glycopeptide antibiotic biosynthesis: Enzymatic assembly of the dedicated amino acid monomer (S)-3,5-dihydroxyphenylglycine. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 14901–14906. [CrossRef] [PubMed]
99. Pfeifer, V.; Nicholson, G.J.; Ries, J.; Recktenwald, J.; Schefer, A.B.; Shawky, R.M.; Schröder, J.; Wohlleben, W.; Pelzer, S. A polyketide synthase in glycopeptide biosynthesis. The biosynthesis of the non-proteinogenic amino acid (S)-3,5-dihydroxyphenylglycine. *J. Biol. Chem.* **2001**, *276*, 38370–38377. [CrossRef]

100. Hatano, K.; Nogami, I.; Higashide, E.; Kishi, T. Biosynthesis of enduracidin: Origin of enduracididine and other amino acids. *Agric. Biol. Chem.* **1984**, *48*, 1503–1508. [CrossRef]
101. Magarvey, N.A.; Haltli, B.; He, M.; Greenstein, M.; Hucul, J.A. Biosynthetic pathway for mannopeptimycins, lipoglycopeptide antibiotics active against drug-resistant Gram-positive pathogens. *Antimicrob. Agents Chemother.* **2006**, *50*, 2167–2177. [CrossRef]
102. Han, L.; Schwabacher, A.W.; Moran, G.R.; Silvaggi, N.R. *Streptomyces wadayamensis* MppP is a pyridoxal 5′-phosphate-dependent l-arginine α-deaminase, γ-hydroxylase in the enduracididine biosynthetic pathway. *Biochemistry* **2015**, *54*, 7029–7040. [CrossRef]
103. Burroughs, A.M.; Hoppe, R.W.; Goebel, N.C.; Sayyed, B.H.; Voegtline, T.J.; Schwabacher, A.W.; Zabriskie, T.M.; Silvaggi, N.R. Structural and functional characterization of MppR, an enduracididine biosynthetic enzyme from *Streptomyces hygroscopicus*: Functional diversity in the acetoacetate decarboxylase-like superfamily. *Biochemistry* **2013**, *52*, 4492–4506. [CrossRef]
104. Stinchi, S.; Carrano, L.; Lazzarini, A.; Feroggio, M.; Grigoletto, A.; Sosio, M.; Donadio, S. A derivative of the glycopeptide A40926 produced by inactivation of the β-hydroxylase gene in *Nonomuraea* sp. ATCC 39727. *FEMS Microbiol. Lett.* **2006**, *256*, 229–235. [CrossRef]
105. Pan, H.X.; Chen, Z.Z.; Shao, L.; Li, J.A.; Chen, J.S.; Zhu, C.B.; Tang, G.L.; Chen, D.J. Production of ramoplanin analogues by genetic engineering of *Actinoplanes* sp. *Biotechnol. Lett.* **2013**, *35*, 1685–1692. [CrossRef] [PubMed]
106. Felnagle, E.A.; Barkei, J.J.; Park, H.; Podevels, A.M.; McMahon, M.D.; Drott, D.W.; Thomas, M.G. MbtH-like proteins as integral components of bacterial nonribosomal peptide synthetases. *Biochemistry* **2010**, *49*, 8815–8817. [CrossRef] [PubMed]
107. Pourmasoumi, F.; De, S.; Peng, H.; Trottmann, F.; Hertweck, C.; Kries, H. Proof-reading thioesterase boosts activity of engineered nonribosomal peptide synthetase. *ACS Chem. Biol.* **2022**, *17*, 2382–2388. [CrossRef] [PubMed]
108. Kaniusaite, M.; Tailhades, J.; Marschall, E.A.; Goode, R.J.A.; Schittenhelm, R.B.; Cryle, M.J. A proof-reading mechanism for non-proteinogenic amino acid incorporation into glycopeptide antibiotics. *Chem. Sci.* **2019**, *10*, 9466–9482. [CrossRef] [PubMed]
109. Kittilä, T.; Kittel, C.; Tailhades, J.; Butz, D.; Schoppet, M.; Büttner, A.; Goode, R.J.A.; Schittenhelm, R.B.; Van Pee, K.H.; Süssmuth, R.D.; et al. Halogenation of glycopeptide antibiotics occurs at the amino acid level during non-ribosomal peptide synthesis. *Chem. Sci.* **2017**, *8*, 5992–6004. [CrossRef]
110. Puk, O.; Huber, P.; Bischoff, D.; Recktenwald, J.; Jung, G.; Süßmuth, R.D.; Van Pée, K.H.; Wohlleben, W.; Pelzer, S. Glycopeptide biosynthesis in *Amycolatopsis mediterranei* DSM5908: Function of a halogenase and a haloperoxidase/perhydrolase. *Chem. Biol.* **2002**, *9*, 225–235. [CrossRef]
111. Yin, X.; Chen, Y.; Zhang, L.; Wang, Y.; Zabriskie, T.M. Enduracidin analogues with altered halogenation patterns produced by genetically engineered strains of *Streptomyces fungicidicus*. *J. Nat. Prod.* **2010**, *73*, 583–589. [CrossRef]
112. Han, J.; Chen, J.; Shao, L.; Zhang, J.; Dong, X.; Liu, P.; Chen, D. Production of the ramoplanin activity analogue by double gene inactivation. *PLoS ONE* **2016**, *11*, e0154121. [CrossRef]
113. Chen, J.S.; Su, M.; Shao, L.; Wang, Y.X.; Lin, H.M.; Chen, D.J. Investigation of halogenation during the biosynthesis of ramoplanin in *Actinoplanes* sp. ATCC33076. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 289–298. [CrossRef]
114. Lairson, L.L.; Henrissat, B.; Davies, G.J.; Withers, S.G. Glycosyl transferases: Structures, functions, and mechanisms. *Annu. Rev. Biochem.* **2008**, *77*, 521–555. [CrossRef]
115. Yushchuk, O.; Zhukrovska, K.; Berini, F.; Fedorenko, V.; Marinelli, F. Genetics behind the glycosylation patterns in the biosynthesis of dalbaheptides. *Front. Chem.* **2022**, *10*, 858708. [CrossRef] [PubMed]
116. Sosio, M.; Stinchi, S.; Beltrametti, F.; Lazzarini, A.; Donadio, S. The gene cluster for the biosynthesis of the glycopeptide antibiotic A40926 by *Nonomuraea* species. *Chem. Biol.* **2003**, *10*, 541–549. [CrossRef] [PubMed]
117. Yushchuk, O.; Vior, N.M.; Andreo-Vidal, A.; Berini, F.; Rückert, C.; Busche, T.; Binda, E.; Kalinowski, J.; Truman, A.W.; Marinelli, F. Genomic-led discovery of a novel glycopeptide antibiotic by *Nonomuraea coxensis* DSM 45129. *ACS Chem. Biol.* **2021**, *16*, 915–928. [CrossRef] [PubMed]
118. Chen, J.S.; Wang, Y.X.; Shao, L.; Pan, H.X.; Li, J.A.; Lin, H.M.; Dong, X.J.; Chen, D.J. Functional identification of the gene encoding the enzyme involved in mannosylation in ramoplanin biosynthesis in *Actinoplanes* sp. *Biotechnol. Lett.* **2013**, *35*, 1501–1508. [CrossRef]
119. Wu, M.C.; Styles, M.Q.; Law, B.J.C.; Struck, A.W.; Nunns, L.; Micklefield, J. Engineered biosynthesis of enduracidin lipoglycopeptide antibiotics using the ramoplanin mannosyltransferase Ram29. *Microbiology* **2015**, *161*, 1338–1347. [CrossRef]
120. Yushchuk, O.; Zhukrovska, K.; Fedorenko, V. Insights into the phylogeny of transporters coded within biosynthetic gene clusters for glycopeptides and related antibiotics. *Visnyk Lviv Univ. Biol. Ser.* **2022**, *86*, 33–46. [CrossRef]
121. Menges, R.; Muth, G.; Wohlleben, W.; Stegmann, E. The ABC transporter Tba of *Amycolatopsis balhimycina* is required for efficient export of the glycopeptide antibiotic balhimycin. *Appl. Microbiol. Biotechnol.* **2007**, *77*, 125–134. [CrossRef]
122. Zhukrovska, K.; Binda, E.; Fedorenko, V.; Marinelli, F.; Yushchuk, O. The impact of heterologous regulatory genes from lipodepsipeptide biosynthetic gene clusters on the production of teicoplanin and A40926. *Antibiotics* **2024**, *13*, 115. [CrossRef]
123. Chen, Y.W.; Liu, X.C.; Lv, F.X.; Li, P. Characterization of three regulatory genes involved in enduracidin biosynthesis and improvement of enduracidin production in *Streptomyces fungicidicus*. *J. Appl. Microbiol.* **2019**, *127*, 1698–1705. [CrossRef]
124. Takano, E.; Tao, M.; Long, F.; Bibb, M.J.; Wang, L.; Li, W.; Buttner, M.J.; Bibb, M.J.; Deng, Z.X.; Chater, K.F. A rare leucine codon in *adpA* is implicated in the morphological defect of *bldA* mutants of *Streptomyces coelicolor*. *Mol. Microbiol.* **2003**, *50*, 475–486. [CrossRef]
125. McCormick, J.R.; Flärdh, K. Signals and regulators that govern *Streptomyces* development. *FEMS Microbiol. Rev.* **2012**, *36*, 206–231. [CrossRef] [PubMed]

126. Yushchuk, O.; Ostash, I.; Mösker, E.; Vlasiuk, I.; Deneka, M.; Rückert, C.; Busche, T.; Fedorenko, V.; Kalinowski, J.; Süssmuth, R.D.; et al. Eliciting the silent lucensomycin biosynthetic pathway in *Streptomyces cyanogenus* S136 via manipulation of the global regulatory gene *adpA*. *Sci. Rep.* **2021**, *11*, 3507. [CrossRef] [PubMed]
127. Chater, K.F.; Chandra, G. The use of the rare UUA codon to define "expression space" for genes involved in secondary metabolism, development and environmental adaptation in *Streptomyces*. *J. Microbiol.* **2008**, *46*, 1–11. [CrossRef] [PubMed]
128. Ohnishi, Y.; Kameyama, S.; Onaka, H.; Horinouchi, S. The A-factor regulatory cascade leading to streptomycin biosynthesis in *Streptomyces griseus*: Identification of a target gene of the A-factor receptor. *Mol. Microbiol.* **1999**, *34*, 102–111. [CrossRef]
129. Du, Y.L.; Li, S.Z.; Zhou, Z.; Chen, S.F.; Fan, W.M.; Li, Y.Q. The pleitropic regulator AdpAch is required for natamycin biosynthesis and morphological differentiation in *Streptomyces chattanoogensis*. *Microbiology* **2011**, *157*, 1300–1311. [CrossRef]
130. Guo, S.; Leng, T.; Sun, X.; Zheng, J.; Li, R.; Chen, J.; Hu, F.; Liu, F.; Hua, Q. Global regulator AdpA_1075 regulates morphological differentiation and ansamitocin production in *Actinosynnema pretiosum* subsp. auranticum. *Bioengineering* **2022**, *9*, 719. [CrossRef]
131. Ostash, B.; Yushchuk, O.; Tistechok, S.; Mutenko, H.; Horbal, L.; Muryn, A.; Dacyuk, Y.; Kalinowski, J.; Luzhetskyy, A.; Fedorenko, V. The *adpA*-like regulatory gene from *Actinoplanes teichomyceticus*: In silico analysis and heterologous expression. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1297–1301. [CrossRef]
132. Bierman, M.; Logan, R.; O'Brien, K.; Seno, E.T.; Nagaraja Rao, R.; Schoner, B.E. Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene* **1992**, *116*, 43–49. [CrossRef]
133. Yushchuk, O.; Homoniuk, V.; Datsiuk, Y.; Ostash, B.; Marinelli, F.; Fedorenko, V. Development of a gene expression system for the uncommon actinomycete *Actinoplanes rectilineatus* NRRL B-16090. *J. Appl. Genet.* **2020**, *61*, 141–149. [CrossRef]
134. Horbal, L.; Kobylyanskyy, A.; Yushchuk, O.; Zaburannyi, N.; Luzhetskyy, A.; Ostash, B.; Marinelli, F.; Fedorenko, V. Evaluation of heterologous promoters for genetic analysis of *Actinoplanes* teichomyceticus—Producer of teicoplanin, drug of last defense. *J. Biotechnol.* **2013**, *168*, 367–372. [CrossRef]
135. Fei, X.; Yin, X.; Zhang, L.; Zabriskie, T.M. Roles of VioG and VioQ in the incorporation and modification of the capreomycidine residue in the peptide antibiotic viomycin. *J. Nat. Prod.* **2007**, *70*, 618–622. [CrossRef] [PubMed]
136. Kieser, T.; Hopwood, D.A.; Wright, H.M.; Thompson, C.J. pIJ101, a multi-copy broad host-range *Streptomyces* plasmid: Functional analysis and development of DNA cloning vectors. *Mol. Gen. Genet.* **1982**, *185*, 223–238. [CrossRef] [PubMed]
137. Healy, F.G.; Eaton, K.P.; Limsirichai, P.; Aldrich, J.F.; Plowman, A.K.; King, R.R. Characterization of γ-butyrolactone autoregulatory signaling gene homologs in the angucyclinone polyketide WS5995B producer *Streptomyces acidiscabies*. *J. Bacteriol.* **2009**, *191*, 4786–4797. [CrossRef] [PubMed]
138. Rodríguez-García, A.; Combes, P.; Pérez-Redondo, R.; Smith, M.C.A.; Smith, M.C.M. Natural and synthetic tetracycline-inducible promoters for use in the antibiotic-producing bacteria *Streptomyces*. *Nucleic Acids Res.* **2005**, *33*, e87. [CrossRef]
139. Muth, G.; Nußbaumer, B.; Wohlleben, W.; Pühler, A. A vector system with temperature-sensitive replication for gene disruption and mutational cloning in streptomycetes. *MGG Mol. Gen. Genet.* **1989**, *219*, 341–348. [CrossRef]
140. Yin, X.; O'Hare, T.; Gould, S.J.; Zabriskie, T.M. Identification and cloning of genes encoding viomycin biosynthesis from *Streptomyces vinaceus* and evidence for involvement of a rare oxygenase. *Gene* **2003**, *312*, 215–224. [CrossRef]
141. Sun, Y.; Zhou, X.; Liu, J.; Bao, K.; Zhang, G.; Tu, G.; Kieser, T.; Deng, Z. "*Streptomyces nanchangensis*", a producer of the insecticidal polyether antibiotic nanchangmycin and the antiparasitic macrolide meilingmycin, contains multiple polyketide gene clusters. *Microbiology* **2002**, *148*, 361–371. [CrossRef]
142. Li, P.; Li, J.; Guo, Z.; Tang, W.; Han, J.; Meng, X.; Hao, T.; Zhu, Y.; Zhang, L.; Chen, Y. An efficient blue-white screening based gene inactivation system for *Streptomyces*. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 1923–1933. [CrossRef]
143. Doumith, M.; Weingarten, P.; Wehmeier, U.F.; Salah-Bey, K.; Benhamou, B.; Capdevila, C.; Michel, J.M.; Piepersberg, W.; Raynal, M.C. Analysis of genes involved in 6-deoxyhexose biosynthesis and transfer in *Saccharopolyspora erythraea*. *Mol. Gen. Genet.* **2000**, *264*, 477–485. [CrossRef]
144. Kieser, T.; Bibb, M.; Buttner, M.; Chater, K. *Practical Streptomyces Genetics*, 2nd ed.; John Innes Foundation: Norwich, UK, 2000; ISBN 0-7084-0623-8.
145. Muth, G. The pSG5-based thermosensitive vector family for genome editing and gene expression in actinomycetes. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 9067–9080. [CrossRef]
146. Miyoshi-Saitoh, M.; Morisaki, N.; Tokiwa, Y.; Iwasaki, S.; Konishi, M.; Saitoh, K.; Oki, T. Dynemicins O, P and Q: Novel antibiotics related to dynemicin A. Isolation, characterization and biological activity. *J. Antibiot.* **1991**, *44*, 1037–1044. [CrossRef] [PubMed]
147. Brunati, M.; Bava, A.; Marinelli, F.; Lancini, G. Influence of leucine and valine on ramoplanin production by *Actinoplanes* sp. ATCC 33076. *J. Antibiot.* **2005**, *58*, 473–478. [CrossRef] [PubMed]
148. Erkan, D.; Kayali, H.A. Replacement of soybean meal with animal origin protein meals improved ramoplanin a2 production by *Actinoplanes* sp. ATCC 33076. *Appl. Biochem. Biotechnol.* **2016**, *180*, 306–321. [CrossRef] [PubMed]
149. Nogami, I.; Shirafuji, H.; Matsumura, S. Production of Enduracidin and Microorganisms Therefor. US 4465771A, 30 October 1981.
150. Nam, J.; Shin, D.; Rew, Y.; Boger, D.L. Alanine scan of [L-Dap2]ramoplanin A2 aglycon: Assessment of the importance of each residue. *J. Am. Chem. Soc.* **2007**, *129*, 8747–8755. [CrossRef]
151. Fang, X.; Nam, J.; Shin, D.; Rew, Y.; Boger, D.L.; Walker, S. Functional and biochemical analysis of a key series of ramoplanin analogues. *Bioorganic Med. Chem. Lett.* **2009**, *19*, 6189–6191. [CrossRef]

152. Waglechner, N.; McArthur, A.G.; Wright, G.D. Phylogenetic reconciliation reveals the natural history of glycopeptide antibiotic biosynthesis and resistance. *Nat. Microbiol.* **2019**, *4*, 1862–1871. [CrossRef]
153. Spohn, M.; Kirchner, N.; Kulik, A.; Jochim, A.; Wolf, F.; Muenzer, P.; Borst, O.; Gross, H.; Wohlleben, W.; Stegmann, E. Overproduction of ristomycin a by activation of a silent gene cluster in *Amycolatopsis japonicum* MG417-CF17. *Antimicrob. Agents Chemother.* **2014**, *58*, 6185–6196. [CrossRef]