International Journal of Antimicrobial Agents 55 (2020) 105910

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Review

Antimicrobial production by strictly anaerobic *Clostridium* spp.

Amila Srilal Nawarathna Weligala Pahalagedara[a,b], Steve Flint[b], Jon Palmer[b], Gale Brightwell[a], Tanushree Barua Gupta[a,*]

[a] Food Assurance Team, AgResearch Ltd., Hopkirk Research Institute, Massey University, Palmerston North, 4474, New Zealand
[b] School of Food and Advanced Technology, Massey University, Palmerston North, 4442, New Zealand

ARTICLE INFO

Article history:
Received 10 September 2019
Accepted 18 January 2020

Keywords:
Clostridium spp.
Strict anaerobe
Antimicrobial
Genome mining

ABSTRACT

Antimicrobial resistance continues to rise on a global scale, affecting the environment, humans, animals and food systems. Use of natural antimicrobials has been favoured over synthetic molecules in food preservation owing to concerns over the adverse health effects of synthetic chemicals. The continuing need for novel natural antimicrobial compounds has spurred research to investigate natural sources, such as bacteria, for antimicrobials. The antimicrobial-producing potential of bacteria has been investigated in numerous studies. However, the discovery of antimicrobials has been biased towards aerobes and facultative anaerobes, and strict anaerobes such as *Clostridium* spp. have been largely neglected. In recent years, genomic studies have indicated the genetic potential of strict anaerobes to produce putative bioactive molecules and this has encouraged the exploration of *Clostridium* spp. for their antimicrobial production. So far, only a limited number of antimicrobial compounds have been isolated, identified and characterised from the genus *Clostridium*. This review discusses our current knowledge and understanding of clostridial antimicrobial compounds as well as recent genome mining studies of *Clostridium* spp. focused at identification of putative gene clusters encoding bacterial secondary metabolite groups and peptides reported to possess antimicrobial properties. Furthermore, opportunities and challenges in the identification of antimicrobials from *Clostridium* spp. using genomic-guided approaches are discussed. The limited studies conducted so far have identified the genus *Clostridium* as a viable source of antimicrobial compounds for future investigations.

1. Introduction

Antimicrobials are widely used in human medicine, veterinary practice, the food industry and agriculture to control harmful or unfavourable micro-organisms. Overuse and misuse of antibiotics has caused the development of antimicrobial-resistant bacterial strains [1,2]. Antimicrobial resistance has become one of the most serious health threats that challenges the effectiveness of many existing antimicrobial agents. The US Centers for Disease Control and Prevention (CDC) has estimated that at least two million people acquire serious infections with antimicrobial-resistant bacteria every year in the USA, with 23 000 deaths due to the ineffectiveness of existing antibiotics [3]. Antimicrobial resistance is a global threat facing every country irrespective of the income of the country, however there is variation in the antimicrobial resistance patterns across different geographical regions [4]. Use of existing antimicrobials in non-medical applications, such as food preservation, has also been compromised by the development of antimicrobial resistance. For instance, the effectiveness of nisin, which is widely used in food preservation as a natural and safe antimicrobial compound, has been challenged by the development of nisin-resistant food spoilage and pathogenic micro-organisms [5,6]. The ineffectiveness of currently available antimicrobials has triggered a more active search for alternative compounds to combat resistant pathogenic bacteria [7,8]. Natural antimicrobials isolated from microbial, plant and animal sources are favoured over synthetic equivalents, particularly in food preservation owing to consumer concerns over the adverse health effects of synthetic chemicals [9,10]. The development of multidrug-resistant bacteria and the consumer demand for natural food preservatives have created an increased interest in the identification and characterisation of novel natural antimicrobial compounds with multiple modes of action [11,12].

Micro-organisms have been a significant source of natural antimicrobial compounds successfully used in a wide range of applications. Many studies have been conducted with a focus on the discovery of new antimicrobial compounds from bacteria and fungi, resulting in many additional antimicrobials being identified. Soil *Streptomyces* spp. and lactic acid bacteria (LAB) commonly

* Corresponding author.
E-mail address: tanushree.gupta@agresearch.co.nz (T.B. Gupta).

https://doi.org/10.1016/j.ijantimicag.2020.105910
0924-8579/

associated with food have been extensively screened for their potential to produce antimicrobial agents [13,14]. The application of LAB strains as probiotic organisms in fermented food products has also been widely studied [15,16].

However, antimicrobial discovery has been biased towards aerobes and facultative anaerobes by selecting potential micro-organisms for antimicrobial studies. Strict anaerobes have been largely overlooked in the search for producers of antimicrobial compounds [17]. In recent years, investigators have started to look into the largely ignored microbial groups, including strict anaerobes and unexploited microbiota such as endophytes and symbiotic microbes from the marine environment as well as humans, insects and nematodes in the search of novel antimicrobial compounds [18–22].

The genus *Clostridium* includes anaerobic spore-forming bacteria belonging to the phylum Firmicutes. This group consists of over 200 known members, including pathogens associated with human and animal diseases [23]. Although most of the *Clostridium* spp. are saprophytes not involved in a disease process, pathogenic species have received the most attention owing to their detrimental effects on humans, animals and food systems. Pathogenic *Clostridium* spp. have been known to be responsible for human diseases such as tetanus, botulism, gas gangrene and pseudomembranous colitis [24]. Mesophilic spore-forming anaerobic bacteria such as *Clostridium putrefaciens, Clostridium estertheticum* and *Clostridium gasigenes* are involved in the spoilage of refrigerated vacuum-packed meat products [25]. *Clostridium botulinum* and *Clostridium perfringens* are two of the most common foodborne pathogens [26]. Nevertheless, despite the harmful activities of some *Clostridium* spp., the non-pathogenic majority may have beneficial effects on human health and food systems through the production of bioactive compounds. Specific *Clostridium butyricum* strains have a protective effect against some pathogens and have been commercialised as probiotics for humans and animals, mainly in Japan, Korea and China [27–29]. With the advent of computational genomics such as genome mining, the genetic potential of anaerobic bacteria to produce secondary metabolites and peptides has been identified and this has encouraged natural product investigations from the genus *Clostridium*.

The purpose of this review was to summarise the current state of knowledge on the antimicrobial compound synthesis potential of bacteria from the genus *Clostridium* and to highlight opportunities, knowledge gaps and challenges in the discovery of antimicrobial agents using genomic-guided approaches. Here we discuss the past and present efforts to identify and characterise antimicrobials from various *Clostridium* spp. and provide examples from the recent literature showing the current genomic efforts of identifying the biosynthetic capability of *Clostridium* spp. to produce potent antimicrobial compounds.

2. Identification of antimicrobials from *Clostridium* spp. using culture-based methods

Obligate anaerobes such as *Clostridium* spp. show diverse metabolic features including the conversion of sugars, proteins, peptides and amino acids into various organic acids (acetic, propionic, butyric) and alcohols [30]. *Clostridium* spp. thrive in soil environments as well as in the human and animal gut where they interact with complex, harsh and/or dynamic physiological and biological environments. Conceivably, the chemical diversity of their secondary metabolites has evolved as a result of mutual interactions with neighbouring micro-organisms and adaptation mechanisms to challenging physiochemical environments [31]. *Clostridium* spp. living in the gut have developed metabolic mechanisms to exploit changes in the ecosystem. Ternan et al. studied the adaptation mechanisms of *Clostridium difficile* to the human gut environment by adding human faecal water to culture media and found that faecal water components upregulated the expression of sporulation-associated genes by 300 times along with downregulation of motility and toxin genes [32]. Moreover, a set of previously unknown metabolic compounds were identified revealing previously hidden metabolic capabilities of *C. difficile*, which might be involved in their adaptation mechanisms. It has been shown that following antibiotic treatment, *C. difficile* utilises succinate to synthesise butyrate, which aids in increasing its population [33]. *Clostridium difficile* also produces *para*-cresol, a bacteriostatic compound, providing a competitive advantage for colonisation over *Escherichia coli, Bacteroides thetaiotaomicron* and *Klebsiella oxytoca* [34]. *Clostridium butyricum* isolated from the root canal system is reported to produce an antagonistic substance against other resident bacteria for their successful colonisation [35]. These studies demonstrate that *Clostridium* spp. possess a variety of metabolic pathways that aid their survival in different environmental conditions. However, still very limited studies have been conducted to explore the metabolic diversity of the genus *Clostridium* and their role in growth and survival under various environmental conditions.

Traditionally, antimicrobial compound discovery has relied on the testing of bacteria cultivated under standard laboratory conditions for antimicrobial activity and isolating the active compounds from their spent culture media. This culture-based method has been successfully used in the discovery of many antimicrobial compounds currently approved for various applications. Nevertheless, standard laboratory conditions may not trigger the production of many specialised metabolites in bacteria, which are likely to be induced in response to abiotic and biotic ecological demands. It is therefore necessary to understand the role of the natural environment and to incorporate of these conditions in laboratory-based experiments for antimicrobial production. Specially, the adaptation pressure of obligate anaerobes appears to have developed a variety of metabolic pathways to produce specialised metabolites such as antimicrobials and their activation may require specific environmental cues [32,34].

Lincke et al. studied the synthesis of secondary metabolites from *Clostridium cellulolyticum* isolated from grass compost and found that it produced no secondary metabolites under standard growth conditions [19] (Fig. 1). However, after introducing various culture conditions to the growth medium, only aqueous soil extract mimicking the natural habitat could induce the production of closthioamide, a secondary metabolite that was identified as the first antibiotic from *Clostridium* spp. This study demonstrated that providing natural growth factors in laboratory culture media may trigger metabolic pathways of *Clostridium* spp. not activated under standard cultivation conditions.

The idea of *Clostridium* spp. as potential antimicrobial producers is not novel, although they have received little attention compared with other bacterial groups such as *Bacillus* spp., which have yielded a considerable number of useful antimicrobials [36–39]. During the 1960–1980s, several research groups worked on the identification of bacteriocins from *Clostridium* spp. (Table 1). In 1968, Hongo et al. described the isolation of four bacteriocins and their properties and activity against a wide range of *Clostridium* and *Bacillus* spp. [40]. However, after the 1980s little attention has been given to the genus *Clostridium* as a potential source of antimicrobials. Lately there is a renewed interest in this subject, particularly with the new genomic knowledge and understanding of strict anaerobes.

Until recently, no clostridial secondary metabolites having antimicrobial activity had been identified. The discovery of three novel antimicrobial metabolites, namely closthioamide, clostrubin and clostrindolin (Fig. 2), are examples of current efforts to discover antimicrobial compounds from strict anaer-

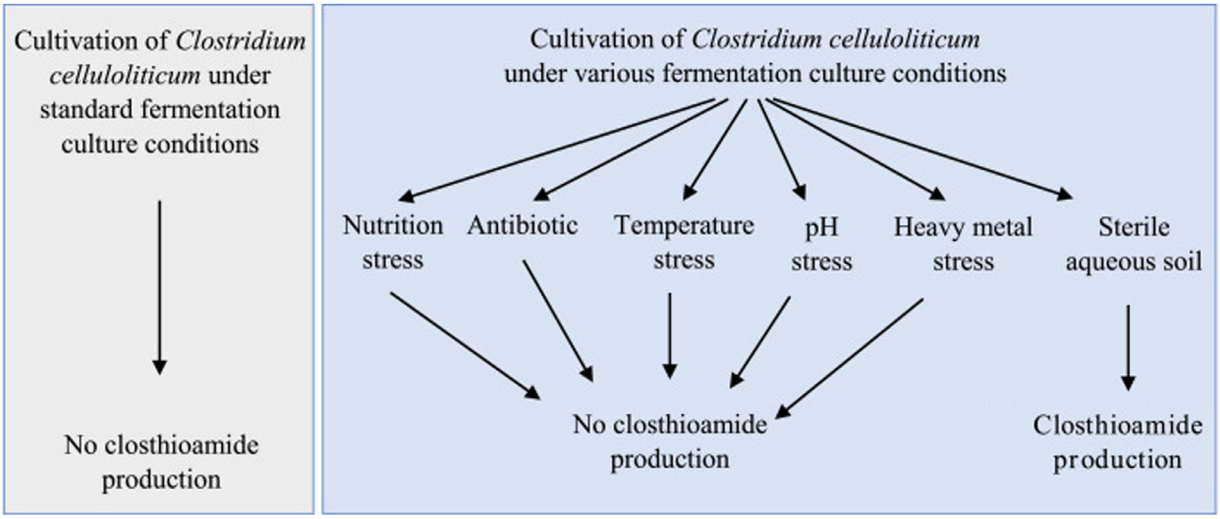

Fig. 1. Effect of various culture conditions on closthioamide production by *Clostridium cellulolyticum*.

Table 1
Antimicrobial compounds identified from *Clostridium* spp.

Compound name	Producing organism	Description	Year reported	Reference
Clostocins A, B, C, D	*Clostridium* spp.	Bacteriocins insensitive to UV light, ribonuclease and deoxyribonuclease. A and D are thermostable (100 °C for 30 min), whereas B and C are relatively thermolabile (started to lose activity at 60 °C after 30 min)	1968	[40]
Clostocin O	*Clostridium saccharoperbutylacetonicum* Nl-4 (ATCC 13564)	Phage tail-like bacteriocin	1968	[40]
Perfringocin 11105	*Clostridium perfringens* type A NCIB 11105	Bacteriocin; amphiphilic peptide with molecular mass of ~76 kDa	1974	[41]
Bacteriocin N5	*Clostridium perfringens* BP6K-N5	Bacteriocin; single polypeptide chain with a molecular mass of ~82 kDa that inhibits the synthesis of DNA, RNA and proteins in sensitive cells	1975	[42,43]
Bacteriocin 28	*Clostridium perfringens* strain 28	Bacteriocin; heat-stable glycoprotein peptide containing 15 AAs with a molecular mass of ~84 kDa	1982	[44]
BCN5	*Clostridium perfringens* CPN50	Bacteriocin whose expression can be stimulated by UV light; molecular mass of ~96.5 kDa	1986	[45]
Butyricin 7423	*Clostridium butyricum* NCIB7423	Bacteriocin; trypsin-sensitive amphiphilic protein whose action appears to be on the cell membrane leading to altered permeability	1974	[41]
Boticin P	*Clostridium botulinum* PM-15, type E	Bacteriocin with phage tail-like structure	1974	[46]
Boticin B	*Clostridium botulinum* 213B	Bacteriocin; heat-stable small peptide with a predicted size of 50 AAs	2000	[47]
Closticin 574	*Clostridium tyrobutyricum* ADRIAT 932	Bacteriocin; synthesised as a pre-protein containing 310 AA residues and subsequently processed to an 82-AA peptide	2003	[48]
Circularin A	*Clostridium beijerinckii* ATCC 25752	Bacteriocin; circular peptide of 69 AA residues and 3-AA leader sequence	2003	[48]
Clostrindolin	*Clostridium beijerinckii* HK1805	Pyrone alkaloid	2019	[49]
Closthioamide	*Clostridium cellulolyticum* DSM 5812 and pSB050	Antibiotic belonging to a new class of natural products, the polythioamides	2010	[19]
Diffocins	*Clostridium difficile*	Bacteriocin; R-type phage tail-like high-molecular-weight peptide	2012	[50]
Perfrin	Necrotic enteritis-associated *netB*-positive *Clostridium perfringens* strain	Bacteriocin; 11.5-kDa C-terminal fragment of a 22.9-kDa protein	2014	[51]
Clostrubin	*Clostridium beijerinckii*	Antibiotic; polyphenolic polyketide antibiotic first reported polyketide from anaerobic bacteria	2014	[52]

UV, ultraviolet; AA, amino acid.

obes. Closthioamide possesses antimicrobial activity against a wide range of Gram-positive bacteria but only partial activity against some *E. coli* strains. It is also found to be effective against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci strains [53]. Clostrubin is the first polyketide discovered from a strict anaerobe and shows antimicrobial activity against MRSA, vancomycin-resistant enterococci and mycobacteria [52]. Clostrindolin is reported to be active against *Mycobacterium vaccae* [49].

However, to date only a limited number of clostridial antimicrobial products have been identified, isolated and characterised. The potential applications of these identified compounds have not been properly investigated in various fields.

3. Genome-based identification of peptide and secondary metabolite gene clusters in *Clostridium* spp

In the last decade there has been a surge in the whole-genome sequencing of micro-organisms and the development of various

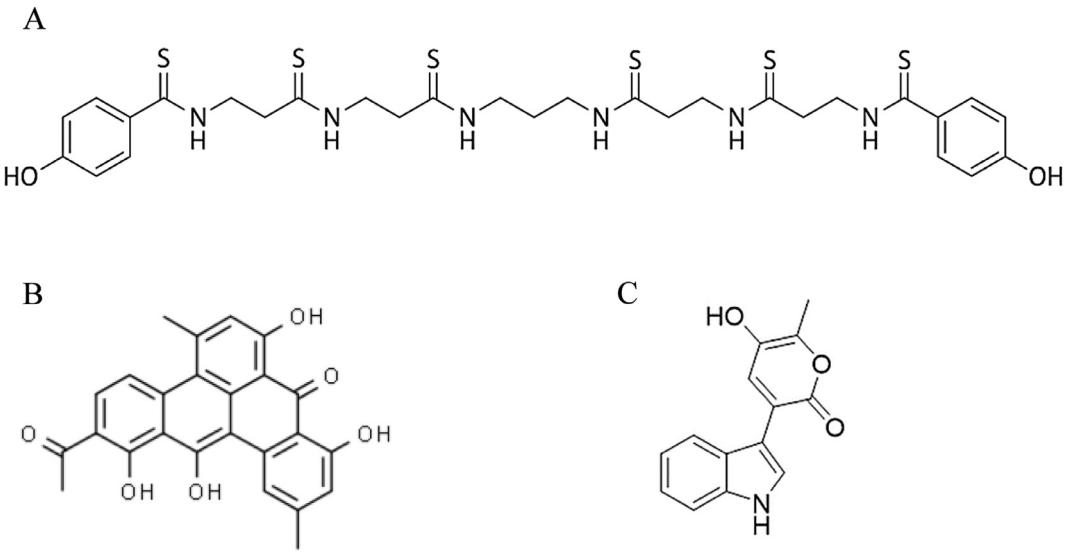

Fig. 2. Structure of (A) closthioamide [53], (B) clostrubin [54] and (C) clostrindolin [49].

bioinformatic tools to analyse increasing genomic information [55]. Analysis of the genomes of a wide range of bacteria, including anaerobes, has revealed the presence of various gene clusters governing the biosynthesis of natural bioactive molecules such as antimicrobials [56,57]. These genetic studies have revealed that the understanding of the potential for the synthesis of bioactive compounds from well-known bacterial groups, such as actinomycetes, is underdetermined and many other bacteria, previously not considered as bioactive compound producers based on conventional approaches, possess genetic capability for secondary metabolite and peptide production [56,58–61].

Clostridium spp. are a group of micro-organisms where the search for antimicrobial compounds has been largely neglected. Antimicrobial compounds are highly diverse in their structures, but many belong to three prominent natural bioactive compound classes: ribosomally synthesised and post-translationally modified peptides (RiPPs); non-ribosomal peptides (NRPs); and polyketides (PKs) [62]. In recent years, the genomes of anaerobic bacteria have been analysed for identification of putative gene clusters responsible for the synthesis of RiPPs, PKs and NRPs using various bioinformatic tools. Biosynthetic gene cluster (BGC)-identifying computational tools can be divided into high confidence/low novelty and low confidence/high novelty techniques [63]. High confidence/low novelty tools such as antiSMASH [55], SMURF [64], NP.searcher [65], ClustScan [66] and CLUSEAN [67] utilise well-defined queries to give a more reliable overview of BGCs of a single bacterial genome sequence. Low confidence/high novelty techniques such as ClusterFinder [68] and EvoMining [69] have been developed to detect gene clusters belonging to unknown metabolite classes that may encode entirely novel chemical compounds [68]. The occurrence of BGCs responsible for the production of RiPPs, PKs and NRPs in the genomes of *Clostridium* spp. indicates the possibility to produce molecules with potential antimicrobial activity.

3.1. Occurrence of ribosomally synthesised and post-translationally modified peptide (RiPP) gene clusters in the genus Clostridium

RiPPs are produced in the ribosome of bacteria as precursor peptides containing a leader peptide and core region, whereupon the core region undergoes different post-translational modifications resulting in mature active peptides [70]. They have been recognised as a predominant group of antimicrobial compounds showing antibacterial, antifungal and antiviral activities [71–74]. Over 20 different RiPP families have been described based on their structural characteristics and biosynthetic machinery [70]. In recent years, genome mining of a wide range of bacteria for RiPP gene clusters has been carried out using homology to genes responsible for the synthesis of precursor peptide or biosynthetic proteins [62,75–77]. However, limited published information is available on the putative RiPP gene clusters detected in *Clostridium* spp. Letzel et al. investigated 211 complete and published genomes of anaerobic bacteria including 35 strains of *Clostridium* for the presence of RiPP-encoding genes and gene clusters and found that >25% of tested anaerobic genomes harboured gene clusters for RiPP synthesis [57]. Among them, 10.4% of tested anaerobic genomes were found to have only RiPP gene clusters and the remainder had RiPP along with non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) gene clusters in their genomes. This study further highlighted the capacity of *Clostridium* spp. to produce a wide range of RiPP classes including lanthipeptides, sactipeptides, thiopeptides, lasso peptides, linear azol(in)e-containing peptides (LAPs), lactococcins and head-to-tail cyclic peptides. However, gene clusters associated with lanthipeptides, sactipeptides and LAP synthesis were the most predominant (Table 2).

Another study conducted by Tushar et al. described the presence of gene clusters responsible for the synthesis of four microcins in the genome of a newly identified *Clostridium* spp. JC272 [78]. These studies demonstrated the potential of *Clostridium* spp. to produce antimicrobial peptides through the presence of RiPP gene clusters.

3.2. Occurrence of non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) gene clusters in the genus Clostridium

NRPs are a diverse group of secondary metabolites synthesised by dedicated NRPS enzymes, mainly in bacteria and fungi. NRPSs are modular multidomain enzyme complexes that serve as templates and biosynthetic machinery for NRP synthesis [79–81]. NRPs are a predominant group of drugs used in human medicine, primarily as antibacterials, followed by antifungals, anti-tumour compounds and immunosuppressants [80,82,83]. PKs are another group of structurally and functionally diverse secondary metabolites mainly produced by bacteria, fungi and plants [84]. PK biosynthesis is initiated from simple carboxylic acid derivatives and they acquire their structural diversity by processing under a series of

Table 2
Different ribosomally synthesised and post-translationally modified peptide (RiPP) families detected in various strains of *Clostridium* spp. by Letzel et al. [57].

Clostridium spp.	Strain	Accession no.	RiPP family
Clostridium cellulovorans	ATCC 35296/743B	**CP002160.1**	Lanthipeptides I & II and thiopeptide
Clostridium kluyveri	DSM 555	**CP000673.1**	Lanthipeptide I and sactipeptide
Clostridium	ATCC 824	**AE001437.1**	Lanthipeptide II and sactipeptide
acetobutylicum	DSM 1731	**CP002660.1**	
	EA 2018	**CP002118.1**	
Clostridium beijerinckii	NCIMB 8052	**CP000721.1**	Lanthipeptide II
Clostridium botulinum	H04402 065	**FR773526.1**	Lanthipeptide II and LAP
Clostridium	A2 Kyoto-F	**AY497358**	LAP
botulinum	A1 ATCC 19397	**CP000726.1**	
	A1 Hall	**CP000727.1**	
	B1 Okra	**CP000939.1**	
	A3 Loch Maree	**CP000962.1**	
	Ba4 657	**CP001083.1**	
	F 230613	**CP002011.1**	
	A ATCC 3502	**AM412317.1**	
	F Langeland	**CP000728.1**	
Clostridium cellulolyticum	H10	**CP001348.1**	Sactipeptide
Clostridium difficile	630	**AM180355.1**	Sactipeptide
Clostridium lentocellum	DSM 5427	**CP002582.1**	Sactipeptide
Clostridium thermocellum	ATCC 27405	**CP000568.1**	Sactipeptide
Clostridium perfringens	13	**BA000016.3**	Lasso peptide
Clostridium perfringens	SM101	**CP000312.1**	Lactococcin-like RiPP and head-to-tail cyclised peptide

LAP, linear azol(in)e-containing peptide.

modular enzymes called PKSs [84–86]. PK compounds such as polyenes, polyethers, polyphenols, macrolides and endiines possess biological functions including immunosuppressive, anticancer and antimicrobial properties [87–90].

It was previously thought that strict anaerobes do not carry genes for NRPs and PKs. However, recent genomic studies revealed the biosynthesis potential of *Clostridium* spp. for NRPs and PKs. Seedorf et al. studied the metabolic capabilities of *Clostridium kluyveri* using its whole-genome sequence and found the presence of four coding sequence (CDS) clusters predicted to encode NRPS and NRPS-PKS hybrids [91]. Letzel et al. mined 211 published genomes of obligate anaerobes in search of specifically putative NRPS and PKS gene clusters and found that 33% of tested genomes harboured PKS and NRPS genes [17]. They also found a correlation between the habitat of the isolate and the secondary metabolite synthesis potential.

Genome mining studies conducted so far with available whole genome data of *Clostridium* spp. have highlighted that non-pathogenic *Clostridium* spp. are more likely to contain secondary metabolite gene clusters than pathogenic species. Behnken and Hertweck explored the occurrence of PKS gene clusters in the genomes of 31 *Clostridium* spp. and detected a wide distribution of putative PKS gene clusters only in non-pathogenic strains [92]. Interestingly, all 26 pathogenic strains included in the study were shown not to possess PKS genes. Relatively small putative PKS gene clusters were found in *Clostridium acetobutylicum, Clostridium cellulovorans* and *Clostridium thermocellum*, whilst putative hybrid PKS-NRPS genes were present in *Clostridium beijerinckii* and *C. cellulolyticum* (Table 3). Furthermore, they developed degenerate primers based on the KS domains of the putative PKS of *Clostridium* spp. and screened 22 non-sequenced strains of non-pathogenic *Clostridium* spp. for the presence of modular type I PKS genes. Only three species (*Clostridium hungatei, Clostridium chartatabidum* and Clostridium akagii) were found to possess PKS genes (Table 3). Another study analysed the complete genomes of 223 bacteria, including 5 from the class Clostridia, for putative gene clusters encoding PKS and NRPS and reported that *C. acetobutylicum* possesses a putative gene cluster for PKS [60].

These available genomic data suggest the secondary metabolite biosynthesis capability of the genus *Clostridium*. These natural products can have different bioactivities, including antimicrobial activity.

3.3. Biosynthetic gene clusters in Clostridium spp. in comparison with other bacterial groups

Bacillus spp. and LAB are known to produce a wide range of antimicrobial compounds, including secondary metabolites and peptides [93–95]. Therefore, we assessed the occurrence of BGCs in *Clostridium* genomes and compared them with that of *Bacillus* spp. and LAB. The antiSMASH database provides BGC information for many publicly available microbial genomes. A complex query search in the antiSMASH database was performed to recover annotated RiPP, PKS/NRPS-PKS and NRPS/NRPS-PKS gene cluster counts on selected *Bacillus* spp., LAB and *Clostridium* spp.

The number of strains annotated in the database for each bacterial species or collectively in the three bacterial groups varied. Table 4 shows that more of *Bacillus* and LAB strains have been subjected to BGC annotation than *Clostridium* spp. This could be due to the availability of higher whole-genome sequences and/or greater interest in *Bacillus* and LAB for the discovery of bioactive molecules. Nevertheless, *Clostridium* spp. were shown to have comparable gene clusters with *Bacillus* spp. and LAB to produce RiPP, PKS, NRPS and NRPS-PKS compounds based on the total BGC and strain counts recovered from the antiSMASH database. These data suggest *Clostridium* spp. to be a viable source of different potential bioactive compounds.

Furthermore, we derived two antiSMASH-annotated core lanthipeptide biosynthetic genes from the *C. cellulovorans* 743B genome and used their amino acid sequences to identify genetic distances with closely related species using NCBI BLAST Tree View. As shown in Figs 3 and 4, type 2 lanthipeptide synthetase gene product sequences (queries) cluster very well with the *C. cellulovorans* sequence showing the presence of the exact gene. On the other hand, homologous sequences from other bacteria including *Bacillus* spp. have demonstrated their high genetic distance from clostridial type 2 lanthipeptide biosynthesis genes. Furthermore, no LAB strains were included in the top 30 sequence matches included in the tree. This demonstrates indirectly the unique ge-

Table 3
Clostridium spp. found to have putative polyketide synthase (PKS), non-ribosomal peptide synthetase (NRPS) and PKS-NRPS biosynthetic gene clusters (BGCs)

Clostridium spp.	Strain	Accession no.	BGC type(s)	Reference
Clostridium acetobutylicum	ATCC 824	**AE001437.1**	PKS	[60,92]
Clostridium kluyveri	DSM 555	**CP000673.1**	NRPS, PKS-NRPS	[91,92]
Clostridium beijerinckii	NCIMB 8052	**CP000721.1**	PKS-NRPS	[92]
Clostridium cellulolyticum	H10	**CP001348.1**	PKS-NRPS	[92]
Clostridium cellulovorans	743B	**CP002160.1**	PKS	[92]
Clostridium papyrosolvens	DSM 2782	**GCF_000175795.2**	PKS, PKS-NRPS	[92]
Clostridium thermocellum	ATCC 27405	**CP000568.1**	PKS	[92]
Clostridium hungatei	DSM 14427	**MZGX00000000.1**	PKS	[92]
Clostridium chartatabidum	DSM 5482	**HE586561**	PKS	[92]
Clostridium akagii	DSM 12554	**KK366007.1**	PKS	[92]

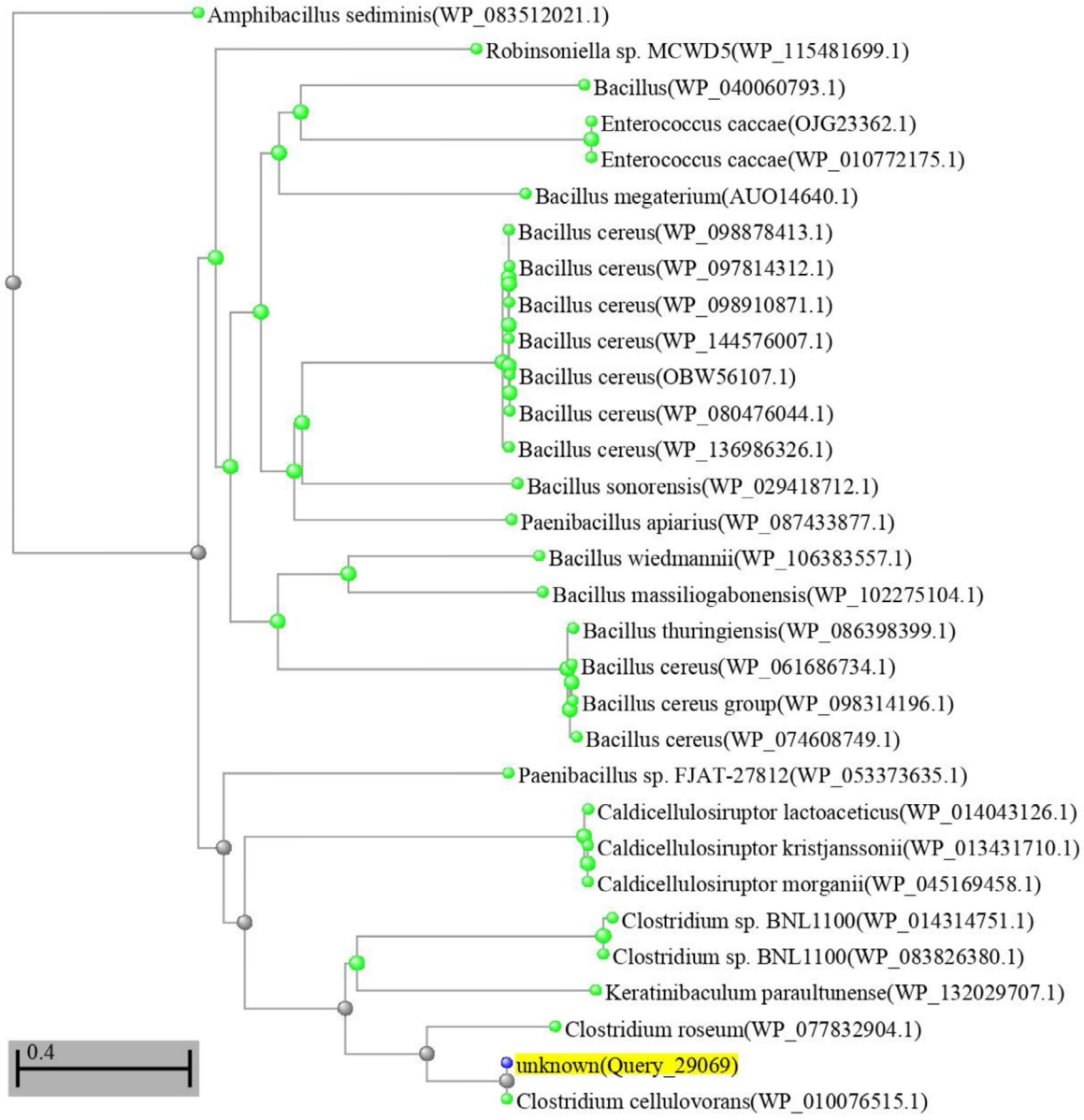

Fig. 3. Rectangular cladogram created from a search using the type 2 lanthipeptide synthetase *lanM* gene (CLOCEL_RS04400) product (Query_29069) against NCBI non-redundant protein database sequences. The tree was built by the Fast Minimum Evolution (FastME) method including 30 homologous sequences. The terminal nodes are labelled using taxonomic names and sequence IDs highlighting taxonomic trends. The horizontal bar represents 0.4 substitutions per amino acid site.

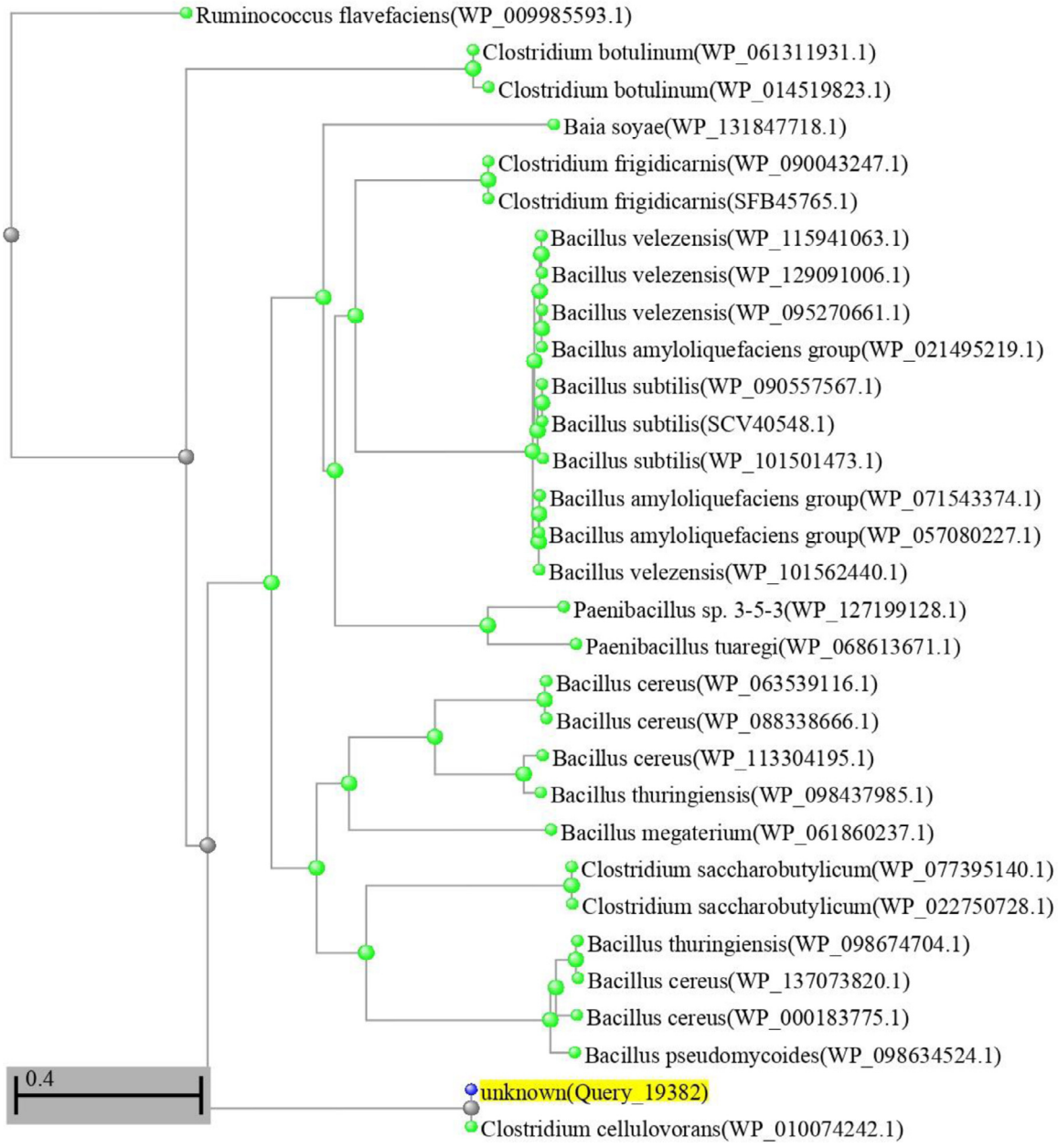

Fig. 4. Rectangular cladogram created from a search using the type 2 lanthipeptide synthetase *lanM* gene (CLOCEL_RS021105) product (Query_19382) against NCBI non-redundant protein database sequences. The tree was built by the Fast Minimum Evolution (FastME) method including 30 homologous sequences. The terminal nodes are labelled using taxonomic names and sequence IDs highlighting taxonomic trends. The horizontal bar represents 0.4 substitutions per amino acid site.

netic makeup of clostridial type 2 lanthipeptide biosynthetic genes, which might be involved in the synthesis of novel types of RiPPs.

4. Opportunities and challenges in identifying antimicrobial compounds through a combination of genomics and culture-based approaches

Over time, natural product discovery efforts, particularly antimicrobial compound discovery, have reduced substantially due to the growing re-discovery rates of identified compounds [96]. However, less explored microbial sources such as obligate anaerobes as well as newly developed genomics, bioinformatics and metabolomics approaches offer exciting opportunities for novel antimicrobial discovery.

As previously described, the conventional culture-based approach has come to a bottleneck in the antimicrobial discovery pipeline with its limited ability to induce various metabolite biosynthetic pathways. Genome mining has overcome this limitation by enabling the detection of cryptic metabolic pathways not activated under standard laboratory conditions. Therefore, sophisticated computational genetic tools and the availability of genomic data have transformed antimicrobial compound discovery. The 'chemical identification first' paradigm is transitioning to a combined effort of both 'genetic and chemical identification' (Fig. 5). Nevertheless, several challenges exist for the discovery of novel antimicrobials using genomics-guided approaches.

The translation of a BGC into an associated chemical compound, providing the real antimicrobial potential of *Clostridium* spp., is the main challenge. So far, most of the putative BGCs identified in the genomes of *Clostridium* spp. have no associated chemical products identified. Genome mining generates information on many potential BGCs, hence prioritisation of BGCs coding for antimicrobial

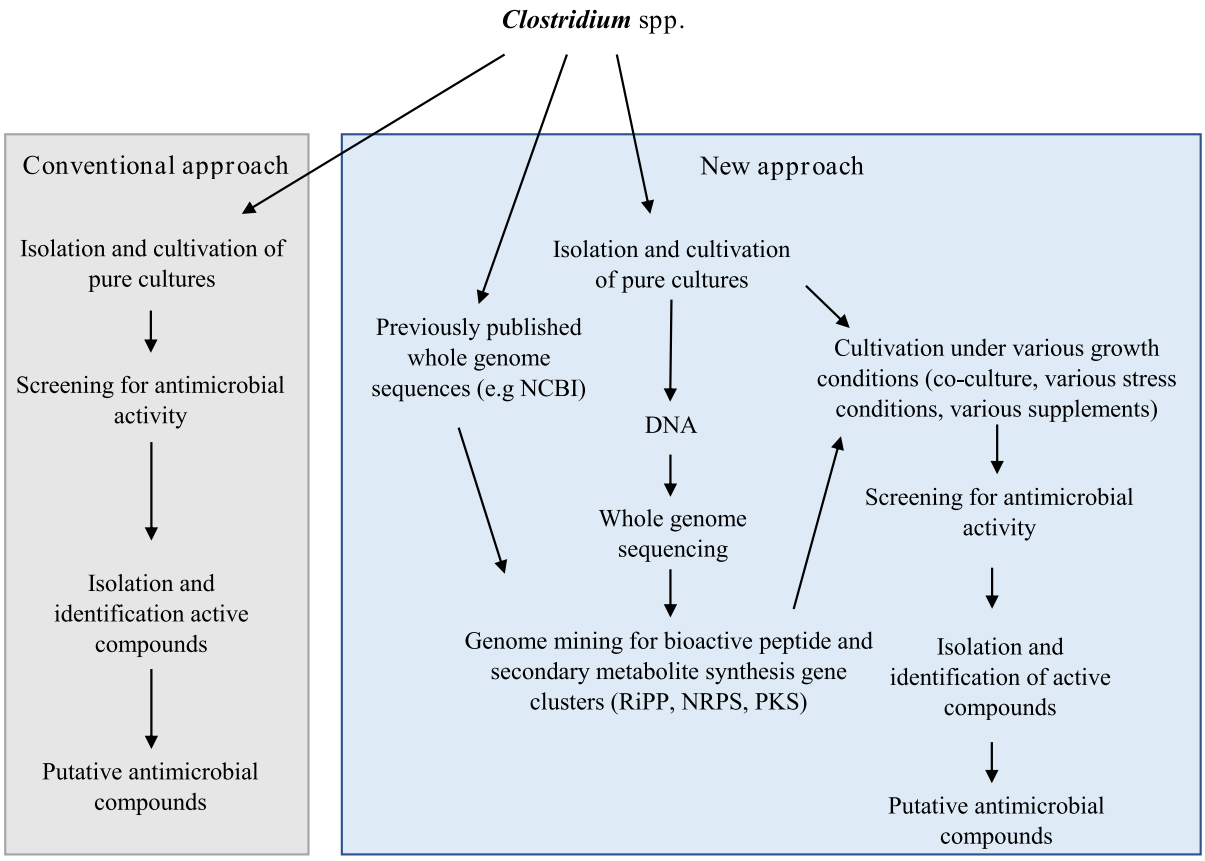

Fig. 5. Conventional and new approaches used in antimicrobial compound research. RiPP, ribosomally synthesised and post-translationally modified peptide; NRPS, non-ribosomal peptide synthetase; PKS, polyketide synthase.

Table 4
RiPP, PKS/NRPS-PKS and NRPS/NRPS-PKS gene cluster counts in the antiSMASH database.

Clostridium spp.	Gene cluster count (strain count)		
	RiPP	PKS/NRPS-PKS	NRPS/NRPS-PKS
Bacillus spp.			
Bacillus subtilis	114 (18)	142 (8)	327 (17)
Bacillus thuringiensis	253 (27)	14 (6)	206 (27)
Bacillus polymyxa	105 (10)	112 (10)	306 (10)
Bacillus brevis	10 (3)	17 (3)	25 (3)
Bacillus licheniformis	63 (5)	9 (2)	52 (2)
Total	545 (63)	294 (29)	916 (59)
Lactic acid bacteria			
Lactococcus lactis	104 (15)	5 (2)	7 (4)
Enterococcus faecium	194 (43)	0 (0)	4 (2)
Lactobacillus plantarum	84 (14)	0 (0)	5 (3)
Lactobacillus paracasei	54 (17)	0 (0)	0 (0)
Lactobacillus brevis	37 (5)	0 (0)	1 (1)
Total	473 (94)	5 (2)	17 (10)
Clostridium spp.			
Clostridium cellulovorans	11 (1)	2 (1)	6 (1)
Clostridium butyricum	18 (8)	2 (2)	3 (2)
Clostridium beijerinckii	30 (9)	13 (4)	23 (5)
Clostridium botulinum	153 (13)	1 (1)	32 (10)
Clostridioides difficile	64 (10)	0 (0)	58 (10)
Total	276 (41)	18 (8)	122 (28)

RiPP, ribosomally synthesised and post-translationally modified peptide; PKS, polyketide synthase; NRPS, non-ribosomal peptide synthetase.

compounds is critical in reducing experimental costs and time. Antibiotic Resistance Target Seeker (ARTS) is a platform that identifies and prioritises BGCs containing known antibiotic genes [97]. Several bioinformatic tools have been developed to predict the chemical structures of RiPPs, NRPs and PKs from putative gene cluster sequence data [75,98]. BGC sequence analysis tools such as Conserved Domain Database (CDD) give substantial understanding about the chemical structure of associated products, allowing dereplication by comparison with known metabolite databases [99]. This helps to emphasise BGCs that have the potential to produce novel bioactive compounds. However, it remains difficult to predict the exact product from genome sequence data. For instance, when a similar gene cluster exists between species, it is difficult to predict RiPP homology since variances in the sequence of the precursor peptide between similar RiPPs may have an influence on the final modified product [57].

Activation of cryptic BGCs and identification of the associated products is very challenging. As previously described, the metabolic activities of obligate anaerobes appear to be highly evolved around their challenging oxygen-free environment. Therefore, in-cooperation ecological niche interactions in the laboratory growth medium could be an effective approach to stimulate silent bioactive compound synthesis pathways in anaerobic bacteria. However, reconstructing their natural ecosystem in the laboratory is relatively challenging because of their oxygen-free conditions and complex natural interactions [100]. Various BGC-specific molecular approaches such as heterologous expression, epigenetic remodelling and activation of pathway regulatory genes together with optimised growth conditions have been considered to promote gene cluster expression in micro-organisms [101].

5. Conclusions and prospects

Antimicrobial resistance has become a global threat in human and veterinary medicine, agriculture and the food sector and, in

parallel, currently available antimicrobials often have reduced effectiveness, necessitating more strict uses of antimicrobials to reduce the selection pressure for resistance. Although restricted and appropriate use of antimicrobials has shown some improvements in antimicrobial resistance trends, as a whole it still contributes to the emergence of new resistance [102,103]. Consequently, the search for novel antimicrobials is an important strategy to combat antimicrobial resistance, particularly against multidrug-resistant bacteria.

Clostridium spp. have been the subject of little scientific interest in terms of investigating their potential for antimicrobial compound synthesis. Genomics knowledge in combination with modified cultural methods may help to explore the potential of *Clostridium* spp. to produce antimicrobial compounds.

The limited research performed thus far has provided some indication of the antimicrobial peptide and secondary metabolite production of *Clostridium* spp. Moreover, a few studies have already shown their capability to produce novel classes of antimicrobial compounds effective against multidrug-resistant bacteria.

Funding

This work was supported by the Strategic Science Investment Fund (SSIF), AgResearch Ltd. (Palmerston North, New Zealand).

Competing interests None declared.

Ethical approval Not required.

References

[1] Sabuncu E, David J, Bernède-Bauduin C, Pépin S, Leroy M, Boëlle P, et al. Significant reduction of antibiotic use in the community after a nationwide campaign in France, 2002–2007. PLoS Med 2009;6:e1000084e.
[2] Meek RW, Vyas H, Piddock LJV. Nonmedical uses of antibiotics: time to restrict their use? PLoS Biol 2015;13:e1002266.
[3] US Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats in the United States, 2013. Atlanta, GA: CDC; 2013.
[4] O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. London, UK: Review on Antimicrobial Resistance; 2014.
[5] Garde S, Ávila M, Medina M, Nuñez M. Fast induction of nisin resistance in *Streptococcus thermophilus* INIA 463 during growth in milk. Int J Food Microbiol 2004;96:165–72.
[6] Zhou H, Fang J, Tian Y, Lu XY. Mechanisms of nisin resistance in Gram-positive bacteria. Ann Microbiol 2014;64:413–20.
[7] Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 2004;10(12 Suppl):S122–9.
[8] Jackson N, Czaplewski L, Piddock LJV. Discovery and development of new antibacterial drugs: learning from experience? J Antimicrob Chemother 2018;73:1452–9.
[9] Gyawali R, Ibrahim SA. Natural products as antimicrobial agents. Food Control 2014;46:412–29.
[10] Pisoschi A, Pop A, Georgescu C, Turcuş V, Olah NK, Mathe E. An overview of natural antimicrobials role in food. Eur J Med Chem 2018;143:922–35.
[11] Lima R, Del Fiol FS, Balcão VM. Prospects for the use of new technologies to combat multidrug-resistant bacteria. Front Pharmacol 2019;10:692.
[12] Quinto EJ, Caro I, Villalobos-Delgado LH, Mateo J, De-Mateo-Silleras B, Redondo-Del-Río MP. Food safety through natural antimicrobials. Antibiotics (Basel) 2019;8 pii: E208.
[13] Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol 2001;176:386–90.
[14] Reis JA, Paula AT, Casarotti SN, Penna ALB. Lactic acid bacteria antimicrobial compounds: characteristics and applications. Food Engineering Reviews 2012;4:124–40.
[15] Harzallah D, Belhadj H. Lactic acid bacteria as probiotics: characteristics, selection criteria and role in immunomodulation of human GI muccosal barrier. In: Kongo JM, editor. Lactic acid bacteria. R&D for food, health and livestock purposes. IntechOpen; 2013. p. 197–216.
[16] Gueimonde M, Frias R, Ouwehand AC. Assuring the continued safety of lactic acid bacteria used as probiotics. Biologia 2006;61:755–60.
[17] Letzel A, Pidot SJ, Hertweck C. A genomic approach to the cryptic secondary metabolome of the anaerobic world. Nat Prod Rep 2013;30:392–428.
[18] Verma VC, Gond SK, Kumar A, Mishra A, Kharwar RN, Gange AC. Endophytic actinomycetes from *Azadirachta indica* A. Juss.: isolation, diversity, and anti-microbial activity. Microb Ecol 2009;57:749–56.
[19] Lincke T, Behnken S, Ishida K, Roth M, Hertweck C. Closthioamide: an unprecedented polythioamide antibiotic from the strictly anaerobic bacterium *Clostridium cellulolyticum*. Angew Chem Int Ed Engl 2010;49:2011–13.
[20] Gebhardt K, Schimana J, Krastel P, Dettner K, Rheinheimer J, Zeeck A, et al. Endophenazines A–D, new phenazine antibiotics from the arthropod associated endosymbiont *Streptomyces anulatus*. I. Taxonomy, fermentation, isolation and biological activities. J Antibiot (Tokyo) 2002;55:794–800.
[21] Zipperer A, Konnerth MC, Laux C, Berscheid A, Janek D, Weidenmaier C, et al. Human commensals producing a novel antibiotic impair pathogen colonization. Nature 2016;535:511–16 Erratum in: Nature 2016;539:314.
[22] Challinor VL, Bode HB. Bioactive natural products from novel microbial sources. Ann N Y Acad Sci 2015;1354:82–97.
[23] Parte AC. LPSN—list of prokaryotic names with standing in nomenclature. Nucleic Acids Res 2013;42:D613–16.
[24] Wells CL, Wilkins TD. Clostridia: sporeforming anaerobic bacilli. Medical microbiology. Baron S, editor. 4th ed.. Galveston, TX: University of Texas Medical Branch at Galveston; 1996.
[25] André S, Vallaeys T, Planchon S. Spore-forming bacteria responsible for food spoilage. Res Microbiol 2017;168:379–87.
[26] Downes FP, Ito K. Compendium of methods for the microbiological examination of foods. 4th ed. Washington, DC: American Public Health Association; 2001.
[27] Nakanishi S, Tanaka M. Sequence analysis of a bacteriocinogenic plasmid of *Clostridium butyricum* and expression of the bacteriocin gene in *Escherichia coli*. Anaerobe 2010;16:253–7.
[28] Takahashi M, Taguchi H, Yamaguchi H, Osaki T, Komatsu A, Kamiya S. The effect of probiotic treatment with *Clostridium butyricum* on enterohemorrhagic *Escherichia coli* O157:H7 infection in mice. FEMS Immunol Med Microbiol 2004;41:219–26.
[29] Woo TDH, Oka K, Takahashi M, Hojo F, Osaki T, Hanawa T, et al. Inhibition of the cytotoxic effect of *Clostridium difficile* in vitro by *Clostridium butyricum* MIYAIRI 588 strain. J Med Microbiol 2011;60:1617–25.
[30] Dürre P. Physiology and sporulation in *Clostridium*. The bacterial spore: from molecules to systems. Driks A, Eichenberger P, editors. Washington, DC: ASM Press; 2016.
[31] Morris JG. Obligately anaerobic bacteria in biotechnology. Appl Biochem Biotechnol 1994;48:75–106.
[32] Ternan NG, Moore ND, Smyth D, McDougall GJ, Allwood JW, Verrall S, et al. Increased sporulation underpins adaptation of *Clostridium difficile* strain 630 to a biologically-relevant faecal environment, with implications for pathogenicity. Sci Rep 2018;8:16691.
[33] Ferreyra JA, Wu KJ, Hryckowian AJ, Bouley DM, Weimer BC, Sonnenburg JL. Gut microbiota-produced succinate promotes *C. difficile* infection after antibiotic treatment or motility disturbance. Cell Host Microbe 2014;16:770–7.
[34] Passmore IJ, Letertre MPM, Preston MD, Bianconi I, Harrison MA, Nasher F, et al. Para-cresol production by *Clostridium difficile* affects microbial diversity and membrane integrity of Gram-negative bacteria. PLoS Pathog 2018;14:e1007191.
[35] Caires NCM, Matos AM, Farias LM, Carvalho MAR, Drummond RMN, Nicoli JR, et al. Partial characterization of antagonistic substance produced by a *Clostridium butyricum* strain. Braz J Microbiol 2007;38:265–9.
[36] Sumi C, Yang B, Yeo I, Hahm Y. Antimicrobial peptides of the genus *Bacillus*: a new era for antibiotics. Can J Microbiol 2014;61:93–103.
[37] Storm DR, Rosenthal KS, Swanson PE. Polymyxin and related peptide antibiotics. Annu Rev Biochem 1977;46:723–63.
[38] Craig LC, Weisiger JR, Hausmann W, Harfenist EJ. The separation and characterization of bacitracin polypeptides. J Biol Chem 1952;199:259–66.
[39] Kawulka KE, Sprules T, Diaper CM, Whittal RM, McKay RT, Mercier P, et al. Structure of subtilosin A, a cyclic antimicrobial peptide from *Bacillus subtilis* with unusual sulfur to α-carbon cross-links: formation and reduction of α-thio-α-amino acid derivatives. Biochemistry 2004;43:3385–95.
[40] Hongo M, Murata A, Ogata S, Kono K, Kato F. Characterization of a temperate phage and four bacteriocins produced by nonpathogenic *Clostridium* species. Agricultural and Biological Chemistry 1968;32:773–80.
[41] Clarke DJ, Robson RM, Morris JG. Purification of two *Clostridium* bacteriocins by procedures appropriate to hydrophobic proteins. Antimicrob Agents Chemother 1974;7:256–64.
[42] Wolff A, Ionesco H. Purification and characterization of '*Clostridium perfringens*' BP6K-N5 strain bacteriocin N5 [in French]. Ann Microbiol (Paris) 1975;126:343–56.
[43] Ionesco H, Wolff A. The mode of action of bacteriocin N5 purified from *Clostridium perfringens* [in French]. C R Acad Hebd Seances Acad Sci D 1975;281:2033–6.
[44] Li AW, Verpoorte JA, Lewis RG, Mahony DE. Characterization of bacteriocin 28 produced by *Clostridium perfringens*. Can J Microbiol 1982;28:860–73.
[45] Garnier T, Cole ST. Characterization of a bacteriocinogenic plasmid from *Clostridium perfringens* and molecular genetic analysis of the bacteriocin-encoding gene. J Bacteriol 1986;168:1189–96.
[46] Lau AHS, Hawirko RZ, Chow CT. Purification and properties of boticin P produced by *Clostridium botulinum*. Can J Microbiol 1974;20:385–90.
[47] Dineen SS, Bradshaw M, Johnson EA. Cloning, nucleotide sequence, and expression of the gene encoding the bacteriocin boticin B from *Clostridium botulinum* strain 213B. Appl Environ Microbiol 2000;66:5480–3.
[48] Kemperman R, Kuipers A, Karsens H, Nauta A, Kuipers O, Kok J. Identification and characterization of two novel clostridial bacteriocins, circularin A and closticin 574. Appl Environ Microbiol 2003;69:1589–97.
[49] Schieferdecker S, Shabuer G, Knuepfer U, Hertweck C. Clostrindolin is an antimycobacterial pyrone alkaloid from *Clostridium beijerinckii*. Org Biomol Chem 2019;17:6119–21.

[50] Gebhart D, Williams SR, Bishop-Lilly KA, Govoni GR, Willner KM, Butani A, et al. Novel high-molecular-weight, R-type bacteriocins of *Clostridium difficile*. J Bacteriol 2012;194:6240–7.

[51] Timbermont L, De Smet L, Van Nieuwerburgh F, Parreira VR, Van Driessche G, Haesebrouck F, et al. Perfrin, a novel bacteriocin associated with *netB* positive *Clostridium perfringens* strains from broilers with necrotic enteritis. Vet Res 2014;45:40.

[52] Pidot S, Ishida K, Cyrulies M, Hertweck C. Discovery of clostrubin, an exceptional polyphenolic polyketide antibiotic from a strictly anaerobic bacterium. Angew Chem Int Ed Engl 2014;53:7856–9.

[53] Chiriac AI, Kloss F, Krämer J, Vuong C, Hertweck C, Sahl HG. Mode of action of closthioamide: the first member of the polythioamide class of bacterial DNA gyrase inhibitors. J Antimicrob Chemother 2015;70:2576–88.

[54] ChemSpider. *Clostrubin*. http://www.chemspider.com/Chemical-Structure.34485440.html [accessed 11 March 2020].

[55] Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, et al. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 2011;39:W339–46.

[56] Machado H, Sonnenschein EC, Melchiorsen J, Gram L. Genome mining reveals unlocked bioactive potential of marine Gram-negative bacteria. BMC Genomics 2015;16:158.

[57] Letzel A, Pidot SJ, Hertweck C. Genome mining for ribosomally synthesized and post-translationally modified peptides (RiPPs) in anaerobic bacteria. BMC Genomics 2014;15:983.

[58] Ikeda H, Ishikawa J, Hanamoto A, Shinose M, Kikuchi H, Shiba T, et al. Complete genome sequence and comparative analysis of the industrial microorganism *Streptomyces avermitilis*. Nat Biotechnol 2003;21:526–31.

[59] Müller CA, Oberauner-Wappis L, Peyman A, Amos GCA, Wellington EMH, Berg G. Mining for nonribosomal peptide synthetase and polyketide synthase genes revealed a high level of diversity in the *Sphagnum* bog metagenome. Appl Environ Microbiol 2015;81:5064–72.

[60] Donadio S, Monciardini P, Sosio M. Polyketide synthases and nonribosomal peptide synthetases: the emerging view from bacterial genomics. Nat Prod Rep 2007;24:1073–109.

[61] Nett M, Ikeda H, Moore BS. Genomic basis for natural product biosynthetic diversity in the actinomycetes. Nat Prod Rep 2009;26:1362–84.

[62] Tracanna V, Medema MH, de Jong A, Kuipers OP. Mining prokaryotes for antimicrobial compounds: from diversity to function. FEMS Microbiol Rev 2017;41:417–29.

[63] Medema MH, Fischbach MA. Computational approaches to natural product discovery. Nat Chem Biol 2015;11:639–48.

[64] Khaldi N, Seifuddin FT, Turner G, Haft D, Nierman WC, Wolfe KH, et al. SMURF: genomic mapping of fungal secondary metabolite clusters. Fungal Genet Biol 2010;47:736–41.

[65] Li MHT, Ung PMU, Zajkowski J, Garneau-Tsodikova S, Sherman DH. Automated genome mining for natural products. BMC Bioinformatics 2009;10:185.

[66] Starcevic A, Zucko J, Simunkovic J, Long PF, Cullum J, Hranueli D. ClustScan: an integrated program package for the semi-automatic annotation of modular biosynthetic gene clusters and in silico prediction of novel chemical structures. Nucleic Acids Res 2008;36:6882–92.

[67] Weber T, Rausch C, Lopez P, Hoof I, Gaykova V, Huson DH, et al. CLUSEAN: a computer-based framework for the automated analysis of bacterial secondary metabolite biosynthetic gene clusters. J Biotechnol 2009;140:13–17.

[68] Cimermancic P, Medema MH, Claesen J, Kurita K, Wieland Brown LC, Mavrommatis K, et al. Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. Cell 2014;158:412–21.

[69] Cruz-Morales P, Martínez-Guerrero CE, Morales-Escalante MA, Yáñez-Guerra L, Kopp JF, Feldmann J, et al. Recapitulation of the evolution of biosynthetic gene clusters reveals hidden chemical diversity on bacterial genomes. bioRxiv 2015 Jun 8. doi:10.1101/020503.

[70] Ortega MA, van der Donk WA. New insights into the biosynthetic logic of ribosomally synthesized and post-translationally modified peptide natural products. Cell Chem Biol 2016;23:31–44.

[71] Essig A, Hofmann D, Münch D, Gayathri S, Künzler M, Kallio PT, et al. Copsin, a novel peptide-based fungal antibiotic interfering with the peptidoglycan synthesis. J Biol Chem 2014;289:34953–64.

[72] Férir G, Petrova MI, Andrei G, Huskens D, Hoorelbeke B, Snoeck R, et al. The lantibiotic peptide labyrinthopeptin A1 demonstrates broad anti-HIV and anti-HSV activity with potential for microbicidal applications. PLoS One 2013;8:e64010.

[73] Mohr KI, Volz C, Jansen R, Wray V, Hoffmann J, Bernecker S, et al. Pinensins: the first antifungal lantibiotics. Angew Chem Int Ed Engl 2015;54:11254–8.

[74] Huo L, van der Donk WA. Discovery and characterization of bicereucin, an unusual D-amino acid-containing mixed two-component lantibiotic. J Am Chem Soc 2016;138:5254–7.

[75] Skinnider MA, Johnston CW, Edgar RE, Dejong CA, Merwin NJ, Rees PN, et al. Genomic charting of ribosomally synthesized natural product chemical space facilitates targeted mining. Proc Natl Acad Sci U S A 2016;113:E6343–51.

[76] Donia MS, Cimermancic P, Schulze CJ, Wieland Brown LC, Martin J, Mitreva M, et al. A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. Cell 2014;158:1402–14.

[77] Hetrick KJ, van der Donk WA. Ribosomally synthesized and post-translationally modified peptide natural product discovery in the genomic era. Curr Opin Chem Biol 2017;38:36–44.

[78] Tushar L, Sasi Jyothsna TS, Sasikala C, Ramana CV. Draft genome sequence of antimicrobial-producing *Clostridium* sp. JC272, isolated from marine sediment. Genome Announc 2015;3:e00650 -15.

[79] Lipmann F. Nonribosomal polypeptide synthesis on polyenzyme templates. Acc Chem Res 1973;6:361–7.

[80] Felnagle EA, Jackson EE, Chan YA, Podevels AM, Berti AD, McMahon MD, et al. Nonribosomal peptide synthetases involved in the production of medically relevant natural products. Mol Pharm 2008;5:191–211.

[81] Laland SG, Zimmer TL. The protein thiotemplate mechanism of synthesis for the peptide antibiotics produced by *Bacillus brevis*. Essays Biochem 1973;9:31–57.

[82] Horwood PF, Burgess GW, Oakey HJ. Evidence for non-ribosomal peptide synthetase production of cereulide (the emetic toxin) in *Bacillus cereus*. FEMS Microbiol Lett 2004;236:319–24.

[83] Di Lorenzo M, Poppelaars S, Stork M, Nagasawa M, Tolmasky ME, Crosa JH. A nonribosomal peptide synthetase with a novel domain organization is essential for siderophore biosynthesis in *Vibrio anguillarum*. J Bacteriol 2004;186:7327–36.

[84] Baerson SR, Rimando AM. Polyketides. A plethora of polyketides: structures, biological activities, and enzymes, 955. Washington, DC: American Chemical Society; 2007. ACS Symposium Series p. 2–14.

[85] Pfeifer BA, Khosla C. Biosynthesis of polyketides in heterologous hosts. Microbiol Mol Biol Rev 2001;65:106–18.

[86] Weissman KJ. Chapter 1. Introduction to polyketide biosynthesis. Methods in enzymology. Academic Press; 2009. p. 3–16.

[87] Park SR, Yoo YJ, Ban Y, Yoon Y. Biosynthesis of rapamycin and its regulation: past achievements and recent progress. J Antibiot (Tokyo) 2010;63:434–41.

[88] Campbell CD, Vederas JC. Biosynthesis of lovastatin and related metabolites formed by fungal iterative PKS enzymes. Biopolymers 2010;93:755–63.

[89] Grimm A, Madduri K, Ali A, Hutchinson CR. Characterization of the *Streptomyces peucetius* ATCC 29050 genes encoding doxorubicin polyketide synthase. Gene 1994;151:1–10.

[90] Cane DE. Programming of erythromycin biosynthesis by a modular polyketide synthase. J Biol Chem 2010;285:27517–23.

[91] Seedorf H, Fricke WF, Veith B, Brüggemann H, Liesegang H, Strittmatter A, et al. The genome of *Clostridium kluyveri*, a strict anaerobe with unique metabolic features. Proc Natl Acad Sci U S A 2008;105:2128–33.

[92] Behnken S, Hertweck C. Cryptic polyketide synthase genes in non-pathogenic *Clostridium* spp. PLoS One 2012;7:e29609.

[93] Venegas-Ortega MG, Flores-Gallegos AC, Martínez-Hernández JL, Aguilar CN, Nevárez-Moorillón GV. Production of bioactive peptides from lactic acid bacteria: a sustainable approach for healthier foods. Compr Rev Food Sci Food Saf 2019;18:1039–51.

[94] Polyudova T, Eroshenko D, Korobov V. The effect of sucrose-induced osmotic stress on the sensitivity of *Escherichia coli* to bacteriocins. Can J Microbiol 2019;65:895–903.

[95] Abriouel H, Franz CMAP, Omar NB, Gálvez A. Diversity and applications of *Bacillus* bacteriocins. FEMS Microbiol Rev 2011;35:201–32.

[96] Li JW-H, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science 2009;325:161–5.

[97] Alanjary M, Kronmiller B, Adamek M, Blin K, Weber T, Huson D, et al. The Antibiotic Resistant Target Seeker (ARTS), an exploration engine for antibiotic cluster prioritization and novel drug target discovery. Nucleic Acids Res 2017;45:W42–8.

[98] Skinnider MA, Dejong CA, Rees PN, Johnston CW, Li H, Webster ALH, et al. Genomes to natural products PRediction Informatics for Secondary Metabolomes (PRISM). Nucleic Acids Res 2015;43:9645–62.

[99] Marchler-Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, et al. CDD: a Conserved Domain Database for the functional annotation of proteins. Nucleic Acids Res 2010;39:D225–9.

[100] Mori K, Kamagata Y. The challenges of studying the anaerobic microbial world. Microbes Environ 2014;29:335–7.

[101] Scherlach K, Hertweck C. Triggering cryptic natural product biosynthesis in microorganisms. Org Biomol Chem 2009;7:1753–60.

[102] Livermore DM. The need for new antibiotics. Clin Microbiol Infect 2004;10(Suppl 4):1–9.

[103] Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob Agents Chemother 2001;45:2054–9.