



Review

# Cyanobacteria and Eukaryotic Microalgae as Emerging Sources of Antibacterial Peptides

Verónica Rojas <sup>1,\*</sup>, Luis Rivas <sup>2</sup>, Constanza Cárdenas <sup>3</sup>  and Fanny Guzmán <sup>3</sup> <sup>1</sup> Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso 2373223, Chile<sup>2</sup> Centro de Investigaciones Biológicas Margarita Salas (C.S.I.C), c/Ramiro de Maeztu 9, 28040 Madrid, Spain; luis.rivas@cib.csic.es<sup>3</sup> Nucleo Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Valparaíso 2373223, Chile; constanza.cardenas@pucv.cl (C.C.); fanny.guzman@pucv.cl (F.G.)

\* Correspondence: veronica.rojas@pucv.cl; Tel.: +56-322274848

Academic Editors: Ana R. Díaz-Marrero and José Javier Fernández Castro

Received: 30 October 2020; Accepted: 7 December 2020; Published: 9 December 2020



**Abstract:** Cyanobacteria and microalgae are oxygen-producing photosynthetic unicellular organisms encompassing a great diversity of species, which are able to grow under all types of extreme environments and exposed to a wide variety of predators and microbial pathogens. The antibacterial compounds described for these organisms include alkaloids, fatty acids, indoles, macrolides, peptides, phenols, pigments and terpenes, among others. This review presents an overview of antibacterial peptides isolated from cyanobacteria and microalgae, as well as their synergism and mechanisms of action described so far. Antibacterial cyanopeptides belong to different orders, but mainly from Oscillatoriales and Nostocales. Cyanopeptides have different structures but are mainly cyclic peptides. This vast peptide repertoire includes ribosomal and abundant non-ribosomal peptides, evaluated by standard conventional methodologies against pathogenic Gram-negative and Gram-positive bacteria. The antibacterial activity described for microalgal peptides is considerably scarcer, and limited to protein hydrolysates from two *Chlorella* species, and few peptides from *Tetraselmis suecica*. Despite the promising applications of antibacterial peptides and the importance of searching for new natural sources of antibiotics, limitations still persist for their pharmaceutical applications.

**Keywords:** cyanobacteria; microalgae; peptide; antimicrobial; antibacterial activity; bioactive compounds

## 1. Introduction

The advent of the antimicrobial era are, together with sanitation and increasing access to safe drinking water, among the greatest milestones in public health [1]. Antibiotic therapy affords the control of many infectious diseases, otherwise highly lethal. In addition, it pushes forward the boundaries of many other medical treatments, such as immunosuppressive treatments or successful surgical procedures.

The notion of total elimination of infectious diseases by antibiotic therapies soon turned out to be utopic. Today, the world is facing a deep global antimicrobial resistance (AMR) crisis, with an alarming decrease of effectiveness in antibiotic treatments due to the rising resistance acquired by pathogens [2,3]. The overuse, and often misuse, of antimicrobials in clinics [3], is one of the main reasons of AMR, but not the only one. The induction of antibiotic resistance outside nosocomial settings, strongly associated to the antibiotic use in livestock farming [4], aquaculture [5], and the uncontrolled dumping of antibiotics into the environment [6,7], account for the horizontal transmission of antibiotic-resistance traits out of the nosocomial setting. Furthermore, zoonosis may act as a reservoir for resistant organisms. Altogether, the term “One Health” was coined as a common umbrella to

encompass all the resistomes, regardless of their biological source, as responsible for induction of resistance [8,9].

This serious situation is worsened by the deficient pipeline for the development of new antibiotic leads, due to the poor return of investments obtained [10,11]. The magnitude of AMR was recognized by the United Nations General Assembly in 2016, that fostered promising initiatives, as the AMR action fund, a multipartner consortium led by the World Health Organization (WHO) expected to put on the market 2–4 new antibiotics by 2020 (<https://www.amractionfund.com/>). Yet, the end of this crisis will not be achieved in a short time range [12], so immediate solutions must resort to drug repurposing [13,14], or combination therapies, with a simultaneous multitarget attack to the pathogen [15,16]. Thus, the development of new approaches for anti-infectious diseases, such as bacteriophages, enzybiotics, the focus on virulence factors as targets, or the potentiality of CRiSPR-Cas13, is mandatory and urgent [17–21], not only because of the current and alarming situation, but also because of the feasibility to fight emerging ongoing threats, as the COVID-19 pandemics, concerning bacterial co-infections [22].

Among these forefront candidates on trial, are the antimicrobial peptides (AMPs) (for recent reviews, see [23–29]), which are ancient chemical weapons in the biological warfare. In unicellular organisms, AMPs help the producer cells to strive against competitors sharing the same ecological niche. In pluricellular organisms, they play a defensive role against invading pathogens. The success of AMPs is endorsed by their ubiquitous presence throughout evolution, crossing taxonomical kingdoms [25], even in those organism endowed with a robust and sophisticated antigen-specific immunity. In pluricellular organisms, AMPs may play additional roles out of their primeval function as deterrent for infection, such as messengers for communication among immune cells, angiogenesis, wound healing, autoimmunity [30,31], their dual role in inflammation [32,33], or even in sleep, among others [34–38].

Until few years ago, the pharmaceutical industry was scarcely receptive to peptide-mediated therapies, mostly due to the high cost for production and their poor ADME (absorption, distribution, metabolism, and excretion) profile, despite their huge potential to cover an extremely broad chemical space, and their structural and functional tuning. This concern was driven by the peptide liability to degradation by proteinases and peptidases present in biological fluids, their sequestration by the cellular matrix and serum components, problematic transport across the membranes, as well as the difficulty of the exogenously administered AMPs to reach an effective concentration at deep tissue or organ locations. Most of these shortcomings were addressed and properly solved in recent years, leading to an increasing number of peptide drugs approved by the Food and Drug Administration (FDA) [39–42].

This turn of the tide underlies new strategies to overcome the limitations described above, converting peptides into valuable drug candidates: firstly, the decrease in cost by implementation of more efficient and cheaper strategies of synthesis [43–47] or, alternatively, the development of improved production of recombinant peptides [48–51]; secondly, the improvement of peptide bioavailability by engineering strategies aimed to prevent proteolytic degradation, either by manipulation of their primary sequence by incorporation of unnatural amino acids [52,53],  $\beta$  and  $\gamma$  amino acid peptides [54,55], enantiomeric peptides [56,57] and peptidomimetics [58–61], or by acquisition of a more stable conformation that secludes or shields the recognition of the cleavage sequence by peptidases (cyclation [46,62,63] and stapled peptides [64]).

In addition, the implementation of nanotechnological vehiculation of the peptides improve their bioavailability by targeting the peptide at the right anatomical or cellular location, preventing peptide waste and off-target effects, as well as avoiding the proteolytic degradation of the peptide. In addition, vehicle degradation may sustain or control a gradual delivery of the peptide at the right site [65–68]. A greater decrease in the number of peptides entering the pipeline for peptide development is achieved by *in silico* selection of new and improved prediction tools for candidate selection based on an expected higher effectiveness or decreased toxicity [69–73].

Yet, despite the increase of eukaryotic AMPs that entered into the pipeline and reached different phases in clinical trials [26,27,74], none of them are currently implemented as an over-the-counter drug in the market. In fact, all the AMPs in clinical use are from bacterial origin [75–77]: colistin, gramicidin, bacitracin, tyrocidine, the two glycopeptides vancomycin and teicoplanin, the lipopeptide daptomycin A), and the lantibiotic nisin extensively applied as a food preservative.

The cost of an AMPs-based anti-infectious therapy is still significantly higher than for classical antibiotics. However, this drawback is blurred in the case of multiresistant bacteria, being AMPs the last resort drug. Although resistance against eukaryotic AMPs can be induced, their frequency is much lower than for classical antibiotics, due to the high loss of fitness associated [78–80]. Nevertheless, a serious clinical concern is the resistance against polymyxin, a lipopeptide used as a last clinical alternative for Gram-negative infections with increase not only in its frequency, but also in its spreading into other bacteria [81,82]. On the other side of the balance, the awareness of the importance of host immune reprogramming by AMPs is a more permanent asset of its overall antimicrobial activity, and presumably, less prone to manipulation by bacterial resistance [27,34].

The search for new natural sources of AMPs has also increased; in this context, microalgae and cyanobacteria have enormous potential as a source of molecules with antimicrobial applications with a high probability of finding new potentially more effective molecules. As a background, these organisms are a source of various chemical substances already characterized, such as peptides, proteins, lipids, vitamins, pigments, carbohydrates, terpenoids, polyunsaturated fatty acids, flavonoids, phenolic compounds, and other organic substances with potential uses as biopharmaceuticals [83].

## 2. Cyanobacteria and Microalgae as Producers of Antibacterial Compounds

These microorganisms are known to be able to survive under all kinds of environmental conditions, terrestrial, saline water and freshwater, and even under extremely competitive environments; moreover, they are exposed to a wide variety of predators and to microbial pathogens, such as bacteria, viruses, and fungi. Their flexible metabolism underlies both their adaptation to a diversity of growth conditions and habitats and their capacity to respond to different environmental stresses and nutrients sources. This versatility can explain the diversity and the number of chemical compounds that have been isolated from them [84,85].

The phylum Cyanobacteria is constituted by photosynthetic bacteria encompassing 1528 species and 1984 taxa grouped under 389 genera [86]. The cyanobacteria are the major oxygen producers and nitrogen fixers, playing an essential role in oceanic phytoplankton, but also, they colonize a wide variety of habitats. They appear as single cells, pluricellular forms, or as symbiotic partners of other animal and plants [87].

There are numerous review articles about marine, freshwater, and terrestrial cyanobacteria, belonging to different families, as a source of antibacterial molecules. This antibacterial activity has been attributed to compounds that belong to quite diverse chemical classes. Those types that present the highest number of antibacterial molecules correspond to alkaloids, fatty acids, pigments, phenolic compounds, and terpenoids; however, the wide range of compounds also includes molecules of another type, such as aromatic compounds, cyclophanes, indole, macrolides, peptides, paracyclophanes, and polyphenyl ethers, among others. Table 1 summarizes the chemical diversity of cyanobacterial molecules with antibacterial activity, and their respective producer cyanobacteria species. Most of the antibacterial assays have been performed *in vitro* by standard conventional methodologies as minimal inhibitory concentration (MIC) and/or zone of inhibition, against Gram-positive and Gram-negative bacteria pathogenic to humans or to other organisms [83,88–95].

Microalgae are photosynthetic eukaryotic microorganisms and the main producers of oxygen, that constitute the basic components of the ecosystem's trophic chains, accounting for approximately 40% of photosynthesis on the planet; moreover, they can efficiently assimilate nutrients in a eutrophic water body. Microalgae are not only interesting for their bioproducts, but also for their application

in bioremediation of waste waters containing inorganic elements and high metal loads, in biological sequestration of CO<sub>2</sub>, and in the production of renewable energy as biodiesel [96].

Microalgae include a great diversity and complexity of strains, as the result of adaptation carried out through billions of years. These microorganisms colonize every known habitat, but are predominantly found in fresh and marine water. The number of microalgal species is not clearly established, AlgaeBase [97] encompasses 159,173 species that include terrestrial, marine and freshwater organisms, but also marine macroalgae (seaweeds) [96,98–101]. The major Phyla/class accounting for commercial microalgae are Chlorophyta, Rhodophyta, Haptophyta, Stramenopiles, and Dinophyta [102].

Metabolites from microalgae are extremely diverse, and some of them have been associated with growth inhibition of pathogenic microorganisms. Pratt et al. [103] were the first to isolate a microalgal antibacterial compound from the genus *Chlorella*; this compound, named chlorellin, is a mixture of fatty acids with inhibitory activity on Gram-negative and Gram-positive bacteria. Then, other microalgal antibacterial substances emerged between 1950s and 1980s, such as two chlorophyll a derivatives [89]. Table 2 summarizes the main molecules isolated from microalgae with antibacterial activity. These active chemical compounds include short chain fatty acids, monounsaturated and unsaturated long chain fatty acids, as well as a diversity of other chemical compounds, such as phenols, terpenes, pigments and indoles, acerogenins, alkaloids, macrolides, peptides, and volatile halogenated hydrocarbons [84,89,90,104–108]. Other works reported antibacterial activities in cyanobacterial extracts, mostly with organic solvents, such as those from the diatoms *Skeletonema costatum* and *Chaetoceros pseudocurvisetus* with anti-mycobacterial activity, absent from aqueous extracts, but the responsible metabolites were not identified [109].

Despite the potential of microalgae to produce antibacterial products as novel antibiotics, their development as a natural antibiotic is jeopardized by the small amount of compounds extracted from the producer organisms, the often cumbersome chemical synthesis, the associated toxicity, and in vivo inactivation [110].

Table 1. Antibacterial compounds from cyanobacteria.

Cyanobacterial Species	Class of Compound	Reference
	<b>Alkaloids</b>	
<i>Fischrella</i> sp.	Eucapsitrione	
<i>Fischrella ambigua</i>	Nostocarboline	
<i>Nostoc</i> sp.	Tjipanazole A and D	
	12-epi-hapalindole E isonitrile	
<i>Nostoc spongiaeforme</i>	Nostocine A	[88,89,92,93,111,112]
	<b>Indole Alkaloids</b>	
<i>Fischrella</i> sp.	Ambiguine A, B, D–I, K and M	
<i>Fischrella ambigua</i>	Fischambiguine B	
<i>Nodularia harveyana</i>	Northarmane	
<i>Nostoc insulare</i>	Norharmane-HCl (9H-pyrido(3,4-b) indole-HCl)	
	4,4-dihydroxybiphenyl	
	<b>Aromatic Compounds</b>	
<i>Fischrella ambigua</i>	Ambigol A and B	[88,92,95,111]
	<b>Carbohydrates</b>	
<i>Anabaena sphaerica</i>		
<i>Chroococcus turgidus</i>	Polysaccharides	[104]
<i>Oscillatoria limnetica</i>		
<i>Spirulina platensis</i>		
	<b>Cyclophanes</b>	
<i>Nostoc</i> sp.	Carbamidocyclophane A–E	[88,89,93,111]
	Nostocyclone	

Table 1. Cont.

Cyanobacterial Species	Class of Compound	Reference
<i>Moorea producens</i> ( <i>L. majuscula</i> )	<b>Dicarboximides</b> Malyngamide C, I and J	[113]
	<b>Fatty Acids and Lipids</b>	
<i>Fischerella</i> sp. <i>Spirulina platensis</i> <i>Phaeodactylum tricornerutum</i> <i>Oscillatoria redekei</i> <i>Scytonema</i> sp. <i>Scytoscalarol</i>	Colioric acid $\alpha$ -dimorphecolic acid $\gamma$ -linolenic acid	[89,90,92,94,108,111]
	<b>Indanes</b>	
<i>Nostoc commune</i>	4-hydroxy-7-methyl indan-1-one	[88]
	<b>Lactones</b>	
<i>Lyngbya majuscula</i>	$\delta$ -lactone malyngolide	[111]
	<b>Macrolides</b>	
<i>Scytonema</i> sp.	Scytophycin A and C Tolytoxin	[88]
	<b>Paracyclophanes</b>	
<i>Nostoc</i> sp.		[92]
	<b>Pigments</b>	
<i>Anabaena cylindrica</i> <i>Nostoc</i> sp. <i>Spirulina platensis</i> <i>Salpa fusiformis</i> <i>Synechocystis</i> sp. <i>Tolypothrix nodosa</i>	Phycobiliproteins Phycocyanins (PC-B and PC-C) Porphyrins (Tolyporphin)	[90–92,111,114,115]
	<b>Phenolic Compounds</b>	
<i>Anabaena sphaerica</i> <i>Chroococcus turgidus</i> <i>Oscillatoria limnetica</i> <i>Spirulina platensis</i>	4,4'-hydroxybiphenyl Polyphenols	[88,111]
	<b>Polyphenyl Ethers</b>	
<i>Leptolyngbya crosbyana</i>	Crossbyanol A–D	[88]
	<b>Porphinoids</b>	
<i>Tolypothrix nodosa</i>	Tolyporphin J	[111]
	<b>Terpenoids</b>	
<i>Nostoc commune</i> <i>Eucapsis</i> sp. <i>Microcoleus lacustris</i> <i>Scytonema</i> sp.	20-nor-3a-acetoxyabieta-5,7,9,11,13-pentaene 8-[(5-carboxy-2,9-epoxy) benzyl]-2,5-dihydroxy-1,1, 4a,7,8-pentamethyl-1,2,3,4,4a,6,7,8,9,10,10 -adodecahydrophenanthrene Abietane Comnostins A–E Norbietane Sesterterpene	[88,92,93]
	<b>Others</b>	
<i>Nostoc</i> sp.	EMTAHDCA 9-ethyliminomethyl-12- (morpholin-4-ylmethoxy)-5,8,13,16-tetraaza- hexacene-2, 3 dicarboxylic acid	[88]
<i>Fischerella ambigua</i>	Parsiguine	[92,111,116]

Table 2. Antibacterial compounds from microalgae.

Microalgae Species	Class of Compound	Reference
<i>Dunaliella salina</i>	<b>Indolic Derivatives</b> β-ionone Neophytadiene	[84,89,104]
	<b>Fatty Acids and Lipids</b>	
<i>Chlorella vulgaris</i>	Chlorellin	
<i>Chlorella pyrenoidosa</i>	Butanoic acid	
<i>Chaetoceros muelleri</i>	Docosa-pentaenoic acid (DPA)	
<i>Chlorococcum</i> sp.	Eicosapentaenoic acid (EPA)	
<i>Dunaliella salina</i>	Hexadecatrienoic acid (HTA)	
<i>Dunaliella primolecta</i>	α-linolenic acid (ALA)	[84,89,90,103–105,107,111]
<i>Haematococcus pluvialis</i>	Methyl lactic acid	
<i>Navicula delognei</i>	Octadecatetraenoic acid	
<i>Phaeodactylum tricornutum</i>	Oleic acid	
<i>Planktochlorella nurekis</i>	Palmitoleic acid	
<i>Scenedesmus obliquus</i>	Triglycerides	
	<b>Macrolides</b>	
<i>Amphidinium</i> sp.	Amphidinolide Q	[117]
	<b>Pigments</b>	
<i>Isochrysis galbana</i>	Carotenoids Chlorophyll a derivatives (Pheophytin a and chlorophyllide a) Phycobiliproteins	[84,89,90,104]
	<b>Terpenoids</b>	
<i>Isochrysis galbana</i> (six classes)	Diterpenoids	[104,106]
	<b>Others</b>	
<i>Phaeocystis</i> sp.	Acrylic acid	[89]
<i>Navicula delognei</i>	Ester	[89]
<i>Dunaliella salina</i>	α- and β-ionone	[104]
	Neophytadiene	[84]
	B-cyclocitral	
	Phytol	
<i>Haematococcus pluvialis</i>	Methyl lactate	[104]
<i>Navicula delognei</i>	Transphytol ester	[84]
<i>Haslea ostrearia</i>	Mareninne	[84]

### 3. Antibacterial Peptides from Cyanobacteria

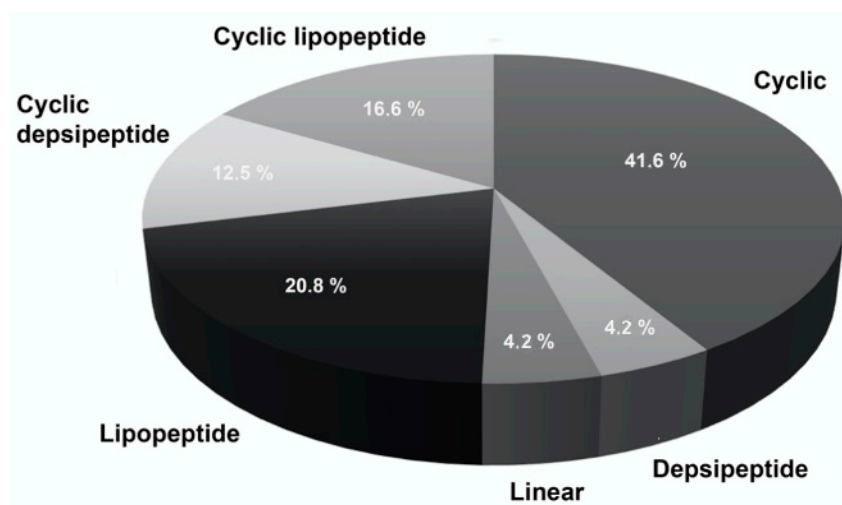
Cyanobacteria are an almost endless source of new peptide scaffolds. The peptides are synthesized as secondary metabolites required for a successful strive with other microorganisms, as well as for their astonishing environmental adaptation [118]. The biotechnological and medical potential of cyanobacterial peptides has been frequently reviewed and updated in the literature [88,119–125].

Since the first description of a cyanobacterial peptide with antibacterial activity, the cyclic peptide schizotrim A isolated from a culture of *Schizothrix* sp. many other peptides have been described. Table 3 summarizes the main cyanobacterial peptides, the producer species, their structure and their effect on known pathogenic target bacteria.

The antibacterial peptides from cyanobacteria are of different types, although those with a cyclic structure are more frequent (Figure 1). The antibacterial peptides identified peptides belong to different orders of cyanobacteria, being Oscillatoriales and Nostocales the most prolific ones. Inside Oscillatoriales, members of the genus *Lyngbya* are important producers of bioactive peptides with a potential therapeutic use. Four cyclic undecapeptides named lyngbyazothrins A, B, C, and D were identified from the freshwater strain *Lyngbya* sp. The mixtures A/B showed antimicrobial activity



only against the Gram-positive bacteria *Micrococcus flavus*, while lynngbyazothrins C/D were active against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Serratia marcescens*), and the Gram-positive *Bacillus subtilis*, although not on methicillin susceptible *Staphylococcus aureus* [92,125–127].



**Figure 1.** Different types of antibacterial peptides isolated from cyanobacteria.

Other antibacterial peptides described for the *Lynngbya* genus are the lipopeptide pahayokolide A, and the depsipeptides pitipeptolide A–F. Furthermore, within the Oscillatoriales order, the *Oscillatoria* and the *Phormidium* genus are known producers of antibacterial peptides although with much lower representation than *Lynngbya*. All of them are cyclic peptides. Concerning the Nostocales order, the *Nostoc* genus stands out, with a variety of cyclic and linear antibacterial peptides; *Anabaena* and *Scytonema* genera produced depsipeptides and lipopeptides, respectively [88,94,125,128–132].

The marine cyanobacterium *Prochlorococcus marinus* produces the lantipeptide prochlorosin, with encoding genes distributed throughout its genome. Lantipeptides are a large family of linear and cyclic peptides, ribosomally synthesized as precursor peptides that underwent post-translational modifications, including the formation of lanthionine bridges, heterocyclization, oxidation, methylation, prenylation, and cyclization. The formation of lanthionine bridges in prochlorosin is catalyzed by LanM, a lanthionine synthetase C enzyme [133].

This vast spectrum of peptides produced by cyanobacteria comprises not only the ribosomal synthesis of peptides, including their posttranslational modifications, but also abundant non-ribosomal peptides (NRPs) synthesized by non-ribosomal peptide synthetases (NRPSs) [85,125,134], modular multienzymatic complexes working as an assembly line for amino acid incorporation into the polypeptide chain and, frequently, to their in situ modification. In addition NRPSs may appear associated to polyketide synthases (PKS), forming NRPS-PKS clusters [135,136] broadening even further the variety of chemical motifs incorporated into the polypeptide chain.

**Table 3.** Cyanobacterial peptides with antibacterial activity.

Peptide Name	Characteristic	Source	Target Bacteria	Activity †	Reference
Aeruginazole A	Cyclic	<i>Microcystis</i> sp.	<i>Bacillus subtilis</i>	MIC = 2.2 µg/mL	[92]
Aeruginazole DA 1497	Cyclic	<i>Microcystis aeruginosa</i> TAU	<i>Staphylococcus aureus</i>	DIZ 7 mm at 25 µg	[92]
Anachelin H	Depsipeptide	<i>Anabaena cylindrica</i> CCAP/2A	<i>Moxarella catharralis</i>	MIC = 32 µg/mL	[139]
Antillatoxin B	Lipopeptide	Hawaii and Caribbean collection of cyanobacteria	<i>Listeria monocytogenes</i> HPB 2812 and <i>Staphylococcus aureus</i> ATCC 29213	MICs = 250 µg/mL	[113]
			<i>Bacillus cereus</i> LSPQ 2872	MIC: 130 µg/mL	
Borophycin	Cyclic	<i>Nostoc linckia</i> and <i>N. spongiaforme</i> ,	ND	ND	[88,94]
Brunsvicamides A B and C	Cyclic	<i>Tychonema</i> sp.	<i>Mycobacterium tuberculosis</i>	IC <sub>50</sub> = 7.3–8 µM	[88]
Kawaguchipectin B	Cyclic undecapeptide	<i>M. aeruginosa</i> (NIES-88),	<i>Staphylococcus aureus</i>	MIC 1 µg/mL	[140]
Laxaphycin A	Lipopeptide	Hawaii and Caribbean collection of cyanobacteria	<i>Listeria monocytogenes</i> HPB 2812 and <i>Bacillus cereus</i> LSPQ 2872	MIC 250 µg/mL	[113]
			<i>Staphylococcus aureus</i> ATCC 29213	MIC = 125 µg/mL	
Laxaphycin B	Lipopeptide	Hawaii and Caribbean collection of cyanobacteria	<i>Listeria monocytogenes</i> HPB 2812, <i>Bacillus cereus</i> LSPQ 2872 and <i>Staphylococcus aureus</i> ATCC 29213	MIC = 250 µg/mL	[113]
Laxaphycin B3	Lipopeptide	Hawaii and Caribbean collection of cyanobacteria	<i>Bacillus cereus</i> LSPQ 2872	MIC = 250 µg/mL	[113]
Lyngbyazothrins mixture A/B	Cyclic undecapeptide	<i>Lyngbya</i> sp 3691 SAG	<i>Micrococcus flavus</i> SBUG 16	DIZ 8 mm at 100 µg (Ref. ampicillin 10 µg, inhibition zone 28 mm)	[92,125,126]
			<i>Bacillus subtilis</i> SBUG 14,	DIZ 18 mm at 125 µg (Ref. ampicillin 10 µg, inhibition zone 14 mm)	
Lyngbyazothrins mixture C/D	Cyclic lipopeptide Cyclic undecapeptide	<i>Lyngbya</i> sp. <i>Lyngbya</i> sp. 3691 SAG	<i>Escherichia coli</i> ATCC 11229	DIZ 18 mm at 100 µg (Ref. ampicillin 50 µg, inhibition zone 26 mm)	[92,125,126]
			<i>E. coli</i> SBUG 13	DIZ 15 mm at 100 µg (Ref. ampicillin 50 µg, inhibition zone 17 mm)	
			<i>Pseudomonas aeruginosa</i> ATCC 27,853	DIZ 8 mm at 100 µg (Ref. gentamycin 25 µg, inhibition zone 26 mm),	
			<i>Serratia marcescens</i> SBUG 9	DIZ 8 mm at 200 µg (Ref. ampicillin 10 µg, inhibition zone 28 mm),	



Table 3. Cont.

Peptide Name	Characteristic	Source	Target Bacteria	Activity ‡	Reference
Microcystin	Cyclic heptapeptide	<i>Synechocystis</i> , <i>Synechococcus</i> and <i>Romeria</i>	<i>Pseudomonas aeruginosa</i> ATCC 27,853 and <i>Staphylococcus aureus</i> ATCC 25923	DIZ 10.5 ± 0.71 to 14.0 ± 1.41 mm (*)	[94,138]
Muscoride A	Linear	<i>Nostoc muscorum</i>	<i>Bacillus subtilis</i>	DIZ = 3–6 mm (streptomycin, 7–10 mm; penicillin G, 7–10 mm)	[88,129]
NRPs, PKs and hybrid NRPS-PKS		Brazilian isolates	<i>Bacillus subtilis</i> and <i>Salmonella typhimurium</i>	34 and 22% of inhibition growth (20 µL/2 mL organic extract)	[137]
Pahayokolide A	Cyclic lipopeptide	<i>Lyngbya</i> sp.	<i>Bacillus megaterium</i> <i>Bacillus subtilis</i>	MIC = 5.5 µg/mL MIC 10 µg/mL	[125,131]
<3 kDa peptide fraction	Hydrolyzed protein	<i>Spirulina platensis</i>	<i>Escherichia coli</i> <i>Staphylococcus aureus</i>	15.2% at 625 µg/mL 19.6% at 625 µg/mL	[141]
Pitipeptolides A–F	Cyclic depsipeptide	<i>Lyngbya majuscula</i>	<i>Mycobacterium tuberculosis</i>	DIZ 40 mm at 100 µg/disk	[88,125]
Pitiprolamide	2,2-diMe-3-Hy-hexanoic acid and Dpv-Pro	<i>L. majuscula</i>	<i>Mycobacterium tuberculosis</i> H37Ra and <i>Bacillus cereus</i>	ND	[125,142]
Portoamide	Cyclic	<i>Phormidium</i> sp. LEGE 05,292	<i>Cobetia marina</i> CECT 4278 <i>Halomonas aquamarina</i> CECT 5000 <i>Pseudoalteromonas atlantica</i> CECT 570	23.3% at 6.5 µM 21.0% at 6.5 µM 21.5% at 6.5 µM	[132]
Schyzotrin A	Cyclic lipopeptide	<i>Schizothrix</i> sp. TAU strain IL.89-2	<i>Bacillus subtilis</i>	DIZ 15 mm at 6.7 nM	[143,144]
Scytonemin A	Lipopeptide	<i>Scytonema</i> sp.	<i>Mycobacterium</i> sp.	MIC = 1 mg/mL (Ref. gentamycin 0.5 mg/mL)	[128]
Tenuecyclamide A to D	Cyclic hexapeptide	<i>Nostoc spongiaforme</i> var tenue	<i>Bacillus subtilis</i> Bs1091-1 <i>Staphylococcus aureus</i> Sau1091-3 Clinical Laboratory, Ministry of Agriculture, Bet-Dagan, Israel	Disk inhibition zone, values not reported	[88,130]
Tiahuramide C	Cyclic depsipeptide	<i>L. majuscula</i>	<i>Aeromonas salmonicida</i>	MIC = 6.7 µM	[125]
Trichormamide C	Cyclic lipopeptide	<i>Oscillatoria</i> sp UIC 10045	<i>Mycobacterium tuberculosis</i>	MIC = 23.8 µg/mL	[125]

‡ MIC: minimal inhibitory concentration; DIZ: diameter inhibition zone (mm); IC<sub>50</sub>: half inhibitory concentration; %: percentage of inhibition. (\*) Methanolic extract of *Romeria gracilis* M6C against *Pseudomonas aeruginosa*: 10.5 ± 0.71; *Synechocystis aquatilis* M62C against *Staphylococcus aureus*: 11.5 ± 0.71. Ethanol extract of *R. gracilis* M6C against *P. aeruginosa*: 11.0 ± 1.41; *Synechococcus* sp M94C and M290C against *P. aeruginosa*: 12.5 ± 0.71 and 14.0 ± 1.41 respectively.

NRPSs represent a major class of secondary metabolites in cyanobacteria, with a broad range of biological and pharmacological properties, mostly as antibiotics. It can be speculated that blue-green algae acquired *nrrps* genes after the first endosymbiotic process that led to the formation of algae, or algae may subsequently have lost *nrrps* genes [101].

The activity on pathogenic bacteria from algae from the orders Chroococcales, Oscillatoriales, Nostocales, and Stigonematales was associated to non-ribosomal pathway involving NRPS, PKS and hybrid NRPS-PKS. Among 50 strains of terrestrial and freshwater cyanobacteria, the species *Cylindrospermopsis raciborskii* 339-T3, *Synechococcus elongatus* PCC7942, *Microcystis aeruginosa* NPCD-1, *Microcystis panniformis* SCP702 and *Fischerella* sp. CENA19 provided the most active extracts, with high activity against *Bacillus subtilis*, and *Salmonella typhimurium* [137].

Microcystins are non-ribosomal cyclic heptapeptides; these toxins are the most commonly found in blooms produced by cyanobacterial genera, such as *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria*, *Nostoc*, *Cylindrospermopsis*, *Aphanizomenon*, *Planktothrix*, *Anabaenopsis*, *Synechocystis*, and *Lyngbya*. Aside from them, the strain *Synechocystis aqualitis* M62C produces a microcystin active against *S. aureus*, but devoid of the *mcyB* gene, one of the genes related to the microcystin synthesis, whereas the strain *S. aqualitis* M204BG showed microcystin production and *mcyB* gene in its genome, but was inactive against *S. aureus* and *P. aeruginosa* [138].

#### 4. Antibacterial Peptides from Microalgae

When compared with the number of cyanobacterial peptides, the number of antibacterial peptides from microalgae is considerably lower. The first report was an antibacterial 30mer peptide purified from the culture of *Stichochrysis immobilis* Pringsheim, active against marine bacteria [145].

The following reports correspond to protein hydrolysates. Sedighi et al. [146], evaluated the antibacterial activity of peptide fractions of the microalga *Chlorella vulgaris*. Protein fraction with 62 kDa were hydrolysates by pepsic digestion and antibacterial activity was determined against *E. coli* CECT 434. The effect of hydrolysate was 8.5 and 1.6 times greater than *Chlorella* biomass and its proteins, respectively, suggesting that *Chlorella* peptides provoked the cell wall destruction and cell growth inhibition. Furthermore, pepsin hydrolysates and peptide fractions from *Chlorella sorokiniana* displayed antibacterial activity against *E. coli* and *S. aureus* using the agar well diffusion method [147].

Guzmán et al. [148], reported antibacterial peptides from the marine microalgae *Tetraselmis suecica*. The AQ-1766 peptide (LWFYTMWH) obtained from acid extract and the 40% acetonitrile eluted fraction was active against the Gram-negative bacteria *E. coli*, *S. typhimurium*, and *P. aeruginosa*, as well as against the Gram-positive bacterial strains *B. cereus*, methicillin-resistant *S. aureus* (MRSA), *L. monocytogenes* and *M. luteus*. Moreover, the substitutions A4Y (AQ-3000: LWFATMWH), (AQ-3001: LWFYAMWH) and T6M (AQ-3002: LWFYTAWH) increased the antimicrobial activity. Additionally, the lysine analogs: K1L (AQ-3369: KWFYTMWH) and tyrosine Y4K (AQ-3370: LWFKTMWH) exhibited the highest antibacterial activity.

On the other hand, microalgae were also used for transgenic production of alien AMPs, as the bovine AMP lactoferricin by transgenic *Nannochloropsis oculata*. This transfected alga was used as biofunctional food for the medaka fish (*Oryzias latipes*) with an improved survival when challenged with *Vibrio parahaemolyticus* infection [149].

The limited knowledge of antibacterial peptides from microalgae will increase through the use of biotechnological tools such as transcriptomics that will help to understand its genome and its pharmacological interactions with bacteria. Transcriptomic sequencing will provide useful data to identify species with antibiotic potential and pathways for the synthesis of new functional metabolites. The transcriptome of the microalgae *Chrysochromulina tobin* revealed the expression of genes involved in the defense of the algae that encode potential antibiotics, antibiotic extrusion proteins, and novel antibacterial peptides [107].

## 5. Mechanism of Antibacterial Action of Peptides and Compounds of Cyanobacteria and Microalgae

In general, the mechanism of action of cyanobacterial and microalgae peptides against bacterial cells has not yet been established, and further studies are needed to elucidate the biological activity of these antimicrobial peptides [90,147]. For those antibacterial peptides with a clear cationic character, e.g., from microalgae, we may surmise a mechanism of action rather similar to those peptides described in higher eukaryotes; that is, the disruption of the cell membrane after specific insertion into the bacterial cell membrane. Specificity is mostly achieved by the different electrical charge of the external hemilayer of the cell membrane, negative for prokaryotes and lower eukaryotes, zwitterionic in higher eukaryotes. This mechanism has two important consequences; first, the negative electrical charge of the membrane is considered as a pathogen associated molecular pattern, as such, common to many microorganisms, that makes them susceptible to a given peptide. Secondly, as the bactericidal mechanism is based on the stoichiometric interaction of the peptide with the phospholipids of the lipid bilayer, physicochemical characteristics of the peptide, such as charge, size, amphipaticity, and even secondary structure, are more important than the primary sequence of the peptide. For others, their mechanism of action differs from membrane disruption, with involvement of intracellular targets, and specificity achieved by subtle recognition between the peptide and its target.

This is the case for some cyanopeptides, as the cyclic peptides brunsvicamides B and C from *Tychonema* sp., reported as inhibitors of phosphatase B of *Mycobacterium tuberculosis*, or the cyclic depsipeptide scyptolin A, isolated from *Scytonema hofmanni*, an inhibitor of a serine protease working as a transpeptidase involved in the bacterial cell wall biosynthesis for certain pathogenic bacteria [88].

Antibiofilm activity is an appealing asset for an antibacterial candidate, as infections in clinical devices are frequently associated to biofilm formation and higher resistance against antibiotics. The cyclic peptides portoamides produced by *Phormidium* sp. display this activity against marine bacteria such as *Cobetia marina*, *Halomonas aquamarina* and *Pseudoalteromonas atlantica*, by inhibition of ATPase H<sup>+</sup>-transport activity [132]. This activity has a straightforward application as antifouling agents, and their test as antibiofilm compounds for relevant clinical bacteria is pending.

In some cases, structure-activity relationships were obtained with a variable degree of success, either by sequence comparison among similar cyanopeptides from the same or different cyanobacteria, by genetic mutation, or by chemical synthesis. The antibacterial activity of the lipopeptide schizotrin A against *B. subtilis* has been associated to the presence of a proline linked to the 3-amino-2,5,7,8-tetrahydroxy-10-methylundecanoic acid (Aound), and their uptake into the bacterial cell facilitated by the presence of the fatty acid [94,126]. The dipeptide motif formed by a proline residue bound to the amino group of a 2-hydroxy-3 amino-long chain acid residue is shared for other cyanobacterial cyclopeptides, such as scytonemin A from *Scytonema* sp [88,143]. The presence of this fatty acid was also identified in lyngbyazothrins A–D, and this acyl chain at position C-5 is relevant for the antibacterial activity of the peptide.

The amino acid analyses of the cyanopeptides lyngbyazothrins A–D reveal three unusual amino acids identified as 4-methoxyhomophenylalanine in A and C, homophenylalanine in B and D, and 3-amino-2,5,7,8-tetrahydroxy-10-methylundecanoic acid (Aound) in A–D; moreover, C and D have an additional *N*-acetyl-*N*-methyltyrosine unit and it seems that the acyl residue at C-5 plays an important role in antimicrobial activity. Schizotrin A and pahayokolides A and B have sequence similarity to lyngbyazothrins. Schizotrin A presents a 4-methoxyhomophenylalanine (Htm) residue, similar to lyngbyazothrins A and C, bound to the Pro-Aound-Gln-Gly-Pro sequence, common to all of the lyngbyazothrins. The same sequential motif is also found in pahayokolides A and B but, in contrast to schizotrin A, it is linked to an homophenylalanine. Significant differences were found for the remaining five residues of the cyclic systems among the three classes of peptides: Phe-Val-Ser-DeHThr-Ser in schizotrin A, Phe-Z-Dhb-Ser-E-Dhb-Thr in pahayokolides and Pro-allo-Ile-Ser-DeHThr-Thr in lyngbyazothrins. The free hydroxyl group at C-5 of the Aound residue in lyngbyazothrins A and B is substituted by *N*-acetyl-*N*-methyltyrosine in C and D instead of the *N*-butyryl-*N*-methylalanine residue in schizotrin A. On the other hand, schizotrin A and pahayokolide A contain the aliphatic amino acids

alanine and leucine, while lyngbyazothrins C and D include the aromatic amino acid tyrosine; it has been proposed that the nature of these amino acids may also account for the activity of lyngbyazothrins against the Gram-negative bacterium *E. coli*, absent in schizotrin and pahayokolide [126].

The ribosomal cyclopeptide aeruginazole A isolated from the cyanobacterium *Microcystis* sp. (IL-323), inhibits the growth of *B. subtilis* and *S. aureus*; in its cyclic structure it contains three subsequent glycine residues plus L-val, L-phe, thiazole-L-val, thiazole-D-leu, D-tyr and thiazole-L-asp. Similarly, aeruginazole DA1497 isolated from *M. aeruginosa*, is a large cyclopeptide with four thiazole (tzl) moieties, having a cyclo-structure of  $(-(\text{tzl-phe})\text{-gly-ala-ile}-(\text{tzl-ala})\text{-ser}-(\text{tzl-val})\text{-pro-gly-val}-(\text{tzl-leu})\text{-pro-gly-})$ . It seems that the larger size and the greater number of thiazole groups of these compounds may be associated with their bioactivity. Only DA1497 out of the one of five aeruginazole peptides tested (DA1304, DA1274, DA1338 and DA1372) was active against *S. aureus*, even when minor differential sequential variations occurred among the five peptides [92]. The cyclic lipopeptide Trichormamide C from *Oscillatoria* sp. UIC 10,045 is characterized by the presence of three non-proteinogenic  $\alpha$ -amino acid residues (*N*-methyl-Ile and two 3-hydroxy-Leu) and one  $\beta$ -amino acid, with a key role on its anti-*M. tuberculosis* activity [125].

The antibacterial mechanisms of microalgal peptides have been scarcely reported to date. The few references on the subject refer to extracts or protein hydrolysates, and not to specific peptides. Microalgal extracts from the species *Leptocylindrus danicus* (FE322) and *L. aporus* (FE332) strongly inhibited the biofilm formation by the bacteria *Staphylococcus epidermidis*, but did not show cytotoxicity by standard antibacterial tests [150]. Tejano et al. [151], reported a higher antibacterial activity on Gram-positive than on Gram-negative bacteria for the pepsin hydrolysate and the peptide fractions from *Chlorella sorokiniana*, likely associated to a hindered penetration of the peptide by the outer membrane.

It has been proposed that microalgal compounds with antibacterial activity are released after the loss of algal integrity, or alternatively induced by the presence of bacteria. For other microalgal compounds involved in a defense mechanism against predators and pathogenic bacteria, it appears that the bacterial cell membrane would be the main site of action. There is some evidence of deleterious effects of fatty acids on the bacterial membrane, causing cell leakage, a reduction in nutrient intake and a reduction in cellular respiration. The antibacterial action of fatty acids can also be mediated by the inhibition of the synthesis of bacterial fatty acids; this effect could be bactericidal or bacteriostatic preventing bacterial multiplication. It has also been reported that antibacterial exometabolites released by *T. suecica* inhibited several *Vibrio* species in vitro causing a rapid decrease in bacterial mobility with cells elongation and vacuolization [84].

Advances in the knowledge of the mechanisms of action underlying the bactericidal activity of peptides from cyanobacteria and microalgae will contribute to the development of these peptides as novel drugs. The role of “omics” techniques in this process, more specially proteomics and peptidomics, will push forward the boundaries for this field [90,147].

## 6. Synergy of Cyanobacterial Peptides

Synergy among AMPs in nature is an important asset in evolution, as it ensures a proper microbicidal function with a spare of components. The issue of microbicidal synergy was extensively addressed for AMPs from higher eukaryotes [152–154], as well as for bacteriocins [155]. This synergism may occur under different modalities. A specific molecular recognition between two different AMPs from *Xenopus laevis*, magainin and PGLa, led to the formation of a functional heterodimer as the forming unit of the pore on the bacterial membrane, with an increased lethal permeation over those formed exclusively by a single AMP species [156]. In this case, the two synergic partners shared a single target, but synergy may also arise from two different AMPs each one acting on their own and specific target in a concerted manner. The disruption of bacterial membranes by the human AMP LL-37 allows the histone H2A to gain access into the bacterial cytoplasm to interact with the bacterial genome, and to halt transcription [157]. Synergism is not limited to AMPs as exclusive partners; other actions or compounds may synergize with a given AMP to improve the final microbicidal aftermath. Any AMPs,

regardless of its biological origin, able to disrupt the outer membrane of Gram-negative bacteria, which is a strong permeability barrier for small size antibiotics, may likely synergize with them, as described for the bacterial polymyxins B and other AMPs from higher eukaryotes. In addition, small antibiotics may also synergize with AMPs acting mostly through host immunomodulation, as the IDR-1018 peptide [158].

Compared to the synergism described for AMPs, there is a dearth of reports on synergy among cyanobacterial peptides, especially those concerning activity against pathogens of putative clinical interest. Synergism in cyanobacteria was mainly approached from an environmental perspective, where peptides were used by cyanobacteria as allelochemicals for a successful striving for survival among other environmental competitors or plankton grazers.

Portoamides A and B, produced by the mat forming *Oscillatoria*, act synergistically against the microalgae *C. vulgaris*, *Ankistrodesmus falcatus*, and *Chlamydomonas reinhardtii*, but also against the cyanobacteria *Cylindrospermopsis raciborskii* [159]. *Planktothrix* sp, other cyanobacteria, produced the ribosomal microviridins by ribosomal synthesis, and microcystin-LR, a peptide inhibitor for protein phosphatases 1 and 2A, as well as anabaenopeptins through the non-ribosomal pathway. When cyanobacterial knock-outs for any of these three peptide families were infected with the chytrid parasitic fungus pNIVA-CYA126/8, its virulence was higher on knockouts than on the parental strain [160].

Beyond this environmental frame, synergy among cyanobacterial peptides has also been reported for antifungal activity on species with clinical or phytological importance. Lobocyclamides A and B from *Lyngbya confervoides* act synergistically against *Candida* spp [161], the cyclic undecapeptides laxaphycins A and B from *Anabaena torulosa* against *Aspergillus oryzae* and *Candida albicans* [162], and the same peptides isolated from *Anabaena laxa* against *A. oryzae* [163].

Interestingly, the synergism of cyanobacterial peptides for antitumoral activity has been also reported on human tumoral cell lines for laxaphycins A and B from *A. torulosa*, and for laxaphycins A and B4 from *Hormothamnion enteromorphoides* [162,164]. Protoamides A and B showed synergism on the human cancer cell line H460 [159].

## 7. Other Relevant Functions of Peptides from Cyanobacteria and Microalgae

In addition to their antibacterial activity, the peptides produced by cyanobacteria and microalgae also have other fields of application, being one of the most prominent treatments of cancer. Some representative examples follow:

### 7.1. Antitumoral Activity

The NRPS and NRPS-PKS described on the cyanobacteria of the genus *Nostoc*, were responsible for the production of bioactive peptides (reviewed by Fidor et al. [120]). Among the extensive peptide armamentarium of this genus are cryptophycins; cyclic 16mer depsipeptides are almost exclusively produced by this genus, with activity against different cancer lines. Other peptides of the genus *Nostoc* are the nostocyclopeptides, cyclic heptapeptides characterized by an imino linkage between the first residue (Tyr) and the aldehyde hydrate of the seventh residues. They induce apoptosis in tumoral cell lines, endorsing their feasible use as anticancer compounds.

The heptapeptide LLAPPER (MW = 796.4) was obtained by in vitro gastrointestinal digestion of *P. lutheri* [165]. After its chemical synthesis, the peptide was tested on HT1080 fibrosarcoma cells, with a decrease of the transcript encoding the matrix metalloproteinase B/MMP9, an enzyme involved in the degradation of collagen type IV, through inactivation of the NFκB pathway, as well as blocking of JNK and p38 phosphorylation. Matrix metalloproteinases are involved in the degradation of the extracellular matrix, to promote metastasis in cancer.

Interestingly other cyanobacterial peptides promotes differentiation, as the peptide dubbed PPLF obtained from *P. lutheri* fermentation by Qian et al. [166]. PPLF induced osteoblastic differentiation in the human cell line MG-63 at 50 and 100 µg/mL.



### 7.2. Antihypertensive Activity

Heo et al. [167] hydrolyzed *Spirulina* with a mixture of digestive enzymes (pepsin, trypsin and chymotrypsin). Afterwards, the heptapeptide TMEPGKP (MW = 759) was isolated from a fraction with inhibitory activity on the angiotensin I converting enzyme (ACE). By molecular modelling and functional assays, this peptide resulted as a non-competitive inhibitor of ACE, and decreased the phosphorylation of p38 MAP Kinase (MAPK), of the expression of the inducible nitric oxide synthase (iNOS) with the concomitant decrease of nitric oxide (NO) production. The levels of reactive oxygen species (ROS), and endothelin-1 (ET-1), also decreased after peptide treatment. Altogether, these results make this heptapeptide a good candidate as antihypertensive compound. In addition, the peptide showed an inhibitory effect on the proliferation of EA.hy926 cells at 125 and 250  $\mu\text{M}$ .

Carrizzo et al. [168] described the fractionation of *Spirulina* lysates by a treatment that simulates gastrointestinal digestion (GID). From these extracts, four peptides were obtained and characterized by mass spectrometry (SP(3–6)). In a further step, these peptides were synthesized by Fmoc chemistry and tested for vasorelaxation in an ex vivo model consisting of mouse mesenteric arteria. SP6 peptide (GIVAGDVTPI, MW = 940.52), showed a dose dependent vasorelaxation effect and antihypertensive activity through the activation of endothelial nitric oxide synthase and NO production.

Suetsuna and Chen [169] searched for peptide fractions from the peptic digest of the microalgae *S. platensis* and *C. vulgaris* and to be tested for their antihypertensive activity through ACE inhibition. A total of ten peptides (three to five residues) were isolated, two of them shared by both algae and the other four sequences specific for each species. The two shared tripeptides FAL and AEL showed  $\text{IC}_{50}$  on ACE of 26.3 and 57.1  $\mu\text{M}$ , respectively. The peptides specific for *S. platensis*: IAE, IAPG, VAF have  $\text{IC}_{50}$  of 34.7, 11.4 and 35.8  $\mu\text{M}$ , respectively, and the  $\text{IC}_{50}$  for those exclusively found in *C. vulgaris*: IVVE, AFL, VVPPA, were 315.3, 63.8 and 79.5  $\mu\text{M}$ , respectively.

Hot water extract of the microalgae *Chlorella sorokiniana* was hydrolyzed with proteinase N, and tested for ACE inhibition [170]. Four dipeptides were isolated and sequenced by automated Edman degradation. In a further step, the four peptides were synthesized by solid phase peptide synthesis (SPPS) and tested for their ACE inhibitory activity: WV, VW, IW, and LW with  $\text{IC}_{50}$  on ACE of 307.6  $\mu\text{M}$ , 0.58  $\mu\text{M}$ , 0.50  $\mu\text{M}$ , and 1.11  $\mu\text{M}$ . Their low  $\text{IC}_{50}$  values plus their small size make them extremely appealing candidates as hypertensive agents. Furthermore, they were impervious to in vitro gastric digestion.

The peptide YMGLDLK (MW = 839) from the microalgae *Isochrysis galbana* hydrolyzed with alcalase, flavourzyme, pepsin, and trypsin, showed an  $\text{IC}_{50}$  on ACE of 36.1  $\mu\text{M}$ . Additionally the peptide was stable after incubation with gastric enzymes (pepsin, chymotrypsin and trypsin) [171].

The hydrolysate of the marine microalga, *N. oculata* obtained by digestion with several enzymes, such as pepsin, trypsin,  $\alpha$ -chymotrypsin, papain, alcalase, and neutralase were used by Samarakoon et al. [172] in the search for ACE inhibitory peptides. The pepsin hydrolysate exhibited the highest ACE inhibitory activity. From this lysate, two ACE inhibitory peptides were purified: GMNNLTP (MW = 728;  $\text{IC}_{50}$  = 123  $\mu\text{M}$ ) and LEQ (MW = 369;  $\text{IC}_{50}$  = 173  $\mu\text{M}$ ). These peptides were proposed as novel inhibitory agents in the functional food industry.

### 7.3. Anti-Inflammatory Activity

The group of Qian and Jung sought to find active compounds from the marine microalga *Paolova lutheri* after fermentation with the yeast *Hansenula polymorpha*. They reported the in vitro antioxidant activity of the fermented microalga [173]. In subsequent works [174], reduction of oxidative stress was achieved by the tetrapeptide MGRY (MW = 526), that works as a scavenger for free radicals, with an  $\text{IC}_{50}$  of 0.285 mM for the DPPH antioxidant test, 0.068 mM for hydroxyl radicals and 0.988 mM for hydrogen peroxide. The peptide also showed inhibitory properties in melanogenesis process when tested in B16F10 melanoma cells. As such, its use was proposed in cosmeceutical and pharmaceutical applications.



#### 7.4. Antiviral Activity

Cyanovirin-V, a lectin of 101 residues and two disulfide bridges, showed potent activity against human immunodeficiency viruses (HIV-1 and 2), simian immunodeficiency virus (SIV), and other enveloped viruses [120].

To note, the inhibition of ACE activity described for some cyanopeptides, make them putative candidates for competitive inhibition of the SARS-CoV-2 virus into ACE, the main receptor used for this pandemic virus to infect human cells.

#### 7.5. Antifouling Activity

Portoamides are cyclic dodecapeptides from the cyanobacterium *Phormidium* sp. They were used for their antifouling activity with an EC<sub>50</sub> of 3.16 µM against the settlement of the larvae of the mussel *Mytilus galloprovincialis* [132]. Portoamides were nontoxic against the mussel larvae, therefore a deterrent effect towards surface colonization was instead proposed. The results of previous reports of portoamides' activity against the microalgae *C. vulgaris* (IC<sub>50</sub> 12.8 µg/mL), *Ankistrodesmus falcatus* (IC<sub>50</sub> 24.7 µg/mL) and *C. reinhardtii* (IC<sub>50</sub> 12.6 µg/mL), and the cyanobacterium *Cylindrospermopsis raciborskii* (IC<sub>50</sub> 28.4 µg/mL) are also described.

### 8. Synthetic Approach

Synthetic approach has become an almost mandatory companion to the discovery of new peptides. The synthesis of peptides in particular is an area of wide development in the current pharmacology. The main goal of this technique for cyanobacterial and microalgal peptides is to overcome the frequent extremely low amount of isolated natural peptides, that limits further studies on their mechanism of action, on the definition of SAR studies, and of a feasible pharmacological development. Peptides have become valuable drug candidates, largely driven by improvements in their synthetic methodology.

Peptide chemistry has developed on two main areas: biosynthesis and chemical synthesis. A special number of Chemical Reviews dealt with the first strategy, with excellent reviews on the subject and some resulting applications [175–178].

However, the chemical synthesis is the first-choice approach as it allows to obtain pure products with good yield, needed to establish its activity in an unambiguous way, and pursue the establishment of its mechanism of action.

Solid phase peptide synthesis is a well-established methodology developed more than 50 years ago [179,180]. Nowadays, the Fmoc/tBu strategy is the most used [181,182]. Several approaches have been carried out to optimize the process, to improve its efficiency, and more recently to develop an ecofriendly approach, more compliant with the green chemistry principles [41,42,183,184].

Peptide synthesis can be carried out manually, with the possibility of simultaneously synthesizing many sequences by using the “tea bag” protocol [185,186], or also in an automated way where developments such as microwave [187,188], and ultrasonication [189] procedures shortened the time of synthesis and reduced solvent consumption as well as waste generation, additionally allowing the synthesis of longer peptides.

These methodologies were straightforwardly applied to peptides derived from microalgae, with a lower complexity, and in general devoid of posttranslational modifications, due to their ribosomal origin. In contrast, non-ribosomal peptides from cyanobacteria have a much higher structural and compositional complexity, broadening their chemical space, due to the versatility and diversity provided by non-ribosomal synthesis. Although the synthesis of cyanobacterial peptides requires more complex strategies, it has been successfully approached for some peptides.

Inguibert's group performed the chemical synthesis of cyclic lipopeptides of the laxaphycin family, isolated from several cyanobacterial species. In a first step, the dodecapeptide laxaphycin B was synthesized [190], and assayed for cytotoxicity against a wide range of cancer cell lines (IC<sub>50</sub> ranging from 0.2 to 6.0 µM). An automated SPPS with an on-resin “head-to-tail” cyclization of the

linear precursor of laxaphycin B (laxaB) was used. Synthesis optimization was achieved by the use of 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) as a coupling reagent. Finally, the head to tail cyclization was carried out with DIC/Oxyma to prevent epimerization. This strategy was also applied for the synthesis of lyngbyacyclamide A. NMR analysis confirmed that the structures of natural and synthetic peptides were identical. In a later work, the same group synthesized other laxaB analogues [191], by replacement of the non-natural amino acids with commercially available counterparts (2-aminodecanoic acid (Ade):  $\beta$ -alanine, the 3-hydroxyleucines (Hle): threonine, and the 3-hydroxyasparagine (HAsn): asparagine. They also implemented an automated microwave SPPS protocol using the same reagents and conditions determined in the previous work. The cyclization residues were selected according to retrosynthetic analysis, and the final product was purified and characterized as before. Although the peptide obtained did not exactly match the expected results, a synthesis and characterization protocol was established.

The synthesis of the peptide mozamide A, produced by a marine sponge of the genus *Theonella* was approached by Junk and Kazamaier [192]. This peptide is a hydroxylated brunsvicamide, a family of peptides isolated from the cyanobacteria *Tychonema*, but differing in the configuration of Val, Lys and Ile amino acids. Interestingly, the right configuration of these residues was confirmed in the synthetic peptide, and established a synthetic scheme for these types of compounds.

Synthesis of lipocyclopeptides, such as the cyclic undecapeptide trichromamide A (TcA) from the cyanobacteria *Trichormus* sp., was performed by Gaillard et al. [193]. SPPS was carried out on a clorotrytil resin to which the uncommon residue aminodecanoic acid (Ada) was anchored and the cyclization step was made in solution with PyOxym/Oxyma. They reported the synthesis of TcA and a second compound, possibly a diastereomer, with a good yield.

Additionally, multiple approaches can be applied to improve the biological performance of peptides, with a special focus to improve their biological stability. This is achieved, by replacement of amino acids that are not susceptible to enzymatic hydrolysis [194], by structural restriction, by cyclization, or by stapling [46,195].

An interesting research area is related with the use of metallic elements in association with peptides to enhance their activity for applications in biomedicine [196–198].

In addition to the development in synthesis, there are also advances in peptide purification and analysis methodologies that include techniques, such as HPLC, MS, circular dichroism (CD), and nuclear magnetic resonance (NMR), which can be helpful in studies of structure-activity relationship or in the determination of mechanisms of action [184,199–201].

## 9. Conclusions

The deep global crisis produced by antimicrobial resistance (AMR) has led to the search for new compounds to provide alternative ways for the development of antibacterial agents that exploits mechanisms of action different from traditional antibiotics.

This report has reviewed the wide variety of peptide compounds produced by cyanobacteria and microalgae, both extremely versatile microorganisms, with activity against human and aquatic pathogens, their structural diversity, and their mechanisms of action.

Cyanobacteria produce a wide variety of peptides, due to their extraordinary synthetic plasticity, endowed by their capacity to synthesize not only ribosomal peptides, but also non-ribosomal and polyketide-associated peptides. Additionally, their adaptability makes them easily cultured in the laboratory, requiring a low amount of inorganic nutrients, producing different compounds under different experimental conditions.

In contrast, there is a scarce knowledge on how microalgae face bacterial infection and their antibacterial peptides involved. Despite the wide diversity of microalgal species, only few of them have been cultivated and explored for their biotechnological potential. The insight on their exploitation as an appealing source for novel antimicrobial peptides has just started.

A major advantage for many antimicrobial peptides is their low propensity to generate resistance, due to their multiple mechanisms of action, and the synergy among them. The implementation of cyanobacterial peptides as candidates for antibacterial drugs, required a careful and rational planning where several factors are involved; among these, the selection of the target bacteria, and MIC or MBC expected or required are essential. Concerning the first one, an especial effort is addressed for those species responsible for a higher a health risk against human health, mostly due to the rise of resistance and depletion of alternative antibiotics, according to WHO criteria [202] as those included in the group called ESKAPE [203]. Particular attention should be given for those peptides with a broad spectrum of activity. Concerning activity, an ideal compound should have an  $IC_{50}$  25  $\mu$ M or 10  $\mu$ g/mL, whereas for extracts at the initial steps of purification, a standard cut-off should be below 100  $\mu$ g/mL, similar to the criteria established for natural compounds [204–207]. In this review, we explored the peptides produced by cyanobacteria and microalgae, mainly as antibacterial, with a special focus on compounds with MIC values below those aforementioned (Table 3), as the most appealing candidates for their further pharmacological development and clinical implementation.

The impressive advances both in isolation and characterization of peptides runs parallel to the development of technologies devoted to these tasks. Among them, new and improved chemical synthesis of peptides, mass spectrometry, “omics” techniques, and bioinformatics tools for in silico selection and identification of feasible starting candidates are crucial. Furthermore, concerning antibacterial assays, the implementation of microfluidics and robotized assays, allow high-throughput screenings with a spare of the peptide required. Likewise, new strategies to improve the stability and bioavailability of peptides were reported, to curb these major shortcuts that jeopardizes the effectiveness of peptides as new drugs. Nowadays, very few peptides derived from cyanobacteria or microalgae overcome the initial steps of the pharmacological development, but it is likely that this is only tip of iceberg for a massive exploitation of these organisms as a new source of antibacterial compounds and a promising alternative for current antibiotics in the future.

**Author Contributions:** Conceptualization, V.R. and L.R.; formal analysis, V.R., L.R., C.C. and F.G.; writing—original draft preparation V.R., L.R., C.C. and F.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by FONDECYT 1170379. LR was supported by a grant (RICET RD16/0027/0010) from the Subdirección General de Redes y Centros de Investigación Cooperativa-FEDER.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## References

1. Hutchings, M.I.; Truman, A.W.; Wilkinson, B. Antibiotics: Past, present and future. *Curr. Opin. Microbiol.* **2019**, *51*, 72–80. [[CrossRef](#)]
2. Dadgostar, P. Antimicrobial Resistance: Implications and Costs. *Infect. Drug Resist.* **2019**, *12*, 3903–3910. [[CrossRef](#)]
3. Malik, B.; Bhattacharyya, S. Antibiotic drug-resistance as a complex system driven by socio-economic growth and antibiotic misuse. *Sci. Rep.* **2019**, *9*, 9788. [[CrossRef](#)] [[PubMed](#)]
4. Zhao, Y.; Yang, Q.E.; Zhou, X.; Wang, F.-H.; Muurinen, J.; Virta, M.P.; Brandt, K.K.; Zhu, Y.-G. Antibiotic resistome in the livestock and aquaculture industries: Status and solutions. *Crit. Rev. Environ. Sci. Technol.* **2020**, 1–38. [[CrossRef](#)]
5. Lulijwa, R.; Rupia, E.J.; Alfaro, A.C. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Rev. Aquac.* **2020**, *12*, 640–663. [[CrossRef](#)]
6. Anthony, E.T.; Ojemaye, M.O.; Okoh, O.O.; Okoh, A.I. A critical review on the occurrence of resistomes in the environment and their removal from wastewater using apposite treatment technologies: Limitations, successes and future improvement. *Environ. Pollut.* **2020**, *263*, 113791. [[CrossRef](#)] [[PubMed](#)]

7. Hassoun-Kheir, N.; Stabholz, Y.; Kreft, J.-U.; de la Cruz, R.; Romalde, J.L.; Nesme, J.; Sørensen, S.J.; Smets, B.F.; Graham, D.; Paul, M. Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. *Sci. Total Environ.* **2020**, *743*, 140804. [[CrossRef](#)] [[PubMed](#)]
8. Hernando-Amado, S.; Coque, T.M.; Baquero, F.; Martínez, J.L. Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nat. Microbiol.* **2019**, *4*, 1432–1442. [[CrossRef](#)]
9. Ogyu, A.; Chan, O.; Littmann, J.; Pang, H.H.; Lining, X.; Liu, P.; Matsunaga, N.; Ohmagari, N.; Fukuda, K.; Wernli, D. National action to combat AMR: A One-Health approach to assess policy priorities in action plans. *BMJ Glob. Health* **2020**, *5*, e002427. [[CrossRef](#)]
10. Beyer, P.; Paulin, S. Priority pathogens and the antibiotic pipeline: An update. *Bull. World Health Organ.* **2020**, *98*, 151. [[CrossRef](#)]
11. Butler, M.S.; Paterson, D.L. Antibiotics in the clinical pipeline in October 2019. *J. Antibiot.* **2020**, *73*, 329–364. [[CrossRef](#)] [[PubMed](#)]
12. Laxminarayan, R.; Van Boeckel, T.; Frost, I.; Kariuki, S.; Khan, E.A.; Limmathurotsakul, D.; Larsson, D.G.J.; Levy-Hara, G.; Mendelson, M.; Outterson, K.; et al. The Lancet Infectious Diseases Commission on antimicrobial resistance: 6 years later. *Lancet Infect. Dis.* **2020**, *20*, e51–e60. [[CrossRef](#)]
13. Cheng, Y.-S.; Williamson, P.R.; Zheng, W. Improving therapy of severe infections through drug repurposing of synergistic combinations. *Curr. Opin. Pharmacol.* **2019**, *48*, 92–98. [[CrossRef](#)] [[PubMed](#)]
14. Farha, M.A.; Brown, E.D. Drug repurposing for antimicrobial discovery. *Nat. Microbiol.* **2019**, *4*, 565–577. [[CrossRef](#)] [[PubMed](#)]
15. Sullivan, G.J.; Delgado, N.N.; Maharjan, R.; Cain, A.K. How antibiotics work together: Molecular mechanisms behind combination therapy. *Curr. Opin. Microbiol.* **2020**, *57*, 31–40. [[CrossRef](#)] [[PubMed](#)]
16. Tyers, M.; Wright, G.D. Drug combinations: A strategy to extend the life of antibiotics in the 21st century. *Nat. Rev. Microbiol.* **2019**, *17*, 141–155. [[CrossRef](#)] [[PubMed](#)]
17. Corsini, B.; Díez-Martínez, R.; Aguinagalde, L.; González-Camacho, F.; García-Fernández, E.; Letrado, P.; García, P.; Yuste, J. Chemotherapy with phage lysins reduces pneumococcal colonization of the respiratory tract. *Antimicrob. Agents Chemother.* **2018**, *62*, e02212–e02217. [[CrossRef](#)]
18. Ghosh, C.; Sarkar, P.; Issa, R.; Haldar, J. Alternatives to conventional antibiotics in the era of antimicrobial resistance. *Trends Microbiol.* **2019**, *27*, 323–338. [[CrossRef](#)]
19. Kiga, K.; Tan, X.-E.; Ibarra-Chávez, R.; Watanabe, S.; Aiba, Y.; Sato'o, Y.; Li, F.-Y.; Sasahara, T.; Cui, B.; Kawauchi, M.; et al. Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria. *Nat. Commun.* **2020**, *11*, 2934. [[CrossRef](#)]
20. Trudil, D. Phage lytic enzymes: A history. *Virolog. Sin.* **2015**, *30*, 26–32. [[CrossRef](#)]
21. Vila, J.; Moreno-Morales, J.; Ballesté-Delpierre, C. Current landscape in the discovery of novel antibacterial agents. *Clin. Microbiol. Infect.* **2020**, *26*, 596–603. [[CrossRef](#)] [[PubMed](#)]
22. Hunter, P. A war of attrition against antibiotic resistance. *EMBO Rep.* **2020**, *21*, e50807. [[CrossRef](#)] [[PubMed](#)]
23. Bhandari, D.; Rafiq, S.; Gat, Y.; Gat, P.; Waghmare, R.; Kumar, V. A review on bioactive peptides: Physiological functions, bioavailability and safety. *Int. J. Pept. Res. Ther.* **2020**, *26*, 139–150. [[CrossRef](#)]
24. Deslouches, B.; Montelaro, R.C.; Urish, K.L.; Di, Y.P. Engineered cationic antimicrobial peptides (eCAPs) to combat multidrug-resistant bacteria. *Pharmaceutics* **2020**, *12*, 501. [[CrossRef](#)] [[PubMed](#)]
25. Lazzaro, B.P.; Zasloff, M.; Rolff, J. Antimicrobial peptides: Application informed by evolution. *Science* **2020**, *368*, eaau5480. [[CrossRef](#)] [[PubMed](#)]
26. Magana, M.; Pushpanathan, M.; Santos, A.L.; Leanse, L.; Fernandez, M.; Ioannidis, A.; Giulianotti, M.A.; Apidianakis, Y.; Bradfute, S.; Ferguson, A.L.; et al. The value of antimicrobial peptides in the age of resistance. *Lancet Infect. Dis.* **2020**, *20*, e216–e230. [[CrossRef](#)]
27. Mookherjee, N.; Anderson, M.A.; Haagsman, H.P.; Davidson, D.J. Antimicrobial host defence peptides: Functions and clinical potential. *Nat. Rev. Drug Discov.* **2020**, *19*, 311–332. [[CrossRef](#)]
28. Newstead, L.L.; Varjonen, K.; Nuttall, T.; Paterson, G.K. Staphylococcal-produced bacteriocins and antimicrobial peptides: Their potential as alternative treatments for *Staphylococcus aureus* infections. *Antibiotics* **2020**, *9*, 40. [[CrossRef](#)]
29. Seyfi, R.; Kahaki, F.A.; Ebrahimi, T.; Montazersaheb, S.; Eyvazi, S.; Babaeipour, V.; Tarhriz, V. Antimicrobial Peptides (AMPs): Roles, functions and mechanism of action. *Int. J. Pept. Res. Ther.* **2020**, *26*, 1451–1463. [[CrossRef](#)]

30. Polcyn-Adamczak, M.; Niemir, Z.I. Cathelicidin—Its Structure, Function and the Role in Autoimmune Diseases. *Adv. Cell Biol.* **2014**, *4*, 83–96. [[CrossRef](#)]
31. Zhang, L.; Zhao, G.X.; Zhao, Y.Q.; Qiu, Y.T.; Chi, C.F.; Wang, B. Identification and active evaluation of antioxidant peptides from protein hydrolysates of Skipjack tuna (*Katsuwonus pelamis*) head. *Antioxidants* **2019**, *8*, 318. [[CrossRef](#)] [[PubMed](#)]
32. Holdbrook, D.A.; Huber, R.G.; Marzinek, J.K.; Stubbusch, A.; Schmidtchen, A.; Bond, P.J. Multiscale modeling of innate immune receptors: Endotoxin recognition and regulation by host defense peptides. *Pharmacol. Res.* **2019**, *147*, 104372. [[CrossRef](#)] [[PubMed](#)]
33. van der Does, A.M.; Hiemstra, P.S.; Mookherjee, N. Antimicrobial Host Defence Peptides: Immunomodulatory Functions and Translational Prospects. In *Advances in Experimental Medicine and Biology*; Springer New York LLC: New York, NY, USA, 2019; Volume 1117, pp. 149–171, ISBN 00652598.
34. Hancock, R.E.W.; Haney, E.F.; Gill, E.E. The immunology of host defence peptides: Beyond antimicrobial activity. *Nat. Rev. Immunol.* **2016**, *16*, 321–334. [[CrossRef](#)]
35. Hilchie, A.L.; Wuerth, K.; Hancock, R.E.W. Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. *Nat. Chem. Biol.* **2013**, *9*, 761–768. [[CrossRef](#)]
36. Lee, E.Y.; Lee, M.W.; Wong, G.C.L. Modulation of toll-like receptor signaling by antimicrobial peptides. *Semin. Cell Dev. Biol.* **2019**, *88*, 173–184. [[CrossRef](#)] [[PubMed](#)]
37. Van Harten, R.; van Woudenberg, E.; van Dijk, A.; Haagsman, H. Cathelicidins: Immunomodulatory Antimicrobials. *Vaccines* **2018**, *6*, 63. [[CrossRef](#)]
38. Xu, D.; Lu, W. Defensins: A double-edged sword in host immunity. *Front. Immunol.* **2020**, *11*, 764. [[CrossRef](#)] [[PubMed](#)]
39. De la Torre, B.G.; Albericio, F. Peptide Therapeutics 2.0. *Molecules* **2020**, *25*, 2293. [[CrossRef](#)] [[PubMed](#)]
40. Fosgerau, K.; Hoffmann, T. Peptide therapeutics: Current status and future directions. *Drug Discov. Today* **2015**, *20*, 122–128. [[CrossRef](#)]
41. Jad, Y.E.; Kumar, A.; El-Faham, A.; de la Torre, B.G.; Albericio, F. Green transformation of solid-phase peptide synthesis. *ACS Sustain. Chem. Eng.* **2019**, *7*, 3671–3683. [[CrossRef](#)]
42. Al Musaimi, O.; de la Torre, B.G.; Albericio, F. Greening Fmoc/ t Bu solid-phase peptide synthesis. *Green Chem.* **2020**, *22*, 996–1018. [[CrossRef](#)]
43. Albericio, F.; El-Faham, A. Choosing the right coupling reagent for peptides: A twenty-five-year journey. *Org. Process Res. Dev.* **2018**, *22*, 760–772. [[CrossRef](#)]
44. El-Faham, A.; Albericio, F. Carpino's protecting groups, beyond the Boc and the Fmoc. *Pept. Sci.* **2020**, *112*, e24164. [[CrossRef](#)]
45. Ramesh, S.; de la Torre, B.G.; Albericio, F.; Kruger, H.G.; Govender, T. Microwave-assisted synthesis of antimicrobial peptides. In *Methods in Molecular Biology*; Humana Press Inc.: Totova, NJ, USA, 2017; Volume 1548, pp. 51–59, ISBN 10643745.
46. Chow, H.Y.; Zhang, Y.; Matheson, E.; Li, X. Ligation technologies for the synthesis of cyclic peptides. *Chem. Rev.* **2019**, *119*, 9971–10001. [[CrossRef](#)] [[PubMed](#)]
47. Lee, A.C.-L.; Harris, J.L.; Khanna, K.K.; Hong, J.-H. A comprehensive review on current advances in peptide drug development and design. *Int. J. Mol. Sci.* **2019**, *20*, 2383. [[CrossRef](#)]
48. Gaglione, R.; Pane, K.; Dell'Olmo, E.; Cafaro, V.; Pizzo, E.; Olivieri, G.; Notomista, E.; Arciello, A. Cost-effective production of recombinant peptides in *Escherichia coli*. *New Biotechnol.* **2019**, *51*, 39–48. [[CrossRef](#)]
49. Kaur, N.; Dilawari, R.; Kaur, A.; Sahni, G.; Rishi, P. Recombinant expression, purification and PEGylation of Paneth cell peptide (cryptdin-2) with value added attributes against *Staphylococcus aureus*. *Sci. Rep.* **2020**, *10*, 12164. [[CrossRef](#)]
50. Sampaio de Oliveira, K.B.; Leite, M.L.; Rodrigues, G.R.; Duque, H.M.; da Costa, R.A.; Cunha, V.A.; de Loiola Costa, L.S.; da Cunha, N.B.; Franco, O.L.; Dias, S.C. Strategies for recombinant production of antimicrobial peptides with pharmacological potential. *Expert Rev. Clin. Pharmacol.* **2020**, *13*, 367–390. [[CrossRef](#)]
51. Wibowo, D.; Zhao, C.-X. Recent achievements and perspectives for large-scale recombinant production of antimicrobial peptides. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 659–671. [[CrossRef](#)]
52. Blaskovich, M.A.T. Unusual amino acids in medicinal chemistry. *J. Med. Chem.* **2016**, *59*, 10807–10836. [[CrossRef](#)]
53. Yao, J.-F.; Yang, H.; Zhao, Y.-Z.; Xue, M. Metabolism of peptide drugs and strategies to improve their metabolic stability. *Curr. Drug Metab.* **2018**, *19*, 892–901. [[CrossRef](#)] [[PubMed](#)]



54. Bandala, Y.; Juaristi, E. Applications of  $\beta$ -Peptides in Chemistry, Biology, and Medicine. In *New Trends in Statistical Physics*; World Scientific: Singapore, 2010; pp. 183–198, ISBN 9789814307543.
55. Shi, Y.; Teng, P.; Sang, P.; She, F.; Wei, L.; Cai, J.  $\gamma$ -AApeptides: Design, structure, and applications. *Acc. Chem. Res.* **2016**, *49*, 428–441. [[CrossRef](#)] [[PubMed](#)]
56. Haney, E.F.; Hancock, R.E.W. Peptide design for antimicrobial and immunomodulatory applications. *Biopolymers* **2013**, *100*, 572–583. [[CrossRef](#)] [[PubMed](#)]
57. Miao, X.; Zhou, T.; Zhang, J.; Xu, J.; Guo, X.; Hu, H.; Zhang, X.; Hu, M.; Li, J.; Yang, W.; et al. Enhanced cell selectivity of hybrid peptides with potential antimicrobial activity and immunomodulatory effect. *Biochim. Biophys. Acta Gen. Subj.* **2020**, *1864*, 129532. [[CrossRef](#)]
58. Kuppusamy, R.; Willcox, M.; Black, D.S.; Kumar, N. Short cationic peptidomimetic antimicrobials. *Antibiotics* **2019**, *8*, 44. [[CrossRef](#)]
59. Mojsoska, B.; Jenssen, H. Peptides and peptidomimetics for antimicrobial drug design. *Pharmaceuticals* **2015**, *8*, 366–415. [[CrossRef](#)]
60. Qvit, N.; Rubin, S.J.S.; Urban, T.J.; Mochly-Rosen, D.; Gross, E.R. Peptidomimetic therapeutics: Scientific approaches and opportunities. *Drug Discov. Today* **2017**, *22*, 454–462. [[CrossRef](#)]
61. Yang, W.; Gadgil, P.; Krishnamurthy, V.R.; Landis, M.; Mallick, P.; Patel, D.; Patel, P.J.; Reid, D.L.; Sanchez-Felix, M. The evolving druggability and developability space: Chemically modified new modalities and emerging small molecules. *AAPS J.* **2020**, *22*, 21. [[CrossRef](#)]
62. Jing, X.; Jin, K. A gold mine for drug discovery: Strategies to develop cyclic peptides into therapies. *Med. Res. Rev.* **2020**, *40*, 753–810. [[CrossRef](#)]
63. Reguera, L.; Rivera, D.G. Multicomponent reaction toolbox for peptide macrocyclization and stapling. *Chem. Rev.* **2019**, *119*, 9836–9860. [[CrossRef](#)]
64. Cromm, P.M.; Spiegel, J.; Grossmann, T.N. Hydrocarbon stapled peptides as modulators of biological function. *ACS Chem. Biol.* **2015**, *10*, 1362–1375. [[CrossRef](#)] [[PubMed](#)]
65. Moorcroft, S.C.T.; Roach, L.; Jayne, D.G.; Ong, Z.Y.; Evans, S.D. Nanoparticle-loaded hydrogel for the light-activated release and photothermal enhancement of antimicrobial peptides. *ACS Appl. Mater. Interfaces* **2020**, *12*, 24544–24554. [[CrossRef](#)] [[PubMed](#)]
66. Parilti, R.; Caprasse, J.; Riva, R.; Alexandre, M.; Vandegaart, H.; Bebrone, C.; Dupont-Gillain, C.; Howdle, S.M.; Jérôme, C. Antimicrobial peptide encapsulation and sustained release from polymer network particles prepared in supercritical carbon dioxide. *J. Colloid Interface Sci.* **2018**, *532*, 112–117. [[CrossRef](#)] [[PubMed](#)]
67. Radaic, A.; de Jesus, M.B.; Kapila, Y.L. Bacterial anti-microbial peptides and nano-sized drug delivery systems: The state of the art toward improved bacteriocins. *J. Control. Release* **2020**, *321*, 100–118. [[CrossRef](#)] [[PubMed](#)]
68. Santos, R.S.; Figueiredo, C.; Azevedo, N.F.; Braeckmans, K.; De Smedt, S.C. Nanomaterials and molecular transporters to overcome the bacterial envelope barrier: Towards advanced delivery of antibiotics. *Adv. Drug Deliv. Rev.* **2018**, *136–137*, 28–48. [[CrossRef](#)]
69. Arif, M.; Ahmad, S.; Ali, F.; Fang, G.; Li, M.; Yu, D.-J. TargetCPP: Accurate prediction of cell-penetrating peptides from optimized multi-scale features using gradient boost decision tree. *J. Comput. Aided Mol. Des.* **2020**, *34*, 841–856. [[CrossRef](#)]
70. Cardoso, M.H.; Orozco, R.Q.; Rezende, S.B.; Rodrigues, G.; Oshiro, K.G.N.; Cândido, E.S.; Franco, O.L. Computer-Aided design of antimicrobial peptides: Are we generating effective drug candidates? *Front. Microbiol.* **2020**, *10*, 3097. [[CrossRef](#)]
71. Minami, A.; Ugai, T.; Ozaki, T.; Oikawa, H. Predicting the chemical space of fungal polyketides by phylogeny-based bioinformatics analysis of polyketide synthase-nonribosomal peptide synthetase and its modification enzymes. *Sci. Rep.* **2020**, *10*, 13556. [[CrossRef](#)]
72. Pupin, M.; Esmaeel, Q.; Flissi, A.; Dufresne, Y.; Jacques, P.; Leclère, V. Norine: A powerful resource for novel nonribosomal peptide discovery. *Synth. Syst. Biotechnol.* **2016**, *1*, 89–94. [[CrossRef](#)]
73. Timmons, P.B.; Hewage, C.M. HAPPENN is a novel tool for hemolytic activity prediction for therapeutic peptides which employs neural networks. *Sci. Rep.* **2020**, *10*, 10869. [[CrossRef](#)]
74. Koo, H.B.; Seo, J. Antimicrobial peptides under clinical investigation. *Pept. Sci.* **2019**, *111*, e24122. [[CrossRef](#)]
75. Bahrami, A.; Delshadi, R.; Jafari, S.M.; Williams, L. Nanoencapsulated nisin: An engineered natural antimicrobial system for the food industry. *Trends Food Sci. Technol.* **2019**, *94*, 20–31. [[CrossRef](#)]



76. Santos, J.C.P.; Sousa, R.C.S.; Otoni, C.G.; Moraes, A.R.F.; Souza, V.G.L.; Medeiros, E.A.A.; Espitia, P.J.P.; Pires, A.C.S.; Coimbra, J.S.R.; Soares, N.F.F. Nisin and other antimicrobial peptides: Production, mechanisms of action, and application in active food packaging. *Innov. Food Sci. Emerg. Technol.* **2018**, *48*, 179–194. [[CrossRef](#)]
77. Hancock, R.E. Peptide antibiotics. *Lancet* **1997**, *349*, 418–422. [[CrossRef](#)]
78. Fodor, A.; Abate, B.A.; Deák, P.; Fodor, L.; Gyenge, E.; Klein, M.G.; Koncz, Z.; Muvevi, J.; Ötvös, L.; Székely, G.; et al. Multidrug Resistance (MDR) and collateral sensitivity in bacteria, with special attention to genetic and evolutionary aspects and to the perspectives of antimicrobial peptides—A review. *Pathogens* **2020**, *9*, 522. [[CrossRef](#)]
79. Maria-Neto, S.; de Almeida, K.C.; Macedo, M.L.R.; Franco, O.L. Understanding bacterial resistance to antimicrobial peptides: From the surface to deep inside. *Biochim. Biophys. Acta Biomembr.* **2015**, *1848*, 3078–3088. [[CrossRef](#)]
80. Nawrocki, K.; Crispell, E.; McBride, S. Antimicrobial peptide resistance mechanisms of gram-positive bacteria. *Antibiotics* **2014**, *3*, 461–492. [[CrossRef](#)]
81. Kaye, K.S.; Pogue, J.M.; Tran, T.B.; Nation, R.L.; Li, J. Agents of Last Resort. *Infect. Dis. Clin. N. Am.* **2016**, *30*, 391–414. [[CrossRef](#)]
82. Nang, S.C.; Li, J.; Velkov, T. The rise and spread of mcr plasmid-mediated polymyxin resistance. *Crit. Rev. Microbiol.* **2019**, *45*, 131–161. [[CrossRef](#)]
83. Kini, S.; Divyashree, M.; Mani, M.K.; Mamatha, B.S. Algae and cyanobacteria as a source of novel bioactive compounds for biomedical applications. In *Advances in Cyanobacterial Biology*; Singh, P.K., Kumar, A., Singh, V.K., Shrivastava, A.K., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 173–194, ISBN 9780128193112.
84. Falaise, C.; François, C.; Travers, M.-A.; Morga, B.; Haure, J.; Tremblay, R.; Turcotte, F.; Pasetto, P.; Gastineau, R.; Hardivillier, Y.; et al. Antimicrobial Compounds from Eukaryotic Microalgae against Human Pathogens and Diseases in Aquaculture. *Mar. Drugs* **2016**, *14*, 159. [[CrossRef](#)]
85. Shah, S.; Akhter, N.; Auckloo, B.; Khan, I.; Lu, Y.; Wang, K.; Wu, B.; Guo, Y.-W. Structural diversity, biological properties and applications of natural products from cyanobacteria. A review. *Mar. Drugs* **2017**, *15*, 354. [[CrossRef](#)] [[PubMed](#)]
86. Hauer, T.; Komárek, J. CyanoDB 2.0—Online Database of Cyanobacterial Genera. Available online: <http://www.cyanodb.cz/> (accessed on 20 November 2020).
87. Gaysina, L.A.; Saraf, A.; Singh, P. Cyanobacteria in diverse habitats. In *Cyanobacteria*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 1–28, ISBN 9780128146682.
88. Swain, S.S.; Paidasetty, S.K.; Padhy, R.N. Antibacterial, antifungal and antimycobacterial compounds from cyanobacteria. *Biomed. Pharmacother.* **2017**, *90*, 760–776. [[CrossRef](#)] [[PubMed](#)]
89. Senhorinho, G.N.A.; Ross, G.M.; Scott, J.A. Cyanobacteria and eukaryotic microalgae as potential sources of antibiotics. *Phycologia* **2015**, *54*, 271–282. [[CrossRef](#)]
90. Pradhan, J.; Das, S.; Das, B.K. Antibacterial activity of freshwater microalgae: A review. *Afr. J. Pharm. Pharmacol.* **2014**, *8*, 809–818.
91. Fan, M.; Liao, Z.; Wang, R.X.; Xu, N. Isolation and antibacterial activity of anabaena phycocyanin. *Afr. J. Biotechnol.* **2013**, *12*, 1869–1873.
92. Nagarajan, M.; Maruthanayagam, V.; Sundararaman, M. SAR analysis and bioactive potentials of freshwater and terrestrial cyanobacterial compounds: A review. *J. Appl. Toxicol.* **2013**, *33*, 313–349. [[CrossRef](#)]
93. Singh, R.K.; Tiwari, S.P.; Rai, A.K.; Mohapatra, T.M. Cyanobacteria: An emerging source for drug discovery. *J. Antibiot.* **2011**, *64*, 401–412. [[CrossRef](#)]
94. Burja, A.M.; Banaigs, B.; Abou-Mansour, E.; Grant Burgess, J.; Wright, P.C. Marine cyanobacteria—A prolific source of natural products. *Tetrahedron* **2001**, *57*, 9347–9377. [[CrossRef](#)]
95. Moore, R.E.; Corbett, T.H.; Patterson, G.M.L.; Valeriote, F.A. The search for new antitumor drugs from blue-green algae. *Curr. Pharm. Des.* **1996**, *2*, 317–330.
96. Lévassieur, W.; Perré, P.; Pozzobon, V. A review of high value-added molecules production by microalgae in light of the classification. *Biotechnol. Adv.* **2020**, *41*, 107545. [[CrossRef](#)]
97. Guiry, M.D.; Guiry, G.M. AlgaeBase. Available online: <https://www.algaebase.org> (accessed on 10 October 2020).
98. Han, P.; Lu, Q.; Fan, L.; Zhou, W. A review on the use of microalgae for sustainable aquaculture. *Appl. Sci.* **2019**, *9*, 2377. [[CrossRef](#)]

99. Duran, S.K.; Kumar, P.; Sandhu, S.S. A review on microalgae strains, cultivation, harvesting, biodiesel conversion and engine implementation. *Biofuels* **2018**, 1–12. [[CrossRef](#)]
100. Boukhris, S.; Athmouni, K.; Hamza-Mnif, I.; Siala-Elleuch, R.; Ayadi, H.; Nasri, M.; Sellami-Kamoun, A. The potential of a brown microalga cultivated in high salt medium for the production of high-value compounds. *Biomed Res. Int.* **2017**, 2017, 4018562. [[CrossRef](#)] [[PubMed](#)]
101. Sasso, S.; Pohnert, G.; Lohr, M.; Mittag, M.; Hertweck, C. Microalgae in the postgenomic era: A blooming reservoir for new natural products. *FEMS Microbiol. Rev.* **2012**, 36, 761–785. [[CrossRef](#)] [[PubMed](#)]
102. Heimann, K.; Huerlimann, R. Microalgal Classification. In *Handbook of Marine Microalgae*; Kim, S.-K., Ed.; Elsevier: Amsterdam, The Netherlands, 2015; pp. 25–41. ISBN 9780128011249.
103. Pratt, R.; Daniels, T.C.; Eiler, J.J.; Gunnison, J.B.; Kumler, W.D.; Oneto, J.F.; Strait, L.A.; Spoehr, H.A.; Hardin, G.J.; Milner, H.W.; et al. Chlorellin, an antibacterial substance from Chlorella. *Science* **1944**, 99, 351–352. [[CrossRef](#)] [[PubMed](#)]
104. Amaro, H.; Guedes, A.; Malcata, F. Antimicrobial activities of microalgae: An invited review. In *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*; Méndez-Vilas, A., Ed.; Formatex Research Center: Badajoz, Spain, 2011; pp. 1272–1280. ISBN 8493984329.
105. Santoyo, S.; Rodríguez-Meizoso, I.; Cifuentes, A.; Jaime, L.; García-Blairsy Reina, G.; Señorans, F.J.; Ibáñez, E. Green processes based on the extraction with pressurized fluids to obtain potent antimicrobials from *Haematococcus pluvialis* microalgae. *LWT Food Sci. Technol.* **2009**, 42, 1213–1218. [[CrossRef](#)]
106. Duff, D.; Bruce, D.; Antia, N. The Antibacterial Activity of Marine Planktonic Algae. *Can. J. Microbiol.* **1966**, 12, 877–884. [[CrossRef](#)] [[PubMed](#)]
107. Shannon, E.; Abu-Ghannam, N. Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications. *Mar. Drugs* **2016**, 14, 81. [[CrossRef](#)] [[PubMed](#)]
108. Bashir, K.M.I.; Lee, J.H.; Petermann, M.J.; Shah, A.A.; Jeong, S.J.; Kim, M.S.; Park, N.G.; Cho, M.G. Estimation of antibacterial properties of chlorophyta, rhodophyta and haptophyta microalgae species. *Microbiol. Biotechnol. Lett.* **2018**, 46, 225–233. [[CrossRef](#)]
109. Lauritano, C.; Martín, J.; de la Cruz, M.; Reyes, F.; Romano, G.; Ianora, A. First identification of marine diatoms with anti-tuberculosis activity. *Sci. Rep.* **2018**, 8, 2284. [[CrossRef](#)]
110. Borowitzka, M.A. High-value products from microalgae—Their development and commercialisation. *J. Appl. Phycol.* **2013**, 25, 743–756. [[CrossRef](#)]
111. Singh, R.; Parihar, P.; Singh, M.; Bajguz, A.; Kumar, J.; Singh, S.; Singh, V.P.; Prasad, S.M. Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: Current status and future prospects. *Front. Microbiol.* **2017**, 8, 515. [[CrossRef](#)] [[PubMed](#)]
112. Volk, R.B.; Furkert, F.H. Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. *Microbiol. Res.* **2006**, 161, 180–186. [[CrossRef](#)] [[PubMed](#)]
113. Dussault, D.; Vu, K.D.; Vansach, T.; Horgen, F.D.; Lacroix, M. Antimicrobial effects of marine algal extracts and cyanobacterial pure compounds against five foodborne pathogens. *Food Chem.* **2016**, 199, 114–118. [[CrossRef](#)]
114. Najdenski, H.M.; Gigova, L.G.; Iliev, I.I.; Pilarski, P.S.; Lukavský, J.; Tsvetkova, I.V.; Ninova, M.S.; Kussovski, V.K. Antibacterial and antifungal activities of selected microalgae and cyanobacteria. *Int. J. Food Sci. Technol.* **2013**, 48, 1533–1540. [[CrossRef](#)]
115. Sarada, D.V.L.; Kumar, C.S.; Rengasamy, R. Purified C-phycoyanin from *Spirulina platensis* (Nordstedt) Geitler: A novel and potent agent against drug resistant bacteria. *World J. Microbiol. Biotechnol.* **2011**, 27, 779–783. [[CrossRef](#)]
116. Ghasemi, Y.; Tabatabaei Yazdi, M.; Shafiee, A.; Amini, M.; Shokravi, S.; Zarrini, G. Parsiguine, a novel antimicrobial substance from *Fischerella ambigua*. *Pharm. Biol.* **2004**, 42, 318–322. [[CrossRef](#)]
117. Kubota, T.; Iwai, T.; Sakai, K.; Gono, T.; Kobayashi, J. Amphidinins C-F, amphidinolide Q analogues from marine dinoflagellate *Amphidinium* sp. *Org. Lett.* **2014**, 16, 5624–5627. [[CrossRef](#)]
118. Janssen, E.M.-L. Cyanobacterial peptides beyond microcystins—A review on co-occurrence, toxicity, and challenges for risk assessment. *Water Res.* **2019**, 151, 488–499. [[CrossRef](#)]
119. Anjum, K.; Abbas, S.Q.; Akhter, N.; Shagufta, B.I.; Shah, S.A.A.; Hassan, S.S. ul Emerging biopharmaceuticals from bioactive peptides derived from marine organisms. *Chem. Biol. Drug Des.* **2017**, 90, 12–30. [[CrossRef](#)]
120. Fidor, A.; Konkel, R.; Mazur-Marzec, H. Bioactive peptides produced by cyanobacteria of the genus *Nostoc*: A review. *Mar. Drugs* **2019**, 17, 561. [[CrossRef](#)]

121. Fotie, J. The potential of peptides and depsipeptides from terrestrial and marine organisms in the fight against human protozoan diseases. In *Bioactive Natural Products*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2015; pp. 279–320, ISBN 9783527684403.
122. Raja, R.; Hemaiswarya, S.; Ganesan, V.; Carvalho, I.S. Recent developments in therapeutic applications of Cyanobacteria. *Crit. Rev. Microbiol.* **2015**, *42*, 394–405. [[CrossRef](#)]
123. Rivas, L.; Rojas, V. Cyanobacterial peptides as a tour de force in the chemical space of antiparasitic agents. *Arch. Biochem. Biophys.* **2019**, *664*, 24–39. [[CrossRef](#)]
124. Xu, J.; Zhang, T.; Yao, J.; Lu, J.; Liu, Z.; Ding, L. Recent advances in chemistry and bioactivity of marine cyanobacteria Moorea species. *Eur. J. Med. Chem.* **2020**, *201*, 112473. [[CrossRef](#)]
125. Xue, Y.; Zhao, P.; Quan, C.; Zhao, Z.; Gao, W.; Li, J.; Zu, X.; Fu, D.; Feng, S.; Bai, X.; et al. Cyanobacteria-derived peptide antibiotics discovered since 2000. *Peptides* **2018**, *107*, 17–24. [[CrossRef](#)]
126. Zainuddin, E.N.; Jansen, R.; Nimtz, M.; Wray, V.; Preisitsch, M.; Lalk, M.; Mundt, S. Lyngbyazothrins A–D, Antimicrobial Cyclic Undecapeptides from the Cultured Cyanobacterium *Lyngbya* sp. *J. Nat. Prod.* **2009**, *72*, 2080. [[CrossRef](#)]
127. Mundt, S.; Bui, H.; Preisitsch, M.; Kreitlow, S.; Bui, H.; Pham, H.; Zainuddin, E.; Le, T.; Lukowski, G.; Jülich, W. Microalgae—A promising source of novel therapeutics. *JSM Biotechnol. Biomed. Eng.* **2014**, *2*, 1032.
128. Helms, G.L.; Moore, R.E.; Niemczura, W.P.; Patterson, G.M.L.; Tomer, K.B.; Gross, M.L. Scytonemin A, a novel calcium antagonist from a blue-green alga. *J. Org. Chem.* **1988**, *53*, 1298–1307. [[CrossRef](#)]
129. Nagatsu, A.; Kajitani, H.; Sakakibara, J. Muscoride A: A new oxazole peptide alkaloid from freshwater cyanobacterium *Nostoc muscorum*. *Tetrahedron Lett.* **1995**, *36*, 4097–4100. [[CrossRef](#)]
130. Banker, R.; Carmeli, S. Tenuencyclamides A–D, Cyclic Hexapeptides from the Cyanobacterium *Nostoc spongiaeforme* var. *tenu*. *J. Nat. Prod.* **1998**, *61*, 1248–1251. [[CrossRef](#)]
131. Liu, L.; Rein, K.S. New peptides isolated from *Lyngbya* species: A review. *Mar. Drugs* **2010**, *8*, 1817–1837. [[CrossRef](#)]
132. Antunes, J.; Pereira, S.; Ribeiro, T.; Plowman, J.E.; Thomas, A.; Clerens, S.; Campos, A.; Vasconcelos, V.; Almeida, J.R. A multi-bioassay integrated approach to assess the antifouling potential of the cyanobacterial metabolites portoamides. *Mar. Drugs* **2019**, *17*, 111. [[CrossRef](#)]
133. Dittmann, E.; Gugger, M.; Sivonen, K.; Fewer, D.P. Natural product biosynthetic diversity and comparative genomics of the cyanobacteria. *Trends Microbiol.* **2015**, *23*, 642–652. [[CrossRef](#)]
134. Kleigrew, K.; Gerwick, L.; Sherman, D.H.; Gerwick, W.H. Unique marine derived cyanobacterial biosynthetic genes for chemical diversity. *Nat. Prod. Rep.* **2016**, *33*, 348–364. [[CrossRef](#)]
135. Galica, T.; Hrouzek, P.; Mareš, J. Genome mining reveals high incidence of putative lipopeptide biosynthesis NRPS/PKS clusters containing fatty acyl-AMP ligase genes in biofilm-forming cyanobacteria. *J. Phycol.* **2017**, *53*, 985–998. [[CrossRef](#)]
136. Micalef, M.L.; D’Agostino, P.M.; Sharma, D.; Viswanathan, R.; Moffitt, M.C. Genome mining for natural product biosynthetic gene clusters in the Subsection V cyanobacteria. *BMC Genom.* **2015**, *16*, 669. [[CrossRef](#)]
137. Silva-Stenico, M.E.; Silva, C.S.P.; Lorenzi, A.S.; Shishido, T.K.; Etchegaray, A.; Lira, S.P.; Moraes, L.A.B.; Fiore, M.F. Non-ribosomal peptides produced by Brazilian cyanobacterial isolates with antimicrobial activity. *Microbiol. Res.* **2011**, *166*, 161–175. [[CrossRef](#)]
138. Barboza, G.; Gorch-Lira, K.; Sassi, C.; Sassi, R. Microcystins production and antibacterial activity of cyanobacterial strains of *Synechocystis*, *Synechococcus* and *Romeria* isolated from water and coral reef organisms of Brazilian coast. *Rev. Biol. Trop.* **2017**, *65*, 890. [[CrossRef](#)]
139. Gademann, K.; Bethuel, Y.; Locher, H.H.; Hubschwerlen, C. Biomimetic Total Synthesis and Antimicrobial Evaluation of Anachelin H. *J. Org. Chem.* **2007**, *72*, 8361–8370. [[CrossRef](#)]
140. Ishida, K.; Matsuda, H.; Murakami, M.; Yamaguchi, K. Kawaguchipectin B, an antibacterial cyclic undecapeptide from the cyanobacterium *Microcystis aeruginosa*. *J. Nat. Prod.* **1997**, *60*, 724–726. [[CrossRef](#)]
141. Sadeghi, S.; Jalili, H.; Ranaei Siadat, S.O.; Sedighi, M. Anticancer and antibacterial properties in peptide fractions from hydrolyzed spirulina protein. *J. Agric. Sci. Technol.* **2018**, *20*, 673–683.
142. Gogineni, V.; Hamann, M.T. Marine natural product peptides with therapeutic potential: Chemistry, biosynthesis, and pharmacology. *Biochim. Biophys. Acta Gen. Subj.* **2018**, *1862*, 81–196. [[CrossRef](#)] [[PubMed](#)]
143. Pergament, I.; Carmeli, S. Schizotrin A; a novel antimicrobial cyclic peptide from a cyanobacterium. *Tetrahedron Lett.* **1994**, *35*, 8473–8476. [[CrossRef](#)]

144. Arif, J.M.; Farooqui, A.; Siddiqui, M.H.; Al-Karrawi, M.; Al-Hazmi, A.; Al-Sagair, O.A. Novel Bioactive Peptides from Cyanobacteria. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier Science: Amsterdam, The Netherlands, 2012; pp. 111–161, ISBN 9780444538369.
145. Berland, B.R.; Bonin, D.J.; Cornu, A.L.; Maestrini, S.Y.; Marino, J.-P. The antibacterial substances of the marine alga *Stichochrysis immobilis* (Chrysophyta). *J. Phycol.* **1972**, *8*, 383–392. [[CrossRef](#)]
146. Sedighi, M.; Jalili, H.; Darvish, M.; Sadeghi, S.; Ranaei-Siadat, S.-O. Enzymatic hydrolysis of microalgae proteins using serine proteases: A study to characterize kinetic parameters. *Food Chem.* **2019**, *284*, 334–339. [[CrossRef](#)]
147. Tejano, L.A.; Peralta, J.P.; Yap, E.E.S.; Panjaitan, F.C.A.; Chang, Y.W. Prediction of bioactive peptides from *Chlorella sorokiniana* proteins using proteomic techniques in combination with bioinformatics analyses. *Int. J. Mol. Sci.* **2019**, *20*, 1786. [[CrossRef](#)]
148. Guzmán, F.; Wong, G.; Román, T.; Cárdenas, C.; Álvarez, C.; Schmitt, P.; Albericio, F.; Rojas, V. Identification of antimicrobial peptides from the microalgae *Tetraselmis suecica* (Kylin) Butcher and bactericidal activity improvement. *Mar. Drugs* **2019**, *17*, 453. [[CrossRef](#)]
149. Li, S.S.; Tsai, H.J. Transgenic microalgae as a non-antibiotic bactericide producer to defend against bacterial pathogen infection in the fish digestive tract. *Fish Shellfish Immunol.* **2009**, *26*, 316–325. [[CrossRef](#)]
150. Lauritano, C.; Andersen, J.H.; Hansen, E.; Albrigtsen, M.; Escalera, L.; Esposito, F.; Helland, K.; Hanssen, K.; Romano, G.; Ianora, A. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. *Front. Mar. Sci.* **2016**, *3*, 68. [[CrossRef](#)]
151. Tejano, L.A.; Peralta, J.P.; Yap, E.E.S.; Chang, Y. Bioactivities of enzymatic protein hydrolysates derived from *Chlorella sorokiniana*. *Food Sci. Nutr.* **2019**, *7*, 2381–2390. [[CrossRef](#)]
152. Cassone, M.; Otvos, L., Jr. Synergy among antibacterial peptides and between peptides and small-molecule antibiotics. *Expert Rev. Anti. Infect. Ther.* **2010**, *8*, 703–716. [[CrossRef](#)] [[PubMed](#)]
153. Hanson, M.A.; Dostálová, A.; Ceroni, C.; Poidevin, M.; Kondo, S.; Lemaitre, B. Synergy and remarkable specificity of antimicrobial peptides in vivo using a systematic knockout approach. *eLife* **2019**, *8*, e44341. [[CrossRef](#)] [[PubMed](#)]
154. Zharkova, M.S.; Orlov, D.S.; Golubeva, O.Y.; Chakchir, O.B.; Eliseev, I.E.; Grinchuk, T.M.; Shamova, O.V. Application of antimicrobial peptides of the innate immune system in combination with conventional antibiotics—A novel way to combat antibiotic resistance? *Front. Cell. Infect. Microbiol.* **2019**, *9*, 128. [[CrossRef](#)] [[PubMed](#)]
155. Mathur, H.; Field, D.; Rea, M.C.; Cotter, P.D.; Hill, C.; Ross, R.P. Bacteriocin-Antimicrobial Synergy: A Medical and Food Perspective. *Front. Microbiol.* **2017**, *8*, 1205. [[CrossRef](#)] [[PubMed](#)]
156. Zerweck, J.; Strandberg, E.; Kukhareno, O.; Reichert, J.; Bürck, J.; Wadhvani, P.; Ulrich, A.S. Molecular mechanism of synergy between the antimicrobial peptides PGLa and magainin 2. *Sci. Rep.* **2017**, *7*, 13153. [[CrossRef](#)] [[PubMed](#)]
157. Doolin, T.; Amir, H.M.; Duong, L.; Rosenzweig, R.; Urban, L.A.; Bosch, M.; Pol, A.; Gross, S.P.; Siryaporn, A. Mammalian histones facilitate antimicrobial synergy by disrupting the bacterial proton gradient and chromosome organization. *Nat. Commun.* **2020**, *11*, 3888. [[CrossRef](#)]
158. Mansour, S.C.; de la Fuente-Núñez, C.; Hancock, R.E.W. Peptide IDR-1018: Modulating the immune system and targeting bacterial biofilms to treat antibiotic-resistant bacterial infections. *J. Pept. Sci.* **2015**, *21*, 323–329. [[CrossRef](#)] [[PubMed](#)]
159. Leão, P.N.; Pereira, A.R.; Liu, W.T.; Ng, J.; Pevzner, P.A.; Dorrestein, P.C.; König, G.M.; Vasconcelos, V.M.; Gerwick, W.H. Synergistic allelochemicals from a freshwater cyanobacterium. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 11183–11188. [[CrossRef](#)]
160. Rohrlack, T.; Christiansen, G.; Kurmayer, R. Putative antiparasite defensive system involving ribosomal and nonribosomal oligopeptides in cyanobacteria of the genus planktothrix. *Appl. Environ. Microbiol.* **2013**, *79*, 2642–2647. [[CrossRef](#)]
161. MacMillan, J.B.; Ernst-Russell, M.A.; de Ropp, J.S.; Molinski, T.F. Lobocyclamides A–C, lipopeptides from a cryptic cyanobacterial mat containing *Lyngbya confervoides*. *J. Org. Chem.* **2002**, *67*, 8210–8215. [[CrossRef](#)]
162. Bonnard, I.; Rolland, M.; Salmon, J.-M.; Debiton, E.; Barthomeuf, C.; Banaigs, B. Total structure and inhibition of tumor cell proliferation of laxaphycins. *J. Med. Chem.* **2007**, *50*, 1266–1279. [[CrossRef](#)] [[PubMed](#)]



163. Frankmölle, W.P.; Larsen, L.K.; Caplan, F.R.; Patterson, G.M.L.; Knübel, G.; Levine, I.A.; Moore, R.E. Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa*. I. Isolation and biological properties. *J. Antibiot.* **1992**, *45*, 1451–1457. [[CrossRef](#)] [[PubMed](#)]
164. Cai, W.; Matthew, S.; Chen, Q.-Y.; Paul, V.J.; Luesch, H. Discovery of new A- and B-type laxaphycins with synergistic anticancer activity. *Bioorg. Med. Chem.* **2018**, *26*, 2310–2319. [[CrossRef](#)] [[PubMed](#)]
165. Ko, S.-C.; Heo, S.-Y.; Choi, S.-W.; Qian, Z.-J.; Heo, S.-J.; Kang, D.-H.; Kim, N.; Jung, W.-K. A heptameric peptide isolated from the marine microalga *Pavlova lutheri* suppresses PMA-induced secretion of matrix metalloproteinase-9 through the inactivation of the JNK, p38, and NF- $\kappa$ B pathways in human fibrosarcoma cells. *J. Appl. Phycol.* **2018**, *30*, 2367–2378. [[CrossRef](#)]
166. Qian, Z.; Ryu, B.; Kang, K.; Heo, S.; Kang, D.; Bae, S.; Park, S.; Kim, J.; Kim, Y.; Kim, Y.; et al. Cellular properties of the fermented microalgae *Pavlova lutheri* and its isolated active peptide in osteoblastic differentiation of MG-63 cells. *Mol. Med. Rep.* **2017**, *17*, 2044–2050.
167. Heo, S.Y.; Ko, S.C.; Kim, C.S.; Oh, G.W.; Ryu, B.; Qian, Z.J.; Kim, G.; Park, W.S.; Choi, I.W.; Phan, T.T.V.; et al. A heptameric peptide purified from *Spirulina* sp. gastrointestinal hydrolysate inhibits angiotensin I-converting enzyme- and angiotensin II-induced vascular dysfunction in human endothelial cells. *Int. J. Mol. Med.* **2017**, *39*, 1072–1082. [[CrossRef](#)]
168. Carrizzo, A.; Conte, G.M.; Sommella, E.; Damato, A.; Ambrosio, M.; Sala, M.; Scala, M.C.; Aquino, R.P.; De Lucia, M.; Madonna, M.; et al. Novel potent decameric peptide of *Spirulina platensis* reduces blood pressure levels through a PI3K/AKT/eNOS-dependent mechanism. *Hypertension* **2019**, *73*, 449–457. [[CrossRef](#)]
169. Suetsuna, K.; Chen, J.R. Identification of antihypertensive peptides from peptic digest of two microalgae, *Chlorella vulgaris* and *Spirulina platensis*. *Mar. Biotechnol.* **2001**, *3*, 305–309. [[CrossRef](#)]
170. Lin, Y.H.; Chen, G.W.; Yeh, C.H.; Song, H.; Tsai, J.S. Purification and identification of angiotensin I-Converting enzyme inhibitory peptides and the antihypertensive effect of *Chlorella sorokiniana* protein hydrolysates. *Nutrients* **2018**, *10*, 1397. [[CrossRef](#)]
171. Wu, H.; Xu, N.; Sun, X.; Yu, H.; Zhou, C. Hydrolysis and purification of ACE inhibitory peptides from the marine microalga *Isochrysis galbana*. *J. Appl. Phycol.* **2015**, *27*, 351–361. [[CrossRef](#)]
172. Samarakoon, K.W.; O-Nam, K.; Ko, J.-Y.; Lee, J.-H.; Kang, M.-C.; Kim, D.; Lee, J.B.; Lee, J.-S.; Jeon, Y.-J. Purification and identification of novel angiotensin-I converting enzyme (ACE) inhibitory peptides from cultured marine microalgae (*Nannochloropsis oculata*) protein hydrolysate. *J. Appl. Phycol.* **2013**, *25*, 1595–1606. [[CrossRef](#)]
173. Qian, Z.-J.; Jung, W.-K.; Kang, K.-H.; Ryu, B.; Kim, S.-K.; Je, J.-Y.; Heo, S.-J.; Oh, C.; Kang, D.-H.; Park, W.S.; et al. In vitro antioxidant activities of the fermented marine microalga *Pavlova lutheri* (Haptophyta) with the yeast *Hansenula polymorpha*. *J. Phycol.* **2012**, *48*, 475–482. [[CrossRef](#)] [[PubMed](#)]
174. Oh, G.-W.; Ko, S.-C.; Heo, S.-Y.; Nguyen, V.-T.; Kim, G.; Jang, C.H.; Park, W.S.; Choi, I.-W.; Qian, Z.-J.; Jung, W.-K. A novel peptide purified from the fermented microalga *Pavlova lutheri* attenuates oxidative stress and melanogenesis in B16F10 melanoma cells. *Process Biochem.* **2015**, *50*, 1318–1326. [[CrossRef](#)]
175. Thompson, R.E.; Muir, T.W. Chemoenzymatic Semisynthesis of Proteins. *Chem. Rev.* **2020**, *120*, 3051–3126. [[CrossRef](#)] [[PubMed](#)]
176. Weeks, A.M.; Wells, J.A. Subtiligase-Catalyzed Peptide Ligation. *Chem. Rev.* **2020**, *120*, 3127–3160. [[CrossRef](#)]
177. Hedges, J.B.; Ryan, K.S. Biosynthetic Pathways to Nonproteinogenic  $\alpha$ -Amino Acids. *Chem. Rev.* **2020**, *120*, 3161–3209. [[CrossRef](#)]
178. Malonis, R.J.; Lai, J.R.; Vergnolle, O. Peptide-Based Vaccines: Current Progress and Future Challenges. *Chem. Rev.* **2020**, *120*, 3210–3229. [[CrossRef](#)]
179. Merrifield, R.B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154. [[CrossRef](#)]
180. Jaradat, D.M.M. Thirteen decades of peptide synthesis: Key developments in solid phase peptide synthesis and amide bond formation utilized in peptide ligation. *Amino Acids* **2018**, *50*, 39–68. [[CrossRef](#)]
181. Carpino, L.A.; Han, G.Y. 9-Fluorenylmethoxycarbonyl function, a new base-sensitive amino-protecting group. *J. Am. Chem. Soc.* **1970**, *92*, 5748–5749. [[CrossRef](#)]
182. Li, W.; O'Brien-Simpson, N.M.; Hossain, M.A.; Wade, J.D. The 9-Fluorenylmethoxycarbonyl (Fmoc) Group in Chemical Peptide Synthesis—Its Past, Present, and Future. *Aust. J. Chem.* **2020**, *73*, 271. [[CrossRef](#)]
183. Varnava, K.G.; Sarojini, V. Making Solid-Phase Peptide Synthesis Greener: A Review of the Literature. *Chem. Asian J.* **2019**, *14*, 1088–1097. [[CrossRef](#)] [[PubMed](#)]

184. Isidro-Llobet, A.; Kenworthy, M.N.; Mukherjee, S.; Kopach, M.E.; Wegner, K.; Gallou, F.; Smith, A.G.; Roschangar, F. Sustainability Challenges in Peptide Synthesis and Purification: From R&D to Production. *J. Org. Chem.* **2019**, *84*, 4615–4628.
185. Houghten, R.A. General method for the rapid solid-phase synthesis of large numbers of peptides: Specificity of antigen-antibody interaction at the level of individual amino acids. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 5131–5135. [[CrossRef](#)] [[PubMed](#)]
186. Guzmán, F.; Gauna, A.; Luna, O.; Román, T.; Álvarez, C.; Albericio, F.; Cárdenas, C. The tea-bag protocol for comparison of Fmoc removal reagents in solid-phase peptide synthesis. *Amino Acids* **2020**, *52*, 1201–1205. [[CrossRef](#)]
187. Kappe, C.O. My Twenty Years in Microwave Chemistry: From Kitchen Ovens to Microwaves that aren't Microwaves. *Chem. Rec.* **2019**, *19*, 15–39. [[CrossRef](#)]
188. Singh, S.K.; Collins, J.M. New Developments in Microwave-Assisted Solid Phase Peptide Synthesis. In *Peptide Synthesis. Methods in Molecular Biology*; Hussein, W., Skwarczynski, M., Toth, I., Eds.; Humana: New York, NY, USA, 2020; pp. 95–109, ISBN 978-1-0716-0227-0.
189. Merlino, F.; Tomassi, S.; Yousif, A.M.; Messere, A.; Marinelli, L.; Grieco, P.; Novellino, E.; Cosconati, S.; Di Maro, S. Boosting Fmoc Solid-Phase Peptide Synthesis by Ultrasonication. *Org. Lett.* **2019**, *21*, 6378–6382. [[CrossRef](#)]
190. Boyaud, F.; Mahiout, Z.; Lenoir, C.; Tang, S.; Wdzieczak-Bakala, J.; Witzak, A.; Bonnard, I.; Banaigs, B.; Ye, T.; Inguibert, N. First total synthesis and stereochemical revision of laxaphycin B and its extension to Lyngbyacyclamide A. *Org. Lett.* **2013**, *15*, 3898–3901. [[CrossRef](#)]
191. Bornancin, L.; Boyaud, F.; Mahiout, Z.; Bonnard, I.; Mills, S.C.; Banaigs, B.; Inguibert, N. Isolation and synthesis of laxaphycin b-type peptides: A case study and clues to their biosynthesis. *Mar. Drugs* **2015**, *13*, 7285–7300. [[CrossRef](#)]
192. Junk, L.; Kazmaier, U. Total synthesis and configurational revision of mozamide A, a hydroxy-brunsvicamide. *J. Org. Chem.* **2019**, *84*, 2489–2500. [[CrossRef](#)]
193. Gaillard, M.; Das, S.; Djibo, M.; Raviglione, D.; Roumestand, C.; Legrand, B.; Inguibert, N. Towards the total synthesis of trichormamide A, a cyclic undecapeptide. *Tetrahedron Lett.* **2018**, *59*, 3713–3718. [[CrossRef](#)]
194. Werner, H.M.; Cabaltea, C.C.; Horne, W.S. Peptide Backbone Composition and Protease Susceptibility: Impact of Modification Type, Position, and Tandem Substitution. *ChemBioChem* **2016**, *17*, 712–718. [[CrossRef](#)] [[PubMed](#)]
195. Skowron, K.J.; Speltz, T.E.; Moore, T.W. Recent structural advances in constrained helical peptides. *Med. Res. Rev.* **2019**, *39*, 749–770. [[CrossRef](#)] [[PubMed](#)]
196. D'Souza, A.; Yoon, J.H.; Beaman, H.; Gosavi, P.; Lengyel-Zhand, Z.; Sternisha, A.; Centola, G.; Marshall, L.R.; Wehrman, M.D.; Schultz, K.M.; et al. Nine-Residue Peptide Self-Assembles in the Presence of Silver to Produce a Self-Healing, Cytocompatible, Antimicrobial Hydrogel. *ACS Appl. Mater. Interfaces* **2020**, *12*, 17091–17099. [[CrossRef](#)]
197. Śmiłowicz, D.; Metzler-Nolte, N. Bioconjugates of Co(III) complexes with Schiff base ligands and cell penetrating peptides: Solid phase synthesis, characterization and antiproliferative activity. *J. Inorg. Biochem.* **2020**, *206*, 111041. [[CrossRef](#)]
198. Conibear, A.C.; Schmid, A.; Kamalov, M.; Becker, C.F.W.; Bello, C. Recent Advances in Peptide-Based Approaches for Cancer Treatment. *Curr. Med. Chem.* **2020**, *27*, 1174–1205. [[CrossRef](#)]
199. Deshpande, D.; Grieshaber, M.; Wondany, F.; Gerbl, F.; Noschka, R.; Michaelis, J.; Stenger, S. Super-Resolution Microscopy Reveals a Direct Interaction of Intracellular Mycobacterium tuberculosis with the Antimicrobial Peptide LL-37. *Int. J. Mol. Sci.* **2020**, *21*, 6741. [[CrossRef](#)]
200. Williamson, M.P.; Waltho, J.P. Peptide structure from NMR. *Chem. Soc. Rev.* **1992**, *21*, 227. [[CrossRef](#)]
201. Hilpert, K.; Elliott, M.R.; Volkmer-Engert, R.; Henklein, P.; Donini, O.; Zhou, Q.; Winkler, D.F.H.; Hancock, R.E.W. Sequence Requirements and an Optimization Strategy for Short Antimicrobial Peptides. *Chem. Biol.* **2006**, *13*, 1101–1107. [[CrossRef](#)]
202. WHO. WHO Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed. Available online: <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed> (accessed on 20 November 2020).
203. Mulani, M.S.; Kamble, E.E.; Kumkar, S.N.; Tawre, M.S.; Pardesi, K.R. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. *Front. Microbiol.* **2019**, *10*, 539. [[CrossRef](#)]



204. Ríos, J.L.; Recio, M.C. Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.* **2005**, *100*, 80–84. [[CrossRef](#)] [[PubMed](#)]
205. Cos, P.; Vlietinck, A.J.; Vanden Berghe, D.; Maes, L. Anti-infective potential of natural products: How to develop a stronger in vitro ‘proof-of-concept’. *J. Ethnopharmacol.* **2006**, *106*, 290–302. [[CrossRef](#)] [[PubMed](#)]
206. Gibbons, S. Phytochemicals for Bacterial Resistance—Strengths, Weaknesses and Opportunities. *Planta Med.* **2008**, *74*, 594–602. [[CrossRef](#)] [[PubMed](#)]
207. Bueno, J. In Vitro Antimicrobial Activity of Natural Products Using Minimum Inhibitory Concentrations: Looking for New Chemical Entities or Predicting Clinical Response. *Med. Aromat. Plants* **2012**, *1*, 1000113. [[CrossRef](#)]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).