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THE BIOTECHNOLOGICAL POTENTIAL OF SECONDARY METABOLITES FROM MARINE BACTERIA

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Abstract

The highly dangerous trend of escalating bacterial resistance to modern antibiotics has evolved in recent decades, with increasingly more drug-resistant strains of pathogens emerging and spreading each year. This poses a threat to not only public health, but also to entire mankind. Marine bioresources, considered as a promising alternative to traditional antibiotics and a valuable source of biologically active compounds with high pharmacological potential, now attract increasing attention of researchers. Modern biotechnology combines the genetic engineering methods and the unusual biosynthetic pathways utilized by marine microorganisms to produce natural antibiotics. The goal of this review is to summarize the latest trends in searching for new natural antimicrobial agents based on secondary metabolites of marine bacteria. The targeted control of biosynthesis mechanisms using the metabolic engineering methods in order to create hybrid peptide synthetases or to obtain hybrid peptides by disrupting the target gene of nonribosomal synthesis becomes a noteworthy trend in modern biotechnology. This pathway is not only one of the most promising approaches to the development of new antibiotics, but also a potential target for controlling the exocrine activity of pathogenic bacteria and, consequently, their viability.

Key words: biotechnologies, marine bacteria, secondary metabolites, nonribosomal biosynthesis, antibacterial strategies.

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Introduction

Despite the significant advances in medicine, diagnostics, and treatment of infectious diseases, pathogenic microorganism still pose a serious threat to the world's human population. Their impact is significant both in developing countries, due to the limited access to medicines there, and in developed countries, where uncontrolled administration of antibiotics has led to a wide distribution of multi-resistant bacteria. The strategy of creating new synthetic antibiotics by

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modifying existing natural ones has not proven effective enough: pathogenic microorganisms adapt to new drugs after their first trials. The world community, represented by the World Health Organization, raises reasonable concern for the future of mankind and encourages searching for novel antimicrobial agents that can become an alternative to modern antibiotics [1, 2]. A number of promising strategies for the search for new antibiotic drugs are associated with the use of products of metabolism of marine bacteria.

The bacterial metagenome synthesizes primary metabolites and transforms small protein molecules into secondary metabolites, also referred to as "specialized metabolites". They play an important role in cell growth, signal transmission, search for nutrients, intra- and interspecies communication, and competition, and are, therefore, of increased interest to researchers considering them as potential alternatives to traditional antibiotics. Of particular importance is the study of antibacterial activity of antimicrobial peptides, which are secondary metabolites of marine microorganisms [3].

Mankind has learnt to use resources of the World Ocean, which covers more than 70% of the earth's surface, since long ago. Despite this fact, marine bacteria aroused researchers' interest only in the middle of the 20th century, although some studies of the biological activity of metabolites of these microorganisms were published as early as in the late 19th century. It was found that the marine environment, including bottom sediments, represents a giant pool of microbial biodiversity, numbering up to approximately 3.67×10^{30} microorganisms [4].

Even the few studies in recent decades have shown that the marine ecosystem, with its unique diversity of habitats and abundant biota, is an inexhaustible resource of biologically active natural chemical substances. Numerous compounds with noteworthy pharmaceutical activities, which can become sources of novel therapeutic agents, have been described from marine organisms over recent decades [5, 6, 7]. In particular, antibacterial substances that are secondary metabolites of marine bacteria attract much researchers' attention due to their high antibacterial potential.

These substances are the subject of extensive research conducted in marine microbiology and chemistry of marine natural compounds, intensively developing nowadays. Due to their unique properties, they have become one of the priorities for modern marine biotechnology.

The goal of the present review is to summarize the current scientific data on the structure and pharmacological activity of secondary metabolites of marine bacteria, as well as on the nonribosomal mechanisms of their biosynthesis, which are a new target for antibacterial strategies.

The search for data sources was carried out in the Cochrane Library data base (at Wiley Online Library), EMBASE (EMBASE.com), PubMed, PubMed Central, EMBASE, and MEDLINE, integrated on the platform of Elsevier, CINAHL, Web of Science, and Health Economic Evaluations. Due to the great scientific attention to the issue of "antibacterial metabolites" and "antibacterial peptides", the strategy of sampling was limited to the search for scientific reviews with the following

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word combinations contained in the title, abstract, and topical catalogs: “marine bacteria and secondary metabolites”, “marine bacteria and antibacterial peptides”, and “marine bacteria and nonribosomal biosynthesis”. The depth of search was 2007–2019.

1. Bacterial metabolites

Bacteria live in the environment of transmitted and received chemical signals, with signal molecules being metabolites, i.e. terminal products of cellular metabolism. The latter is a combination of two opposite but interrelated processes: energetic (catabolism) and constructive (anabolism). This is a continuous and multi-component biochemical process that occurs in every bacterial cell throughout its lifecycle [8, 9, 10].

Terminal products of metabolism, being small peptide molecules, are used as substrates for biochemical reactions or are utilized by microorganisms to support their life processes. This is a wide range of molecules extremely diverse in their structures and functions, with their registered number exceeding 25,000, which accounts for less than 2% of the total number of natural metabolites of microorganisms not yet available for research [9, 10, 11].

Depending on the functional properties and biosynthesis mechanisms, metabolites are divided into primary and secondary. Primary metabolites serve as the main energy source for providing various biochemical reactions and performing physiological functions to support life processes of bacterial cells such as growth and development. Secondary metabolites are organic compounds with a complex chemical structure and a variety of physiological functions. They are required to implement the survival strategies of bacteria in adverse conditions, acting as mediators with the external environment and means of intercellular communication (Table 1).

Table 1

Key biochemical and physiological properties of primary and secondary metabolites of bacteria [8]

Primary metabolites	Secondary metabolites
Small-sized molecules	Small-sized molecules
Produce several intermediate and terminal product	Participate in the synthesis of new compounds and a multitude of molecules
Terminal products involved in the synthesis of macromolecules, coenzyme	Not vitally important for cell growth
Important for cell growth and viability	Have unusual chemical structures
Have a simple chemical structure	Terminal products are used as antibacterial agents
Synthesized during the lag phase of bacterial growth	Synthesized at the beginning of stationary phase of bacterial growth
Used in food and feed industry	Used in medicine, cosmetics, and agriculture as preservatives
Provide energy reserve for communication of cells	Protect bacteria during the period of adverse conditions
Main source of energy for cellular metabolism and life support	Participate in intercellular communication, cell protection, and competition for food and space

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Since this review is focused on the bioactive properties of secondary metabolites of marine bacteria, the main emphasis will be on these complex molecules.

1.1 Secondary metabolites of bacteria

This group of metabolites is an essential component supporting life processes of marine bacteria, fungi, archaea, and other microorganisms, which are rich sources of these compounds. Substances with various biological properties, including antibacterial, antifungal, antiviral, and antiproliferative agents, exotoxins, metal carriers, hormones, immunomodulators, pigments, and enzyme inhibitors, have been found among these complex biomolecules synthesized by marine prokaryotes [8–10].

Many of these compounds, exhibiting high biological activity, play an important role in life functions of bacteria and are widely used in pharmacology, cosmetics, food industry, and agriculture. Nevertheless, some bacteria (such as *Clostridium botulinum, Vibrio cholerae, Escherichia coli, Yersinia* sp., etc.) synthesize exotoxins, which are secondary metabolites and cause diseases in humans [10].

As a rule, each bacterial species produces several antibiotics, the profile of which depends on the genus of microorganism. For instance, more than 5,000 antibiotics referred to secondary metabolites have been identified to date from the genus *Actinobacteria* [8], including traditional antibiotics discovered in the 1950s–1960s, as well as new antibiotics. According to forecasts, these bacteria may produce up to 150,000 different chemical antimicrobial agents [8, 9].

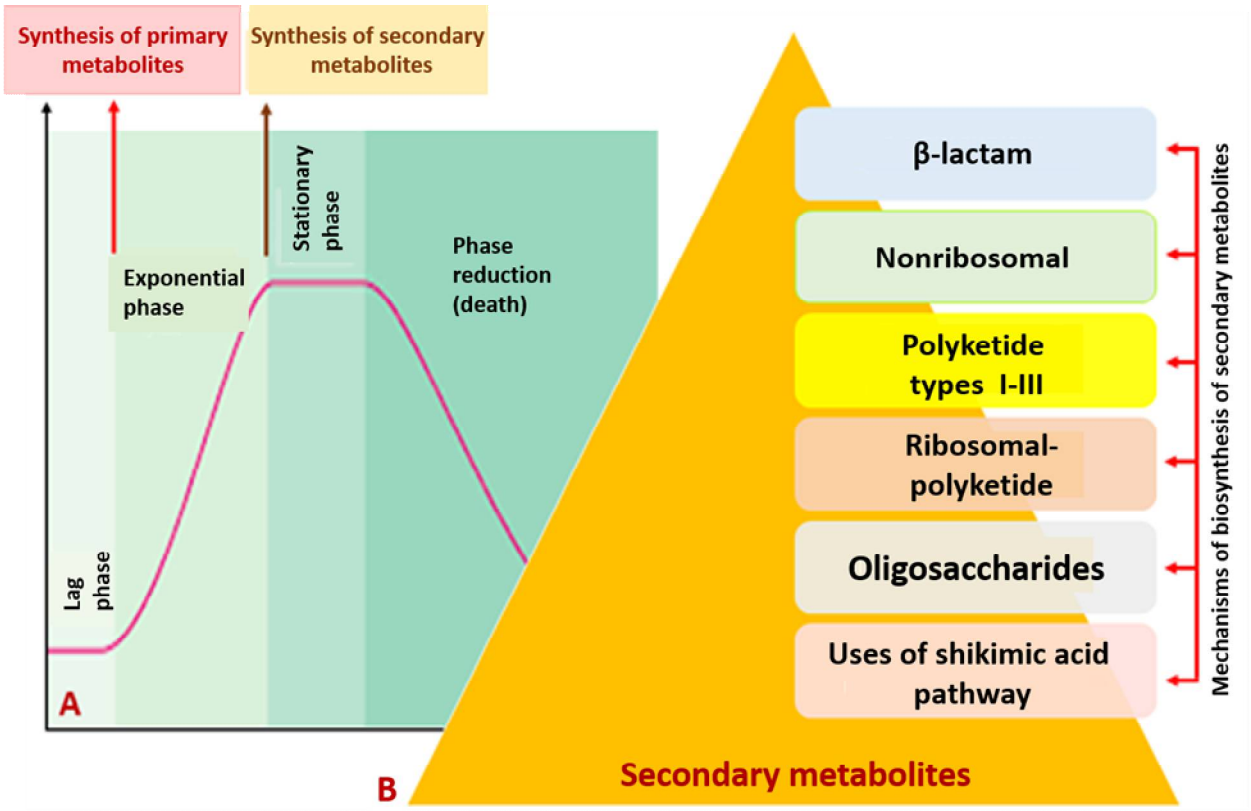

Fig. 1. Production of secondary metabolites occurs at the end of the exponential and at the beginning of the stationary phases of bacterial growth (A). Various mechanisms of biosynthesis of secondary metabolites in bacteria (B).

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Modern science considers secondary metabolites as a group of low-molecular-weight, structurally diverse, and complex bioactive compounds. It has been found that the active stage of synthesis of these molecules in microorganisms occurs at the end of the exponential and the beginning of the stationary phases of their growth (Fig. 1-A). Their production is induced by depletion of nutrients and adverse habitat conditions; the genes responsible for the biosynthesis of secondary metabolites are grouped together in a small number of clusters [10, 12].

Unlike primary metabolites, the biosynthetic pathways utilized to produce these molecules are numerous and have not been fully understood [8, 10]. For biosynthesis, bacteria use multi-stage biosynthesis pathways, which involve specific enzymes or multi-enzyme complexes, being intermediate or end products of intracellular metabolism. Biosynthesis includes cascade regulations, the mechanisms of which have been studied at the transcription level [12].

Among the key pathways of biosynthesis of secondary metabolites with antibacterial activities, the best characterized are nonribosomal (with peptide synthetase as the key enzyme), β-lactam, polyketide (types I–III, with polyketide synthase as the key enzyme), ribosomal-polyketide, oligosaccharide, and shikimate pathways (Fig. 1-B).

The significantly increased interest in obtaining new antibiotic agents derived from secondary metabolites of marine bacteria is associated with the advances in biotechnology that have been made in recent decades [12, 13]. They are based on the revealed mechanism of synthesis of major microbial metabolite classes by means of polyketide synthase [14, 15], nonribosomal peptide synthetase [16–18], which are biosynthetic pathways extensively utilized by marine microorganisms for producing antimicrobial substances.

2. Antimicrobial substances of marine microorganisms

Microorganisms from terrestrial ecosystems and their metabolites have always been a source of many biologically active compounds applied in medicine, pharmaceutical industry, and agriculture. After years of intensive studies of terrestrial microorganisms, attention was focused on aquatic ecosystems of the World Ocean. Temperature conversions, hydrostatic pressure, variable salinity and oxygen concentration are the factors that cause the rich taxonomic diversity of marine biota, in which bacteria and fungi constitute a substantial part, provide a rich resource of chemical products, and are considered a promising source of a large number of biologically active compounds [3, 7, 20, 21].

One of the first researchers to reveal the antagonistic interactions of some marine bacteria with causative agents of dangerous infections (*Bacillus anthracis* and *Vibrio cholerae*) was V. de Giaxa in 1889. In his work “Veber das Verhalten einiger pathogener Mikroorganismen im Meerwasser”, he showed that in the case of combined cultivation with marine bacteria, these terrestrial pathogens lost their

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ability to cause infection in the experiment [cited by 22, 23]. However, in those years, this article did not receive due attention of researchers.

The issue of competitive interaction of marine bacteria and some members of the family Enterobacteriaceae was raised again only in the 1940s by S. Kiribayashi, T. Aida (1941), B.D. Rosenfeld, C.E. ZoBell (1947), and others. The results of the studies conducted in that period showed for the first time that the mortality of pathogenic enterobacteria in sea water was a consequence of the toxic effect of "*antibiotics produced by marine microorganisms*" (ZoBell, 1947) and, to a lesser extent, due to the salinity and osmotic pressure of water [cited by 22]. At the same time, an attempt was made to isolate these substances. A total of 58 species of marine bacteria, members of the genera *Actinomyces*, *Bacillus*, *Micrococcus*, and *Serratia*, were tested; of them 9 strains were identified as producers of antibacterial substances antagonistically interacting with Gram-positive microorganisms [19, 22, 23].

The growing worldwide interest in the study of biologically active metabolites produced by marine bacteria resulted from the accumulation of knowledge about true marine microorganisms. The modern scientific paradigm is consistent with the concept proposed in the middle of the 20th century by the academician B.L. Isachenko (1871–1948) and Claude E. ZoBell (1904–1989), the founders of marine microbiology who explained the autochthonous existence of marine bacteria and their taxonomic uniqueness [cited by 23]. Subsequent discoveries have shown that marine biota is comprised of specific taxa of prokaryotes, fungi, and other microorganisms distributed ubiquitously. They are active participants in the cycle of matter in water and bottom sediments of the ocean, as well as sources for the production and isolation of specific peptide-based metabolites [20, 23–25].

The history of study of secondary metabolites from marine bacteria is an example of the joint efforts and achievements of microbiologists, chemists, biochemists, molecular biologists, and geneticists. The discovery of the phenomenon of unusual peptides synthesized in microorganisms independently of ribosomes and RNA was followed by a long series of findings and evidence of extremely diverse natural bacterial metabolites exhibiting antibiotic and antitumor activities (Table 2).

Table 2

Promising secondary metabolites with antimicrobial activity isolated from marine bacteria

Metabolite	Producer	inhibiting	Active concentration	References
Bogorol A	*Bacillus sp.*	Methicillin-resistant *S. aureus* (MRSA)	2 µg/mL (MIC)	26, 27
Loloatin B	*Bacillus sp.*	Methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus*	1–2 µg/mL (MIC)	28, 29

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		faecium (VRE)		
Tauramamide	*Brevibacillus laterosporus*	*Enterococcus sp.*	0.1 µg/mL (MIC)	30, 31
Halobacillin	*Bacillus sp. CND-914*	*S. aureus, P. vulgaris, and E. faecalis*. Human HCT-116 cancer cells	0.98 µg/mL (IC50)	4, 32
Macrolactin S	*B. amyloliquefaciens*	*E. coli, S. aureus*	0.1– 0.3 µg/mL (MIC)	33, 34
Macrolactin V	*B. amyloliquefaciens*	*E. coli, B. subtilis, S. aureus*	0.1 µg/mL (MIC)	33, 34
Bacillistatins	*Bacillus silvestris*	*Streptococcus pneumonia*	0.5–2 µg/mL (GI_{50})	35
Triopeptid TP-1161	*Nocardiopsis sp*	Vancomycin-resistant *Enterococcus faecium* (VRE)	1.0 µg/mL (MIC)	36–39
Halocintin	*Halocynthia papillosa*	*Micrococcus luteus, Bacillus megaterium, Aerococcus viridans, S. aureus, Enterococcus faecalis*	0.39–50 µM (MBC)	40, 41
Indigoidin	*Phaeobacter sp.*	*Vibrio fischeri*	n/d	42, 43
Unnarrmicins A, C	*Photobacterium* sp.	*Pseudovibrio* sp.	7–18 µg/disk	44, 45
Ngercheumicins A–D	*Photobacterium* sp.	Gram (-) bacteria	n/d	31, 46
Solonamidin A	*Photobacterium* sp	*S. aureus*, methicillin-resistant *S. aureus* (MRSA)	n/d	3, 47
Cyclo-peptides	*Pseudomonas sp.*	*S. aureus, M. luteus, B. subtilis, E. coli, V. anguillarum*	n/d	4, 3, 48, 49
Ariakemicins A, B	*Rapidithrix* sp.	*Brevibacterium* sp., *S. aureus, B. subtilis*	0.46–80 µg/mL (MIC)	44, 45
Turnagainolides A, B	*Bacillus* sp. RJA *2194*	MRSA, VRE, and penicillin-resistant *S. pneumoniae*	*1–2* µg/mL (MIC)	50
Anthramycin	*Streptomyces* sp.	*B. anthracis, E. faecalis, S. pneumonia, S. aureus,* MSSA, MRSA, *S. aureus* (VRE)	0.03125–0.25 µg/mL (MIC)	51, 52

3. Secondary metabolites of marine bacteria are products nonribosomal synthesis

During their life cycle, marine microorganisms actively synthesize secondary metabolites which are low-molecular-weight peptides. They represent specific protein fragments that, in addition to being sources of nitrogen and amino acids, perform numerous biological functions [5, 7, 11, 53]. These substances were derived from algae, marine bacteria, and fungi. The anti-infection activity of marine peptides has been shown to depend on their structural properties, amino acid composition and sequence, as well as on the habitat conditions for producer bacteria [7, 53, 54] (Table 2).

The major part of marine bacteria are exposed to extreme conditions of high pressure, salinity, low temperature, and lack of sunlight. These factors caused them to develop the unique properties and the ability to biosynthesize substances with unusual characteristics, different from their terrestrial counterparts. To date, biological properties of only a small number of these peptides have been studied, but the proportion of described substances increases each year, attracting increasingly more attention of researchers [11, 54–56].

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Most marine bacteria and other microorganisms use numerous gene clusters for metabolite biosynthesis [7, 56]. Studies of genome sequences have shown that a significant part of them is responsible for the biosynthesis of secondary metabolites. For example, among marine microorganisms, isolates of the genus *Bacillus* are referred to as phylogenetically heterogeneous groups of marine bacteria. They need much nutrients and space, and, in order to compete with other bacteria, they synthesize a significant amount of secondary metabolites with pronounced antimicrobial activity, encoded by genes constituting up to 8% of the genome [54, 57].

To date, dozens of metabolites, which are peptides consisting of 20–40 amino acids and used for inter- and intraspecific competition, have been isolated from various marine microorganisms [5, 7, 54]. Most of them are capable of quick inhibition or kill of a wide range of microbes. Other antimicrobial metabolites (proteins consisting of 100 or more amino acids) disrupt the structure or function of microbial cell membranes by binding to specific targets [58, 59]. In the framework of the global program of search for antimicrobial alternatives to traditional antibiotics, results of more than 40 studies on finding new antimicrobial compounds isolated from marine bacteria and fungi have been published over the past 15 years [53, 55, 57].

In recent years, much of the attention of researchers has been focused on secondary metabolites of marine bacteria, which are products of nonribosomal biosynthesis considered a new class of natural antimicrobial agents that can potentially become an alternative to traditional antibiotics [54, 56, 59, 60].

The capability of nonribosomal peptide synthesis is widely distributed among bacteria. As a rule, these metabolites show a wide range of biological activities (antimicrobial, antitumor, antiviral, and antifungal), a variety of pharmacological properties, and an extremely high structural diversity [58–60]. Marine ecosystem provides an inexhaustible source of diverse classes of nonribosomally synthesized secondary metabolites. These substances (lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, lipoamides, and isocoumarins) are cyclic branched peptide compounds with unusual structure and structural templates of novel natural antibiotics [59, 61–63].

In the second half of the 20th century, this biosynthesis mechanism was simulated in a laboratory to obtain antimicrobial peptides produced by marine isolates of *Bacillus* sp., in which the pharmacologically-induced inhibition of ribosomes or RNA removal did not prevent protein synthesis [64].

It has been established that the ATP-dependent synthesis of nonribosomal peptides (NRP) occurs by means of peptide synthetase, an enzyme complex independent of messenger RNA, transmitting the genetic information from DNA to ribosomes, where the amino acid sequence of protein products of gene expression is determined [61, 63, 65].

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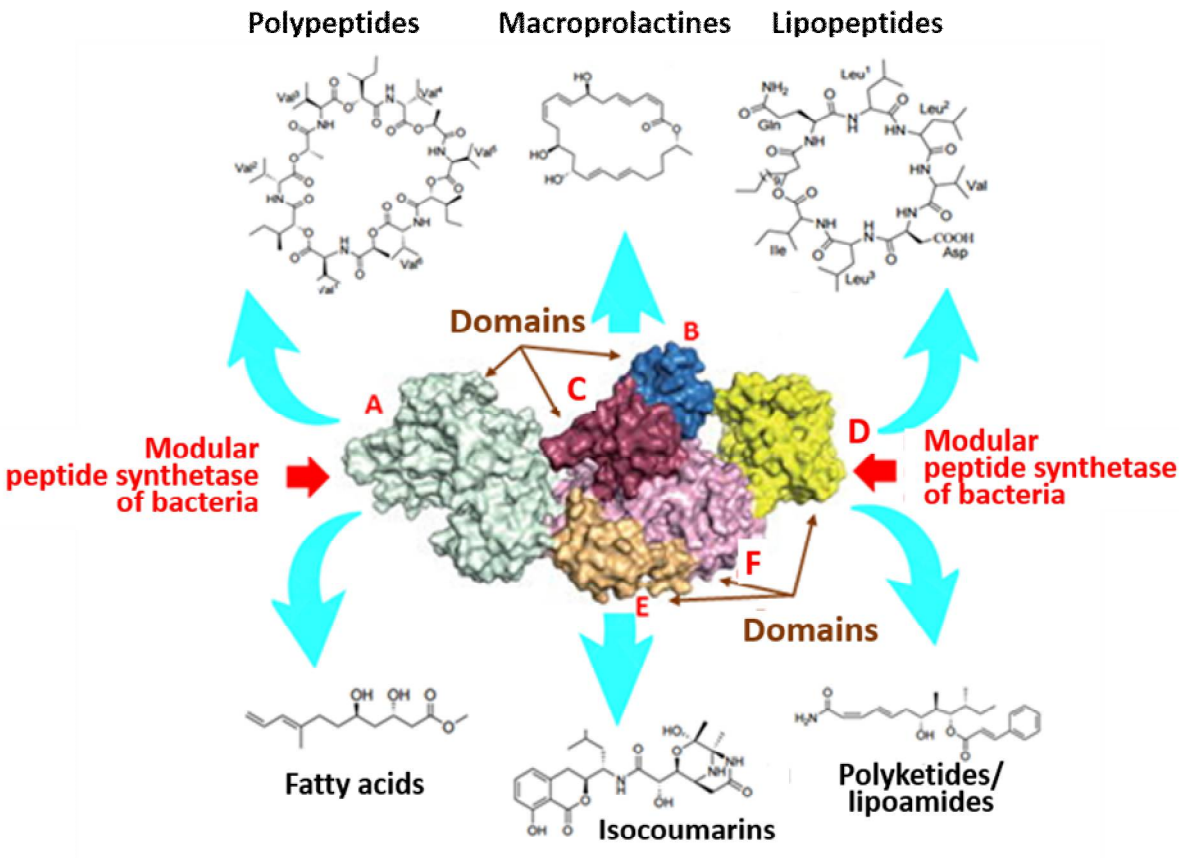

Fig. 2. Structure of Macrolactin of bacteria constituting the basis of nonribosomal synthesis and structural diversity of synthesized metabolites.
Domains are as follows: (A) carrier peptide; (B) acetylation; (C) condensation;
(D) thyosterase domains; (E, F) formyltransferase domains.

Operation of this “assembly line” depends solely on the activity of peptide synthetase, which is a multi-domain modular enzyme complex. It catalyzes the ATP-dependent synthesis of important peptide products with antimicrobial activity from specific sequences of proteinogenic and non-encoded amino acid substrates. The process includes three key sequential stages: acetylation, thyolization, and condensation with the involvement of the same-name key domains and peptide carrier protein [18, 59, 62] (Fig. 2).

Unlike ribosomal synthesis, where the sequence of 20–22 natural amino acids is determined by the primary structure of RNA, the ribosome-independent mechanism provides assembly of relatively short NRPs. They consist of a set of non-encoded (non-proteinogenic) amino acids, the sequence of which is strictly determined by the structure of the polyenzyme complex. To date, almost 150 such amino acids and dozens of thousands of their combinations are known, which explains the wide structural variety of NRPs, as well as their physical and chemical stability and conformational plasticity [58, 60, 63].

Due to the high pharmacological activity of the nonribosomal synthesis products, much effort has been applied in recent years to the study of the promising and unusual biosynthesis

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mechanisms and the diversity of pharmacological properties of NRPs. To date, several pathways of nonribosomal synthesis of peptides have been characterized from both terrestrial (human commensals and pathogens) and marine species of bacteria, which are enough comprehensively considered in recent reviews [59, 60, 64].

It should be noted that, from the evolutionary aspect, the understanding of nonribosomal biosynthesis mechanisms has evolved from the erroneous view of peptide synthetase as a precursor of ribosomes, as well as from the discovery of the "thiotemplate" mechanism [62, 65] and its revision in connection with the advent of the modern modular-domain "multiple carrier model" [58, 63, 64]. In the present review, we consider only some of the antimicrobial peptide substances that are products of this biosynthesis pathway, being components of secondary metabolites of marine bacteria.

3.1 *Cyclic lipopeptides* (cLPs)

Cyclic lipopeptides (cLPs) are common metabolites synthesized by various bacterial genera and are of interest as substances having various biological activities (Fig. 2). Lipopeptides of marine bacteria consist of a short cyclic oligopeptide (backbone) bound to fatty acids (tail) and show strong antibacterial activity against common human, animal, and plant pathogens, due to which these metabolites have attracted attention as potential natural antibiotic agents (Table 2).

Lipopeptides are divided into three families: iturins, fengycins, and surfactins [66–68]. In the chemical structure, the peptide backbone is represented by seven (iturins and surfactins) or ten (iturins) amino acids bound to β-hydroxy- (fengycins and surfactins) or β-amino- (iturins) fatty acids with the number of carbon atoms being from C-10 to C-16 (surfactins), from C-14 to C-17 (iturins), and from C-14 to C-18 (fengycins). Each family is subdivided into homologous subfamilies depending on the position of a certain amino acid in the peptide ring [66, 68]. The examples of well-characterized lipopeptide antibiotics, which are metabolites of marine bacteria, are tauromamid, halobacillin, and methylhalobacillin [4, 30–32].

Tauromamid is a relatively new nonribosomally biosynthesized antibiotic [30, 31] (Table 2), belonging to the group of cyclic lipopeptides (like daptomycin, the first permitted antibiotic of this class). It is produced by the marine bacterial isolate of *Brevibacillus lateosporus* PNG276, inhabiting the Gulf of Papua [31]. Tauromamid has a strong and selective inhibitory action on the Gram-positive pathogen *Enterococcus* sp., as well as exhibits unexpressed activity against the methicillin-resistant *Staphylococcus aureus* (MRSA; MIC = 200 μg/mL) and *Candida albicans* (MIC = 50 μg/mL) [30, 31].

Halobacillin and methylhalobacillin are two cyclic lipopeptides isolated from bacteria inhabiting deep-sea sediment in the Gulf of California, Mexico [32]. Halobacillin is also one of

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the most effective known biosurfactants [4]. This antibiotic inhibits the growth of human colon tumor cells (HCT-116) at IC_{50} 0.98 µg/mL and exhibits analogous, but lower than surfactin (a known surfactant antibiotic isolated from terrestrial strains of *Bacillus subtilis*), antimicrobial activity against *S. aureus*, *Proteus vulgaris*, and *Enterococcus faecalis* [4, 32] (Table 2).

The wide distribution of cLPs among secondary metabolites of marine bacteria is evidenced by the fact that they make up the major part of products of marine isolates from *Bacillus* sp., one of the most common inhabitants of the World Ocean [3].

3.3 Polyketides / lipoamides

Polyketides are extremely large classes of secondary metabolites that contain acyl-coenzyme A and constitute the basis of many pharmaceutical, agrochemical, and veterinary drugs. The biosynthesis of these metabolites occurs with the involvement of multimodular megasynthases known as polyketide synthases [14, 15]. Thanks to this biosynthesis mechanism, polyketides show an amazing structural and antimicrobial diversity. Several metabolites with antibiotic action from marine isolates of B. lateosporus, belonging to the family of polyketides, such as basiliskamide A and B, as well as tupusleiamide A and B, have been characterized recently. These antibiotics exhibited antifungal activity against *Candida albicans* (MIC = 1.0 and 3.1 µg/mL) and *Aspergillus fumigatus* (MIC = 2.5 and 5.0 µg/mL) [14, 15, 45].

Two polyketides with unique antimicrobial and antitumor properties were isolated in 2012 from the marine bacterium *B. licheniformis* from a sediment core sample collected on the southern Iodo reef, Republic of Korea. Antibiotics ieodoglucomides A and B *in vitro* showed antimicrobial activity against Gram-positive and Gram-negative pathogenic bacteria (MIC = 8–32 µg/mL). In addition, ieodoglucomide B exhibited cytotoxic activity against lung cancer cell line (GI_{50} = 25.18 µg/mL) and gastric cancer cell line (GI_{50} = 17.78 µg/mL) [14].

The thiopeptide antibiotic TP-1161, isolated from the marine Gram-positive bacterium *Nocardiopsis* sp., belongs to the same structural group [36–38]. This antibiotic showed high antibacterial activity *in vitro* against clinical isolates of Gram-positive bacteria (with MIC varying from 0.25 to 4 µg/mL), i.e., at concentrations comparable or lower than that of the reference antibiotic vancomycin. TP-1161 also inhibited growth of vancomycin-resistant bacterial strains, including *E. faecalis* and *E. faecium*, at MIC = 1 µg/mL [38].

4. Mechanisms of antimicrobial action of antibacterial peptides

In recent years, a large group of secondary metabolites of different types, structures, and mechanisms of antibacterial action has been isolated from a number of other marine bacteria [3–5, 7, 8]. Unlike those from terrestrial ecosystems, producers of antimicrobial peptides were mainly

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strains of Gram-positive marine bacteria [7, 9, 10], despite Gram-negative prokaryotes dominate the marine environment [21, 22, 68, 69].

Most of the isolated antimicrobial metabolites are capable of rapidly killing a wide range of microbes. Large-sized antimicrobial proteins (>100 amino acids) are often lytic proteins binding nutrients [68, 71] or destroying specific cell patterns [71–73], causing DNA degradation [73, 74], and inhibiting intracellular synthesis of peptidoglycan [75–77] and specific proteins by disrupting the structure or function of microbial cell membranes [7, 54, 78, 79] (Fig. 3).

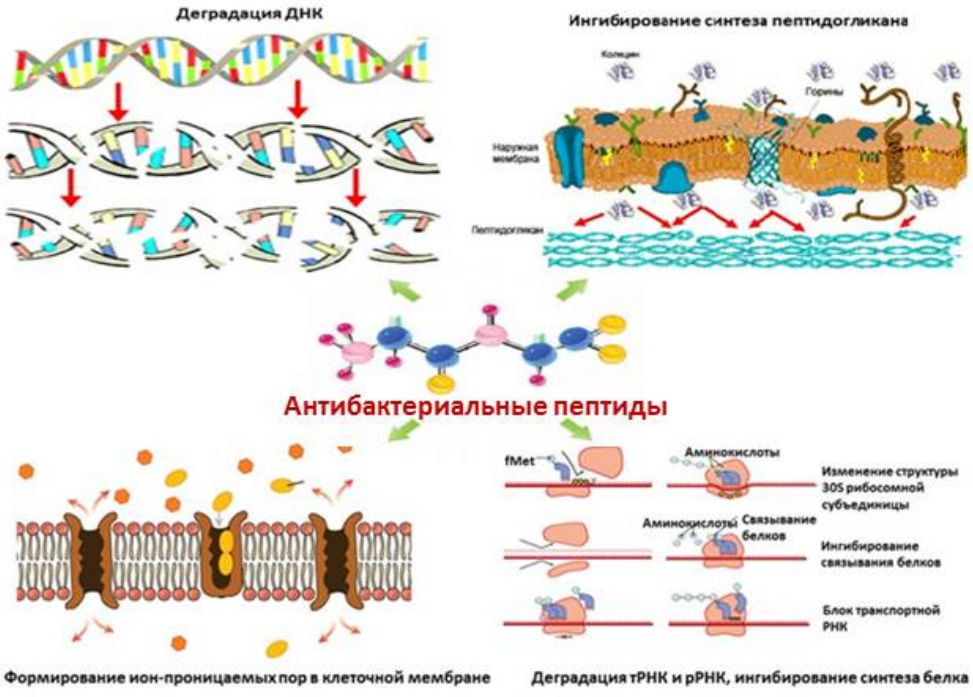

Fig 3. Key mechanisms of antimicrobial action
of antibacterial peptides, which are secondary metabolites
of marine bacteria (diagram by authors).

In recent years, two accessible databases have been created for storing and querying information on almost two hundred antibacterial peptides: BACTIBASE [80] and BAGEL [81, 82]. Moreover, members of this metabolite class are mentioned in other relevant databases, such as APD3 [83], ANTIMIC [84], CyBase [85], or StraPep [86], the use of which in the mode suggests the antibacterial activity of the substances obtained.

Despite the isolation and study of secondary metabolites from marine bacteria is currently at the initial stage, the obtained results show them as the most promising agents to control infectious diseases of fish. This is especially important in the light of the current trend of increasing proportion of marine aquaculture in the global seafood production and its increasing role in world's fisheries [87–89].

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In addition, marine-derived metabolites have demonstrated their immense potential to be used as natural preservatives of foods, medical and veterinary therapeutic drugs, or phytosanitary agents for plant protection [4, 88, 90, 91]. Their antitumor resource [4, 32, 66, 92] and anti-virus [5, 64] and antifungal activities [3–5] are very noteworthy and promising.

5. Conclusions and prospects to the future

Marine bacteria are an extremely rich source of structurally diverse classes of protein-based secondary metabolites. In recent years, significant progress has been made in our understanding of the complex mechanisms of their nonribosomal biosynthesis. These natural metabolic byproducts of marine bacteria have a wide range of antimicrobial activities, low rate of elimination from the organism, high specificity to cell targets, and reduced risk of undesirable side effects. Due to these properties, they are already considered as a source of effective biologically active therapeutic agents that can become an alternative to traditional antimicrobial drugs.

Modern biotechnologies of invention and development of novel antibiotics having a medical value are based on application of natural strategies of nonribosomal peptide synthesis [4, 9, 10]. Revealing the mechanisms and potential of this type of metabolite production in terrestrial and marine bacteria is not only of fundamental, but also of great practical importance. In human pathogens, this biosynthetic pathway probably causes the emergence of microbial isolates with multidrug resistance. Under these conditions, the activity of peptide synthetase and clusters of the genes responsible for nonribosomal synthesis become a new target for the strategy of treatment of infections caused by drug-resistant forms of bacteria [3, 5, 91, 92].

As regards marine bacteria and their secondary metabolites, the targeted control of biosynthesis mechanisms by using the metabolic engineering methods to create hybrid peptides or obtain hybrid peptide synthetases by disrupting the target gene of nonribosomal synthesis is now one of the noteworthy trends in modern biotechnology. This pathway becomes not only one of the most promising approaches to the development of novel antibiotics, but also a potential target for controlling the exocrine activity of pathogenic bacteria and, consequently, their viability [5, 93–95].

The range of active antimicrobial agents derived from marine bacteria, discovered and tested in recent years, indicate a high pharmacological potential of their secondary metabolites, and the study of these peptides is expected to be an interesting and fruitful activity in the coming years.

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