

ANTI-BIOFILM AGENTS FROM MARINE BIOTA

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ABSTRACT

Microbial biofilm forms on any living or non-living material surface contacting with microbial species. It is a persistent world-weight spread problem with very high prize. This explains the exclusive interest to development of anti-biofilm material surfaces. The continuously increasing microbial resistance to currently used antimicrobial agents requires looking for new ones. Marine biota is a rich source of biologically active substances with anti-biofilm potential that is scarcely studied.

The aim of this review is to outline the variety of marine sources delivering antimicrobial agents and their ability to inhibit different stages of the biofilm development, expecting to give some ideas for their utilization in the creation of improved antibiofilm material surfaces.

It includes short information about the negative impact and cost, mode of development and composition of the microbial biofilms, as well as principle approaches to the inhibition with focus on the sources of biologically active substances and anti-biofilm agents from marine biota.

The main conclusion is that the antibiofilm activity of many marine biota derived biologically active substances is not enough investigated although their strong bactericidal, antioxidant, surfactant and other activities are already proved and utilized in the medicine, cosmetic, food industry and others. This review is an update of the known to day on the marine sources derived anti-biofilm agents.

Keywords: microbial biofilms, marine biota, anti-biofilm agents.

INTRODUCTION

Microbial biofilm formation is a persistent world-weight spread problem at any living or non-living material surface contacting with microbial species: bio- and food contacting materials; water based industrial processes; membrane technologies; underwater constructions; sensors; ship hulls; pipes; water desalination systems; fishing and aquaculture farms; heat exchangers, etc. Biofilm formation is the initial step of the complex marine biofouling process contributing to the increased fuel consumption and having own negative impact on many submersed marine constructions and underwater devices, for example, underwater sensors and others. It shorts the exploitation life of membranes and

causes health problems such as inflammatory reactions, rejection and blood coagulation at biomaterials, etc. Biofilm formation is a main reason for bacterial infections related to indwelling medical devices. Since one device is colonized, the infection is almost impossible to eliminate [1]. In many cases, urinary catheters and stents closes and should be removed; some settled implants also should be removed and replaced. Biofilm-related infections in prosthetic devices affect more than 4.1 million patients a year. In Europe, around 7 billion euros per year is the total cost of the treating the complications due to infections [2, 3]. A report of the UK government commission predicts that by 2050 antimicrobial resistance will cause 300 million premature deaths and cost the global economy over

\$100 trillion [4]. The cost of biofilm negative effects and protection against such is very high. This explains the outstanding interest to the combat with the microbial biofilms.

Challenges and novel strategies in the treatment of microbial biofilm using nanoparticles, plant diterpenoids, biomacromolecules (polysaccharides: hyaluronic acid, heparin, chitosan; organic acids; antimicrobial peptides, etc.) as well as a development of novel anti-biofilm technologies are largely discussed. However, the reduction and removal of microbial biofilms remains a challenge [5]. The discovery of new anti-biofilm agents from marine biota is expected to contribute to the treatment of the increasing drug-resistant infectious and to reduce the negative impact of marine and industrial biofilms [6]. Marine microorganisms (fungi, bacteria, microalgae, etc.) producing substances with unique properties for a wide variety of industrial and biotechnological applications were already isolated [7]. In some cases, the biological activity of marine organisms produced substances is higher than that of those from terrestrial sources [8]. The benefits under the sea are outside of doubts [9].

The aim of this review is to update the identified in the last five years antibiofilm agents from marine biota, their advantages and ability to inhibit development of mono- and multispecies biofilms. A special attention is given on resistant to current treatments biofilms and the opening of new ways to mitigate the problems with their negative impact of medical, marine and technical devices.

MODE OF DEVELOPMENT AND COMPOSITION OF MICROBIAL BIOFILM

Microbial biofilm is a complex, surface attached community of microorganisms, engage in extracellular polymeric substances (EPSs) that create a gel matrix providing enzymatic interactions, exchange of nutrients, protection against environmental stress and increased resistance to biocides. In a biofilm state, the microbes are significantly more resistant to antimicrobial treatment [10, 11].

The mode of development and composition of the microbial biofilm are intensively studied to understand the molecular- and macroscale interactions between microbial species and surfaces as a base of current

approaches to the biofilm control [8, 12]. A generalized concept of biofilm formation includes several stages. Initially, planktonic cells reversibly attach to a surface (reversible adhesion) and remain in this transient state until signaling by an environmental cue to form a less ephemeral relationship - a phenomenon known as a quorum sensing, QS. Once microorganisms begin to secrete EPSs, biofilm develops to an irreversible process due to a cross-linking. In the mature biofilm, the cells are already engaged in an extracellular matrix composed mainly of proteins, exopolysaccharides and extracellular DNA (eDNA) [8, 12 - 14].

The primary mechanism in the attachment of microorganisms to surfaces usually involves secretion of protein or glycoprotein adhesives. Protein adsorption, that happens within seconds to minutes following immersion, acts as a “conditioning layer”, altering the physical-chemical properties of the surface and providing a nutrient source for attachment of microbes. While the biological cascade of “conditioning layer” and following biofilm formation begins with deposition of proteins, low protein adsorption is accepted as the most important pre-requisite for biofouling resistance. The identification of the type and amount of adsorbed proteins provide important information for the rational development of new material surfaces resisting biofilm development [15].

PRINCIPLE ANTI-BIOFILM APPROACHES AND ANTI-BIOFILM AGENTS

The development of anti-biofilm strategies should take account for all possible interactions (including the competitive relationships in multispecies biofilms) used by the microbes to adhere and develop a biofilm. Any event included in the biofilm formation could be a target to its control but it seems that combating biofilms is much easier at the initial stages of its formation [16, 17].

The current antibiofilm strategies are based on the knowledge about the composition and mechanism of marine biofilm development; the microbial adhesion and protein adsorption as mediator of this process; cell-surface and cell-cell interactions; characteristics of microbes living in biofilms; EPS production and cross-linking as well as intercellular communication via quorum sensing [8, 18]. The settled microorganisms in turn can modify biofilm composition and thus change

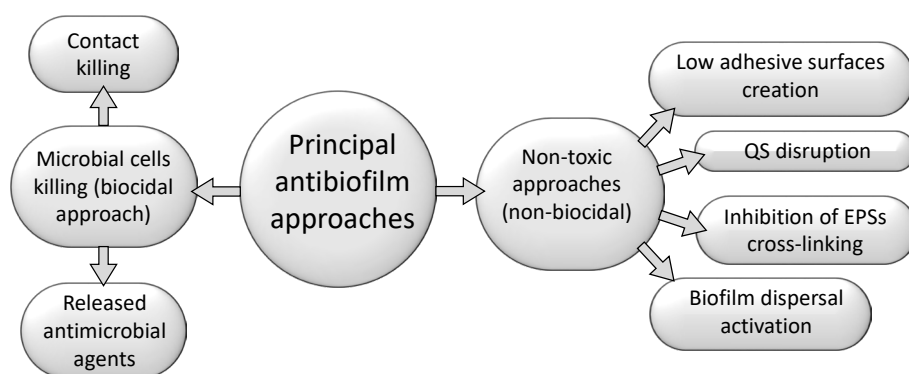


Fig. 1. Principle antibiofilm approaches.

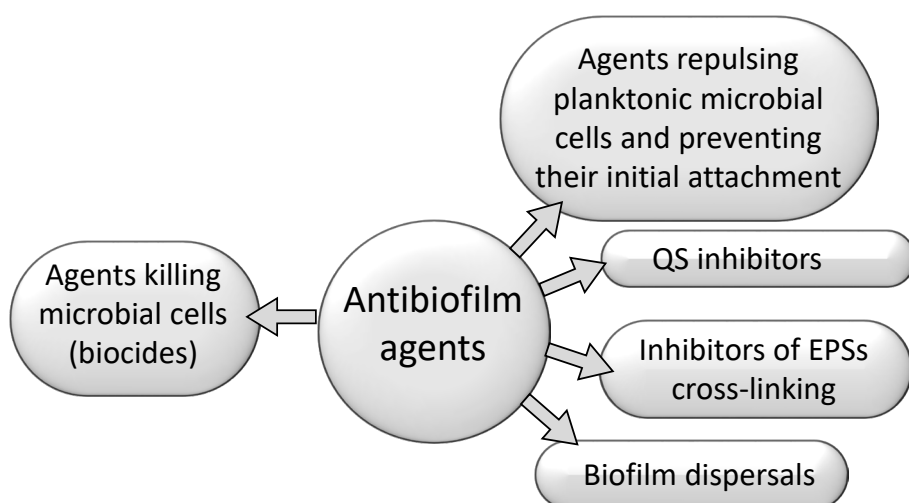


Fig. 2. Antibiofilm agents.

its properties and dynamics [19]. Generally, the more in depth understanding of the molecular-scale and macro-scale events between the fouling species and surfaces is expected to support the combat with the marine biofilm [8]. A general current trend in the combat with microbial biofilms (medical, marine and industrial) is the development of non-toxic approaches based on physical, chemical and biological methods, most of them inspired by nature. The biofilm formation is complicated process which inhibition needs in a complex approaches. Therefore, the combining of two or more approaches promises to be more beneficial.

The principle antibiofilm approaches can be split in two main groups: 1) non-biocidal approaches including minimizing the reversible attachment; disruption the QS; inhibition the EPS cross-linking; biofilm dispersal activation and 2) biocidal approaches, based on the use of

natural biocides from marine biota to develop contact-active systems: biocide-releasing or non-releasing (Fig. 1) [15].

The antibiofilm agents include: i) agents, minimizing the initial adhesion (lowering the initial surface/microbial cell interaction); ii) QS inhibitors; iii) inhibitors of the EPS cross-linking; iv) biofilm dispersals and v) biocides/enzymes (Fig. 2).

Non-biocidal approaches

Minimizing the initial reversible microbial attachment could be achieved by development of low adhesive surfaces (strong hydrophobic, water like or strong/super hydrophobic) by utilization of suitable polymers, biosurfactants and other microbial metabolites [11, 20, 21]. However, in practice synthetic materials, which are capable of preventing microbial adsorption and following microbial adhesion, are still elusive,

despite a large volume of research carried out up to now. Different biosurfactant groups exhibit diverse properties and variety of physiological functions, including not only the reduction of bacterial cell adhesion and aggregation, but also influencing the QS and biofilm formation.

QS (a form of cell-to-cell communication) attracts increasing interest as a target potentially substitutive or complementary to traditional treatments for reduction the biofilm formation [22]. The QS in microbial biofilms could be disrupted/inhibited by different strategies, including employment of derived from marine organisms compounds [23, 24]. Two main groups of compounds are currently known inhibiting the QS transcriptional regulation in Gram-negative bacteria: 1) molecules comprising structural analogs of the native signaling and 2) compounds comprising lacking structural resemblance [11, 21, 25, 26]. An example of enzymatic QS signals obstruction is the acylase degradation that reduces bacterial fouling via hydrolyzing the acyl-amide bonds between carboxylic acids and amines/amino acids, i.e. [27]. The practical application of enzymes in anti-biofilm approaches limits by some their features: i) any enzyme needs in water and structural mobility for keeping the own natural activity, both difficult for providing in antifouling coating; ii) the optimal balance between enzyme activity and stability is another challenge because with the increasing of temperature the enzyme activity increases whereas its stability decreases. Different biosurfactant groups (lipids and glycolipids from marine microalgae; polysaccharides from sponge-associated bacteria and others) exhibit diverse properties including influence on quorum sensing [11].

Once the microorganisms begin to secrete EPSs, biofilm development progresses to an irreversible process due to a cross-linking and thus attaching the microfouling species to the surface [10, 28].

Inhibition of the EPS cross-linking is another non-biocidal way to reduce marine biofilm development. Antioxidants and other inhibitors of EPSs cross-linking are known and well presented in the special literature [14, 17, 29]. A large variety of marine organisms and their metabolites are reported to deliver antioxidant products in a form of biomass, crude or sequential extracts and pure substances [30]. It is expected that a nonconventional, optimized derivation will provide sustainable alternative to preserve the potency of extracted antioxidant compounds, together with many

other benefits [31]. Enzymes also could be utilized in the inhibition of EPSs crosslinking. Targeting any event in the complex biofilm formation they affect not only the intercellular communication, but also degradation of adhesives used by microorganism for settlement; biofilm matrix disruption; release of antifouling compounds (deterrents/biocides) from the surface and activation of biofilm dispersal [27].

Activation of biofilm dispersal is a relatively new mode of action for anti-biofilm compounds. Natural derivatives using non-microbiocidal mechanism, such as bio-surfactants/dispersals attracts improved attention with their unique properties: high biodegradability, low toxicity and effectiveness at extreme temperatures, pH and salinity. Biosurfactants are mainly comprised of lipids and lipopeptides. Bio-surfactants reduce the surface tension and induce swarming or coagulation of cells. Some bio-surfactants were isolated from marine sponges, bacteria, brown algae, and others. Identification of marine micro-organisms producing biosurfactants is going on [17, 32]. Current review of Amankwah et al. [33] is focused on bacterial biofilm destruction by the use of phages combined with other antibiofilm agents. It is concluded that despite successful use of phages and phage-derived products they are mostly not adequate to eradicate all bacterial cells. Nevertheless, a combined use of phages and/or phage-derived products with other antimicrobial agents, like antibiotics, nanoparticles and antimicrobial peptides, may be effective to remove microbial biofilms from medical device surfaces.

Biocidal approaches

Killing the microbial cells by marine biota derived biocides and enzymes happens by their including in contact-active systems: biocide-releasing or biocide-non releasing. In the second case, the natural biocide or enzyme is somehow attached to the protected surface [11, 15, 17]. Many of extracts from marine sponges, algae, soft corals, anemones, sea weeds, fungi and bacteria demonstrate own biocide effect. A number of derived from them substances and their synthetic analogues (like terpenes and pyrrole-imidazole alkaloids, succinic acid, taurine acid, substituted bromo-pyrrole alkaloids and many others) display monospecific or bread bactericidal activity [11, 17]. The marine biota is an inexhaustible source of natural biocides and enzymes but they are produced in scare amounts; their including in antifouling

paints is complicated; and some of them are toxic for aquatic organisms. The identification of new marine sources and antibiofilm agents, simultaneously with a development of new derivation and microbial strains cultivation technologies continue with the expectation to be mitigated some of above-mentioned problems.

ANTI-BIOFILM AGENTS FROM MARINE BIOTA

An ideal anti-biofilm agent (medical, marine or technical) should have ability to effectively kill or inhibit microbial adhesion and/or growth; to have suitable solubility (in water or other solvent); reasonable period of time storage without significant loss of antimicrobial activity; if possible to have cleaning properties; and to be easy for preparation [35]. The natural chemical defense of the non-fouling marine organisms is due to production of secondary metabolites. Biological extracts of secreted metabolites are anticipated of the toxic biocides to act as environmentally safe anti-foulants and anti-biofilm agents [34, 35]. The obtaining of marine anti-biofilm agents usually starts with crude extracts preparation, followed by separation, purification and identification of antifouling compounds. In some cases, it continues with attempts to obtain synthetic analogues and rarely reach a preparation of antifouling compositions.

Large variety of marine organisms such as marine sponges, algae, sea weeds, bacteria, fungi, etc. are capable of biosynthesizing a broad variety of secondary metabolites. Largely unexplored sources for isolation of new microbes (bacteria, fungi, actinomycetes, microalgae-cyanobacteria and diatoms) exist that are potent producers of bioactive secondary metabolites [36]. The identified so far natural antibiofilm agents from marine flora and fauna involve natural biocides and enzymes together with biosurfactants/dispersals, and quorum-sensing inhibitors [36, 37]. Based on their chemical type, the antimicrobial compounds from marine biota are categorized as terpenes (sesquiterpenes, diterpenes, sesterterpenes, and triterpenes), steroids (sterols), alkaloids (indole, quinoline, pyridoacridone, and amine alkaloids), aromatics (flavonoids, chalcones, coumarins, lignans, xanthenes, anthracenes, anthraquinones, naphthalene), polyketides (acetylenic fatty acids, polycyclic esters and quinones), and peptides [11, 17, 38].

Ameen et al. discuss the discovery of bioactive

molecules from marine microorganisms reported from 2018 onwards and highlight the huge potential of marine microorganisms (fungi, myxomycetes, bacteria, and microalgae) for obtaining of highly valuable bioactive compounds [7]. Problems such as accessibility of the natural sources, too low ratio bioactive compound/biomass and other limit their commercialization and practical application. According to Singh et al., secreted by marine microorganisms (bacteria, fungi and algae) bioactive molecules (in traceable amount as their secondary metabolites) can be converted into antimicrobial agents, using modern tools of molecular biology and drug discovery, like metagenomic tools, genetic recombinant techniques and chemo enzymatic synthesis [39]. Tortorella et al. report novel antibacterial agents isolated from deep-sea microorganisms using recent advances in techniques and strategies for the exploitation of the deep-sea microorganisms [40]. Blue biotechnology provides opportunities for a wide range initiatives of commercial interest for the pharmaceutical, biomedical, cosmetic, nutraceutical, food, feed, agricultural, and related industries including anti-biofilm treatments [41]. In relevant form, all marine biota derived antimicrobial agents could be utilized in the anti-biofilm protection, if they have enough strong activity against the corresponding microbial species. However, the reports about anti-biofilm protection with their participation are scarce.

In the year 2000, Armstrong et al. report the first formulation of anti-fouling paints (acrylic) that incorporate marine natural products [36]. Lately, Costa et al. presented a broad-spectrum antiadhesive coatings based on an extracellular polymer from a marine cyanobacterium [42]. The development of this anti-adhesive coating is an important step towards the establishment of a new technological platform capable of preventing medical device-associated infections, without inducing thrombus formation in blood-contacting applications. Nasri et al. report past and current progress in the development of antiviral/antimicrobial polymer coating towards COVID-19 prevention enjoying of improved interest lately [43]. Various antiviral/antimicrobial polymer coatings are presented in this review. It is expected an improvement of their antiviral activity by exploring of derived from marine biota antibiofilm agents. Combined with current nanotechnology and progressive derivation techniques an

accelerated development of successive antiviral material surfaces is expected [44, 45]. Here are summarized different potential antibiofilm agents from marine biota although their antibiofilm activity was not tested yet.

Marine bacteria in the anti-biofilm protection

Marine bacteria attract attention with their ability to synthesize structurally diverse classes of bioactive secondary metabolites with high biotechnological potential. The marine bacteria are one of the main sources of antimicrobial agents. They could be also a “living” anti-biofilm protector.

Cetina et al. report bacteria (isolated in the Gulf of Mexico from Campeche, Mexico) producing substances inhibiting the growth of human pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa* [46]. Schinke et al. report over 50 antibacterial compounds derived from marine bacteria during the period 2010 - 2015 including some broad-spectrum antibacterial compounds with activity against antibiotic resistant Gram-positive and Gram-negative bacteria [47]. Cita et al. find that metabolites of bacteria associated with marine sponge *Xestospongia testudinaria* (from Tanjung Kasuari, Sorong, Papua) hide promise for development of new antibacterial agents [48]. The marine bacteria extracts demonstrate antimicrobial activity against two Gram-positive bacteria (*Staphylococcus aureus* *Staphylococcus epidermidis*) and three Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*) determined by disk diffusion test. Chianese et al. isolate linear aminolipids with moderate antimicrobial activity from the Antarctic Gram-negative bacterium *Aequorivita sp.* using a miniaturized culture chip technique [49]. Leon-Palmero et al. report the diversity and antimicrobial activity of bacteria derived from the microbiota of two sea anemones (*Anemonia sulcata*, *Actinia equina*) and two holothurians (*Holothuria tubulosa*, *Holothuria forskali*) [50]. The antimicrobial activity was tested against pathogens of interest for human health, agriculture and aquaculture. From the isolated 27 strains with antibacterial activity, 12 show also antifungal activity. However, more sophisticated culture techniques are necessary to explore these rare symbiotic bacteria and to determine their biotechnological potential [50]. The biotechnological potential of secondary metabolites from marine bacteria is a subject of Andryukov et al. study [51]. Identifying

the mechanisms and potential of this type of metabolite production in marine bacteria become one of the noteworthy trends in modern biotechnology. A review of Ramesh et al. emphasizes marine pigmented bacteria as a potential alternative source of natural compounds with good antibacterial potential [52].

Stincone and Brandelli review the current state of art of antimicrobial molecules from marine bacteria. They found that during the last two decades, the most studied group of bacteria producers of substances with antimicrobial activity is the *Firmicutes phylum*, in particular strains of the *Bacillus* genus [53]. The reason for that is maybe the difficult cultivation of typical *Actinobacteria* from marine sediments, whose members are the major producers of antimicrobial substances in land environments. However, a reversed trend observes in recent years with an increasing number of reports settling on *Actinobacteria*. Great diversity of chemical structures was identified, such as fijimicyns and lynamycin from *Actinomycetes* and macrolactins produced by *Bacillus* [53]. Four new uncommon 20-nor-isopimarane di-terpenoid epimerase, aspewentins I-L (1-4), together with a new methylated derivative of 3, aspewentin M (5), were isolated from the deep sea sediment-derived fungus *Aspergillus wentii* SD-310 by Li et al. [54]. Magdalena et al. perform screening and characterization of bacteria with antibiofilm activity, isolated from waterfall and marine environment of several locations in Indonesia [55]. Their antimicrobial activity against six bacterial pathogens were evaluated, followed by antibiofilm screening. Eleven isolates show quorum sensing or quorum quenching activity, one of them showing both activities. Supernatants of 11 isolates inhibit biofilm formation of at least one pathogen (by static biofilm assay). Different bioactive compounds, such as carbohydrates, proteins, and nucleic acids were presented in five selected isolates [55]. In a screening program for antibiofilm compounds against marine biofilms, Long et al. discover the potential antibiofilm activity of elasnin. Elasnin effectively inhibits the biofilm formation of seven strains of bacteria isolated from marine biofilms [56]. With high productivity, elasnin-based coatings were prepared in an easy and cost-effective way, which shows a great performance in inhibiting the formation of multi-species biofilms and the attachment of large biofouling organisms in the marine environment with low toxic effects. These

findings indicated the potential application of elasin in the marine biofilm control [56].

Anti-biofilm agents from marine microalgae

Microalgae, suspended in the oceans, are a promising source of organic material for deriving of new bioactive compounds with novel structures and potential biological functions. Activity of these compounds against bacterial pathogens and biofilm formation is under intense study now. NoMorFilm is a Horizon 2020 EU-funded project to investigate how these compounds act on bacterial pathogens and biofilm formation. The biosynthesis of the targeted bioactive compounds is addressed to diminish the cultivation costs by mimicking natural aquatic ecosystems. Industrially interesting anti-biofilm molecules are incorporated into nanoparticles for the application on prosthetic devices. Four compounds with antibacterial and anti-biofilm activity were already patent protected. A new coating method was developed by use of solid-like gel (as antimicrobial agent carrier) and prototype of a releasing antibiotic prostatic device was developed. Development of new antibacterial and antibiofilm compounds under NoMorFilm is expected to support the EU blue biotechnology [57, 58].

A number of studies describe compounds produced by microalgae species with antimicrobial activity. However, studies on the antibiofilm activity of extracts and/or molecules produced by these microorganisms are scarce. Lopez and Soto discuss the usefulness of microalgae compounds for preventing biofilm infections [59]. Quorum-sensing inhibitor and anti-adherent agents, among others, were isolated from microalgae and cyanobacteria species. The use of tools such as nanotechnology is expected to increase their activity for preventing and treating of biofilm-related infections [59]. Iglesias et al. [60] investigate the chemical composition of five marine microalgae (*Dunaliella sp.*, *Dunaliella salina*, *Chaetoceros calcitrans*, *Chaetoceros gracilis* and *Tisochrysis lutea*) through nuclear magnetic resonance (NMR) of the soluble material obtained by sequential extraction with hexane, ethyl acetate (AcOEt) and methanol of biomass from stationary phase cultures and evaluate its antibacterial and anti-biofilm activity. The AcOEt extract from *C. gracilis* shows a moderate antibiofilm activity. The antimicrobial and antibiofilm activities of sulfated polysaccharides from several types' marine algae (Fucoidans) against dental

bacteria and plaque are the subject of the review of Achmad et al. [61]. They show that these Fucoidans are effectively working as antimicrobial and antibiofilm agents. Selvekesavan and Franklin present prospective application of nanoparticles green synthesized using medicinal plant extracts as novel nanomedicines, nanotechnology, science and applications that probably could be approach to use marine biota derivatives [62].

Anti-biofilm agents from invertebrates

Marine invertebrates include several species across different taxa (*Porifera*, *Annelids*, *Coelenterates*, *Mollusks*, *Echinoderms* and etc.) that produce bioactive natural products [11]. Variety of studies identify natural products from marine invertebrates and their biological activities as well as carry out biosynthetic studies to modify their structures. Sterols from marine invertebrates demonstrate antimicrobial/antibiofilm, antiinflammatory, antiHIV, and anticancer activities [63]. Rozirwan et al. undertake study to find most active inhibitors of pathogen bacteria on pond shrimp between extracts of marine biota, collected from Maspari island (South Sumatera, Indonesia): soft coral *Sarcophyton sp.*, *Sponge Aaptos sp.*, seaweeds *Sargassum sp.* and *Halimeda sp.* and mangroves *Avicennia sp.* and *Rhizophora sp. species* [64].

Anti-biofilm agents from marine sponges

Nowadays, it is supposed that the solution of the problem with the growing resistance of many type bacteria to the known antimicrobial agents may come from the sea microorganisms and especially from sea sponges. Sponges are the most studied marine invertebrates in terms of secondary metabolites. Stowe et al. discuss several sets of compounds derived from marine sponges to be utilized against the persistent biofilm problem [1]. They discuss discovery/synthesis of natural products and their analogues, including marine sponge derived compounds, initial adjuvant activity and toxicological screening of the novel antibiofilm compounds. More than 5300 different bioactive substances were already discovered, presenting in sponges, with pharmaceutical activities against human diseases. These substances are classified as alkaloids, lipids, steroids, and terpenoids, some of them exhibiting cytotoxic activities (polyacetylenic lipid derivatives, glycerol ethers, and linear alcohols). Some secondary

metabolites from sponges are cytotoxic to human tumor and cancer cells; ovarian sarcoma, pancreatic cancer, and colorectal adenocarcinoma cell lines [65, 66]. Natural products with antioxidant, antimicrobial, and other activities were also found in sponges [67]. With the great potential of their secondary metabolites, the marine sponges are considered as a container of compounds with a wide spectrum biological activity including antibacterial, antiviral and antifungal that could be utilized in different anti-biofilm approaches [68]. For over 30 years, researchers of the Florida Atlantic University (USA), created collection of 19 000 different kinds of marine microorganisms, 11 000 of which are found more than 45 meters deep these (along the US East Coast, the Gulf of Mexico, the Caribbean and the European and African deep waters) creating a huge biological archive, called Harbor Branch Marine Microbial Collection [69].

Anti-biofilm agents from sea stars

A number of studies report isolation of about 2000 (to the year 2017) biologically active secondary metabolites from sea stars. The metabolites include: saponins from *Culcita novaeguineae* (cytotoxic against carcinoma cells); biologically active substances, preventing initiation of carcinogenesis by enzymes, from *Asterina pectinifera*; carotenoids from *Marthasterias glacialis* (cytotoxic against rat basophilic leukemia); polysaccharides from the starfish *Asterias rollestoni* as well as steroidal glycosides and glucocerebrosides from *L. maculata* (demonstrating antioxidant activity); crude extracts of *Astropecten polyacanthus* (inhibiting inflammatory components); sterol compounds from *Tremaster novaecaledoniae*, *Asterias amurensis*, *Styracaster caroli*, and *Echinaster brasiliensis* (inactivating HIV 6) and others [70]. Several studies report the isolation of marine natural products from secondary metabolites of sea stars with several bioactivities utilizable in the anti-biofilm approaches. Crude and fractioned ethanolic, n-butanol and methanolic extracts of the sea stars *Luidia maculate*, *Stellaster equestris*, *Astropecten indicus*, *Protoreaster lincki*, *Pentacaster regulus* demonstrate antibacterial and antifungal activity against human pathogens. Although only few biologically active substances were identified and isolated, the marine sea star *Echinoderm* is considered as an exceptional source of polar steroids with large structural diversity showing a wide range of bioactivities [71].

Anti-biofilm agents from other invertebrates

Antimicrobial, antioxidant and other activities were found in other marine invertebrates: significant inhibitory effects against *T. brucei* of the extract of soft corals in Vietnam; non-selective anti-cancer activity of several secondary metabolites isolated from the marine crinoid invertebrate *Colobometra perspinosa*; a broad spectrum antimicrobial activity of dolabellin (33 amino acid residue peptide) from a mollusk sea hare (Japan); antimicrobial activity of secondary metabolites from digestive glands and mantle of nudibranchs; antibacterial activity of extracts from a bivalve *P. viridis* against *V. cholera* [70, 72].

Anti-biofilm peptides

Antimicrobial peptides (AMPs) are group of low molecular proteins showing broad-spectrum antimicrobial activity against a variety of pathogens. This class of compounds contributes to solving the microbial resistance limiting the use of many potential antimicrobial agents [73]. The marine environment is one of the richest but not fully explored sources for antimicrobial peptides. This section is an update of new identified antimicrobial peptides from marine sources during the last 5 years.

Among the unique features of the AMPs is their ability to kill rapidly bacteria (often both Gram-positive and Gram-negative) and other microorganisms without toxicity to other cells that is a base of the exclusive interest to them during the last decades. AMPs with high antimicrobial potential were found in the edible sea urchin *Echinus esculentus* that kill bacteria at low μM concentrations and fungi at somewhat higher concentrations [74]. Pérez et al. present the major compounds with antimicrobial activities found in microalgae and their most promising applications [75]. The antimicrobial and antibiofilm active agents isolated from green, brown and red algae are peptides, polysaccharides, fatty acids, phlorotannins, pigments, lectins, alkaloids, terpenoids and halogenated compounds. Cationic antimicrobial peptide, Epinecidin-1, identified from *Epinephelus coioides* possesses multiple biological functions, including antibacterial anti antifungal [76]. N-acetyl cysteine (NAC) is a greatly applied antioxidant *in vivo* and *in vitro*. It acts directly as a scavenger of free oxygen radicals [77]. Costa et al. report N-acetylcysteine-functionalized coating avoiding

bacterial adhesion and biofilm formation [78]. They immobilize NAC covalently in order to obtain long-lasting high local concentration of the drug onto a chitosan (Ch) - derived implant-related coating. For the development of NAC-functionalized Ch films, water-based carbodiimide chemistry is applied to avoid the use of toxic organic solvents. Surface characterization by ellipsometry, measurement of water contact angle and X-ray photoelectron spectroscopy (XPS), demonstrate the success of NAC immobilization. Quartz crystal microbalance with dissipation (QCM-D) demonstrates that surface immobilized NAC decreases protein adsorption to Ch coatings. Biological studies confirm that immobilized NAC avoids methicillin-resistant *Staphylococcus aureus* adhesion to Ch coating, impairing biofilm formation, without cytotoxic effects [78]. Antibacterial and antiendotoxic peptides or proteins, composed of metabolically tolerable residues present in many marine species, including marine vertebrates, invertebrates and microorganisms. Many studies report that these marine peptides and proteins or their derivatives exhibit potent antibacterial activity and antiendotoxic activity *in vitro* and *in vivo*. However, their heterologous expression in microorganisms, physicochemical factors affecting peptide or protein interactions with bacterial LPS and LPS-neutralizing mechanism are not fully understood [79]. Aldini et al. discuss N-acetylcysteine (NAC), as an antioxidant and disulphide breaking agent. The main molecular mechanisms explaining the well-established antioxidant and reducing activity of NAC the N-acetyl derivative of the natural amino acid l-cysteine are summarized and critically reviewed [80]. The antioxidant effect is due to the ability of NAC to act as a reduced glutathione (GSH) precursor; GSH is a well-known direct antioxidant and a substrate of several antioxidant enzymes. Under some conditions NAC can act as a direct antioxidant for oxidant species such as NO₂ and HOX. The antioxidant activity of NAC could also be due to its effect in breaking thiolate proteins, thus releasing free thiols as well as reduced proteins, which in some cases, such as for mercaptoalbumin, have important direct antioxidant activity. As well as being involved in the antioxidant mechanism, the disulphide breaking activity of NAC also explains its mucolytic activity which is due to its effect in reducing heavily cross-linked mucus glycoproteins [80].

Anti-biofilm biosurfactants

Biosurfactants (BSs) that are amphiphilic molecules with hydrophilic and hydrophobic moieties play a key role in emulsification, foam formation, detergency and dispersal, the last two of especial interest for anti-biofilm applications. The BSs are already discussed as multifunctional biomolecules of the 21st century [81].

BSs could affect all stages of microbial biofilm development: the initial reversible microbial cells attachment, QS process, EPSs cross-linking and biofilm dispersal. Many BSs contain lipids or lipopeptides. Microbial produced BSs are less toxic compared to the synthetic ones, biodegradable and have potential uses in large variety of areas. Makovitzki et al. report ultrashort antibacterial and antifungal lipopeptides [82]. Composed of specific lipophilic moieties attached to anionic peptides (six to seven aminoacids), they are produced only in bacteria and fungi during cultivation. The following is interesting for these ultrashort lipopeptides: (i) The attachment of an aliphatic chain to, otherwise inert, cationic D,L-tetra peptides that makes them potentially active against various microorganisms (including antibiotic resistance strains); (ii) The cell specificity determined by the sequence of the short peptide chain and the length of the aliphatic moiety; (iii) The mode of action of the very short peptide chains involving permeation and disintegration of membranes, similarly to the action of many long antimicrobial peptides [82].

Rufino et al. demonstrate the antimicrobial and anti-adhesive potential of the crude BS extract, isolated from *C. lipolytica* UCP0988, against several pathogenic and nonpathogenic bacteria, yeasts and filamentous fungi [83]. The results suggest that this biosurfactant could be a suitable alternative to conventional antibiotics [83]. Hashada study the potential antimicrobial activity of BSs derived from bacterial samples collected from Uran sea coast, Mumbai, local Garage and Petrol pump [84]. The antimicrobial activity of the purified surfactants was shown to be effective against *E. coli* (NCIM 2065), *B. subtilis* (NCIM 2063), and *S. aureus* (NCIM 5021). Cochrane et al. report a non-ribosomal lipopeptide, Tridecaptin A₁ (TriA₁), produced by *Bacillus* and *Paenibacillus* species [85]. This acylated tridecapeptide demonstrates strong and selective antimicrobial activity against Gram-negative bacteria, including multidrug-resistant strains of *Klebsiella pneumoniae*, *Acinetobacter*

baumannii, and *Escherichia coli*. It is believed that Tridecaptin A₁ could be an excellent antibiotic candidate. Despite being non-pathogenic, marine-derived BSs are unexploited commercially due to their low yields, insufficient structural elucidation and uncharacterized genes. Therefore, optimization the conditions of BSs production in marine bacteria, characterization of the produced compounds and the genes involved in the biosynthesis are necessary to improve cost-efficiency and realizing in industrial demands [86]. Emerging trends and promising strategies in BSs production are a subject of the review of Singh et al. [87]. Use of nanoparticles and co-production of BSs along with other commercially important compounds like enzymes, are other upcoming bioprocess intensification strategies on the long way that should be past in making of BSs a commercially successful compounds.

Two papers report studies on the mechanism of interaction between the BSs and microbial cells. Kathka et al. study lipopolysaccharide-dependent membrane permeation and lipid clustering caused by cyclic lipopeptide colistin (polymyxin E), having for the importance of the polyanionic lipopolysaccharides (LPS)-enriched outer membrane (OM) in initiating the bactericidal activity of polymyxins [88]. The experimental results of LPS-dependent membrane restructuring provide useful insights into the mechanism that could be used by polymyxins in impairing the permeability barrier of the OM of Gram-negative bacteria [88]. Shahane et al. study the interaction of antimicrobial lipopeptides (AMLPS) with bacterial lipid bilayers. In contrast to traditional antibiotics, AMLPs act by physically disruption of the cell membrane (rather than targeting specific proteins), thus reducing the risk of inducing bacterial resistance [89]. Microsecond timescale atomistic molecular dynamics simulations are used in this investigation to quantify the interaction of a short AMLP with model bacterial lipid bilayers. Potential implications on membrane function and associated proteins are discussed.

Ackbary et al. discuss the BSs as a relatively new frontier for social and environmental safety. BSs are widely known as multi-functional compounds due to their non-harmful properties [90]. Industries such as pharmaceutical, cosmetic, food, textile, agricultural and other employ BSs for different purposes. Some challenges, like biological sources, high prize, etc.

limit the production of BSs. Various studies reveal that these problems can be solved by further investigating of different microorganisms and plants as alternative sources. The possible application in antibiofilm approaches is not discussed here [90]. Pirog et al. are focused on antimicrobial activity of surfactants from microbial origin. The recent data about the antibacterial and antifungal activity of microbial surfactants are presented: lipopeptides from *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Brevibacillus*, rhamnolipids bacteria and *Rhodotorula*, as well as of *Acinetobacter calcoaceticus*, *Rhodococcus erythropolis* and *Nocardia vaccini* [91]. The advantages of the natural antimicrobial glycolipids are the possibility of their synthesis on industrial waste and the high concentration of synthesized surfactants. Fenibo et al. present production, classifications, properties and characterization of microbial surfactants [92]. Plaza and Achal highlight the recent findings on the application of BSs as eco-friendly and innovative biocides against biocorrosion as alternative of the most of the used now synthetic biocides that are not effective against the biofilms, toxic and not degradable [93].

CONCLUSIONS

The combat with microbial biofilms remains a significant challenge despite the enormous efforts of many researchers across all over the world.

The current antibiofilm approaches are based on knowledge about the mode of development and composition of the microbial biofilm. They include all possible interactions of the microbial cells used to adhere and develop biofilm: cell/surface interactions, inter- and intracellular communication via quorum sensing, EPSs secretion and their cross-linking as well as microbial adhesion and protein adsorption as mediator of this process.

Replacement of the toxic biocides with marine biota derived, degradable and repellent antibiofilm agents is in the focus of the current antibiofilm strategies. The most marine biota derived highly active antimicrobial agents were not investigated as potential antibiofilm agents.

The marine derived biologically active substances are biosurfactants, antioxidants, antimicrobial peptides or other microbial metabolites that target all steps of the biofilm formation: from the initial adhesion inhibition to the matrix degradation, cell to cell communication

disruption and biofilm dispersion induction.

Unfortunately, no one-antibiofilm agent is known that is able to stop totally the initial reversible adsorption of microbial cells. The activation of biofilm dispersal rises as a relatively novel mode of action for anti-biofilm compounds. The biofilm development is a complex process and seems that its inhibition requires a complex strategy.

Produced by marine biota antimicrobial agents that show excellent biological activity remain unexploited commercially due to their low yields, insufficient structural elucidation and uncharacterized genes. Optimized antimicrobial agents production in marine conditions, optimized bacterial culture and coating technologies, characterization of the produced compounds and the genes involved in the biosynthesis are necessary to improve the cost efficiency and realizing in industrial demands.

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