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Review

Recent Progress in Terrestrial Biota-Derived Anti-Biofilm Agents for Medical Applications

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Abstract: The terrestrial biota is a rich source of biologically active substances whose anti-biofilm potential is not studied enough. The aim of this review is to outline a variety of terrestrial sources of antimicrobial agents with the ability to inhibit different stages of biofilm development, expecting to give some ideas for their utilization in improved anti-biofilm treatments. It provides an update for the last 5 years on anti-biofilm plant products and derivatives, essential oils, antimicrobial peptides, biosurfactants, etc., that are promising candidates for providing novel alternative approaches to combating multidrug-resistant biofilm-associated infections. Based on the reduction in bacterial adhesion to material and cell surfaces, the anti-adhesion strategy appears interesting for the prevention of bacterial attachment in combating a broad range of mono- and multispecies bacterial biofilms. So far, few studies have been carried out in this direction. Anti-biofilm coatings made by or containing biologically active products from terrestrial biota have scarcely been studied although they are of significant interest for a reduction in infections associated with medical devices. Combination therapy with commercial antibiotics and natural products is accepted now as a promising base for future advances in anti-biofilm treatment. In vivo testing and clinical trials are necessary for clinical application.

Keywords: terrestrial biota; anti-biofilm agents; targeted stage; biocidal and non-biocidal approaches

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1. Introduction

Biofilms are communities of microorganisms attached to biotic or abiotic surfaces embedded in a self-elaborated extracellular matrix (ECM). Non-desirable biofilm formation as a reservoir of microbial contamination is a hazard to human health, and a persistent problem for the healthcare, marine, and water-based industrial productions. The need to control biofilm formation promotes international interest in the development of mitigating strategies that include anti-biofilm agents and the redesign of material surfaces.

Providing an environment for the most stable microbial life, medical biofilms cause extensive antibiotic resistance, morbidity, and mortality, and substantial economic loss. Biofilms could be the leading cause for the failure of antibiotic treatments and play an important role in infections associated with both medical devices and tissues, many of them chronic [1].

The use of orthopedic implants, cardiovascular and ureteral tract devices, orthodontia prosthetics, and other devices is sometimes associated with infections due to the detachment

of microorganisms from the biofilm on the medical device. The very common urinary tract infections are an example. Microorganisms may colonize the outside surface of the catheter by direct inoculation during catheterization or by migration through the surrounding mucous sheath. The microbial biofilm on the inner catheter surface in patients with long-term catheterization hampers antibiotic treatment and causes chronic infection [2]. Biofilm formation on long-term medical implants, such as pacemakers and heart valves, prosthetic joints, and breast implants, leads to postoperative complications. Infections could cause inflammation and tissue destruction around the implant that sometimes are life-threatening. The use of large doses of antibiotics that may cause toxicity in the host, and/or removal of the infected device, are the only possible options, which may have a lethal impact for some patients [3]. Furthermore, infections associated with medical devices due to microbial attachment and biofilm formation on their surface have a high economic cost. For example, biofilm infections associated with prosthetic devices affect more than 4.1 million patients per year, with a total cost of the treated complications of around 7 billion euros in Europe [4,5].

Microorganisms adhering to biotic surfaces form biofilms in different tissues of the host, like epidermal cells and teeth, mucus on mucosal membranes, and chronic wounds. Multispecies biofilms could be formed in host intestines. Oral multispecies biofilm formation is very common. Gastrointestinal infections could also be caused by multispecies biofilm formation. Biofilm-associated persistent inflammations and chronic infections hide an increased risk of cancer development, like gallbladder cancer [6].

Numerous reports highlight alarming antimicrobial resistance rates [6,7]. The role of biofilms in antimicrobial resistance (AMR) is complex and significantly increasing resistance. The cells in a biofilm have intrinsic characteristics different from those of planktonic cells. Bacteria living in a biofilm can cause an increase of about 10- to 1000-fold in antibiotic resistance as compared to similar bacteria living in a planktonic state [8].

Biofilms act as physical barriers against drugs and host immune responses. They reduce the possibility of eradicating infections and cause relapses after the traditional appropriate treatment. The eradication of biofilms is challenging and more complicated in the predominant multispecies microbial biofilms. Many researchers are working to address biofilm-related infections. Some anti-biofilm strategies were developed and identified as promising. However, after about one hundred years since the first evidence of the mode of development and decades of intensive investigations, there are no therapeutic solutions to eradicate bacterial biofilms and their biomedical-related issues [9]. The failure of conventional antibiotic therapies and multidrug treatments indicates that biofilm control needs a significant upgrade [10].

Utilization of natural biologically active substances having relatively low toxicity, high efficiency, and little or no capacity to induce antimicrobial resistance rose as a new trend in combating biofilms during recent decades. Our former review [11] presented a five-year update, up to 2022, of marine organism-derived anti-biofilm agents. The terrestrial biota is also rich in biologically active substances whose anti-biofilm potential has not been studied enough up to now. The focus of this review is on medical bacterial biofilms and anti-biofilm approaches based on the utilization of natural products from the terrestrial biota. Its aim is to outline the variety of terrestrial sources and new antibacterial agents with the ability to inhibit different stages of biofilm formation, expecting to give birth to some ideas for their utilization in improved anti-biofilm treatments. This review is an update of the previously published review, from 2019 until now, on anti-biofilm plant products and derivatives, essential oils, antimicrobial peptides, biosurfactants, etc., and their anti-biofilm ability. A brief presentation is also included here of the anti-biofilm strategies based on the current knowledge about the mode of development and composition of the bacterial biofilm.

2. Composition and Mode of a Bacterial Biofilm Development

A bacterial biofilm is a complex, surface-attached community of microorganisms engaged 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. The EPSs produced by microorganisms are a mixture of biopolymers consisting of polysaccharides, proteins, nucleic acids, lipids, and humic substances. EPSs make up the intercellular space of microbial aggregates and form the structure and architecture of the biofilm extracellular matrix (ECM). Key functions of the EPSs comprise the mediation of the initial attachment of microbial cells to different substrates and protection against environmental stress and dehydration. In a biofilm state, the microbes are more resistant to antibiotic and multidrug treatments [12–14].

A generalized concept of microbial biofilm formation includes several stages (Figure 1). Initially, planktonic cells reversibly attach to a surface (reversible adhesion) and remain in this transient state until signaling by an environmental cue. This is a cell-to-cell communication mechanism, a phenomenon known as quorum sensing (QS). It plays an important role in biofilm development and balances the environment when the bacterial density becomes high. Once microorganisms begin to secrete EPSs, the biofilm develops into an irreversible process due to a cross-linking. In the mature biofilm, the cells are already engaged in an ECM composed mainly of proteins, exopolysaccharides, and extracellular DNA (eDNA).

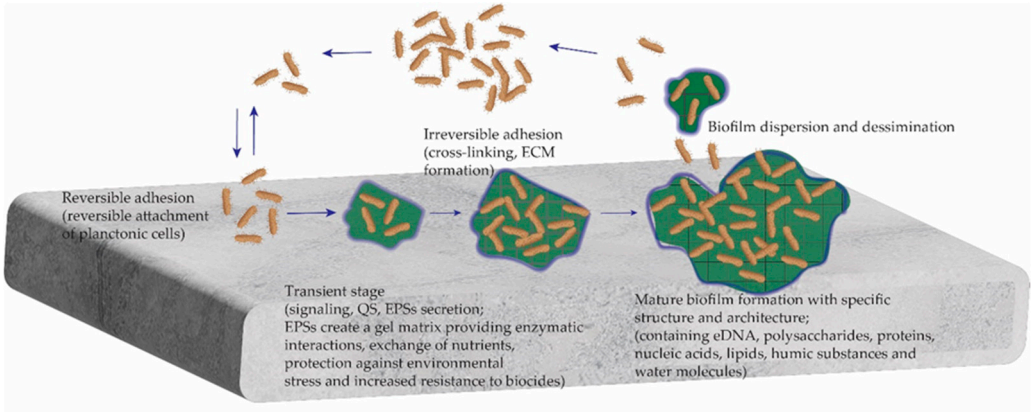

Figure 1. General mode of bacterial biofilm formation.

The development of anti-biofilm strategies should take into account all possible interactions used by the microbes to adhere and develop a biofilm, including the competitive relationships in multispecies biofilms. Any event included in the biofilm formation could be a target of its control, but combating the biofilm seems to be easiest at the initial stage of its development (reversible attachment, reversible adhesion), and this could be an interesting opportunity to limit microbial growth prior to human infection [15–20]. As the bacterial biofilm is perceived as a dynamic multistage process in which bacterial attachment on solid surfaces is the prerequisite for biofilm formation, the interference with the attachment is the most promising environmentally benign option to be inhibited [15].

3. Principal Anti-Biofilm Approaches

The principal anti-biofilm approaches can be divided into three main groups (Figure 2):

(i) Biocidal, including killing of pathogenic, biofilm-forming microbes and development of both contact killing or biocide-releasing surfaces, in the case of infections associated with medical devices;

(ii) Non-biocidal approaches, including mechanical removal of the biofilm, if it is possible; minimizing the reversible microbial cell attachment and development of anti-adhesive material surfaces in the case of infections associated with medical devices; QS disruption; inhibition of the cross-linking of the EPSs; ECM disruption; and activation of biofilm dispersal;

(iii) Combination treatments, including treatment by combinations of different anti-biofilm agents; combinations of antibiotics and terrestrial biota-derived anti-biofilm agents;

utilization of multi-functional anti-biofilm agents; and development of anti-adhesive material surfaces that deliberate antimicrobial agents [16–18].

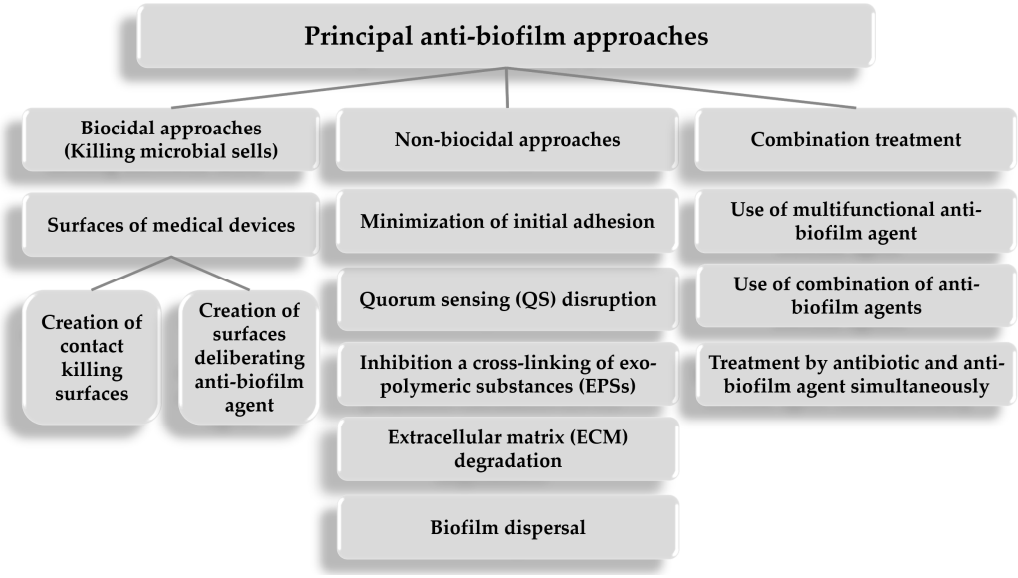

Figure 2. Principal anti-biofilm approaches.

The focus of this review is on bacterial biofilms and anti-biofilm approaches (biocidal, non-biocidal, and combinatorial) based on the use of different natural products: plant-derived products, essential oils, natural quorum sensing inhibitors, enzymes and other biologically active ECM-degrading substances, anti-biofilm peptides, biosurfactants, etc., called by some authors "biological approaches", unlike the chemical and physical ones [20].

3.1. Biocidal Approaches

The biocidal approaches are based on the use of antimicrobial agents that kill bacterial cells (biocides). They include development of contact killing or antimicrobial agent (biocide)-deliberating biomaterial surfaces of medical devices. Contact killing surfaces are usually created by grafting of an antimicrobial agent or deposition of a coating containing such an agent; the surfaces of both types kill microbial cells in contact with them. Antimicrobial agent (biocide)-deliberating biomaterial surfaces are usually created by deposition of gel or another type of coating that deliberates biocide and kills microbial cells in contact with them. Anti-biofilm agents that kill pathogenic microbial cells are enzymes, antimicrobial peptides inspired by natural chemical compounds, and other natural biocides [21].

Examples of enzymes that kill microbial cells are peptidoglycan-degrading enzymes and phage-derived polysaccharide depolymerase [22]; combined DNase and proteinase, which are effective against multispecies oral biofilms [23]; proteinase K and lysozyme, with proven anti-biofilm activity; and others. Some researchers believe that the complete elimination of heterogeneous biofilms needs amalgamation of hydrolytic enzymes that can degrade proteins, polysaccharides, eDNA, and QS molecules [24]. Several features of the enzymes limit their practical application as anti-biofilm agents in the creation of anti-biofilm biomaterial surfaces: (i) Carrying out the function while keeping the own natural activity. Any enzyme should be structurally stable and have some structural mobility in aqueous solutions. Simultaneously, the enzyme should be released in water media, which is difficult to provide when it is included in an anti-biofouling coating. (ii) The enzyme activity increases, whereas its stability decreases, with a temperature increase. Finding an optimal balance between enzyme activity and stability is a challenge in enzyme-based anti-biofouling coatings [18].

Immobilization of antimicrobial peptides or other biologically active substances that kill microbial cells, and deposition of antimicrobial coatings and deliberating anti-microbial

agents, are largely studied approaches for the creation of contact killing or biocide-releasing surfaces in the case of biofilms associated with medical devices [13].

3.2. Non-Biocidal Approaches

The non-toxic control of biofouling is based on approaches that exclude the killing of microbial cells. The non-biocidal approaches include mechanical removal of biofilm, if possible, and affecting the biofilm development stages, i.e., minimization of cell–surface adhesion; hampering and disruption of cell-to-cell signaling, i.e., quorum sensing; inhibition of the cross-linking of EPSs; disruption of the formed ECM; and biofilm dispersion. All these are studied to combat medical biofilms and infections associated with medical biofilms, and have lately used a variety of terrestrial biota-derived biologically active substances [25].

3.2.1. Mechanical Removal of Biofilms

Mechanical removal of dental and some other biofilms has achieved limited success. Irrigation with water jets and debridement followed by aggressive antimicrobial therapy have been widely carried out for oral, wound, and prosthetic joint biofilm-related infections, although it was found that biofilms spread across the surface and cause a persistence of bacteria on surfaces after these treatments [26]

3.2.2. Minimization of Initial Adhesion

Bacterial attachment on solid surfaces is a prerequisite for biofilm formation, and minimization of the initial cell adhesion is accepted as the most promising environmentally benign option to antifouling [15]. Minimization of the initial adhesion could be achieved by creation of a strong hydrophilic (with a water contact angle (WCA) approaching zero) or a strong/super hydrophobic surface (with surface tension, γ, approaching zero) [16,18,27,28]. The presence of a surfactant/biosurfactant on such a surface contributes to additional minimization of the reversible microbial cell's attachment [29,30]. ECM-degrading enzymes, i.e., D Nase I, dispersin B, and lysostaphin, also inhibit microbial adhesion to surfaces [1]. Attempts were made to coat biomaterials or to immobilize biologically active substances aimed at decreasing the adhesion of pathogenic microorganisms to the surface of indwelling medical devices. Some examples are the anti-adhesive coatings based on cyanobacterium *Cyanothece* spp. derivatives, which are active against *Proteus mirabilis*, *E. coli*, and *Candida albicans* biofilms in urinary tract infections [31].

Generally, terrestrial biota-derived anti-biofilm agents are not sufficiently studied as anti-adhesive coatings, or a component of composite coatings, although phyto-compounds and plant extracts are known to play a significant role in bacterial adhesion. For example, the crude extract of *Adiantum philippense*, which shows a promising role in decreasing the content of biofilm exopolysaccharides, restrains biofilm formation at the initial stage by targeting adhesive proteins [32]. The inhibition of bacterial adhesion and anti-biofilm effects of biosurfactants (BSs) extracted from *Bacillus cereus* and *Serratia nematodiphila* were found against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The combination of BSs from *B. cereus* and *S. nematodiphila* demonstrates synergistic effects, e.g., a decrease of about 60% in the adhesion of *S. aureus* and *P. aeruginosa*, and a decrease of about 80% in the biofilm mass, which indicate an increased inhibitory potential against pathogenic microbial attachment and biofilm eradication [33].

3.2.3. Quorum Sensing Inhibition

Pathogenic microbial cells forming biofilms on indwelling medical devices (orthopedic implants, urinary catheters and stents, cardiovascular devices, etc.), human skin, oral cavities, and other surfaces communicate through quorum sensing (QS) signals that regulate both microbial biofilm development and pathogenesis. Numerous natural bioactive agents that are able to hamper cell-to-cell communication have been identified so far, including enzymes degrading autoinducer compounds, blocking autoinducer production, or blocking their receptors through addition of some "inhibitor" compounds mimicking them [34].

Interference with QS was already identified as a novel potential therapeutic approach to the treatment of bacterial infections. The effect of QS molecules, like Farnesol (a natural 15-carbon organic compound which is an acyclic sesquiterpene alcohol), was demonstrated on mixed biofilms of *C. albicans* and *S. aureus* [35]. The first compound identified as a broad-spectrum QS inhibitor is a sulfur-rich molecule from garlic Ajoene. It decreases the expression of small regulatory RNAs in both Gram-negative (*P. aeruginosa*) and Gram-positive (*S. aureus*) bacteria [36]. Metalloproteinase AHL-lactonase from cell-free lysate of endophytic *Enterobacter* species blocks cell-to-cell communication in pre-formed biofilms and regulates virulence factor production, and thus significantly inhibits biofilm formation by *Aeromonas hydrophila* [37].

Diversity of bacteria, bacterial enzymes, and secondary metabolites known to interfere with the QS mechanisms of pathogenic bacteria are studied as anti-biofilm and anti-QS drugs against pathogenic bacteria [38]. Natural products, based on quorum quenching (QQ) enzymes, i.e., AiiA, AHL lactose, AHL acylase, and others, could block cell-to-cell communication and regulate virulence factor production. They disrupt QS systems in two main ways: (i) inhibiting and degrading signal molecules, and (ii) mimicking the signal molecules to inhibit their binding to corresponding receptors. QS inhibitors are discussed by some researchers as the best anti-biofilm alternative to antibiotics [39]. Lactobacillus crustorum ZHG 2-1 is reported as a QQ bacterium that functions as a *P. aeruginosa* anti-biofilm agent [40].

Essential oils (EOs) and other plant-based natural products are found to demonstrate anti-QS activities against pathogenic bacteria and are accepted as promising anti-biofilm agents [36,37]. A number of BSs of microbial origin were found to destroy cell-to-cell communication [41]. Two main types are rhamnolipieds, produced predominantly by the Gram-negative bacteria *P. aeruginosa*, and sophorolipieds, produced mainly by *Candida bombicola* species [42]. In 2023, Adnan et al. [43] demonstrated that a BS derived from probiotic strain *Lactobacillus acidophilus* inhibits QS-regulated virulence against three Gram-negative bacteria and exhibits broad-spectrum anti-biofilm activity.

3.2.4. Inhibition of the Cross-Linking of Exopolymeric Substances, Degradation of the Extracellular Matrix (ECM), Biofilm Dispersal

A complex mixture of biopolymers secreted by the pathogenic bacteria, EPSs mediate the initial attachment of cells to different substrates and form the extracellular matrix (ECM) after cross-linking [12–15,44]. Many anti-biofilm approaches are focused on the inhibition of EPS cross-linking, degradation of the ECM, and finally biofilm dispersal. All these can be achieved via the use of natural products, such as antioxidants and enzymes. Supposing that the cross-linking of bacterial EPSs is carried out by an oxidative mechanism (as was demonstrated earlier for some macro-biofoulers), the effect of several antioxidants was tested as a component of low-adhesion siloxane coatings. It was found that some antioxidants significantly inhibit, whereas others stimulate, the biofilm formation on the coated surface. The effect depends on the chemical nature of the antioxidant, the type of microfouler, and the conditions under which the mono-spaces or multispecies biofilm develop [29,30,45]. The complexity and heterogeneity of the microbial EPSs and the ECM composition require hydrolases and leases in order to achieve efficient enzymatic degradation [18].

Biofilm dispersal is often assisted by surfactants or/and other substances, like enzymes, that are able to break already established cross-links [16]. Enzymes, such as beta-N-acetylglucosaminidase and dispersin B, secreted by the Gram-negative periodontal pathogen *Actinobacillus actinomycetemcomitans*, disintegrate mature biofilms of *S. epidermidis* [24]. The design of enzymatic biofilm dispersal using nanotechnology demonstrates improved anti-biofilm activity and achieves good results [46,47].

3.3. Combination Approaches

Combination approaches include simultaneous application of antibiotics and anti-biofilm agents, the combination of several anti-biofilm agents, or minimizing the cell–surface adhesion and application of antibiotics and/or anti-biofilm agents.

Biofilm formation is a dynamic and complex process starting with the initial reversible attachment of microbial cells, and this stage is accepted as the easiest for the inhibition of the biofilm development. However, the large diversity of both microbial species and adhesive substances secreted by them, and the variety of material surfaces on which a biofilm develops, make the complete elimination of the initial (reversible) adhesion difficult. In addition, the same microorganisms secrete different EPSs against different surfaces [16]. For example, EPSs produced by *E. coli* demonstrate a predominant amount of chemically bound free EPSs under different growth conditions, with over 500 proteins identified in the free EPSs [48]. Combating an already developed biofilm is much more complicated and requires a complex strategy. Creation of surfaces that reduce the adhesion of microbes and provide biocidal activity or combinatory effects emerges as a major global strategy in combating bio-contamination. The function of natural anti-biofilm agents appears to be more effective in combination approaches as compared with their single use in terms of antibacterial activity, decreased toxicity, etc. [10].

3.3.1. Antibiotic Treatment Simultaneous with Natural Medicine

Antibiotic treatment simultaneous with natural medicine seems to be a promising therapy for bacterial biofilm infection. For example, combined with levofloxacin, a plant-derived anti-neuropeptidesodium, houttuyfonate better inhibits *P. aeruginosa* biofilm formation [49]. A pre-treatment by naringin (a flavanone glycoside, extracted from citruses) followed by ciprofloxacin and tetracycline antibiotic-treatment is more effective against *P. aeruginosa* biofilms than individual treatment with the same antibiotics. The effect of naringin is due to the depletion of biofilm EPSs and facilitation of the diffusion of antibiotics, thereby reducing pellicle formation and decreasing the flagella movement of bacteria on medical device surface [50]. Nisin, an FDA-approved AMP, produced by certain Gram-positive bacteria *(Lactococcus* and *Streptococcus* spp.), and found in plants and in dairy environments, acts as anti-biofilm agent synergistically with conventional antibiotics against methicillin-resistant *S. aureus*, *S. pneumoniae, Enterococci*, and *Clostridium difficile* [51]. AMPs produced by insects can act synergistically with classical antibiotics for a double-pronged attack on infections [52]. Although combinations of plant-based products and antibiotics have been studied and discussed widely, they are currently poorly explored and for a reduced number of bacterial species only [9].

3.3.2. Natural Products with Multiple Anti-Biofilm Activities

Some natural products demonstrate multiple anti-biofilm activities. Significant anti-QS and biofilm dispersal activities were found in honey against multi-species bacterial biofilms [53]. Disruption of established bacterial and fungal biofilms by a nutraceutical enzyme and botanical extract blend (NEBB) was demonstrated in vitro for five microbial strains, *C. albicans*, *S. aureus*, *S. simulants* (coagulase-negative, penicillin-resistant), *Borrelia burgdorferi*, and *P. aeruginosa*, causing chronic human illnesses. The mass of post-treated biofilm (evaluated by crystal violet staining) and the metabolic activity (measured by MTT assay) were significantly influenced by NEBB treatment [54].

3.3.3. Nanoparticles Containing Natural Products

Unlike antibiotics, which are used to treat infected patients, nanomaterials provide an interesting opportunity to limit microbial growth prior to human infection. This led to the development of engineered antimicrobial materials, targeting different applications with combinations of natural products and biogenic synthetized metal, metal oxide, or composite nanoparticles as the active antimicrobial agents [55,56].

The pandemic coronavirus disease 2019 (COVID-19) motivated a survey focused on nanotechnology-based strategies to develop functional antimicrobial and antiviral surface coatings that include combinations of biologically active compounds [57]. Use of biologically active polymers, such as chitosan in combination with silver, zinc oxide, and others, attracts attention to combat persistent, antimicrobial-resistant bacterial biofilms [58].

3.4. Some Considerations for the Design of Anti-Biofilm Surfaces

Based on a large number of literature sources and the authors' own experience, some conclusions about the design of anti-biofilm surfaces were made. Since biofilm formation on a medical device is a complex phenomenon, it requires a complex strategy. The most important prerequisite for a material surface to be clean from biofilm is to be low-adhesion, i.e., strongly hydrophilic, water-like or strongly/super hydrophobic, flat and slippery, or with special micro/nanotopography, that is, a protein-rejective surface [16,27].

When microbial cells contact any surface, they start to secret EPSs containing adhesive proteins, which support their attachment to the surface. If one is able to stop the protein and EPS adsorption, the biofilm development can be stopped. Unfortunately, the proteins have a versatile nature and, in front of a complementary surface, they attach to a different mechanism. This makes it almost impossible to completely stop the protein adsorption. Therefore, a sharp reduction in biofilm, but not a complete lack, is observed on such surfaces [27,28,59]. In addition to low adhesion, it will be beneficial for the anti-biofilm surface to contain a relevant surfactant and/or inhibitor of both QS and EPS cross-linking [30], as well as agents breaking already established cross-links and biofilm dispersal, to avoid the insufficient microbial settlement of the low-adhesion surfaces [59].

4. Anti-Biofilm Agents from the Terrestrial Biota

Anti-biofilm agents from the terrestrial biota are a subject of numerous reviews, original papers, and books aiming to highlight new prospects for the development of efficient alternatives to the currently used but insufficiently effective antibiotic and multidrug treatments. The potential anti-biofilm agents from the terrestrial biota include plants and herbal extracts, essential oils, biosurfactants (BSs), and antimicrobial peptides (AMPs), which target different stages of biofilm formation on different mechanisms [1]. With or without considerable structural alterations, they could be applied to the treatment of biofilm-associated infections, as well as to the development of anti-biofilm surfaces. The design of effective anti-biofilm molecules requires their minimum inhibitory concentrations to eradicate biofilm-associated infections without causing toxic effects. The knowledge accumulated in the last decades demonstrates that many natural antibacterial products are more efficient for inhibition of biofilm development and have less side effects than their chemically synthesized counterparts. Natural anti-biofilm compounds can attack one or more stages of biofilm formation in combination, or not, with antibiotics [60].

Identified natural products with anti-biofilm activity include elligic acid glycosides, hama melitannin, carolacton, skyllamycins, promysalin, phenazines, bromoageliferin, flustramine C, meridianin D, and brominated furanones. Medicinal chemistry programs facilitate structure confirmation, identification of critical structural motifs, better understanding of mechanistic pathways, and the development of more potent, more accessible, or more pharmacologically favorable derivatives of anti-biofilm natural products [61]. Natural polymers in different forms are reported to help antibiotics transport to the targeted site, mainly for periodontal biofilm-forming pathogens [62]. the anti-biofilm potential of *Actinobacterial* compounds from different terrestrial or aquatic microbial species against various pathogenic bacteria is supposed to be due to the cell–surface and cell–cell interaction [63]. Xu et al. [64] recently reported targeting only biofilm-forming pathogenic microorganisms without influencing the normal microflora. Chi et al. [65] summarized the importance, functions, and anti-biofilm effects of natural products from traditional medicine, targeting multi-species oral biofilms. The anti-biofilm activity of 11 natural compounds and their derivatives (phenyl propenes and phenolic aldehydes, eugenol, ferulic acid, sinapic acid, salicyl aldehyde, vanillin, cinnamoyl acid, and aldehydes) was tested against 32 clinical isolates of *S. aureus.* Using both qualitative and quantitative assays, followed by qPCR analysis to examine the differences in the expression levels of biofilm-forming genes *(ica-A*, *fnb-B*, *clf-A*, and *cna*) in treated and untreated clinical isolates, it was found that the tested natural compounds and their analogues exhibit significant antimicrobial and anti-biofilm activity against *S. aureus* [66].

Lately, the potential value of new neutral deep eutectic solvents (NADESs) as anti-biofilm agents was demonstrated. NADESs are considered "natural" because their constituent components are primary metabolite groups (which are naturally used by the plants for their self-survival), such as sugars, organic acids and bases, and amino acids. A potential use of NADESs as anti-biofilm agents is proposed due to their ability to solubilize and stabilize biological macromolecules [67]. The potential capacity of a huge number of natural and synthetic compounds as inhibitors of bacterial biofilm formation is widely presented. This includes the natural chemical compounds resisting bacterial biofilm growth by different mechanisms; the stage of biofilm formation at which the chemical compound is introduced into the biofilm system; polymer matrix formation inhibition; suppression of the cell adhesion and cell attachment to itself or to an external surface; and interruption of the extracellular polymeric matrix generation and decrease in virulence factor production [68].

4.1. Plant Products and Derivative Compounds

Long-known medicinal plants rich in bioactive compounds with diverse structures and functional groups are presented now as demonstrating anti-biofilm properties. Plant-based products are usually crude or fraction extracts, derived anti-biofilm compounds, and secondary metabolites [69,70].

A crude extract of *Adiantum philippense* L. was found to decrease the content of biofilm exopolysaccharides. It restrains biofilm at the initial stages by targeting adhesion proteins, deforming the pre-formed biofilms and obstructing EPS production [32]. The anti-biofilm activity of extracts from the *Moringa oleifera* plant and *Citrus sinensis* fruit (known to treat multiple human infections) was evaluated against pathogenic *P. aeruginosa* and *S. aureus* biofilms. The experimental results show that the peel extract of *C. sinensis* and the flesh extract of *M. oleifera* efficiently inhibit the formation of such biofilms by sub-inhibitory concentrations. The comparative study reveals a better anti-biofilm activity of the *M. oleifera* extract than that of *C. sinensis* against mixed cultures [71].

Several classes of natural compounds with anti-biofilm properties are known: phenolics, essential oils, terpenoids, lectins, alkaloids, polypeptides, and polyacetylenes [72]. Malaysian plant species are known to control biofilm infection by QS pathway inhibition, disruption of the ECM, alteration of cell permeability, and reduction in the hydrophobicity of the cell surface. The phytocompounds having a curlicide and pilicide nature can be exploited in therapeutic strategies against *Enterobacterales* biofilm development [73]. Guzzo et al. [74] presented the anti-biofilm activity and molecular mechanisms of action of plant-derived natural products against *P. aeruginosa* and *S. aureus*, together with an update of anti-biofilm properties of terpenes, flavonoids, alkaloids, and phenolic compounds. A survey of natural and synthetic plant compounds as anti-biofilm agents against seropatotype *E. coli* (STEC) biofilm was directed to a development of antibacterial drugs against STEC-induced infections [75]. In search of alternatives to the conventional antibiotics and safe therapies, natural plant-derived phytomedicines are discussed now as promising candidates to combat microbial biofilms. The efficacy, characteristics, and corresponding mechanisms of action of phytochemicals, biosurfactants, antimicrobial peptides, and their sources were included [76].

Plant secondary metabolites are also studied as anti-biofilm agents. Secondary metabolites from seeds of *Annona senegalensis* were tested for their antimicrobial, anti-biofilm, and anti-QS activities using three test microorganisms (*S. aureus*, *E. coli*, and *Chromobacterium violaceum*). Column chromatography of the seed extract affords N-cerotoyltryptamine, asimicin and ent-19-carbomethoxykauran-17-oic acid. The testing of their antimicrobial, anti-biofilm, and anti-QS activities demonstrates that these compounds inhibit biofilm formation of the test microorganisms to different extents in a dose-dependent manner [77]. For the first time, the activity of 20 secondary metabolites produced by pathogenic fungi of forest plants (belonging to different classes of natural compounds) was evaluated against clinical isolates of antibiotic-resistant Gram-negative and Gram-positive bacteria. It appears that epiepoformin, sphaeropsidone, and sphaeropsidin A show antimicrobial activity against

all test strains. Furthermore, sphaeropsidin A decreases biofilm formation of methicillin-resistant *S. aureus* and *P. aeruginosa* at sub-inhibitory concentrations (as quantified by crystal violet staining). Mixtures of sphaeropsidin A and epiepoformin show a synergistic effect and a reduction in cytotoxicity against human immortalized keratinocytes [78]. Small carbohydrate derivatives are discussed as potential anti-biofilm agents that hold promise in addressing the problem of biofilm-related infections. Sugar scaffolds and potent synthetic carbohydrate-based molecules are accepted as valuable entities for the development of anti-biofilm agents because of their structural diversity and specificity [79].

The biological activity of honey has been known for a long time. Recently, the activity of honey samples from two Pakistan bees (*Apis dorsata* and *Apis cerana)* was studied in the context of an anti-QS and dispersal agent against multispecies bacterial biofilms. Multi-species biofilms were grown in batch cultures of *P. aeruginosa*, *E. coli*, *S. aureus*, *M. morganii*, and *K. pneumonia* by a mixture of equal volumes. Significant anti-biofilm, anti-QS, and biofilm dispersal potential was found for the two honey samples, by crystal violet staining and the culture supernatant method, respectively [53].

Based on published studies, it can be concluded that plant-based products and isolated compounds that detach or dismantle biofilms on the World Health Organization's list of priority human pathogens (ESKAPE pathogens: *E. faecium, S. aureus, K. pneumoniae, Acinetobacter baumannii, P. aeruginosa*, and *Enterobacter* spp.) could be promising potential therapeutics for biofilm-associated infections. Flavonoids and phenolic compounds seem to be the most effective anti-biofilm agents [9].

Essential Oils

Essential oils (EOs) are volatile mixtures of compounds found in medicinal herbs. All parts of the medicinal herbs, like roots, stems, leaves, buds, flowers, seeds, and fruits, are used to extract essential oils by hydro or steam distillation and other technologies. For centuries, EOs have been used in medicine and lately they have been studied as anti-biofilm agents because they have fewer side effects, broad-spectrum biological activity, and a diverse range of pharmacological characteristics [80]. Aromatic plants and derivatives prepared from them are known for their antiseptic, bactericidal, fungicidal, anti-inflammatory, antioxidant, antiviral, and smooth muscle relaxant properties. EO components easily penetrate biological membranes and exert therapeutic effects due to their lipophilicity and small molecular size. Currently research is underway on the use of EOs for the treatment of inflammatory diseases, viral infections, and cancer prevention [81,82]. Of interest is also the scarcely studied anti-biofilm potential of EOs and their compounds. Purkait et al. [83] evaluated the anti-biofilm efficacy of EO components β-caryophyllene, cinnamaldehyde, and eugenol alone and in combination against *Listeria monocytogenes* and *Salmonella typhimurium* biofilm formation. The results demonstrate that a cinnamaldehyde/eugenol combination has potential for the design of a novel, more efficient natural anti-biofilm blend at sufficiently low concentrations. Lobos et al. [84] studied anti-biofilm and antimicrobial properties of *Laurelia sempervirens* (Chilean laurel) EO on human pathogenic strains *C. albicans* and *S. aureus*. Their results indicate the ability for the domestic use of Chilean laurel EO as an antimicrobial agent and provide knowledge about the anti-biofilm properties of *L. sempervirens*. EOs obtained from clove, oregano, thymus, cinnamon bark, rosemary, eucalyptus, and lavender have been shown to present significant inhibitory effects on bacteria, fungi, and viruses. Enhanced efficacy was exhibited by the combinations EO–EO or EO–antibiotic [85].

4.2. Antimicrobial Peptides

Natural antimicrobial peptides (AMPs) are extracted from different kinds of live organisms, including vertebrates, invertebrates, plants, and bacteria. AMPs can also be produced by chemical synthesis. Compared to conventional antibiotics, both natural and synthetic AMPs play a broad range of antimicrobial roles without inducing development of antibiotic resistance. Only a few AMPs can affect biofilms, and some of them show anti-biofilm activity

below the MIC, such as the human cathelicidin peptide LL-37. The latter presents very weak anti-planktonic cell activity, while its anti-biofilm activity is much higher [86].

The anti-biofilm actions of AMPs include (i) membrane-associated activity through pore formation and/or membrane disruption; (ii) penetration into the cytoplasm of bacteria and suppression of cell wall, enzyme, or protein synthesis; (iii) degradation or destabilization of the extracellular matrix; and (iv) prevention of cell attachment and promotion of existing cell dispersion in the early stages of biofilm development [1,86,87].

Terrestrial plants and animals have protective factors to defend themselves against constant attacks by a wide range of pathogens. Their innate immunity is provided from different AMPs that are categorized in families according to the sequence similarity, the number and order of amino acid residues, and the tertiary structure of the mature peptide. AMPs are used by the terrestrial biota for fine-tuning their responses to biotic and abiotic factors. Along with activity against planktonic bacteria, many AMPs show anti-biofilm activity. AMPs are accepted now as new potential antibiotics that are less vulnerable to microbial resistance and that could be used individually or in combinations with conventional antibiotics or other antimicrobial agents. AMPs are already being discussed as anti-biofilm agents for human therapy and prophylaxis because they have a wide range of inhibitory effects against both Gram-positive and Gram-negative bacteria, fungi, parasites, and viruses [88]. Efforts have been made to design peptide mimetics as potential therapeutics targeting oral bacteria and oral biofilms, i.e., a novel specifically targeted multi-domain AMP composed of a species-targeting peptide linked to a broad-spectrum antimicrobial killing peptide domain [89]. Numerous AMPs with anti-biofilm properties have been represented lately that are potentially good candidates for the development of new anti-biofilm drugs because they can act at different stages of biofilm formation, including initial bacterial adhesion, QS factor regulation, and pre-formed biofilm disruption. It is thought that the QS inhibiting potential of natural AMPs could be utilized in an alternative antibiotic-free approach to overcome biofilm-associated infections [90].

Bacteriocins are ribosomally synthesized peptides that are produced by lactic acid bacteria. Some bacteriocins produced by almost all groups of bacteria, such as the colicins and microcins, present antibacterial activity against *S. aureus*, *P. fluorescens*, *P. aeruginosa*, *E. coli*, *Salmonella typhi*, *Listeria monocytogenes*, and others. Colicins and colicin-like bacteriocins are highly effective at killing target strains growing in a biofilm state. Microcins were used to fight a *P. aeruginosa* biofilm, and the killing activity of microcins against planktonic and mature biofilm cells was proven. The use of bacteriocins is expected to become a new strategy for biofilm treatment. However, they could interact with eukaryotic host cells, inducing some degree of host DNA damaging, and this effect might limit their application in anti-biofilm therapy [91,92].

Different combination strategies based on AMPs were evaluated, such as AMPs combined with nanoparticles that could penetrate the barrier of biofilms, and low doses of AMPs could be used to overcome their disadvantages and potential toxicities [93]. Some AMPs combined with traditional antibiotics show a synergistic effect via the promotion of improved antibiotic uptake [94].

Bose et al. [95] developed machine learning models to identify the distinguishing characteristics of known anti-biofilm peptides, and to mine peptide databases from diverse habitats to classify new peptides with potential anti-biofilm activities. Thus, a new in silico approach was demonstrated for predicting anti-biofilm efficacy, and for identifying promising new candidates for biofilm eradication.

Until the year 2020, more than 3000 AMPs had been discovered, but the FDA [96] has approved only 7 of them. Terrestrial sources of AMPs are different plants, worms, amphibians, and others.

4.2.1. Plant Antimicrobial Peptides

Several plant AMPs are reported as being studied for anti-biofilm activity. The effect of plant-derived neuropeptide sodium houttuyfonate (SH) was investigated against the

dispersion of *P. aeruginosa* biofilm by an in vitro model. The results show that it can penetrate into and repress the biofilm life cycle [49]. The relationship between the charge of corn-derived AMPs and their activity in media with elevated salt concentrations was investigated using plant defensins. A recombinant defensin ZmD32 derived from *Zea mays*, (with a predicted charge of +10.1 at pH 7, the highest of any defensins) demonstrates that it is active against *C. albicans* and both Gram-negative and Gram-positive bacterial biofilms in the presence of salt, whereas the anti-biofilm activity of many other defensins is lost under these conditions [97].

Peptides are common components in the seeds of *Capsicum baccatum* (red pepper). Gomes Von Borowski et al. [98] reported an anti-biofilm strategy based on the development of capsicumicine, a natural peptide that strongly controls biofilm formation by the most prevalent pathogen in device-related infections, *S. epidermidis*. Capsicumicine prevents *Staphylococcal* biofilm in vitro and in vivo via a new matrix anti-assembly mechanism of action. Since capsicumicine is not cytotoxic, it is a promising candidate for complementary treatment of infectious diseases.

4.2.2. Skin Antimicrobial Peptides

Amphibian skin is a source of many AMPs effective against various biofilm-developing microorganisms. Yuan et al. isolated an AMP, Japonicin-2LF, from skin secretions of the Fujian large-headed frog (*Limnonectes fujianensis*), which inhibits multidrug-resistant biofilms by membrane permeabilization [99].

The frog-skin-isolated AMP, esculentin 1a, and its D-amino acid-containing diastereomer, inhibit *P. aeruginosa* biofilm formation by membrane perturbing activity and show potential activity against chronic lung *Pseudomonas* infections of cystic fibrosis patients [100]. In addition, it was reported that the human skin AMP β-defensin 2 inhibits biofilm production of *P. aeruginosa* without compromising the metabolic activity [101]. The inhibitory effect of the human β-defensin 2 is due to structural changes in the biofilm, alteration in the outer membrane protein profile, and interference with the transfer of biofilm precursors into the extracellular space. The anti-biofilm peptide human cathelicidin LL-37 affects the bacterial cell signaling system and inhibits *P. aeruginosa* biofilm formation at 0.5 μg/mL by downregulating genes of the QS system [90]. The anti-biofilm potential of AMPs from other terrestrial sources has also been investigated.

4.2.3. Honey Bee and Insect Antimicrobial Peptides

Khozani et al. [102] performed a kinetic study of simultaneous biofilm degradation and eradication of multidrug-resistant *P. aeruginosa* isolates by melittin (26-amino-acid, α-helical peptide from the honeybee *Apis mellifera* venom). The experimental results show that the melittin degrades about 90–95% of *P. aeruginosa* biofilm biomass at 50 μg concentrations in 24 h. This gives a reason for its further investigation as a novel drug in an animal model of biofilm-associated burn infection.

AMPs present in insect venom prevent the spread of infections that may be a source of pathogens [103]. The insect AMP cecropin A is reported as destroying a planktonic and sessile biofilm forming uropathogenic *E. coli* cells, either alone or in combination with the antibiotic nalidixic acid, synergistically influencing the infection without cytotoxicity [104]. Sahoo et al. [52] discussed insect-derived AMPs as a novel therapeutic option focused on anti-biofilm-based strategies. Existing antibiotics cannot eradicate most biofilms, especially of ESKAPE pathogens (*E. faecium*, *S. aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* spp.). Generally, AMPs, produced by most insects, have broad-spectrum activity and a potential to bypass the resistance mechanisms of classical antibiotics. Such AMPs may well act synergistically with classical antibiotics for a double-pronged attack on infections.

4.2.4. Others

Although many AMPs share common structural characteristics, for example, having an overall size between 10 and 100 amino acids, a net positive charge, a γ-core motif, or a high content of cysteines, they greatly differ in coding sequence because of multiple parallel evolutions in the face of pathogens. This is especially the case of annelids (ringed worms). Bruno et al. [105] surveyed the primary structures and functions of AMPs encountered to date in annelids and nematodes, and presented a selection of AMPs that are promising sources of antibiotics.

Surface modification of plasma pre-treated polydimethylsiloxane (PDMS) was performed by cataractous eye protein isolate, aimed at decreasing the PDMS hydrophobicity. Antimicrobial activity was not tested and the measurements of the dielectric properties of the modified samples indicate that they behave as capacitors at a lower frequency range, while demonstrating resistive characteristics at a higher frequency. They provide preliminary ideas for developing flexible devices for potential applications in diverse areas such as thermo-elective wireless switching devices and energy storage materials [106].

The AMPs studied during the last 5 years that can affect biofilms are summarized in Table 1.

Table 1. Antimicrobial peptides from the terrestrial biota.

Name	Origin	Effect on Biofilm In Vitro	Reference
Cathelicidin peptide LL-37	Human	Anti-biofilm activity	[96]
Neuropeptide sodium houttuyfonate	Plants	*P. aeruginosa*	[49]
Defensin ZmD32	*Zea mays*	Gram-negative and Gram-positive bacteria	[97]
Capsicumicine	seeds of red pepper Capsicum baccatum	*Staphylococcal* biofilms (also in vivo)	[98]
Japonicin-2LF	Frog Skin *Limnonectes fujianensis*	Multidrug-resistant biofilms *P. aeruginosa*	[107]
Esculentin1a and its D-amino acid containing diastereomer	Frog skin	*P. aeruginosa*	[100]
β-defensin 2 and cathelicidin LL-37	Human skin		[101]
Melittin (26-amino-acids α-helical peptide)	Venom of honeybee *Apis mellifera*	Uropathogenic *E. coli*	[102]
Cecropin A	Insect	ESKAPE pathogens	[52,103]
Bacteriocines colicins and microcins	Lactic bacteria	*S. aureus*, *P. fluorescens*, *P. aeruginosa*, *E. coli Salmonella typhi*, *Listeria monocytogenes*, and other	[91,92]

4.3. Biosurfactants

Biosurfactants (BSs) are amphiphilic compounds synthesized by plants and microorganisms, and are capable of lowering the surface tension of liquids. Their hydrophilic moieties are made of acids, cationic peptides, anions, or sugar (monosaccharide, disaccharide, or polysaccharide). The hydrophobic moieties are made of hydrocarbon or fatty acid chains. Classification of the BSs is based on their charge (non-ionic, cationic, and anionic) and molecular structure. Plants and microorganisms generate numerous simple glycolipids, comprising hydrophobic groups linked directly to complex carbohydrate moieties via C-, N-, O-, and S-glycosidic bonds. Owing to the amphiphilic nature of the natural glycolipids, they remain an integral part of the membrane in cell walls and other functional parts. The BSs sophorolipids and rhamnolipids are widely studied because of their high biological activity, inherent biodegradability, low toxicity, and sustainable synthesis. In addition, sophorolipids and rhamnolipids are active under extreme temperatures, pH, and salinity [108]. Recently, BSs have been discussed as promising new-generation biocompatible

anti-adhesive and antimicrobial agents that could be used for coating implantable medical devices to improve their anti-biofilm properties. BSs hinder biofilm formation by altering the cell attachment ability through alteration of the hydrophilic/hydrophobic balance on the cell surface, cell membrane disruption, and inhibition of the electron transport chain [1].

Biosurfactants from Microbes

Due to their great metabolic versatility, bacteria are the most traditional and well-known microbial BS producers, with *Bacillus* and *Pseudomonas* spp. being their typical representatives. BSs obtained from microbes are generally anionic or neutral, while a few are cationic. Bacterial surfactants show interesting properties for a range of applications, including in the oil industry, food, agriculture, pharmaceuticals, cosmetics, bioremediation, and, more recently, nanotechnology and as anti-biofilm agents [109]. Different BS classes of microbial origin exhibit anti-biofilm activity by destroying the QS. Rhamnolipid BSs are produced by several types of microbes such as bacteria, fungi, and yeast. Rhamnolipids are predominantly produced from the Gram-negative bacteria *P. aeruginosa*, whereas sophorolipids are produced mainly by *C. bombicola* species [42]. BSs are discussed as appropriate coating agents for indwelling medical devices, such as bone implants and urinary catheters, to inhibit pathogenic organism biofilm formation without using synthetic drugs. Rhamnolipids and sophorolipids are reported to have the potential to inhibit the development of Gram-negative and Gram-positive microbial biofilms [110]. Abdullahi et al. [111] found that the rhamnolipids produced from *P. aeruginosa MN1* have higher anti-adhesive and anti-biofilm activity than those of the trade product surfactin.

A variety of BSs have been derived from *Lactobacilli*. Their potential as an anti-adhesive agent on the surface of various biomedical devices is the focus of numerous studies. The production, purification, and properties of key components of the anionic, glycolipoprotein-type, cell-associated BS from *L. acidophilus* have been reported. It was found that this BS inhibits biofilm formation of *P. vulgaris* and *S. aureus* on polydimethylsiloxane (PDMS) implants [112]. Liposomes loaded with BS derived from *Lactobacillus* demonstrate greater ability to inhibit methicillin-resistant *S. aureus* biofilm formation as compared to the free BS [113]. Yan et al. [107] evaluated the effect of BSs from *L. plantarum* and *Pediococcus acidilactici* on QS signaling molecules and expressions of biofilm-linked genes in *S. aureus.* They found that the studied BSs reduce the growth in *S. aureus* biofilm by regulating the expression of the biofilm-related genes *dltB*, *icaA*, *cidA*, etc. Recently, it was demonstrated that the BS derived from the probiotic strain *L. acidophilus* exhibits broad-spectrum anti-biofilm activity and inhibits the QS-regulated virulence. The testing against three Gram-negative bacteria clearly demonstrates that the BS derived from *L. acidophilus* significantly inhibits virulence factors of Gram-negative pathogenic bacteria, thus providing an effective method to inhibit the formation of biofilms by Gram-negative bacteria [43]. Anti-biofilm activity was shown for an exopolysaccharide BS (a hetero-polysaccharide produced by *Pandoraea pnomenusa* MS5, with two functional carbonyl and hydroxyl groups) against *Burkholderia cepacia* [114].

Lipopeptide BSs are also investigated as anti-biofilm agents. An anionic lipopeptide BS from *A. junii* was identified that self-aggregates to form β-sheet rich vesicles. Its antimicrobial, anti-biofilm, and antiproliferative activities were studied. It was found that, simultaneous with high anti-biofilm activity, this BS has high thermostability and low toxicity [115]. In vitro and ex vivo anti-biofilm activity testing of lipopeptide BS produced by the entomo-pathogenic *Beauveria bassiana* strain demonstrates good activity against the dermatophyte *Microsporum canis* biofilm, which is otherwise very difficult to eradicate. The results show that it this due to disruption of the cell membrane integrity and interference with the cell membrane permeability [116]. Janek et al. [117] demonstrated the in vitro efficacy of the cyclic lipopeptide BS surfactin-C15 from *B. bassiana*, and its complexes with divalent counter ions, to inhibit *C. albicans* biofilm and hyphal formation. This BS controls the expression of hyphal specific genes, acts mainly by decreasing cell surface hydrophobicity, and is effective against *C. albicans* biofilm-related infections. Surfactin C15

is promising for recalcitrant treatment of dermatophytosis because, in addition, it is cheap as it is produced from corn steep liquor. Achita et al. [118] characterized and evaluated the antibacterial and anti-biofilm potential of BS produced by the endophyte *Burkholderia* spp. WYAT7 (from *Artemisia Nilagirica Pamp*) and reported that it has significant activity against *S. aureus* biofilm formation. The anti-biofilm potential of a BS extracted from the *A. indicus M6* strain, which contains broad-spectrum glycolipoproteins rich in Leu-His-Trp amino acids, was found to have the capacity to remove 82.5% of a biofilm at a concentration of 500 µg/mL [119,120].

The inhibitory effects of lipopeptides and glycolipids (lipopeptide AC7BS, rhamnolipid R89BS, and sophorolipid SL18) was studied against clinically relevant fungal/bacterial dual-species biofilms (*C. albicans*, *S. aureus*, and *S. epidermidis*) through quantitative and qualitative in vitro tests. *C. albicans* and *Staphylococcus* spp. cultures produce a dense biofilm on the surface of polystyrene plates and on medical grade silicone discs. All tested BSs demonstrate an effective inhibitory activity against the formation of the studied dual-species biofilm on the studied surfaces, in terms of total biomass, cell metabolic activity, microstructural architecture, and cell viability up to 72 h. In co-incubation conditions, in which BSs were tested in soluble form, rhamnolipid R89BS appears to be the most effective. The obtained results indicate that the coating of implant surfaces by BSs may be a promising strategy for the prevention of *C. albicans–Staphylococcus* spp. colonization of medical devices. Thus, it can potentially contribute to the reduction in the high economic impact on healthcare systems for the treatment of complex fungal–bacterial infections [119].

Biosurfactants studied during the last 5 years that can affect biofilms are summarized in Table 2.

Table 2. Biosurfactants from terrestrial biota.

Name	Origin	Effect on Biofilm of	Reference
Rhamnolipid BSs Sophorolipid BSs	*P. aeruginosa* *C. bombicola*	*P. aeruginosa;* *S. aureus*	[42,43] [42]
Lactobacillus BS	from *L. acidophilus*	*Enterobacterial* biofilms	[112]
Lactobacillus derived BS loaded liposomes	*Lactobacilus*	Anti-adhesive and anti-biofilm	[113]
BSs from	*L. plantarum* and *Pediococcus acidilactici*	against *P. vulgaris* and *S. aureus* biofilms on PDMS implants	[107]
BS, derived from probiotic strain	*L. acidophilus*	Methicillin-resistant *S. aureus* biofilm	[43]
Exopolysaccharide BS	*Pandoraea pnomenusa* MS5	*Burkholderia cepacia*	[114]
Lipopeptide BS	*A. junii*	Anti-biofilm	[115]
Lipopeptide BS	entomo-pathogenic *Beauveria bassiana* strain	*Microsporum canis* biofilm (in vitro, ex vivo)	[116]
Cyclic lipopeptide BS, surfactin-C15	*B. bassiana*	*C. albicans*	[117]
Endophyte BS	endophyte *Burkholderia* spp. WYAT7 (from *Artemisia Nilagirica Pamp*	*S. aureus*	[118]
Glycolipoprotein BS	*A. indicus M6* strain	High anti-biofilm activity; low toxicity; thermostability	[119,120]
Lipopeptides and glycolipids (lipopeptide AC7BS, rhamnolipid R89BS, and sophorolipid SL18)	–	clinically relevant fungal/bacterial dual species biofilms (*C. albicans*, *S. aureus*, and *S. epidermidis*)	[119]

4.4. Enzymes

Khan et al. [38] reviewed the diversity of bacteria and bacterial products as anti-biofilm and anti-QS drugs against pathogenic bacteria. Interfering with the QS mechanisms of

pathogenic bacteria, many bacterial enzymes and secondary metabolites disrupt biofilm formation. The impact of DNase I and/or proteinase K on the formation of a simulated supragingival biofilm was investigated in vitro. Six-species biofilms were grown anaerobically in the presence of DNase I and proteinase K. The results show that neither DNase I nor proteinase K has an impact on the total colony forming units (CFUs) compared to the control without the enzymes. However, DNase I significantly suppresses the growth of *Actinomyces oris*, *Fusobacterium nucleatum*, *S. mutans*, *S. oralis*, and *C. albicans*. Proteinase K treatment induces a significant increase in *S. mutans* and *S. oralis* CFUs, whereas *C. albicans* shows lower CFUs compared to the control. It was concluded that the enzymatic treatment should be combined with conventional antimicrobial agents aiming at both bactericidal effectiveness and biofilm dispersal [23,97].

4.5. Bacteriophages

Bacteriophages (phages) are viruses that specifically infect and kill bacteria. Reuter et al. [121] showed that engineered phage-derived enzymes, polysaccharide depolymerase, or peptidoglycan-degrading enzymes, are promising therapeutic anti-biofilm candidates. Two lytic bacteriophages, vB_SauM_ME18 and vB_SauM_ME126, were studied for their ability to reduce biofilm formation, and it was shown that they could be potential natural antimicrobials for inhibiting biofilms of multidrug-resistant *S. aureus*. Phage therapy first received FDA approval in 2019, when patients received phage treatment at the School of Medicine, University of California San Diego (UCSD) phage therapy center [122].

5. Concluding Remarks

Due to their high resistance to the current antibiotic and multidrug therapies, medical biofilms and biofilm-associated infections are remarkably difficult to eradicate and remain a major challenge for human health and healthcare systems.

Utilization of natural biologically active substances in combating biofilms has increased during recent decades, showing a new trend, because of their relatively low toxicity, high efficiency, and little or no capacity to induce antimicrobial resistance. During the last 5 years, anti-biofilm activity has been experimentally shown for a variety biologically active products derived from the terrestrial biota, such as plant extracts and compounds, essential oils, antimicrobial peptides, and biosurfactants.

Many studies during recent years have categorized novel anti-biofilm agents and strategies as promising candidates for treatments of biofilm-associated infections based on in vitro testing. However, in vivo tests are few and there are no reports about their clinical application. More in vivo tests and clinical trials are necessary for clinical application.

Most biologically active products (phytocompounds, antimicrobial peptides, enzymes, biosurfactants, quorum sensing inhibitors, etc.) demonstrate significant activity against monospaces, but only some are active against multispecies biofilms.

Natural products have structural and functional specificity, which inspires development of modified compounds with increased anti-biofilm activity.

Combinations of different anti-biofilm agents or combinations of antibiotics and other anti-biofilm agents that demonstrate improved anti-biofilm activity and lower toxicity compared to their individual application are accepted as a base for development of new advanced therapeutic strategies.

The efficacy of antimicrobial peptides against drug-tolerant pathogenic biofilms, without disturbing the natural microflora, is the focus of some current investigations, but the clinical studies are insufficient.

An anti-adhesion approach can be a novel strategy for the treatment of a broad range of microbial biofilms, and especially of multispecies bacterial biofilms, as it targets and prevents attachment of bacteria to the material and cell surface. So far, few studies have been conducted in this direction. Future research targeting adhesive substances secreted by microbes may lead to the discovery of unique natural anti-biofilm agents.

Biologically active products from the terrestrial biota have scarcely been studied as antimicrobial coatings or as a component of composition coatings to develop anti-biofilm material surfaces. The latter is very important for the reduction in infections associated with medical devices.

By combining promising approaches with natural anti-biofilm agents and antibiotics, the eradication of biofilms may be possible in the future.

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