Chapter 10

Natural Products: Strategic Tools for Modulation of Biofilm Formation

Tomáš Řezanka*, Alena Čejková† and Jan Masák†

*Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
†Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology Prague, Prague, Czech Republic

INTRODUCTION

Microorganisms tend to adhere to biotic and abiotic surfaces and to form a structured community—biofilm. Growth in such systems is accompanied by a change of cell phenotype manifested by different metabolic pathways, increased virulence, resistance to antibiotics, etc. [1]. Biofilm formation and its maturation and stability are influenced by physical, chemical, and biological factors. The course of colonization of the surfaces can be divided into several interconnected phases.

The phase prior to cell adhesion is called conditioning of the surface and includes an adsorption of inorganic and organic molecules presented in the bulk flow leading to changes of the physicochemical properties of the surface [2]. The conditioning film can affect biofilm formation both positively and negatively [3]. Initial phase of cell adhesion is often described by DLVO theory as an interaction of colloidal particles with a surface [4].

Similarity of microbial cells with a colloidal particle is, however, only partial. The process of microbial adhesion is considerably more complex.

A microbial cell interacting with the conditioned surface exhibits a high heterogeneity of envelope layers. The individual chemical substances in cell envelopes provide local areas with different surface hydrophobicity, charge, etc.; structures such as pili and flagella also come into play. Presence of extracellular polymeric substances (EPS), produced by biofilm microorganisms, is of considerable importance for the construction of biofilm architecture [5].

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Active participation of microbial cells leading to dynamic changes of interacting structures is essential for the adhesion process.

Regulatory mechanisms of microorganisms that are involved in biofilm formation and the development of a new phenotype are often controlled by chemical signals in the environment of cells. Such signaling molecules are produced specifically by the microorganism itself and then are included in the quorum-sensing (QS) regulatory system, or they are products of other microorganisms and can interfere with this regulatory system [6].

Natural products can affect biofilm formation in all its phases, and they can interact with the mature biofilm. They are able to change physicochemical properties of the environment (e.g., surface tension) or interfere with the structures of cell envelope layers, which are involved in adhesion. Natural products are able to modulate very effectively biofilm formation at the level of regulatory mechanisms [7].

Special and often unique properties of microbial cells growing in biofilm are preferably used in many technological processes; on the other hand, they cause important economic losses (e.g., biofouling) and serious health problems.

For these reasons, considerable attention is devoted to tools that can specifically affect biofilm formation, its stability, and structure. Research aimed at using natural products in the role of such tools occupies one of the leading positions.

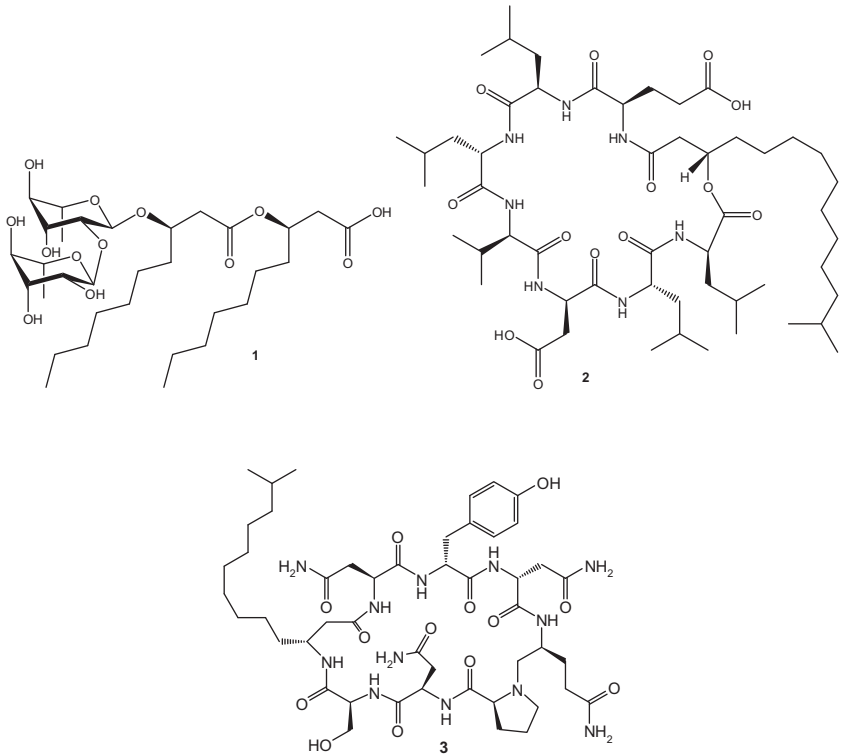
The following sections are devoted to such natural substances, produced by unicellular and higher organisms, which affect the physicochemical properties of the surfaces (surface activity) or interfere with the microbial regulatory mechanisms that are important for biofilm formation. The greatest attention is given to compounds that primarily affect adhesion of microbial cells or the stability of the biofilm structure but not the compounds which primarily kill microbial cells and thereby also cause biofilm breakdown.

SURFACE ACTIVE NATURAL PRODUCTS

Adhesion of microbial cells and the subsequent biofilm formation depends on physicochemical properties of surfaces and interfacial tension. The surface-active compounds produced by living organisms (biosurfactants) are, apart from antimicrobial activity, able to significantly influence the microbial adhesion or biofilm formation. Many structurally different biosurfactants are currently known, including glycolipids, lipoproteins, polysaccharides, or proteins [8]. Fungi and bacteria are the most common producers.

It appears that surfactants are necessary for their producers to initiate biofilm formation, as well as for migration of subpopulations within the biofilm. The participation of *Pseudomonas aeruginosa* surfactants in maintaining channels between multicellular structures was also confirmed [9]. Al-Tahhan *et al*. [10] found that very low concentrations of rhamnolipids (**1**)

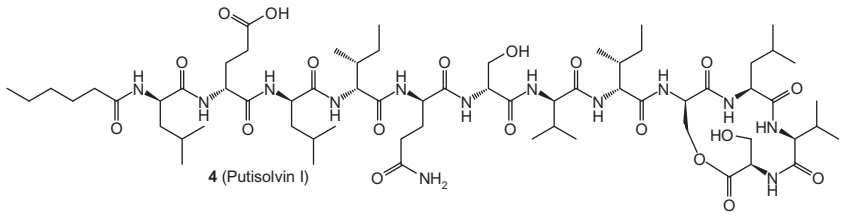
(amphipathic anionic glycolipids composed of rhamnose and β-hydroxy-fatty acids) in the environment increase cell surface hydrophobicity due to the release of lipopolysaccharide from the outer membrane and thus promote cell adhesion. Direct interaction (adsorption) of surfactant molecules with cell envelopes can change their hydrophobicity as well, depending on orientation of adsorbed molecules [11]. Zhong *et al*. [12] indicate that rhamnolipids (mono- or di-) at low concentration occupy adsorption sites on the cell surface of *P. aeruginosa* with hydrophobic tail extending to the environment and increase the cell surface hydrophobicity. A higher surfactant concentration saturates the adsorption sites and multilayer adsorption or accumulation of hemimicelles occurs. Under such conditions, the cell surface hydrophobicity can fall again. Likewise, surfactin (**2**) and iturin A (**3**), lipopeptides produced by *Bacillus subtilis*, increase cell surface hydrophobicity and are responsible for enhanced ability to colonize surfaces [13]. Lopez *et al*. [14], however, showed that surfactin has also the function of a signal molecule that triggers cannibalism and biofilm formation in *B. subtilis*.



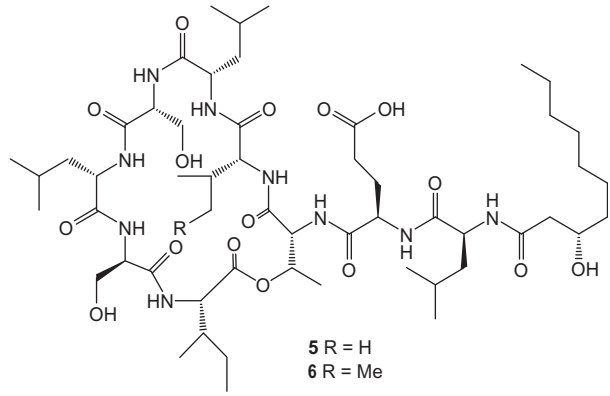
Because they possess the ability to reduce the surface and interfacial tension, biosurfactants are very often referred to as the "antibiofilm" active compounds toward other microorganisms. A lot of works using rhamnolipids as

antiadhesive substances were published in this context [9,11,15–17]. Rhamnolipids caused an effective decomposition of the biofilm of marine bacteria *Bacillus pumilus* [18], as well as the respiratory pathogen *Bordetella bronchiseptica* [19]. Interest in commercial applications of rhamnolipids led to the development of biotechnological processes, allowing their industrial production.

Antibiofilm activity was also demonstrated in the case of lipopeptides. The above-mentioned surfactin prevents biofilm formation of *Listeria monocytogenes* and *Enterobacter sakazakii*, important pathogens in food [20], as well as *Salmonella enterica* sv. *typhimurium* [21]. Inhibition of biofilm formation by putisolvins (**4**), cyclic lipopeptides, produced by *Pseudomonas putida* was described by Kuiper *et al*. [22]. Putisolvins inhibited *P. aeruginosa* PA14 and *Pseudomonas fluorescens* WCS365 biofilms.

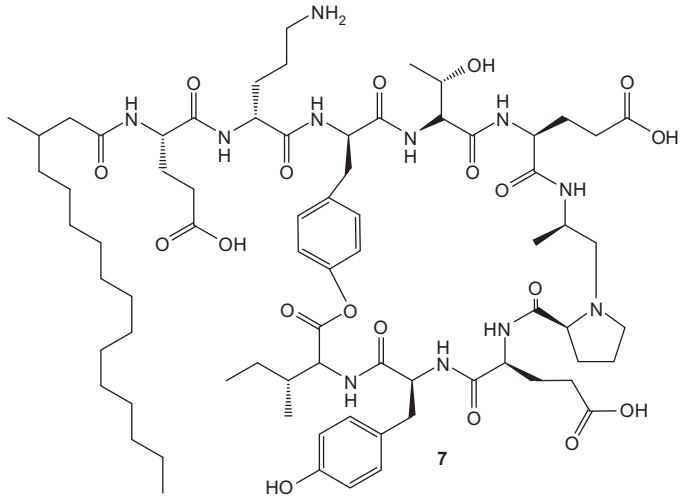


Other cyclic lipopeptides acting as biosurfactants/antibiotics, viscosin (**5**) and massetolide A (**6**), from *P. fluorescens* prevent the formation of *P. aeruginosa* PAO1 microcolonies [23].



Rivardo *et al*. [24] found in *B. subtilis* and *Bacillus licheniformis* biosurfactants containing two separate fractions, each containing the surfactin-like and the fengycin-like (**7**) biosurfactant families in varying proportion. The reactions showed selective antiadhesive activity in biofilm inhibition of pathogenic strains G^- *Escherichia coli* CFT073 and G^+ *Staphylococcus aureus* ATCC 29213. The V9T14 biosurfactant, active against the G^- strain, was ineffective against the G^+ one. The V19T21 fraction acted in an opposite

manner. Subsequent studies carried out by these authors [25] demonstrated synergistic activity of biosurfactant V9T14 with some antibiotics on the destruction of mature biofilm of *E. coli* CFT073. The authors hypothesize that the action of V9T14 biosurfactant lies in its interaction with the bacterial membrane, increasing the activity of antimicrobial agents by forming pores in the outer membrane and thus facilitating the entrance of antibiotics into biofilm cells.



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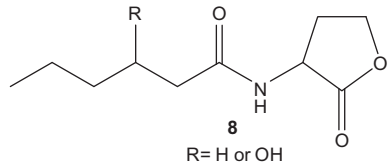
A lipopeptide biosurfactant isolated from marine bacterium *Bacillus circulans*, which was not exactly specified, suppressed adhesion and caused destruction of mature biofilm of several potentially pathogenic strains. Applications of 10 g/l had a nearly 90% efficiency [26]. Rodrigues *et al*. [27] isolated a biosurfactant from probiotic lactic acid bacteria *Lactococcus lactis* 53, which after adsorption on the surface of silicone rubber reduced its hydrophobicity and very effectively suppressed the deposition of several strains of staphylococci and yeasts C*andida albicans* and *C. tropicalis* on this carrier. Also other lactic acid bacteria such as *Lactobacillus acidophilus* [28,29] and *L. fermentum* RC-14 [30] produce surfactants whose importance is associated with an effective ability to suppress the adhesion of uropathogenic bacteria in the urogenital tract.

NATURAL PRODUCTS IN BIOFILM REGULATION MECHANISMS

Current knowledge of the regulatory processes controlling microbial cell adhesion, biofilm formation and maintenance, or its dispersal was very well summarized by Landi *et al*. [31]. A relatively universal regulatory process that occurs in the development of a biofilm phenotype is QS, a process based on

the ability of cells to identify the cell population density by certain threshold concentration of an autoinducer in the environment. There are a lot of different extracellular signal molecules in G^+ and G^- bacteria and their number continuously grows.

N-Acyl homoserine lactones (**8**) (AHLs) are the common signaling molecules (autoinducers) of G^- bacteria. The side chain lengths vary from 4 to 18 carbon atoms.



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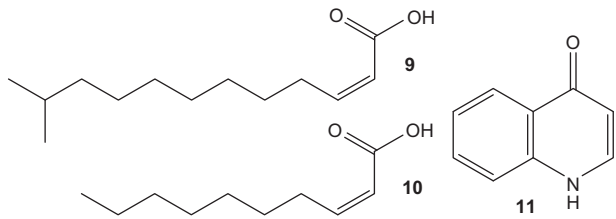
R= H or OH

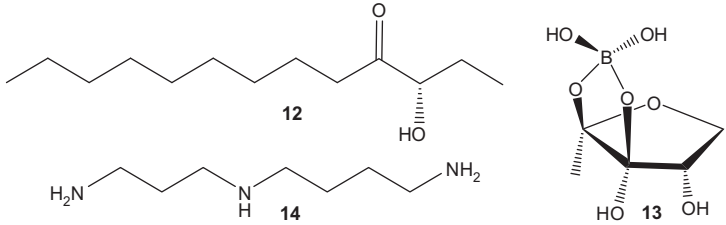
They regulate the production of EPS, one of the most important components in the biofilm [32]. AHLs are further involved in regulatory mechanisms of both virulence factors and synthesis of rhamnolipids (pathogens) and pili and flagella, that is, substances (structures) that directly affect the biofilm formation [33,34]. The autoinducers have a variety of functions not only in intraspecies communication but also they are also involved in mutual interactions (relationships) at the level of interspecies and interkingdoms. Autoinducers can act in such cases as agonists or antagonists. In connection with our topic, they are able to stimulate or suppress biofilm formation [35].

AHLs are not the only autoinducers in G^- bacteria. For example, *Xanthomonas campestris* uses substituted fatty acid messenger, *cis*-11-methyl-2-dodecanoic acid (**9**), and called diffusible signal factor [36]. Another small messenger fatty acid, *cis*-2-decenoic acid (**10**), is produced by *P. aeruginosa* [37]. This compound induces biofilm dispersion and inhibits its formation in a number of G^+ and G^- bacteria and yeast, as well as in the producers themselves. *P. aeruginosa* produces 4-quinolones (**11**) that serve as another type of QS signals. Some of these compounds play an important role in the formation and maintenance of the biofilm by a number of different mechanisms [38–40].



Vibrio fischeri is a bioluminescent bacterium, in which regulation by QS was observed in 1970 for the first time [41]. The genus *Vibrio* is being constantly studied in this context. *Vibrio cholerae* uses two autoinducers that control pathogenicity and biofilm formation. Cholerae autoinducer-1

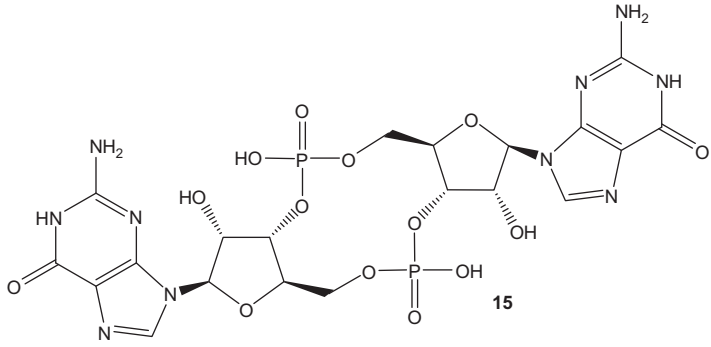
(CAI-1)—(*S*)-3-hydroxytridecan-4-one (**12**) and autoinducer-2 (AI-2)—furanosyl borate diester (**13**) function synergistically to control gene regulation [42]. Polyamines are required for normal growth of both eukaryotes and prokaryotes. They are involved in numerous and diverse cellular processes including the regulation of biofilm formation. McGinis *et al*. [43] confirmed the regulatory effects of spermidine (**14**) on the formation of *V*. *cholerae* biofilms.



Indole is a common natural product. Furthermore, it is important interspecies signal molecule produced by a variety of both G^+ and G^- bacteria. Indole affects many regulatory mechanisms and is also involved in biofilm formation/inhibition [44]. Extracellular indole suppresses *E*. *coli* adhesion but induces pseudomonad adhesion in dual-species biofilm [45].

Ribosomally synthesized short peptides are common signal molecules in QS in G^+ bacteria. Posttranslational modification of the peptides is often of great importance [46].

The regulatory cascade involving the cyclic-di-guanosine monophosphate (**15**) (c-di-GMP) as a second messenger is another important regulation process associated with the multicellular (biofilm) behavior of microbial populations. The GGDEF and EAL domain proteins involved in c-di-GMP biosynthesis and degradation are widespread in bacterial genomes [47]. Intracellular level of c-di-GMP plays a central role in regulation of production of extracellular polysaccharides (EPS), adhesines, pili and flagella, and in this connection influences biofilm formation or destruction [48].



The complexity of natural regulatory processes provides a large number of targets that can interfere with chemical compounds of various origins, which

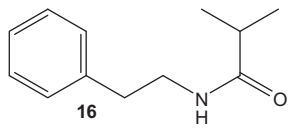
in turn leads to positive or negative changes in cell adhesion and the ability of microorganisms to form biofilm. Moreover, a great importance is attributed to enzymes that are able to degrade AHLs and other signal molecules, which have been found in some bacteria and also in plant and animal tissues [49–51].

A more detailed overview of natural products affecting mechanisms of microbial adhesion in the following sections is subdivided according to the producers of these substances.

MICROBIAL NATURAL PRODUCTS

Microorganisms are undoubtedly one of the most important groups of organisms producing natural products. In the early stages of research, the most attention has been devoted to compounds with antibiotic activity or to toxins. Recent years saw a marked increase in the research of the regulatory functions of natural products. Study of interferences of these substances with mechanisms that are directly or indirectly involved in the adhesion and/or formation of a biofilm cell phenotype is an integral part of this upswing.

The considerable versatility of signaling molecules directly involved in regulation of microbial adhesion renders their wide impact across the microbial world quite logical. However, signaling by AHLs may be diminished (eliminated) by competitive antagonists, which do not have their own signaling activity, but bind to the same receptor site as the native agonists. Metabolites of marine G^+ bacterium *Halobacillus salinus* can be one of many examples. *H. salinus* produces two compounds, phenylethylamides (*N*-(2-phenylethyl)-isobutyramide (**16**) and 3-methyl-*N*-(2-phenylethyl)-butyramide) (**17**), which resemble in their structure AHLs, that is, they possess a ring system with a side chain connected via an amide bond [52].



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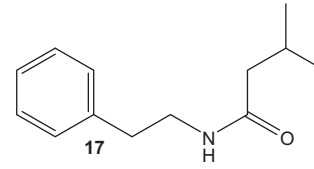

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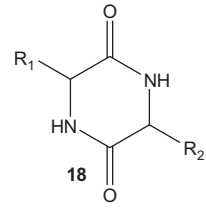
Some pseudomonads produce substances that activate or antagonize LuxR-based QS systems in other G^- bacteria. However, the structure of these substances greatly differs from the AHL molecules. They are diketopiperazines (**18**), the smallest possible cyclic peptides (cyclo(D-Ala-L-Val), cyclo (L-Pro-L-Tyr), and cyclo(L-Phe-L-Pro)), which are capable of mimicking the action of AHLs [53].



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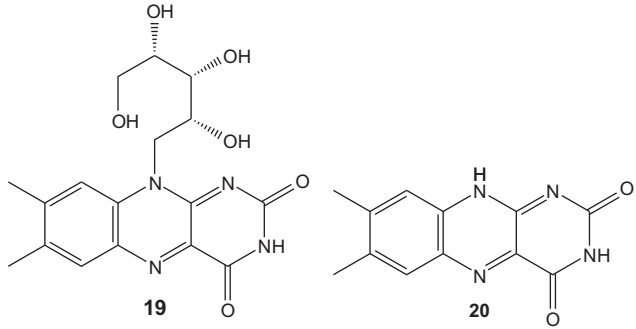
Examples of other compounds that can mimic AHLs and thereby manipulate QS in bacteria by interacting with the Las-R receptors are riboflavin (**19**) and lumichrome (**20**). It should be noted that these substances are commonly secreted by bacteria, algae, and plants, and so their activity can reach across the kingdoms.



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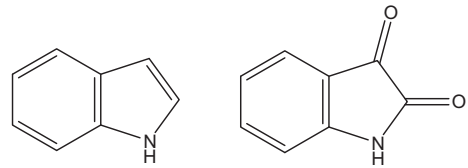
Indole (**21**) is a substance produced by many microorganisms, whose activities associated with the induction of virulence, cell cycle regulation, resistance to acid and biofilm formation were summarized by Hu *et al*. [54]. The ability of indole to stimulate/inhibit biofilm formation is not entirely clear, and one explanation could be strain specificity [55,56]. Many nonindole-producing bacteria have oxygenases which can oxidize indole and the resulting products have antibiofilm activity. In this context, it is interesting to note the role of isatin (**22**) (indole-2,3-dione), a stress-related biological substance in animals. The presence of isatin represses indole biosynthesis in pathogenic *E. coli* O157:H7 and thus enhances biofilm formation [57].



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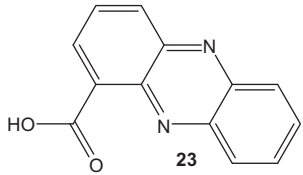
Phenazines represent a very large group of nitrogen-containing secondary metabolites of bacteria. Some affect the regulation of biofilm, while the specific effect is determined by the molecular structure [58]. Pierson and Pierson [59] found that *Pseudomonas chlororaphis* produces phenazine-1-carboxylic

acid (**23**) which is partly converted into 2-hydroxy-phenazine-1-carboxylic acid (**24**). The former may facilitate growth within the biofilm while the latter may facilitate cell adhesion.



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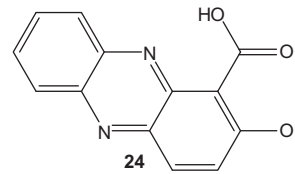

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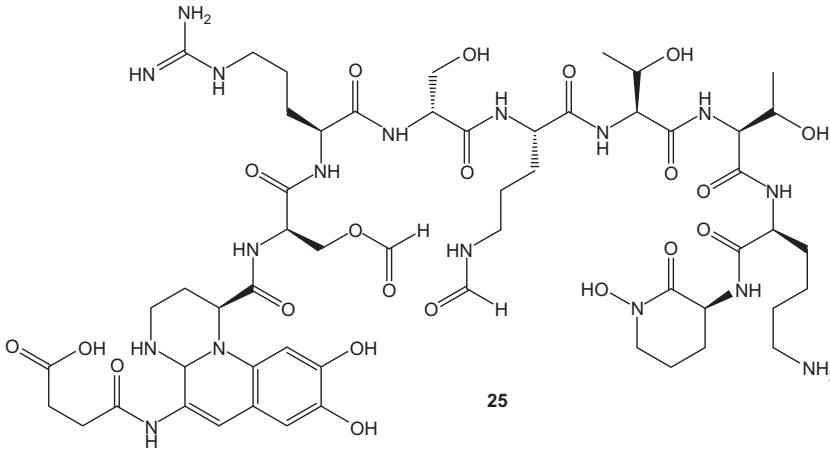
Siderophores as iron chelators are also involved in *P. aeruginosa* biofilm formation, albeit indirectly. Iron serves as a signal in *P. aeruginosa* and, for example, pyoverdine (**25**) can ensure its sufficiently high intracellular level in an iron-poor environment [60].



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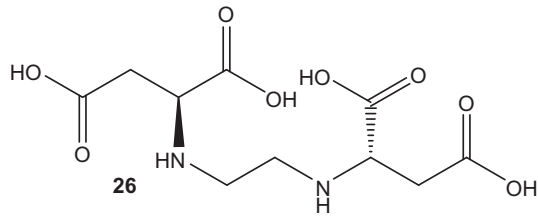
Aminopolycarboxylic acid ethylenediaminedisuccinate (**26**) was isolated from culture filtrate of the actinomycete *Amycolatopsis orientalis* [61]. Wen *et al*. [62] have shown that this natural chelator was able to enhance the treatment of sulfate-reducing bacteria biofilms by glutaraldehyde.



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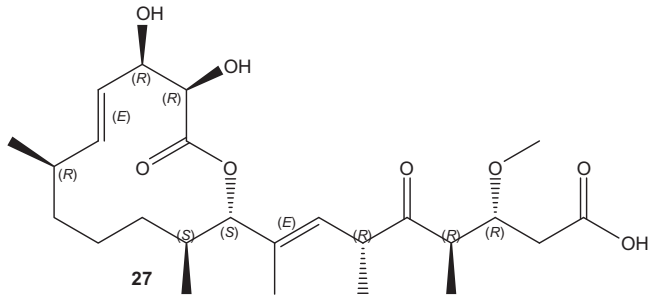
Bacteria produce D-amino acids in the stationary phase of growth [63]. Some of them may contribute to the destruction of the formed biofilm. Kolodkin-Gal *et al*. [64] demonstrated that D-tyrosine, D-leucine, D-tryptophan, and D-methionine

are able to inhibit biofilm formation by *B. subtilis*, while a combination of these acids showed synergistic effect. The authors also found that mixtures of these D-amino acids or D-tyrosine itself can inhibit the biofilm of pathogenic bacteria such as *S. aureus* and *P. aeruginosa*.

Carolacton (**27**) is a macrolide metabolite of the myxobacterium *Sorangium cellulosum*. This compound exhibits significant inhibitory activity against *Streptococcus mutans*, a major pathogen in human dental caries. An important characteristic of this compound is its ability to damage *S. mutans* biofilm at nanomolar concentrations under anaerobic conditions [65].



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In addition to studies on individual products of microorganisms, a number of papers deal with the effects of variant types of extracts of microorganisms on biofilm formation.

Actinomycetes are known for extensive production of secondary metabolites. You *et al.* [66] screened 88 marine actinomycetes and found that extracts from 33 of them dispersed the mature biofilm. Strain A66, identified as *Streptomyces albus*, was rated as the most effective with regard to the biofilm attenuation and AHLs inhibition.

The antibiofilm activity against *P. aeruginosa* PAO1 and *Vibrio* spp. was also demonstrated in extracts of culturable bacteria isolated from sediments in the Palk Bay [67,68]. None of the extracts showed any antibacterial activity. Some extracts have shown QS inhibition, others showed biofilm dispersion and disruption of the biofilm architecture by reducing the EPS production and hydrophobicity index.

Similarly, numbers of bacteria isolated from the marine environment produce substances that inhibit QS [69]. Marine bacterial extracts appear as valuable sources for developing biofilm inhibitors against pathogens and biofouling. Although the participation of bacterial biofilms in biofouling is important, microorganisms with antibiofilm effects can be found even in this complex system. So epiphytic bacterium *Pseudoalteromonas tunicata* growing on green algae *Ulva lactuca* synthesizes yellow pigmented substances which block biofilm formation [70].

Released exopolysaccharides (r-EPS) produced by probiotic *L. acidophilus* A4 have shown antibiofilm activity against a number of G^+ and G^- pathogens [71]. Biofilm formation of certain bacteria is stimulated by the presence of highly adhesive curli fibers on the cell surface [72]. As found in the case of

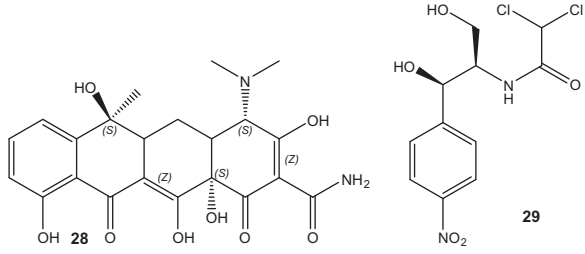
E. coli O157: H7, r-EPS significantly repressed genes responsible for production of curli [71].

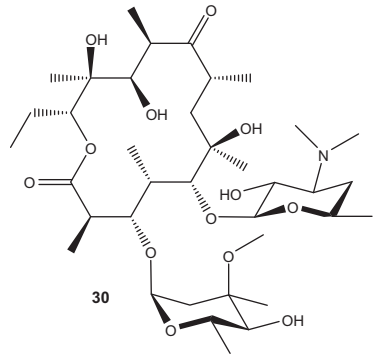
In addition to bacteria, imperfect fungi are important producers of secondary metabolites. Screening of a wide set of *Penicillium* species yielded two compounds with a significant effect on *P. aeruginosa* QS [73]. Both penicillic acid and patulin affect QS-controlled gene expression. Expectedly, patulin was found to enhance biofilm susceptibility of *P. aeruginosa* to tobramycin treatment.

Antibiotics

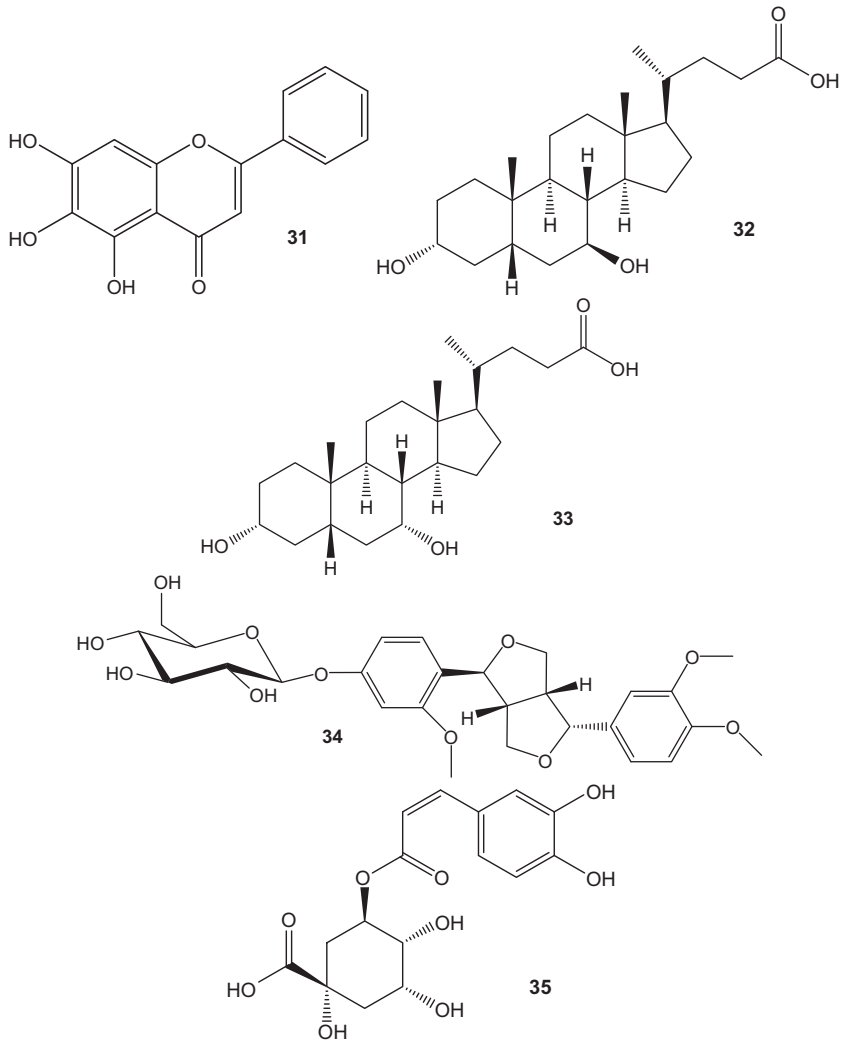
Antibiotics are an inherent group of microbial natural products that are essential for man. Accurate separation of their activities suppressing microbial growth and activities associated with cell adhesion is rather difficult. Nevertheless, it was shown that some substances, originally isolated as antibiotics, can exhibit signal activity based on an interference with the microbe communication.

The number of microorganisms resistant to the "old" antibiotics has been growing over the years. The importance of these substances may, however, lie in the suppression of microbial colonization. Tetracycline (**28**) and chloramphenicol (**29**) demonstrated the ability to effectively inhibit the biofilm formation of such pathogens as *Achromobacter* sp. *P. aeruginosa*, *K. pneumoniae*, and *B. pumilis* [74]. Heerden *et al.* [75] found that chloramphenicol and the steroid antibiotic fusidic acid used for coating the surface of silicone implants were able to inhibit the adhesion of pathogenic bacterium *Staphylococcus epidermidis in vitro*. Erythromycin A (**30**) as a natural product and some other semisynthetic macrolides inhibit EPS production in *P. aeruginosa* even at subinhibitory concentrations [76].

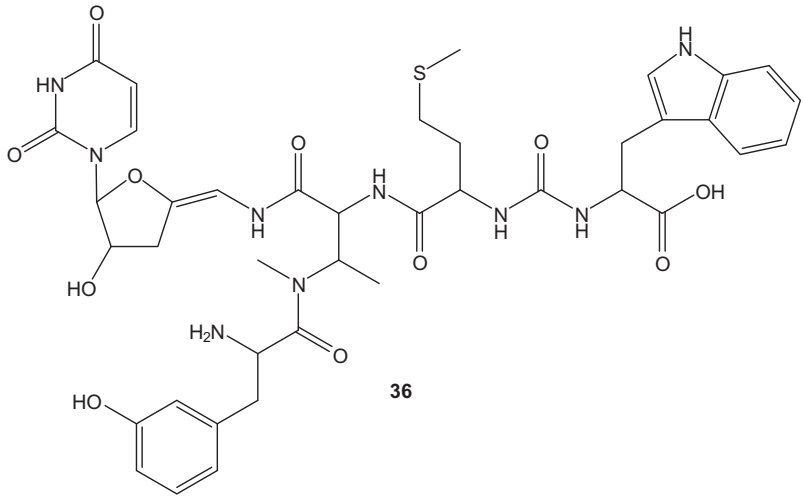




Traditional Chinese medicine uses TanReQuin (TRQ) as an antibacterial agent to treat infections of upper airways. Baicalein (**31**), ursodeoxycholic acid (**32**), chenodeoxycholic acid (**33**), forsythin (**34**), and chlorogenic acid (**35**) are the main active compounds in the formulation. Good efficiency of TRQ to infections caused by *S. aureus*, for example, lies in its ability to inhibit biofilm formation [77]. Baicalein is also able to regulate biofilm formation in the pathogenic yeast *C. albicans*. Yeast has *CSH1* gene, which encodes the synthesis of protein responsible for the rise of cell surface hydrophobicity. Baicalein reduces the *CSH1* expression and thus can reduce the cell surface hydrophobicity and, consequently, reduce cell adhesion [78].



Sansanmycin (**36**), a novel narrow-specific antibiotic, was isolated from not otherwise specified *Streptomyces* sp. SS (CGMCC No. 1764). Chemically, it is classified as a nucleosidyl-peptide antibiotic [79]. Sansanmycin, especially in combination with macrolides, inhibits *P. aeruginosa* biofilms [80].



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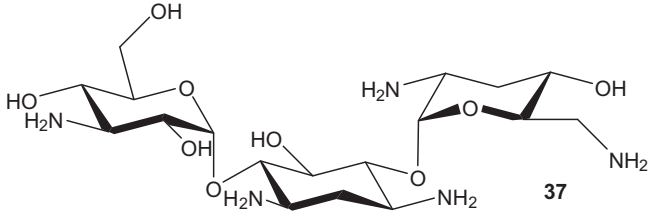
Interactions of tobramycin (**37**) (product of *Streptomyces tenebrarius*) with signal systems regulated by AHLs are not fully understood. Linares *et al.* [81] proved that subinhibitory concentration of tobramycin induces swarming and biofilm formation in a clinical isolate of *P. aeruginosa* PAO1. On the other hand, this antibiotic suppresses formation of C4-HSL in environmental *P. aeruginosa* strain PUPa [82].

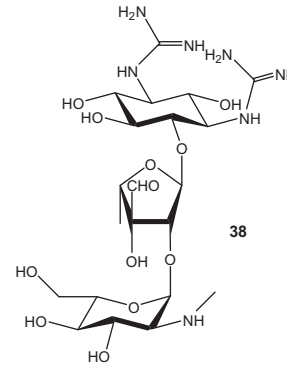


37

Positive effect on biofilm formation was also identified during the testing of other antibiotics. In this context, the hypothesis was expressed that in nature antibiotic-producing microorganisms could serve as an organizing force in integrated microbial communities, helping, for instance, nonantibiotic members of the community to colonize surfaces and form biofilm [81].

Subinhibitory concentrations of aminoglycoside antibiotics are able to induce biofilm formation in pathogenic strains *P. aeruginosa* and *E. coli*. The aminoglycoside streptomycin (**38**) interferes with the regulation of c-di-GMP (second messenger) biosynthesis whose intracellular level is

essential for cell surface adhesiveness [83]. It was shown that the loss of potassium from intracellular space of *B. subtilis* stimulates the activity of protein kinase KinC, which governs the expression of genes involved in biofilm formation. The polyene antibiotic nystatin A1 (**39**) or the nonribosomally encoded peptide gramicidin S (**40**) are causing cation leakage and it is therefore not surprising that both substances enhance the biofilm formation [14].



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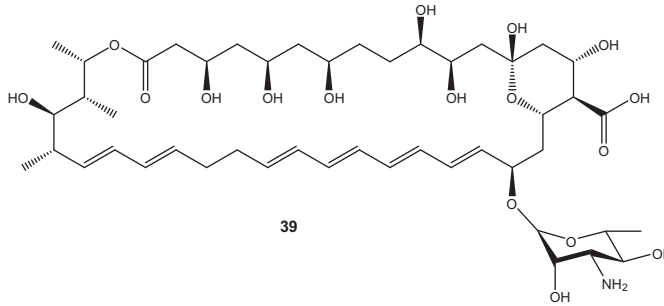

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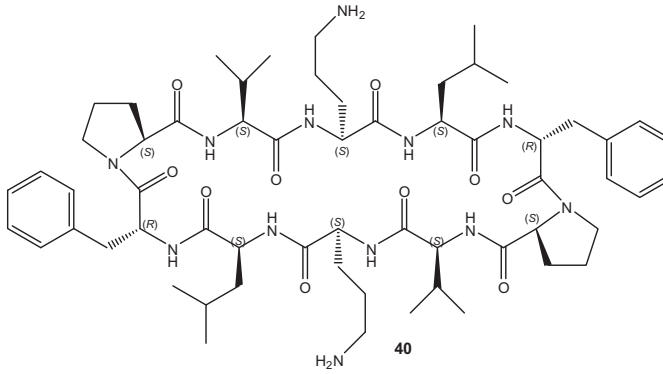
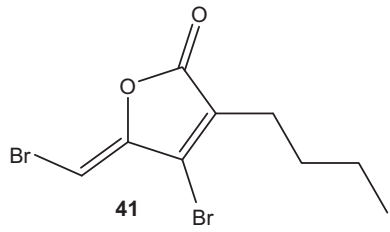


40

MARINE ORGANISMS

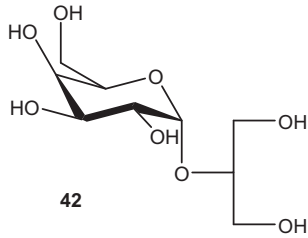
Marine ecosystems are in many aspects little explored regions, which is also confirmed by numerous new discoveries concerning the metabolism of and metabolites produced by their inhabitants. Marine microorganisms producing antiadhesive substances were described above.

Natrah *et al*. [84] published a comprehensive study devoted to the negative effects of marine organisms on bacterial cell-to-cell communication, which often led to suppression of adhesion ability and bacterial biofilm formation. A very important group of these compounds, halogenated furanones, was identified in marine red algae *Delisea pulchra* by Manfield *et al*. [85] in 1999. The compounds are structurally similar to AHLs and can specifically interfere with AHL-dependent gene transcription at the level of the LuxR-like regulatory protein (they displace the AHL signal from its receptor protein). Further studies then showed that one of the *D. pulchra* furanones ((5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2-(5*H*)-furanone) (**41**) significantly inhibited *E. coli* and *B. subtilis* biofilms [86–88]. Zang *et al*. [89] found that a naturally occurring brominated furanone covalently modifies and inactivates LuxS (*S*-ribosylhomocysteine lyase, EC 4.4.1.21), the enzyme which produces AI-2. The activity of natural furanones has led to the preparation of synthetic analogues with a higher ability to suppress QS. 5-(Bromomethylene)-4-ethyl-3-methyl-2-pyrrolinone, a strong inhibitor of bacterial swarming and biofilm formation of *P. aeruginosa*, can be mentioned as an example [90].



41

The Korean red alga *Ahnfeltiopsis flabelliformis* produces three structurally different compounds, which, though different from the AHLs structure, interfere with *N*-octanoyl-DL-homoserine lactone (OHL) [91]. A mixture of floridoside (**42**) and isethionic acid (**43**) exhibited a dose-dependent inhibitory effect on the function of the OHL. Conversely, the third compound, betonicine (**44**), exhibited dose-dependent stimulatory effect [92].



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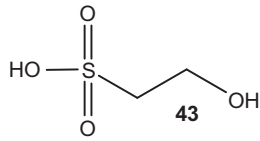

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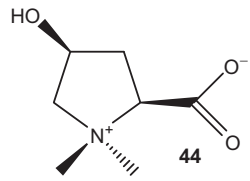
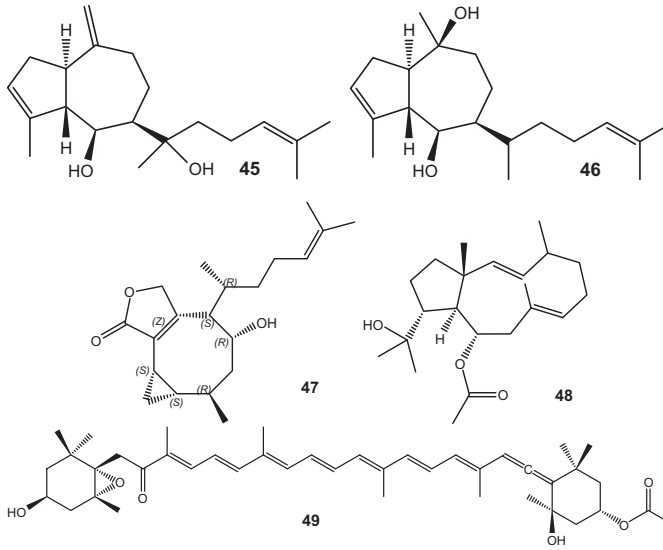
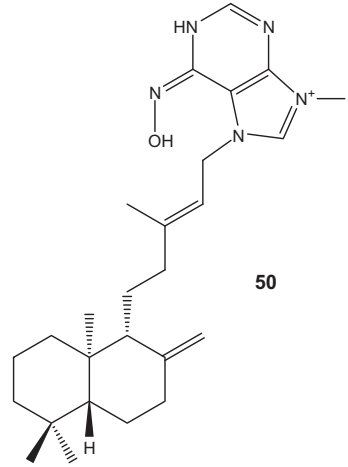


44

Species of Mediterranean brown alga *Dictyota* are important producers of secondary metabolites. Significant portions are diterpenoids. Viano *et al*. [93] isolated 11 compounds from the algae of which 4 were new diterpenes. Dictyols E (**45**) and C (**46**), hydroxycrenulide (**47**), 9-acetoxy-15-hydroxy-1, 6-dollabelladiene (**48**), fucoxanthin (**49**), and two of the new diterpens (dolabellanes) showed antibiofilm activity, while dictyol C and fucoxanthin were the most efficient.

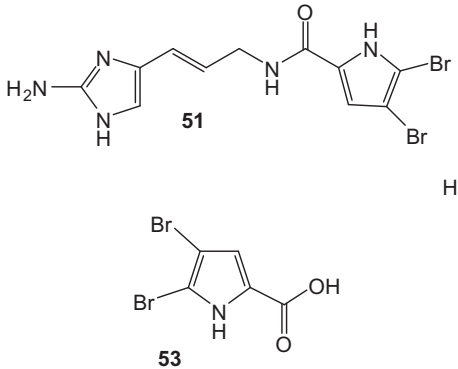


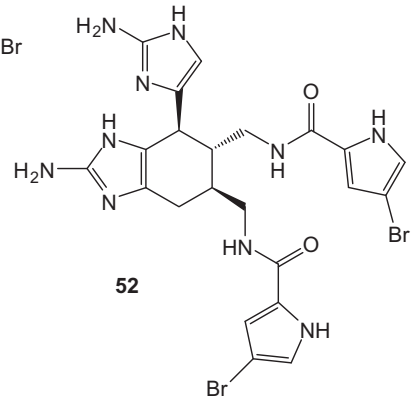
Agelas nakamurai also produces diterpenes; the diterpenoid alkaloid ageloxime D (**50**) prevents bacteria *S. epidermidis* from forming the biofilm [94].

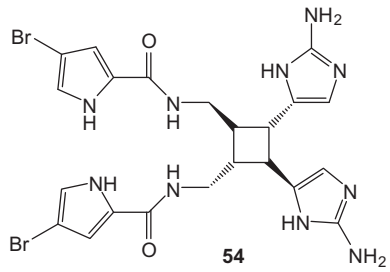


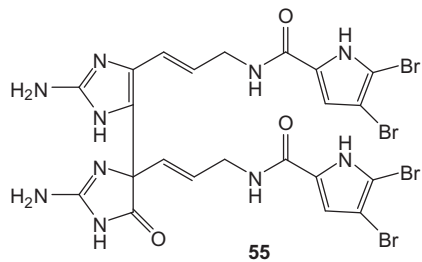
Similarly, other bromoalkaloids such as oroidin (**51**), ageliferin (**52**) and 4,5-dibromopyrrole-2-carboxylic acid (**53**) isolated from *Agelas clathrodes*, and sceptrin (**54**) isolated from *A. conifera* inhibited bacterial attachment at natural concentrations. Tsukamoto *et al*. [95] found an oroidin dimer

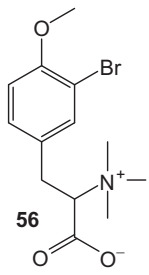
mauritiamine (**55**) in *A. mauritinia* with the same antibiofilm property. Marine sponge *Ailochroia crassa* synthesizes bromotyrosine-derived natural products—for instance (**56**). They are often able to influence the growth and motility of bacteria. Ianthellin (**57**) was identified as the metabolite that inhibited bacterial attachment [96].

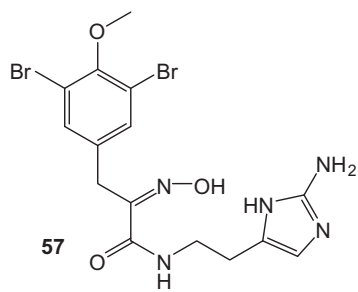




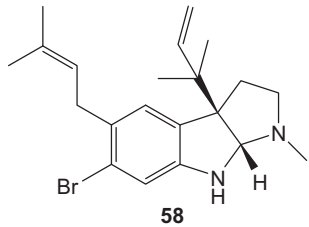








The North Sea bryozoan *Flustra foliacea* produces several secondary metabolites and thus prevents the colonization of their surfaces. Two of these compounds have been identified as bromoalkaloids, that is, flustramine D (**58**) and dihydroflustramine C (**59**) and are effective antagonists of AHL-dependent QS signaling systems [97].



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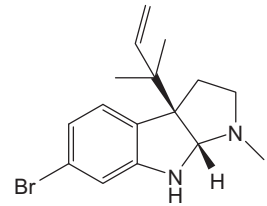
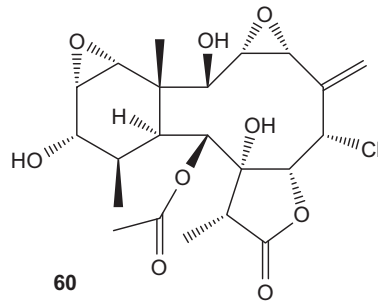


59

Yamada *et al*. [98] isolated from the marine sponge *Psammaplysilla purpurea* the chlorinated polycyclic metabolite bis (deacetyl) solenolide D (**60**) possessing antibiofilm activity, expressed as a decrease of the acidic EPS produced by biofilm-forming marine bacterium *Rhodospirillum salexigenes* SCRS 113.



60

PLANTS

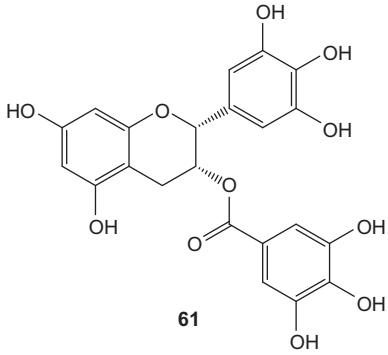
Plants produce an enormous number of natural products. Many of them have been used by people in the form of whole plants, their parts or extracts since ancient times. Despite the long-term research, plants seem to be an inexhaustible treasure of new compounds in the present.

Extracts of many plants contain components (phenol derivatives, terpenes, etc.) that have an ability to suppress microbial cell attachment and a biofilm growth [99].

Natural polyphenols are a group of substances exhibiting extensive biological activities. In particular, plants are rich sources of these compounds. It was shown [100] that epigallocatechin-3-gallate (**61**) (EGCG), a rich source of which is green tea, and ellagic acid (**62**), which is contained in various fruits (blackberries, raspberries, strawberries, cranberries), act as antagonists of AHLs-dependent microorganisms. The latter compound had a high antibiofilm activity.

Antibiofilm activity of green tea polyphenols was also demonstrated on attached pathogenic yeast *C. albicans* [101], EGCG being more effective than epigallocatechin (**63**) or epicatechin-3-gallate (**64**). This study [101] suggests that the metabolic instability produced by the catechin-induced proteasome

inactivation was a contributor to the decrease in the growth rate constant as well as biofilm formation and maintenance.



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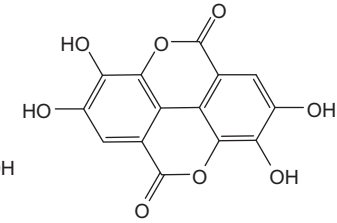

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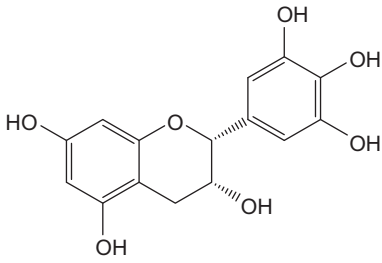

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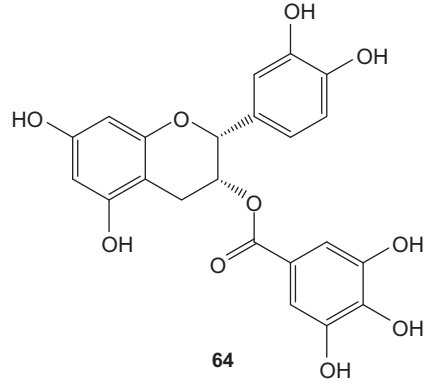
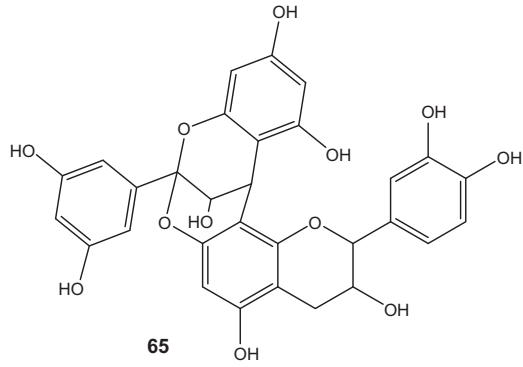


64

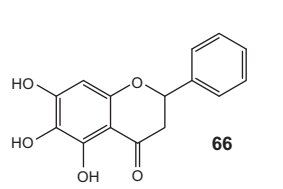
Daglia *et al*. [102] found that dealcoholised red wine is able to inhibit *S*. *mutans* biofilm formation on human teeth. Proanthocyanidins, for example, proanthocyanidin A1 (**65**), were the components most involved in the antiadhesion and antibiofilm activity. Cranberry (*Vaccinium macrocarpon*) contains less frequent A-type linked proanthocyanidins. In contrast to the more frequent proanthocyanidins containing B-type linkages, the A-type showed far greater antiadhesive activity to uropathogenic *E*. *coli* [103]. It was proven that the cranberry juice cocktail significantly decreases nanoscale adhesion forces between P-fimbriated *E*. *coli* and uroepithelial cells [104].



65

Zeng *et al*. [105] carried out an analysis of 51 active compounds used in traditional Chinese medicine. Five of them had a proven ability to inhibit biofilm formation, the flavonoid baicalein (**66**) being the most effective. This substance is contained, for example, in *Oroxylum indicum* or in the roots of *Scutellaria baicalensis*. Baicalin (**67**), the glucuronide of baicalein (**31**), has significant antibiofilm activity against *Burkholderia cenocepacia* or *B. multivorans* [106]. Many flavonoids are prominent secondary metabolites present in citrus species. So naringenin (**68**), kaempferol (**69**), quercetin (**70**), and apigenin (**71**) have shown the ability to inhibit biofilm formation in *Vibrio harvey* and *E. coli* O157:H7 [107]. *Combretum albiflorum* (Tul.) Jongkind, which is endemic in Madagascar, contains flavonoids in bark. The flavan-3-ol catechin (**72**) has quorum-quenching activity and partly suppresses biofilm formation [108].

Hamamelitannin (**73**) (2′,5-di-*O*-galloyl-D-hamamelose) is a natural product found in the bark of *Hamamelis virginiana* (witch hazel). Kiran *et al*. [109] have been found that this compound acted as nonpeptide analog of the QS inhibitor RNAIII-inhibiting peptide, and that it effectively prevented an attachment of *S. aureus* and *S. epidermidis*.



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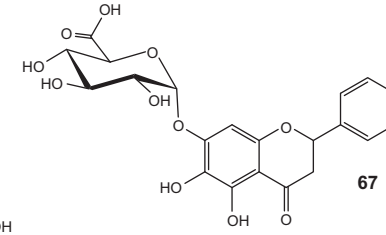

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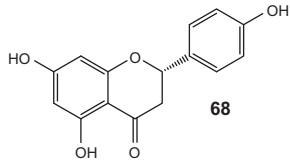

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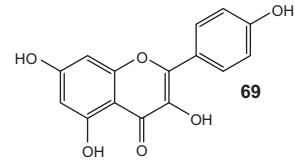

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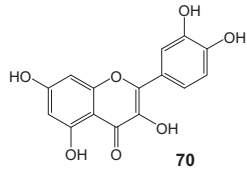

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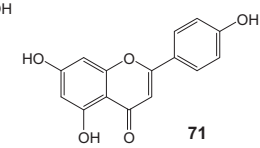

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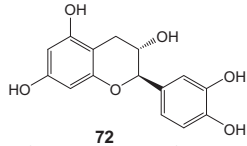

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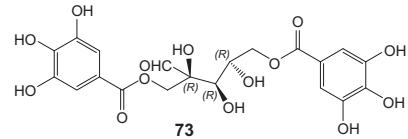
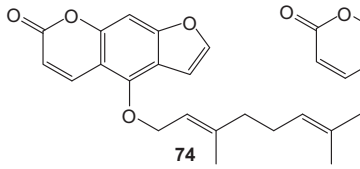


73

Plant furanocoumarines are substances known as mediators of the activities of certain drugs [110]. In this context, grapefruit juice was mainly studied because of the content of bergamottin (**74**) and dihydroxybergamottin (**75**). Both of these substances are also potent inhibitors of G^- bacteria autoinducers and suppress the formation of biofilms of *E. coli* O157: H7, *S. typhimurium*, and *P. aeruginosa* [111].

6,7-Dihydroxycoumarin (**76**) (aesculetin) present in horse chestnut, *Aesculus hippocastanum*, was proved to be efficient in preventing biofilm formation by *S. aureus* [112].



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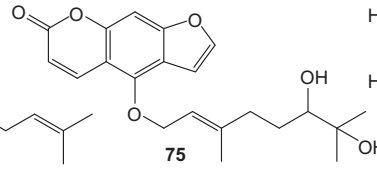

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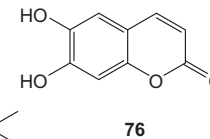
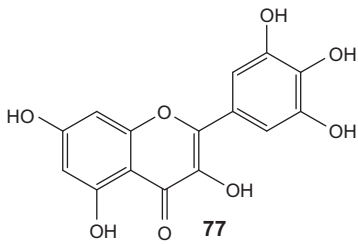


76

Jeon *et al.* [113] studied antibiofilm activity of myricetin (**77**) (flavonol) and *tt*-farnesol (**78**), compounds ubiquitously found in fruits (cranberries and red wine grapes), and propolis (a resinous mixture collected from tree buds, sap flows, or other botanical sources by honey bees), against *S. mutans* causing dental caries. They showed that the mixture of the natural products in combination with fluoride disrupted the accumulation and structural organization of EPS and bacterial cells in the matrix, which affected the biochemical and physiological properties of the biofilms.



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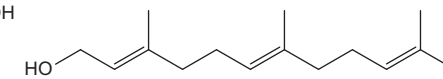
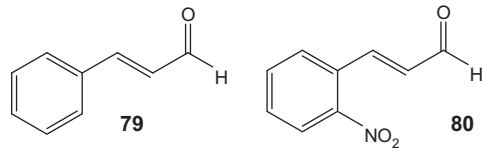


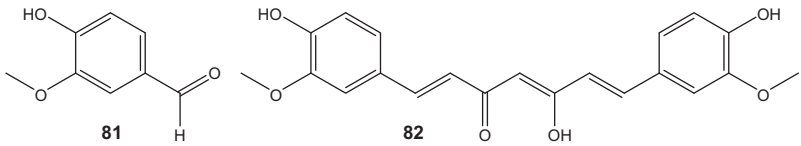
78

Certain compounds contained in the commonly used spices can affect the adhesion of microorganisms. The flavor essence in the bark of cinnamon trees,

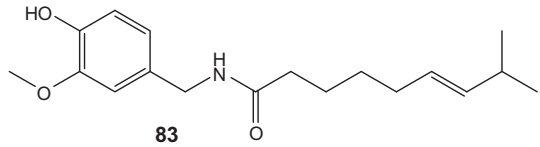
cinnamaldehyde (**79**), has an inhibitory effect on target protein LuxR in *V. harvey* [114]. Some semisynthetic derivatives such as 2-nitrocinnamaldehyde have the same effect (**80**). The authors also showed that these compounds affect the production of EPS and thereby suppress biofilm formation.



Similarly, vanilla, a spice popular for its distinctive aroma, can inhibit bacterial QS [115]. It has long been known that aldehyde vanillin (**81**), the primary flavoring component of the vanilla, inhibits many bacterial strains [116]. Later, it was found that vanillin is an important inhibitor of C4-HSL and 3-Oxo-C8-HSL in *Aeromonas hydrophila*. A significant effect of vanillin in suppression of the bacterial biofilm was also confirmed [117]. Kannan *et al*. [118] found that the interaction efficiency of vanillin with AHLs is dependent on the molecular structure of AHL, mainly on the length of the side chain. Weak antibiofilm activity has been also found in peppermint (*Mentha piperita*) extract against biofilms of *P. aeruginosa* and *C. albicans* [119]. Turmeric (*Curcuma longa*), commonly used as spice, contains the active substance curcumin (**82**). Rudrappa and Bais [120] found that this substance downregulates genes associated among others with the QS and biofilm initiation in *P. aeruginosa* strain PAO1.

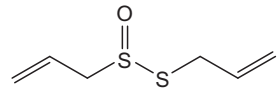


Capsaicin (**83**) (8-methyl-*N*-vanilly-6-nonenamide), the natural product from chili pepper responsible for the "hotness" of the pepper, also effectively prevents bacterial attachment and biofilm formation [121,122].



The prophylactic role of garlic (*Allium sativum*) was evaluated as an alternative to conventional antibiotics in the treatment of infections caused by biofilm-forming bacteria [123]. Garlic contains a large amount of highly biologically active compounds. Persson *et al*. [124] found six sulfur compounds in a garlic extract, which were not toxic to bacteria, but had high activity

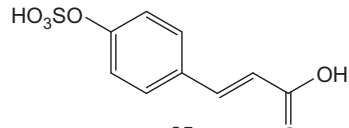
toward the LuxR QS system. Interestingly, these inhibitors were structurally different from the AHLs. Allicin (**84**), applied at subinhibitory concentration, is involved in specific enzymatic inhibition of polysaccharide intracellular adhesin (PIA) synthesis. Suppression of PIA production, the main substance in *S. epidermidis* agglutination, leads to prevention of biofilm formation by this pathogen [125].



84

Simultaneous application of the garlic extract and the antibiotic tobramicin enable effective destruction of *P. aeruginosa* biofilm [126,127].

Eelgrass *Zostera marina* produces another sulfur compound, zosteric acid (**85**), which was studied in the light of its ability to act as a nontoxic antifoulant [121,122,128].

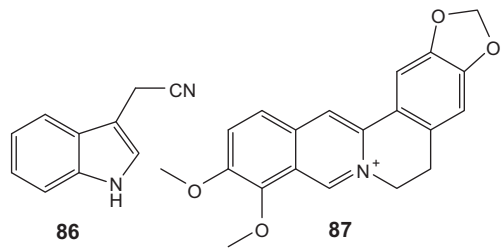


85

Nitrogenous natural substances produced by plants did not remain ignored in the context of microbial adhesion, either.

Auxin 3-indolylacetonitrile (**86**) produced by cruciferous vegetables is a potent inhibitor of pathogenic *E. coli* O157:H7 biofilm formation. As mentioned above, *E. coli* produces in the stationary growth phase indole, which suppresses biofilm formation. The 3-indolylacetonitrile deregulates synthesis of the indole and thus increases its concentration [129].

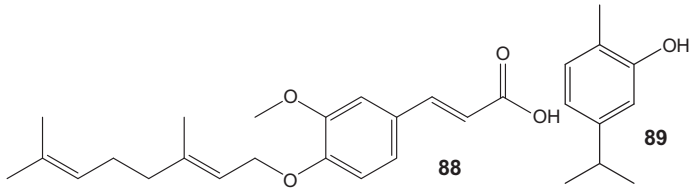
Herbal isoquinoline-type alkaloid, berberine (**87**), has a number of medically usable properties. Very important is the finding of Wang *et al.* [130] that subinhibitory concentration of berberine can effectively block biofilm formation of *S. epidermidis*.



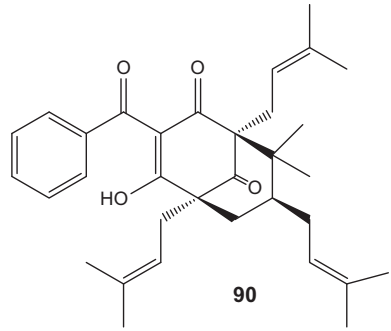
86 **87**

Plants produce a variety of substituted terpene compounds. Also this group includes compounds with significant antibiofilm activity. The bark of *Acronychia baueri* Schott (an Australian small tree belonging to the family of

Rutaceae) contains 3-(4′-geranyloxy-3′-methoxyphenyl)-2-*trans* propenoic acid (**88**) in which geranyl chain is attached to phenolic group [131].This compound, as well as some of its synthetic derivatives, effectively inhibits biofilm formation, which was documented on two oral pathogens *Porphyromonas gingivalis* and *S. mutans* [132]. Essential oil of Summer Savory (*Satureja hortensis*) has limited antibiofilm effect in a subinhibitory concentration against *Prevotella nigrescens*, commonly isolated from endodontic infections [133]. *Satureja khuzistanica* Jamzad (Lamiaceae) is an endemic plant widely distributed in the southern parts of Iran. Iranians have been using it as an analgesic and antiseptic. Essential oil from this plant (called "Dentol"), whose main component is similar to *S. hortensis*, carvacrol (**89**), has anti-inflammatory, antinociceptive, antidiabetic, and antioxidant effects [134]. This essential oil also caused changes in the structure of *P. aeruginosa* biofilm, causing lowered levels of jelly appearance. This phenomenon indicates that Dentol could block polysaccharide production [135].

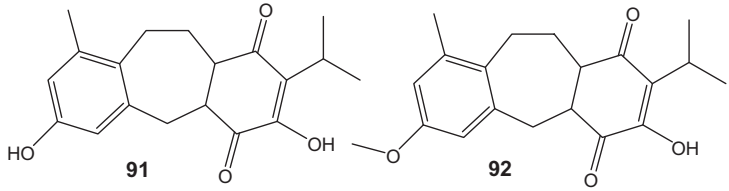


Almeida *et al.* [136] evaluated the antimicrobial activity of extracts obtained from *Rheedia brasiliensis* fruit. They characterized the polyprenylated benzophenone 7-*epi*-clusianone (**90**) as a new agent to control *S. mutans* biofilms.

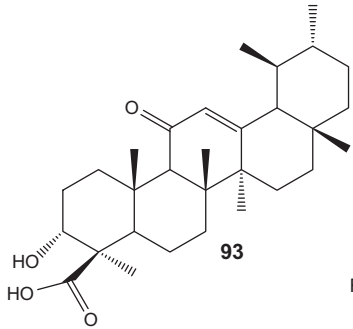


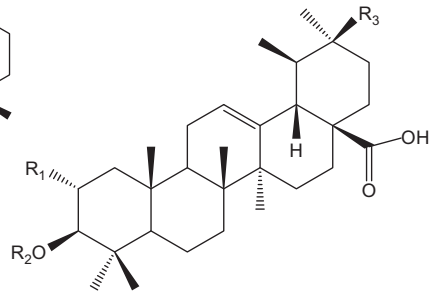
Exudate from the aerial parts of *Salvia corrugata* contains, among others, two diterpenoid compounds, quinones demethylfruticuline A (**91**) and fruticuline A (**92**). Both compounds exhibit different levels of antibiofilm activity on pathogenic bacteria such as *S. aureus*, *S. epidermidis*, and *Enterococcus faecalis*. The authors [137] assume that this effect could depend not only on

inhibition of EPS synthesis but also on their chelating activity and on changes in the microorganism's surface, including cell hydrophobicity.



Boswellia serrata, also known as Indian frankincense, has been used in traditional Ajurvedic medicine for the treatment of inflammatory diseases. The gum resin from this plant contains among others pentacyclic triterpenes called boswellic acids [138]. The 11-keto-β-boswellic acid (**93**) is a biologically very active substance, which can also inhibit formation and cause eradication of biofilm of pathogens such as *S. aureus* or *S. epidermis* [139].





	R_1	R_2	R_3
94	H	*cis-p*-coumaroyl	OH
95	OH	*trans-p*-coumaroyl	H

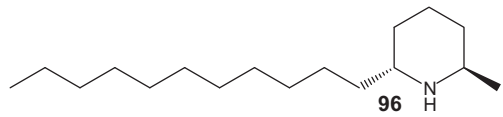
This genus *Diospyros* is known to yield triterpenes. Ursene triterpenes, for example, 3β-*O*-*cis*-*p*-coumaroyl-20β-hydroxy-12-ursen-28-oic acid (**94**) and 2α-hydroxy-3β-*O*-*trans*-*p*-coumaroyl-12-ursen-28-oic acid—jacoumaric acid (**95**) from *D. dendo* inhibited the formation of bacterial biofilm *P. aeruginosa* PAO1 [140].

Extracts from various plants are the subject of growing interest to find compounds with anti-QS or antibiofilm activity [141,142]. Adonizio *et al.* [143] studied the anti-QS activity of aqueous extracts of several terrestrial plants from Florida. Suppression of QS in *P. aeruginosa* PAO1 by extracts of *Callistemon viminalis* (Myrtaceae), *Quercus virginiana* (Fagaceae), and *Tetrazygia bicolor* (Melastomataceae) resulted in more than 80% reduction in biofilm formation.

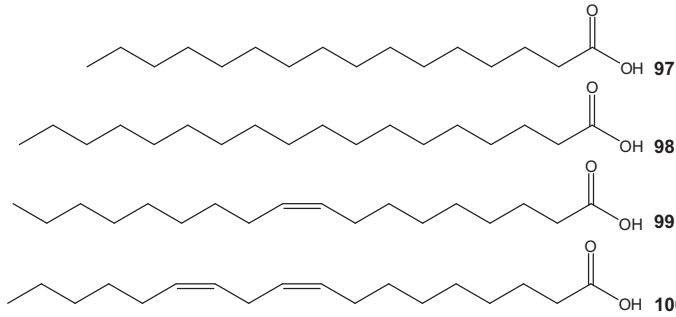
ANIMALS

Animal kingdom does not belong to the priority groups synthesizing natural products. Still, compounds are described that have the ability to influence microbial QS.

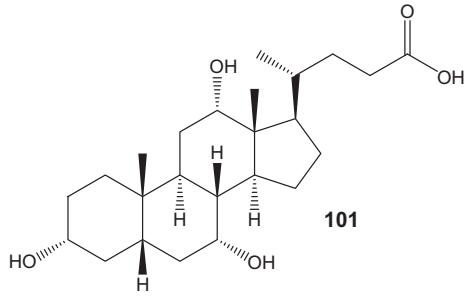
Solenopsin A (**96**), a venom alkaloid from the fire ant *Solenopsis invicta*, efficiently disrupted *P. aeruginosa* QS signaling [144]. Biofilm formation in *P. aeruginosa* was gradually reduced in the presence of solenopsin A in a dose-dependent manner.



Ground beef extract contains several fatty acids such as palmitic acid (**97**), stearic acid (**98**), oleic acid (**99**), and linoleic acid (**100**) that were able to inhibit AI-2 activity. A mixture of these fatty acids, prepared at concentrations equivalent to those present in the ground beef extract, negatively influenced *E. coli* K-12 biofilm formation. Similarly, fatty acids isolated from poultry meat added in the appropriate ratio had concentration-dependent effect on the AI-2 inhibition of *V. harvey* BB 170 [145].

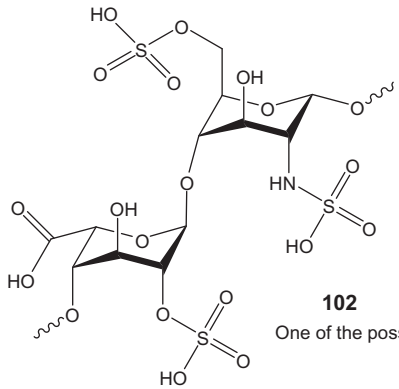


Bile acids (cholic acid (**101**), etc.) promote *V. cholerae* biofilm formation. The reason is probably an effort to reduce bacteria toxicity of this substance, which is quite significant against planktonic cells. Bile acid induction of biofilms was found to be dependent on the *vps* genes, which are responsible for the synthesis of EPS [146].



101

S. aureus is a versatile human and animal pathogen, commonly associated with catheter-related bloodstream infections. Biofilm formation by several *S. aureus* strains is stimulated by heparin (**102**), highly sulfated glycosaminoglycan. The exact mechanism of its effect on *S. aureus* adhesion is unknown [147].



102

One of the possible disaccharide units

Traditional Chinese medicine extensively uses extracts from *Nidus Vespae* (the honeycomb of *Polistes olivaceous*, *P. japonicus*, and others). The chloroform/methanol fraction of these materials showed the highest antibiofilm activities against *S. mutans*, the principal etiological agent of dental caries [148].

Application of honey in traditional medicine is also widely known. Alnaqdy *et al*. [149] studied its effect on adhesion of *Salmonella interitidis* to isolated intestinal epithelial cells *in vitro*. Bacteria pretreated with dilute solutions of honey up to a ratio of 1:8 had a significantly reduced ability of adhesion to epithelial cells. Chestnut honey and especially its aqueous extract have the ability to both degrade AHLs and inhibit the AHLs production of a number of bacterial strains. The result of such exposure was a significant suppression of biofilm formation by *Erwinia carotovora*, *Yersinia enterocolitica*, and *Aeromonas hydrophyla* [150].

CONCLUSION

The ability to control biofilm growth is becoming increasingly important for several reasons. First, biofilms cause considerable economic losses associated with, for example, biofouling; second, they give rise to health problems caused by increasing drug resistance of populations colonizing the tissue, implants, and medical instruments. On the other hand, biofilms are increasingly applied as biocatalysts in biotechnological processes, where their stability is one of the key factors. From the previous text, it is evident that natural compounds are very promising tools for coping with these situations. Substances, whose mechanism of action on the biofilm formation was identified, form a basis for creation of modified structures with higher activity or selectivity. It is also clear that the ongoing screening of natural materials brings many other structures which are able to interact with the regulatory mechanisms involved in adhesion of microbial cells and biofilm formation. New drugs based on a combination of substances with antibiofilm and antibiotic activity, paints, or covering films containing antiadhesion natural substances are just some examples of the expected practical applications.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AHL	N-Acyl homoserine lactone
AI-2	autoinducer 2
c-di-GMP	cyclic di-guanosine monophosphate
DLVO	Derjaguin, Landau, Verwey and Overbeek theory
DNA	deoxyribonucleic acid
EGCG	epigallocatechin-3-gallate
EPS	extracellular polymeric substances
G−	Gram negative bacteria
G+	Gram positive bacteria
HSL	homoserine lacton
m-RNA	messenger ribonucleic acid
OHL	N-octanoyl-DL-homoserine lactone
PIA	polysaccharide intracellular adhesin
QS	quorum-sensing
r-EPS	released exopolysaccharides
TRQ	TanReQuin

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