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Quorum Sensing

A Novel Target for the Treatment of Biofilm Infections

Morten Hentzer,[1] *Leo Eberl*,[2] *John Nielsen*[3] and *Michael Givskov*[1]

1 Molecular Microbiology, BioCentrum-DTU, Technical University of Denmark, Lyngby, Denmark
2 Department of Microbiology, Technical University Munich, Freising, Germany
3 Department of Chemistry, The Royal Veterinary and Agricultural University, Frederiksberg C, Denmark

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Abstract

Present-day treatment of chronic infections is based on compounds that aim to kill or inhibit growth of bacteria. Two problems are recognised to be intrinsically associated with this approach: (i) the frequently observed development of resistance to antimicrobial compounds; and (ii) the fact that all therapeutics are considerably less effective on bacteria growing as biofilms when compared with planktonic cells. The latter point is of particular importance as evidence has accumulated over the past few years that most chronic bacterial infections involve biofilms. The discovery of bacterial communication systems (quorum sensing systems) in Gram-negative bacteria which are believed to orchestrate important temporal events during the infectious process, including the production of virulence factors and the formation of biofilms, has afforded a novel opportunity to control the activity of infecting bacteria by other means than interfering with growth. Compounds that interfere with communication systems are present in nature. Such compounds should not only specifically attenuate the production of virulence factors but should also affect biofilm formation in a manner that is unlikely to pose a selective pressure for the development of resistant mutants.

One of the greatest achievements of modern medicine has been the development of pharmaceuticals for the treatment of various infectious diseases. Alexander Fleming discovered the first antibiotic, penicillin in 1928, and after half a century of intense antibacterial research, most acute bacterial infections can be treated effectively. Conventional antibiotics are designed to have bactericidal or static properties. In most cases, the ability of bacteria to develop resistance to such compounds lies in mutations. In brief, this can cause modification of the antibiotic target site by a single amino acid change or can result in an increased activity of enzymes capable of degrading the antibacterial compound. Such enzymes may in the first place be encoded on mobile genetic elements. Alternatively, cells may exhibit a decreased permeability or increase the activities of multiple efflux pumps that efficient-

ly reduce the internal concentration of the antibacterials. As a consequence, the frequent occurrence of resistant pathogenic strains is gradually rendering traditional antimicrobial treatment increasingly ineffective.

Most antibacterial research and development in academia and in industry has assessed drug efficiency on planktonic cells in pure cultures, basically complying with the procedures laid out by Robert Koch more than a century ago. Within the last decade it has become clear that in nature bacteria predominantly exist as sessile, surface-associated communities, commonly referred to as biofilms (for recent reviews[1-3]). In clinical microbiology, the biofilm mode of bacterial growth has attracted particular attention as many persistent and chronic bacterial infections, including periodontitis, otitis media, biliary tract infection and endocarditis are now believed to be intrinsically linked to the formation of biofilms.[4] Another well recognised medical problem involving biofilms is the colonisation of medical implants by pathogenic bacteria.[4] Bacterial biofilm infections are particularly problematic as sessile bacteria can withstand host immune responses better than planktonic bacteria and are drastically more tolerant to antibiotics and biocides than cells grown in suspension.[5,6]

In conclusion, the increasing problems associated with the use of traditional antibiotics calls for the development of novel therapeutic approaches to the treatment of bacterial infections. The most obvious alternative to antibiotic-mediated killing or growth inhibition would be to attenuate bacterial virulence such that the organism fails to establish a successful infection. This might in turn suffice to reverse the delicate balance in favour of the host clearance mechanism and thereby reverse the severity of infection. Key regulatory systems controlling bacterial virulence and colonisation are obvious prime targets for the development of such new therapeutics, which might be termed antipathogenic drugs as opposed to antibacterial drugs (i.e. traditional antibiotics).

1. Microbial Biofilms and Chronic Infections

According to the prevailing conceptual model, bacterial biofilms consist of microcolonies as the basic unit (figure 1). In the initial stages of biofilm development, bacteria attach to a surface, aggregate to each other and then proliferate to form microcolonies. These microcolonies are hydrated structures in which bacterial cells are enmeshed in a matrix of self-produced exopolymeric substances (EPS). However, as substrate availability becomes limiting as a result of increased diffusion distances, growth will decrease and biofilm development will reach a steady state. Such mature biofilms typically consist of 'towers' and 'mushrooms' of cells enmeshed in EPS, which are separated by channels and interstitial voids to allow convective flow to transport nutrients to interior parts of the biofilm and remove waste products. The biofilm mode of growth might be considered a predestined survival strategy for bacteria in hostile environments. It allows bacteria to withstand a variety of environmental stresses, including the

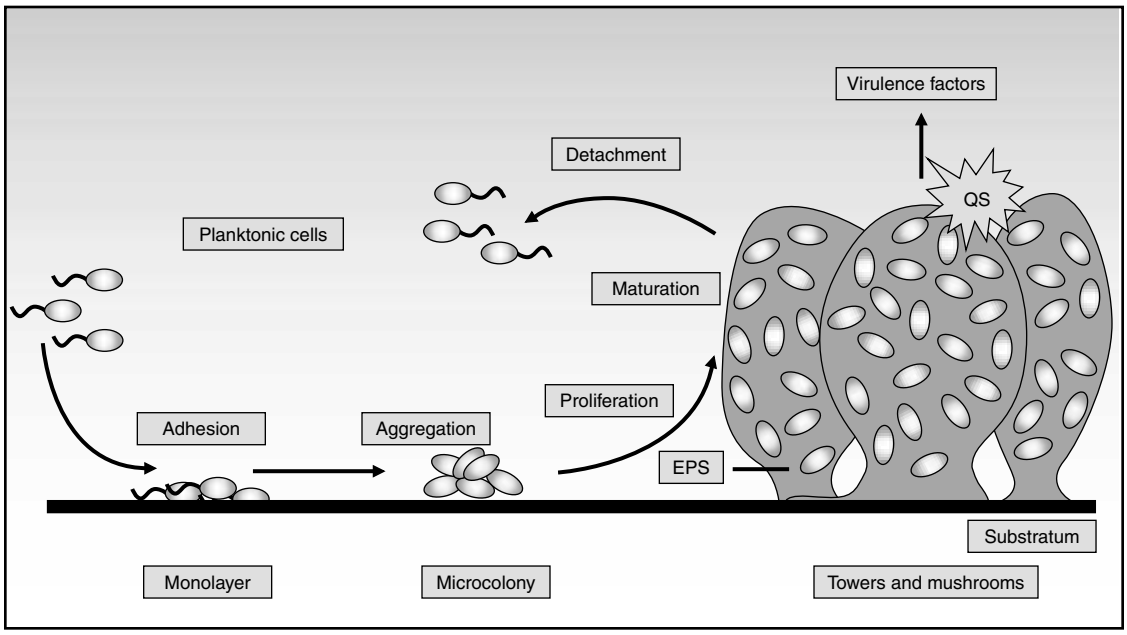

Fig. 1. Temporal biofilm development. **EPS** = exopolymeric substances; **QS** = quorum sensing.

lethal action of antibiotics, disinfectants and the action of the immune system.[7] In general, biofilm cells are 100- to 1000-fold more tolerant to antibiotic treatment than growing, planktonic bacteria. Several factors contribute to the enhanced resistance of biofilms. For instance, the EPS matrix surrounding biofilm bacteria can act as a diffusion barrier and retard the penetration of some (not all) antibacterials into the biofilm. Furthermore, biofilms are composed of bacteria in a multitude of physiological states due to nutrient and oxygen gradients in the biofilm. Consequently, a fraction of the biofilm population will be in a 'dormant state' and less sensitive to most antibacterials that target processes involved in growth or protein synthesis.[7] Also, Lewis[6] has suggested the existence of so-called persister cells in all types of bacterial cultures. Persisters are the small fraction of cells that are never killed by a lethal, antimicrobial treatment, and biofilms might be particularly rich in those.[6]

Biofilms have become evident in many environmental, industrial and medical problem areas, in particular chronic infections. A recent public announcement from the US National Institutes of Health stated that more than 60% of all microbial infections involve biofilms.[6] The best investigated example of a disease in which biofilms are thought to play a prominent role is the occurrence of chronic lung infections by the opportunistic bacterial pathogen *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF), the most common lethal, inherited disease among the Caucasian population.[8-10] During chronic infection, *P. aeruginosa* produces copious amounts of alginate, which forms a matrix completely embedding the bacterial cells and becomes highly resistant to antibacterial treatment. These observations led to the suggestion that *P. aeruginosa* exists as a biofilm in the lungs of patients with CF.[4,11] Recently, a study has demonstrated that *P. aeruginosa* biofilms in the bronchi of patients with CF establish in hypoxic mucus and respond to the anaerobic microenvironment by increasing alginate production.[12] This new understanding raises concern as many front-line antibacterials used in the treatment of CF are either ineffective or significantly reduced in efficacy under anaerobic conditions.[13] This hypothesis was recently corroborated through profiling of signal molecules involved in quorum sensing (QS).[14]

2. Quorum Sensing (QS)

QS is a generic regulatory mechanism used by many Gram-negative bacteria to sense and respond to microbial population density by expressing specific sets of genes. The QS system consists in most cases of a four-component circuit: a LuxI-type signal synthase, a N-acylhomoserine lactone (AHL) signal molecule, a LuxR-type signal receptor and the target gene(s). The AHL signal is synthesised constitutively at a low basal level by the AHL synthase. AHL signals diffuse out of the bacteria and into the surrounding environment. An increased bacterial population density leads to an increased local AHL concentration, and at a threshold concentration the signal interacts with a cognate receptor (LuxR-type response regulator) that in turn becomes active as a positive transcription factor to modulate expression of QS-regulated genes. Occasionally, the quorum sensor is subject to autoinduction because the gene encoding the signal synthase is among the target genes; hence, a positive feedback regulatory loop is created. This positive feedback regulation allows a rapid increase in signal production and dissemination, which in turn induces QS-controlled phenotypes throughout the entire bacterial population.

One environment that contains a large number of bacteria in close proximity is a bacterial biofilm. The dense and diffusion-limited biofilm matrix provides ideal conditions for the accumulation of signal molecules, and a protected environment for bacteria to induce QS-regulated virulence factor production and launch an attack on the host. Recent research by Dr E.P. Greenberg and colleagues, as well as our laboratory, have pointed out that the expression of QS genes forms a continuum throughout the growth cycle with some genes being induced during the exponential phase of growth (immediate responders) whilst others only are induced in the early stationary phase (late responders).[15-17] All in all, QS is thought to afford pathogenic bacteria with a mechanism to minimise host immune responses. It achieves this by delaying the production of tissue-damaging virulence factors until sufficient bacteria have been amassed to overwhelm host defence mechanisms and to establish a successful infection.

Recent findings suggest that QS also regulates biofilm formation and surface motility in the opportunistic pathogens *Serratia liquefaciens*, *P. aeruginosa*, *Burkholderia cepacia* and *Aeromonas hydrophila* (unpublished data).[18-21] These observations suggest that QS provides a regulatory link between surface motility, biofilm formation and production of virulence factors. Furthermore, other studies have demonstrated that QS is also required for biofilms to withstand sheer force (sloughing), antibiotics and sodium dodecyl sulphate treatment.[15,18,22]

In our laboratory we also view QS as a mechanism by which bacteria expose part of their genetic repertoire to other organisms

(prokaryotes as well as eukaryotes), a phenomenon referred to as cross-talk. AHL-mediated QS systems are found in more than 50 Gram-negative bacterial species.[23] Given that many bacteria colonising the same ecological niche utilise a similar chemical language it appears likely that AHL signal molecules are not only used as population density sensors of one species but also for communication between bacteria of different species. In fact, bacterial cross-talk has been demonstrated between *P. aeruginosa* and *B. cepacia*[24] and between *S. liquefaciens* and *P. aeruginosa.*[25]

3. QS Systems as Targets for Antibacterial Drugs

The role of QS systems as central regulators of virulence and biofilm formation in many pathogenic bacteria make QS systems highly attractive targets for the development of novel therapeutics.[26,27] Disruption of the cell-to-cell signalling cascade may be a particularly valuable strategy for the treatment of chronic biofilm infections. In this respect, it has to be emphasised that the rationale behind this approach is to specifically interfere with the expression of pathogenic traits rather than to impede growth of the bacteria. However, as the production of virulence factors is inhibited, the pathogens can no longer adapt to the host environment and will consequently be cleared by the innate host defences. Therefore, the major advantage of this novel strategy for anti-infective therapy is that it circumvents the problem of resistance, which is intimately connected to the use of conventional antibacterial agents. Hence, QS inhibitory (QSI) compounds show great promise as a novel class of antimicrobial agents with applications in many fields, including medicine (human and veterinary), agriculture and aquaculture. Indeed, in the recent year a number of novel biotech companies, which specifically aim at developing anti-QS and anti-biofilm drugs, have emerged. No such drugs have yet been approved for release, but several drugs are claimed to be in late-phase clinical trials.

4. Inhibition of QS

Disruption of QS systems may be accomplished in several ways: (i) blockade of AHL signal synthesis; (ii) AHL signal molecule degradation; and (iii) inhibition of AHL signal reception. Recent work has shown that LuxI-type proteins catalyse the formation of AHLs from the appropriately charged acyl carrier protein that acts as the major acyl chain donor and *S*-adenosyl methionine, which provides the homoserine lactone moiety. Although it is conceivable that AHL biosynthesis could be effectively obstructed by the blockade of AHL synthases, no specific inhibitors for this class of enzymes have yet been derived. In a recent study, Dong et al.[28] isolated an enzyme, named AiiA, from *Bacillus* spp. that inactivates AHLs by hydrolysing the lactone bond of the molecules. It was shown that transgenic plants expressing AiiA exhibited enhanced resistance to *Erwinia carotovora* infections.[28] Since this bacterium employs an AHL-dependent QS system to control expression of plant pathogenic traits, the attenuated virulence is likely to be a direct consequence of signal degradation. However, whether AiiA or a related enzyme is applicable for the treatment of human infections remains to be seen.

At present, the most promising strategy for the successful disruption of QS appears to be the blocking of the perception of the signal molecule by its cognate receptor protein. Interference of QS signal transduction can be achieved by a molecule capable of antagonising binding of the native AHL signal to the LuxR-type receptor (see figure 2 for AHL structures). Competitive inhibitors are likely to be structurally related to the native AHL signal, in order to bind to and occupy the AHL binding-site, but will fail to activate the LuxR-type receptor. Noncompetitive inhibitors may show little or no structural similarity to AHL signals as these molecules are thought to bind to different sites on the receptor protein.

Several studies have been performed to demonstrate the feasibility of AHL-analogues to inhibit QS circuits of various bacteria in *in vitro* experimental scenarios. These studies have generated substantial knowledge about structure-function relationships of AHL signals, which is of great value for the continued search for potent QS inhibitors. Based on competitive inhibition studies,[29] it appears that a tight structure-function relationship exists and thus only AHL analogues with rather conservative changes are capable of binding to the receptor and functioning as agonists of the cognate AHL signals.

From studies in which the acyl side chains of analogues were modified in several ways it was concluded that the length of the acyl side chain plays a critical role for activity. For *E. carotovora* it has been reported that increasing the acyl side chain by one methylene unit reduced activity by 50%, whereas a two units' extension reduced activity by 90%. Decreasing the chain length by one methylene unit decreased activity by 10%.[30] Interestingly, long-chain AHLs appear to be better inhibitors of QS systems that utilise short-chain AHLs as the native signal, than vice versa. This observation might suggest that a minimum acyl side chain length

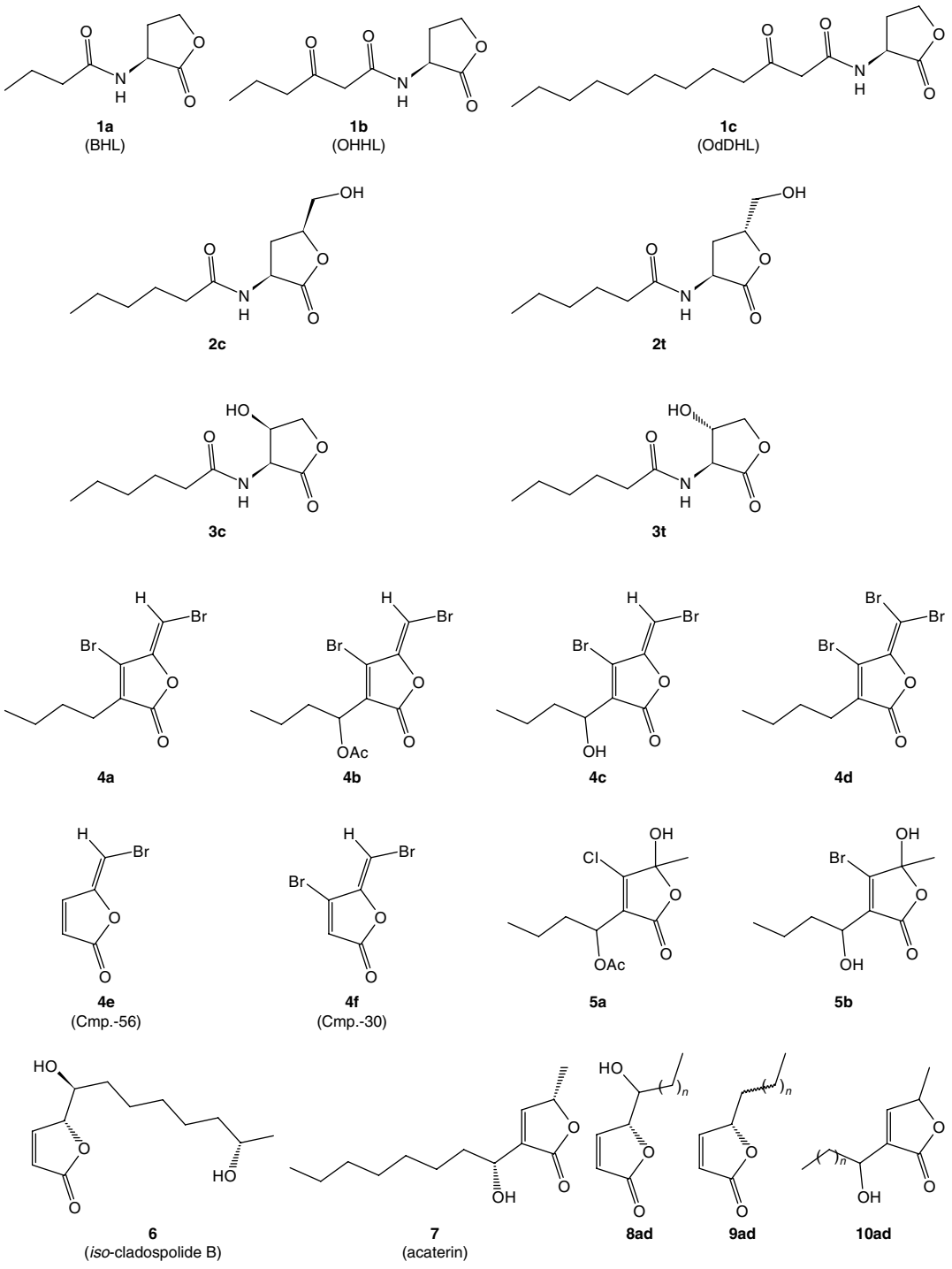

Fig. 2. Molecular structures of N-acylhomoserine lactones (AHLs), **1a**, BHL ([N-buturyl]-L-homoserine lactone), **1b**, OHHL (N-[3-oxo-hexanoyl]-L-homoserine lactone) and **1c**, OdDHL (N-[3-oxo-dodecanoyl]-L-homoserine lactone) and selected quorum sensing (QS) inhibitors. BHL and OdDHL are the cognate signal molecules of the *Pseudomanas aeruginosa* Rhl and Las QS systems, respectively. OHHL is the cognate signal of *Vibrio fischeri*. **2c** and **2t** are cis- and trans-5-hydroxymethyl analogues of HHL (N-hexanoyl-L-homoserine lactone), respectively. Similarly, **3c** and **3t** are cis- and trans-4-hydroxy analogues of HHL. **4ad** and **5ab** are natural furanone compounds isolated from *Delisea pulchra*. Structures **4ef** are synthetic analogues (frequently termed 'Cmp.-56' and 'Cmp.-30'). **6** and **7** are the natural product template structures *iso*-cladospolide B (**6**) and acaterin (**7**). **8ad** and **9ad** are synthetic analogues of *iso*-cladospolide B and **10ad** are analogues of acaterin (n = 0, 2, 4 or 6 for all analogues).

determined by the native AHL signal is required for binding to LuxR-homologues and longer acyl chains can apparently be accommodated in the AHL binding site of LuxR-type receptors. The flexibility of the acyl side chain also appears to be important for binding to LuxR-type proteins. For instance, reduction of the chain rotation by the introduction of an unsaturated bond close to the amide linkage almost completely abolished binding to the receptor.[29-32] In support of this hypothesis, no natural AHL signal has so far been reported to contain an α-, β-unsaturated bond. A study on the *P. aeruginosa* LasR receptor suggested that the fully extended chain geometry is necessary for activation, whereas constrained analogues locked into different conformations showed no activity.[33] The substitution at the β-position (3 position) is important for the agonistic activity of AHLs, but there is no clear rule to the importance of this substitution to the antagonistic activity.

The homoserine lactone moiety of AHL signals is generally very sensitive to modifications. The chirality is crucial to biological activity. Natural AHL signals are L-isomers whereas D-isomers generally are devoid of biological activity[30,34,35] (figure 2). Importantly, L-isomer activities were not inhibited by D-isomers, therefore suggesting that the D-isomers do not bind to the LuxR-type receptor.[34] The homoserine lactone moiety also appears to be very sensitive to changes in composition and ring size. Conversion of the homoserine lactone ring to a homoserine lactame ring results in a molecule without agonistic or antagonistic properties.[30,31] Interestingly, change to a homoserine thiolactone ring appears permissible for several QS systems.[29-31,34,35]

In two recent studies from our research groups, the biological impact of chain length variation and hydroxylation of hexanoyl homoserine lactone (HHL) has been investigated[36] (figure 2). Inspired by the halofuranone structure from *Delisea pulchra* (discussed in detail in section 6), a small combinatorial library of 4-hydroxy and 5-hydroxymethyl derivatives of HHL and their corresponding ethylcarbamylates were synthesised. Interestingly, only the molecules carrying the free hydroxyl groups proved active. Thus, both the cis-4-hydroxy and the trans-4-hydroxy HHL analogues were able to antagonise a LuxR-based QS system. Indeed, the cis-4-hydroxy homoserine lactone (figure 2, 3c) was a more potent activator than HHL. Among the HHL derivates tested, only the cis-5-hydroxymethyl homoserine lactones were able to antagonise the LuxR-type receptor.

5. QS Inhibitors from Natural Sources

As AHL-mediated QS is utilised by a large number of bacteria it may not be too surprising that higher organisms are capable of perceiving and responding to these molecules. Recently, Joint et al.[37] demonstrated that motile zoospores of the green seaweed *Enteromorpha* was attracted by AHL molecules to settle on bacterial biofilms of *Vibrio anguillarum.* The best investigated example of an eukaryotic organism, which produces metabolites that specifically interfere with bacterial signalling, is the Australian macroalga *D. pulchra* and this example will be described in better detail in the following section. Recently, another example was provided by Teplitski et al.[38] who showed that several plants secrete substances that mimic bacterial AHLs and affect QS regulated behaviours in respective plant-associated bacteria. Exudates from pea (*Pisum sativum*) were demonstrated to contain several separable activities that mimicked bacterial AHL signals. While some of these activities stimulated AHL-dependent phenotypes, others inhibited expression of AHL-regulated traits.[38] Many plants and fungi have coevolved and established carefully regulated symbiotic associations with bacteria. Interestingly, the percentage of AHL producers is particularly high among plant-associated bacteria[39,40] and recent results have provided evidence that AHL signal molecules may serve as a universal language for communication between the different bacterial populations of the rhizosphere consortium.[41,42] A recent study showed that the legume *Medicago truncatula* can detect very small concentrations of bacterial AHL molecules and mount a specific response involving over 150 proteins. Interestingly, the response included changes in the secretion of AHL-mimicking molecules that have the potential to disrupt bacterial QS.[43]

It is worthwhile noting that both plants and fungi are devoid of active immune systems, as seen in mammals, but rather rely on chemical defence systems to deal with bacteria in the environment. For these reasons, it might be expected that plants and fungi utilise chemical compounds to inhibit (or in other cases, to stimulate) bacterial AHL-mediated communication. We are currently surveying various plants (including traditional herbal medicine) and fungal extracts for QSI activity. Preliminary results indicate that a surprisingly large number produces compounds with QSI activity. Not surprisingly, we found AHL-producing bacteria (which secrete hydrolytic exoenzymes) associated with these plants and their roots. On the basis of these data, we hypothesize that the interplay of signals and signal inhibitors enables a stable coexis-

tence of the eukaryotic host and its pathogen, as long as the plant produces sufficient amounts of inhibitor to intefere with the QS systems of the potential pathogen. Work is currently under progress to characterise and isolate the pure compounds responsible for the QSI activity.

6. Halogenated Furanone Compounds Are Potent QS Inhibitors

The Australian red macroalga *D. pulchra* produces a range of halogenated furanone compounds,[44] which display antifouling and antimicrobial properties[45-47] (figure 2). This particular alga originally attracted the attention of marine biologists because the alga was devoid of surface colonisation, i.e. biofouling, unlike other plants in the same environment. Biofouling is primarily caused by marine invertebrates and plants, but bacterial biofilms are believed to be important for providing an initial conditioning stratum to which other marine organisms may attach.[48]

The *D. pulchra* furanone compounds consist in general of a furan ring structure with an alkyl chain at the C-3 position and a halogen, often bromine, at the C-4 position. The substitution at the C-5 position may vary but the structures frequently possess an exocyclic brominated double bond. The natural furanones are halogenated at various positions by bromine, iodide or chloride.[44] *D. pulchra* produces at least 30 different species of halogenated furanone compounds that are stored in specialised vesicles and are released at the surface of the thallus at a concentration ranging from 1–100 ng/cm^2.[48] Field experiments have demonstrated that the surface concentration of furanones is inversely correlated to the degree of colonisation by marine bacteria.[49]

Givskov et al.[50] hypothesised that furanones of *D. pulchra* constitute a specific mean of eukaryotic interference with bacterial signalling processes. Extensive experimental evidence in support of this model has accumulated during the last couple of years. This includes the observations that furanones repress AHL-dependent expression of *V. fischeri* bioluminescence,[49] displace radiolabelled signal molecules from LuxR,[51] inhibit AHL-controlled biofilm development and virulence factor production,[15,22] surface motility and colonisation of *S. liquefaciens*,[25,50] accelerate the degradation of the LuxR receptor,[52] inhibit QS controlled luminescence and *in vivo* virulence of the black tiger prawn pathogen *V. harveyi*[53] and finally carbapenem antibiotic synthesis and exoenzyme virulence factor production in *E. carotovora*.[54] In addition, work in progress has identified a number of food-relevant bacteria[55] that employ QS to control the process of food deterioration. Our recent results suggest that these functions can also be controlled by furanone compounds (our unpublished data).

The natural furanone compounds have little or no effect on the QS systems of *P. aeruginosa*. These natural compounds were modified by means of chemistry and screened for increased efficacy as described by Manefield et al.[52] In a recent study, we have investigated the biological consequences of the chain length and substitution pattern of furanones (butenolides).[56] To clarify the biological consequences of 5-hydroxymethyl alkylated structures, iso-cladospolide B served as our target structure (figure 2). The natural product iso-cladospolide B is a fungal metabolite possessing, among others, antimicrobial and nerve growth factor enhancing activities. Along these lines, another natural product acaterin, a 3-hydroxyalkylated *Pseudomonas* spp. metabolite and an inhibitor of CoA cholesterol acetyltransferase (ACAT), served as our target structure to investigate the influence of substitutions at the 3-position. From these studies, it was evident that we were able to synthesise new inhibitors of QS. In general, compounds carrying the shorter chain lengths were more active than compounds with longer chain lengths at inhibiting QS. Interestingly, the strongest inhibitor found from these studies originated from the 3-alkylated series (the acaterin series) and was originally identified as an ACAT-inhibitor rather than as an antibacterial agent.

Some of the derivates of the *D. pulchra* furanone compounds, in particular compound C-56 and C-30, were able to inhibit QS in *P. aeruginosa* and reduce virulence factor expression.[15,22] Compared with growing, planktonic cells, biofilm bacteria exhibit an increased tolerance to antibiotic treatment,[4,9,57] and it has been proposed that diffusion barriers and the special physiological condition of the cells may contribute to this phenomenon. Hence, synthetic compounds that function well on planktonic cells might be less efficient on biofilm cells. For that reason the analysis of the efficiency of QSI compounds was extended to include cells in the biofilm mode of growth. This was accomplished by the construction of a novel GFP (green fluorescence protein)-based AHL biosensor in *P. aeruginosa*.[22] This sensor contains a translational fusion of the QS-regulated *lasB* elastase gene of *P. aeruginosa* to GFP together with the constitutively expressed *lasR* gene, which encodes the cognate LuxR-type receptor protein. This construct enabled us to develop a simple fluorescence-based high-throughput assay to determine the efficacies of the various potential QSI compounds. More importantly, however, the use of the GFP-based single cell technology in combination with laser confocal micro-

scopy allowed the analysis of QS and its inhibition in biofilms. Estimates of penetration efficacies and half-lives of the QSI compounds became possible and these investigations led to the identification of synthetic compounds that not only inhibited the quorum sensors in the majority of the cells but also blocked biofilm development, such that only flat, undifferentiated biofilms were formed which eventually sloughed off.[22] A significant finding is that the synthetic furanones, in concentrations that significantly lower QS-controlled gene expression in planktonic cells, were equally active on biofilm bacteria.[22] Classical antibiotics used for the treatment of *P. aeruginosa* infections, such as tobramycin and piperacillin, are required in 100- to 1000-fold higher concentrations to eradicate biofilm bacteria when compared with their growing, planktonic counterparts.[7] Most interestingly, the furanones exhibited synergistic effects with tobramycin, an aminoglycoside antibacterial agent routinely used in the treatment of patients with CF, as this agent eradicated biofilm cells much more efficiently when the biofilm was pretreated with furanones.[15]

Recent technical developments allow for the entire bacterial transcriptome to be overviewed by DNA array technology and offers the unprecedented possibility to precisely identify the cellular targets of a particular drug. We have employed the Affymetrix *P. aeruginosa* GeneChip®[1] to demonstrate that furanone compounds specifically target QS in this organism.[15] Interestingly, we observed that the *snr-1* gene, encoding the shared nitrate reductase, was repressed by the furanone compounds. The *snr-1* gene has previously been shown to be controlled by QS.[17] Snr-1 is a soluble cytochrome c and catalyses the first step in nitrate reduction and therefore determines the rate of denitrification. Hence, furanone drugs might prove to be unique in their ability to work under anaerobic conditions while at the same time inhibiting anaerobic respiration in *P. aeruginosa* biofilm lung infections.

To assess the potential of these furanone compounds in the treatment of *P. aeruginosa* infections we used a mouse pulmonary infection model. Groups of mice infected with *P. aeruginosa* as described by Wu et al.[58] received subcutaneous furanone injections for 3 days and this treatment was found to significantly reduce the bacterial content compared with the placebo group.[15] Furthermore, the efficiency of bacterial clearing was positively correlated to the concentration of the furanone compound. The concentration used was equal to or less than the concentrations required to inhibit expression of virulence factors in planktonic cultures, promote sloughing of *in vitro* biofilms[15,22] and make biofilms grown in the presence of furanone compounds more susceptible to antibiotic treatment. It is therefore likely that furanones, in contrast to classic antibiotics, might comprise a biofilm defence system developed through the course of evolution to penetrate biofilm structures and target and inactivate receptors of bacterial communication systems, which in turn, control virulence factor production, surface colonisation and biofilm formation.

7. Discussion

Results of the past few years have provided clear evidence that AHL-mediated communication plays a major role in many Gram-negative pathogens to coordinate and fine tune the infection process in the host. Blocking communication between the cells may render the aggressors uncoordinated and thus highly prone to host immune defence systems. However, the compounds able to paralyse communication do not affect any vital function of the bacterium and thus will not interfere with its growth. In nature, several eukaryotic organisms have been shown to employ this approach to prevent bacterial colonisation and infection. It is therefore very promising to tap natural resources for the identification of novel anti-infectives that do not *per se* inhibit growth but instead directly interfere with the expression of pathogenic traits.

Knowledge of the molecular details of bacterial communication and its role in the control of virulence, biofilm development and pathogenicity opens up a completely new perspective in the control of microbial activity. The halogenated furanones are reactive molecules and therefore not well suited for the treatment of infections in humans. However, their ability to control *P. aeruginosa* infections in animal models is 'a proof of concept' that demonstrates that QS is in fact a useful and promising novel drug target.[15] Moreover, the furanones may serve as lead compounds for the development of novel pharmaceutically relevant drugs with less adverse effects.

One complication associated with AHL-based QS antagonists in therapy is the fact that long-chain AHL signal molecules function as virulence factors *per se*, as they possess immune modulatory activity[59-61] and affect muscle tissue[62] and tracheal cells.[63] This might, however, prove advantageous if it is possible to develop antagonists that simultaneously inhibit virulence factor expression, as well as reduce the immune response which in turn leads to reduced inflammation.

1 The use of tradenames is for product identification purposes only and does not imply endorsement.

In addition to being an important opportunistic pathogen, *P. aeruginosa* is a highly attractive model organism as a DNA GeneChip® is available from Affymetrix. For the first time scientists can now overview the activity of the entire *P. aeruginosa* chromosome. We have used this technique to demonstrate the target specificity of our first generation anti-infectives.[15] We envision that this approach will be used in many scientific and pharmaceutical laboratories in the future for the identification of drugs as well as their target specificity. Given the large number of bacteria (>50 species) that employ QS systems, chemical attenuation of undesired bacterial activities rather than bacteriocidal or static strategies may find application in many different fields, e.g. medicine, agriculture and food technology. This new concept is highly attractive because it is unlikely to pose a selective pressure for the development of resistance. Furthermore, since the compounds are non-toxic they are not expected to eliminate communities of helpful and beneficial bacteria present in the host (for example, the gut flora). The present approach is generic in nature and highly interesting for a wide range of professionals such as microbiologists and medical doctors working in the field of infectious diseases and artificial implants and for those engineers who are involved in the maintenance of industrial facilities and water pipelines.

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Correspondence and offprints: Dr *Michael Givskov*, Centre for Biomedical Microbiology, BioCentrum-DTU, Bldg. 301, Technical University of Denmark, DK-2800, Denmark.

E-mail: immg@pop.dtu.dk