

Bacterial Signals and Antagonists: The Interaction Between Bacteria and Higher Organisms

Scott A. Rice^{1,2}, Michael Givskov³, Peter Steinberg^{2,4}, and Staffan Kjelleberg^{1,2} *

¹The School of Microbiology and Immunology, The University of New South Wales, Sydney NSW 2052, Australia

²The Centre for Marine Biofouling and Bio-Innovation, The University of New South Wales, Sydney NSW 2052, Australia

³The Department of Microbiology, The Technical University of Denmark, DK-2800 Lynby, Denmark

⁴The School of Biological Sciences, The University of New South Wales, Sydney NSW 2052, Australia

Abstract

It is now well established that bacteria communicate through the secretion and uptake of small diffusible molecules. These chemical cues, or signals, are often used by bacteria to coordinate phenotypic expression and this mechanism of regulation presumably provides them with a competitive advantage in their natural environment. Examples of coordinated behaviors of marine bacteria which are regulated by signals include swarming and exoprotease production, which are important for niche colonisation or nutrient acquisition (e.g. protease breakdown of substrate). While the current focus on bacterial signalling centers on N-Acylated homoserine lactones, the quorum sensing signals of Gram-negative bacteria, these are not the only types of signals used by bacteria. Indeed, there appears to be many other types of signals produced by bacteria and it also appears that a bacterium may use multiple classes of signals for phenotypic regulation. Recent work in the area of marine microbial ecology has led to the observation that some marine eukaryotes secrete their own signals which compete with the bacterial signals and thus inhibit the expression of bacterial signalling phenotypes. This type of molecular mimicry has been well characterised for the interaction of marine prokaryotes with the red alga, *Delisea pulchra*.

Introduction

Bacteria have developed numerous adaptations which help them to sense and respond to their environment; some classic stimuli which have been extensively studied include temperature shifts, nutrient availability, hydrostatic pressure, oxygen, and pH. Importantly, it is this ability to respond or interact with the local environment which determines the overall success or failure of an organism. Microorganisms are also capable of sensing and responding to the presence of other organisms. Clearly it is essential

for an organism to sense the levels of its own population as well as other members of the community so that the organism can take the appropriate action. One mechanism by which bacteria sense and respond to members of the community is through the secretion and uptake of small diffusible chemical cues or signalling molecules. This includes density dependent population monitoring, in which the organism takes a population census, determined by the concentration of signalling molecules present, and when the required cell density, or quorum, is detected, the phenotype is expressed (Fuqua *et al.*, 1996; Fuqua *et al.*, 1994; Swift *et al.*, 1996).

It is becoming increasingly clear that many different microorganisms rely on signal sensing mechanisms, including quorum sensing, to regulate a variety of adaptive phenotypes. Signalling molecules come in a wide range of classes and types from a large variety of bacteria including but not restricted to: amino acids, *Myxococcus xanthus* (Kuspa *et al.*, 1992a; Kuspa *et al.*, 1992b); cAMP, *Dictyostellium discoideum* (Van Haastert, 1991), short peptides, *Enterococcus faecalis* (Clewell and Weaver, 1993); cyclic dipeptides (CDPs), *Pseudomonas aeruginosa* (Chhabra *et al.*, unpublished); butanolides, *Streptomyces* (Yamada *et al.*, 1987); fatty acid derivatives, *Ralstonia solonacerum* (Flavier *et al.*, 1997); acylated homoserine lactones (AHLs) e.g. *V. fischeri*, *V. anguillarum* and *Aeromonas hydrophila* (Eberhard *et al.*, 1981; Milton *et al.*, 1997; Swift *et al.*, 1997), and there are some organisms with as yet uncharacterised signalling molecules, e.g. *V. harveyi*, *V. vulnificus*, and *V. angustum* (Bassler *et al.*, 1994; deNys *et al.*, 1999). Moreover, it should be pointed out that bacteria are not limited to one single signalling type. In the case of *V. harveyi* (see below), there are two signalling systems which interact to regulate bioluminescence and *R. solonacerum* uses 3-hydroxy palmitic acid methyl ester as a density dependent signal to regulate exopolysaccharide production through the regulation of at least three two component regulatory systems (Huang *et al.*, 1998).

As expected, just as the list of signalling systems is wide and varied, so too are the phenotypes which are regulated by those signalling systems. Examples of those phenotypes include: sporulation (butanolides), fruiting body formation (amino acids, cAMP), development of competence (peptides), starvation adaptation (generally uncharacterised), bioluminescence (AHLs), pigment production (AHLs), colonisation phenotypes (AHLs), virulence factor production (AHLs and an unknown factor in *Salmonella typhimurium*), and for some signals, the associated phenotypes are poorly described (CDPs). While most of the sensing systems described above seem to be associated with a single organism or phenotype, two systems, the AHL and CDP systems, appears to be broadly disseminated in a vast number of bacteria. A study by Cha *et al.* (1998) demonstrated that approximately 60% of all soil isolates, including *Agrobacterium*, *Rhizobia*, *Pseudomonas*, *Pantoea*, and *Erwinia* species, produce extracellular molecules which activate AHL bioassays. Marine isolates

*For correspondence. Email s.kjelleberg@unsw.edu.au.

are no exception, as studies have demonstrated that approximately 10% of marine bacteria from the surface of the red alga *Delisea pulchra* produce AHLs (Maximilien *et al.*, 1998; England *et al.* unpublished). We have identified CDPs in supernatants from *P. aeruginosa*, *Proteus mirabilis*, and *Citrobacter freundii* (Chhabra *et al.* unpublished). Furthermore, it has been previously reported that numerous organisms produce CDPs (Prasad, 1995), and in particular, many marine bacteria have been associated with CDP production, including a *Micrococcus sp.* (Stierle *et al.*, 1988) and *Vibrio parahaemolyticus* (Bell *et al.*, 1994).

AHL Mediated Sensing

Besides AHLs being widely prevalent among Gram negative bacteria, the AHL family of quorum sensing systems share some highly conserved features. Some details of AHL sensing systems are included here as background to the subsequent discussion of cross-talk of signals produced from other sources, with a specific focus on signals from the marine environment. AHLs consist of a lactone ring covalently linked to an acylated fatty acid chain through an amide bond (Figure 1A). The various AHLs produced differ in the length and the degree of saturation of the acyl chain. *Vibrio fischeri* serves as the paradigm for AHL mediated quorum sensing. Central to the AHL family of quorum sensing genes are a pair of genes, *luxI* and *luxR* and their homologues, which respectively code for the AHL synthase, LuxI, and the membrane bound AHL receptor, LuxR, which also acts

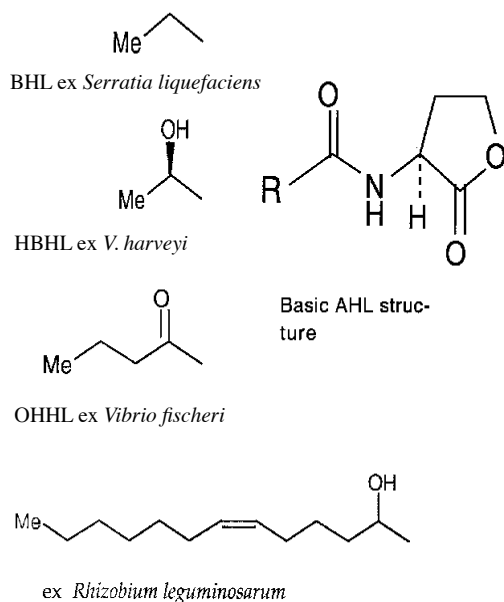
as a transcription factor. In essence, *luxI* is transcribed at a low-basal rate, which allows for a constant low rate of production of the AHL signal. As the population density increases, the AHL concentration increases until a critical threshold concentration is reached, usually during late log or early stationary phase, at which time, there is sufficient AHL present to bind to LuxR. The AHL-LuxR complex is then capable of stimulating transcription of *luxI* which in turn leads to even more AHL production, thus forming a positive feedback or autoinduction circuit. This autoinduction circuit is the key to quorum sensing as it allows for a rapid increase in signal production and dissemination and therefore leads to a rapid induction of the phenotype by all members of the population. In addition to stimulating transcription of the *luxI*, the AHL-LuxR complex also stimulates transcription of the quorum sensing phenotypic genes.

The interaction of the AHL with the cognate receptor shows a range of specificity and current data indicate that there is a structure-function relationship for cross reaction of AHLs with the receptors. For *V. fischeri* the interaction is very specific, the LuxR protein optimally responds to the cognate AHL, OHHL. In contrast, the LuxR homologues in *Chromobacterium violaceum* and *Agrobacterium tumefaciens* can bind to a number of different AHLs in addition to their own native signals. This property also makes these strains useful as bioassays for the detection of signals produced by other organisms (McClellan *et al.*, 1997a; 1997b; Shaw *et al.*, 1997).

However, there are exceptions to all models, and AHL quorum sensing is no exception. Bioluminescence is also regulated by a quorum sensing mechanism in a closely

A

R Groups of the Four Classes of AHLs



B

Four furanones

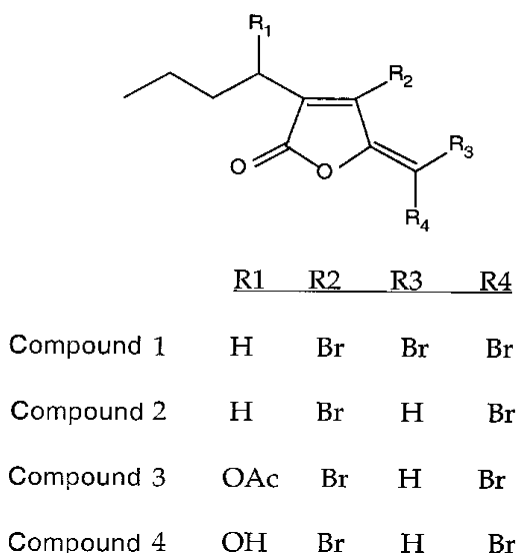


Figure 1. The Structures and Molecular Comparison of Bacterial Signals (AHLs) and Eukaryotic Signal Antagonists (Furanones)

A. AHLs can be grouped into four classes based on the substitutions to the acyl chain. Group 1, represented by BHL, have no substitutions. Group 2, represented by HBHL, have a hydroxy group at the 3' position. Group 3, represented by OHHL, have an ester linked oxygen at the 3' position. Group 4 have an unsaturated bond in the acyl chain. B. The basic furanone structure is presented with R groups which represent different substitutions which are indicative of the members of the chemical family.

related bacterium, *V. harveyi*, although the mechanism of quorum sensing is quite different. *V. harveyi* has two signalling systems, one of which produces and responds to the AHL, HBHL, while the second system responds to an as yet unidentified chemical cue. In addition, the response regulator, LuxN, that binds HBHL is not a homologue of the *V. fischeri* LuxR, but rather is the membrane bound component of a two component signal transduction pathway (Bassler and Silverman, 1995). The second signalling system appears to also be sensed by another two component regulatory pair, LuxQ and LuxP (Bassler and Silverman, 1995). Signalling information from the two pathways is integrated through a common transcriptional regulator, LuxO. Phosphorylation of LuxO, allows for derepression of the bioluminescence genes, which are then transcriptionally activated by LuxR; it should be noted that the LuxR of *V. harveyi* shares no sequence homology to the LuxR of *V. fischeri* (Bassler and Silverman, 1995).

Regulation of AHL phenotypes is primarily controlled by binding of the LuxR-AHL complex to the promoter region of the genes involved, however, this is not the only level of regulation for either the phenotypic genes or the *luxIR* homologues. There is a growing body of evidence indicating that many global regulators are important in the control of quorum sensing genes. Catabolite repression (Ulitzur and Dunlap, 1995) has been implicated in the regulation of *lux* genes of *V. fischeri* as has the histone-like protein, HNS (Ulitzur *et al.*, 1997) and the heat shock sigma factor, σ^{32} (Ulitzur and Kuhn, 1988). In *Pseudomonas* spp. regulation of the *las* system is controlled by a host of global regulators including: catabolite repression (Albus *et al.*, 1997) and a two component regulatory pair, *gacA* and *lemA* (Kitten *et al.*, 1998). In addition, the second quorum sensing system in *P. aeruginosa*, the *rhl* system, which is partially regulated by the *las* system, regulates the expression of the global and stationary phase sigma factor, *rpoS* (Latifi *et al.*, 1995; Latifi *et al.*, 1996). Thus, it seems that signalling systems, like many bacterial responses, are regulated through multiple pathways and in this way can fine tune their expression of the relevant phenotypes.

Implicit in the model is the ability of the AHLs to rapidly diffuse into or out of the cell across the membrane. It was previously demonstrated in *V. fischeri* that AHLs can freely diffuse across the bacterial membrane (Kaplan and Greenberg, 1985). Therefore, if diffusion of AHLs out of the cells is not impeded and AHL synthesis occurs at a particular constant rate prior to autoinduction, then quorum regulation is directly correlated to the cell density of the population or more correctly, in a given environment (*e.g.* a culture flask), the threshold concentration of AHL required for quorum sensing will be achieved at a reproducible cell density. However, the suggestion has been put forth that not all AHLs diffuse rapidly across the membrane. It is conceivable that some of the AHLs such as OdDHL which have longer acyl chains are less likely to diffuse across the membrane and therefore may require a transport mechanism to cross the cell membrane. To date, there have been no demonstrations of an AHL transport system, although Evans *et al.* (1998) demonstrated that overexpression of a MexAB-OprM multi-drug efflux pump in *P. aeruginosa* will transport autoinducer out of the cell and repress AHL mediated phenotypes. However, as this is over expression of a non-specific transporter, it is doubtful

that it normally plays a role in transport of AHLs. If indeed there is some diffusion barrier which slows or limits export of AHLs, then it may be possible to induce the density dependent phenotype at low cell densities or independent of cell density. One possible environment where low cell density induction might occur is in a population of cells growing as a biofilm where there may be differences in autoinducer accumulation in the biofilm or diffusion of the AHL out of the cell into the biofilm. Similarly, changes in the cell membrane which effect its fluidity, as in response to stress conditions such as osmotic or heat shock or starvation may also decrease the rate of diffusion of AHLs out of the cell and cause induction at low cell density.

Using mathematical models, we have predicted a number of factors which might lead to early autoinduction at low cell densities (Nilsson *et al.*, 1999). Some of these factors include low growth rates, a very high rate of autoinduction, and the biofilm acting as a diffusion barrier between the cell membrane and the biofilm. In some scenarios, such as low diffusion rates and slow growth, we predict that maximum autoinduction could occur at low cell densities when cells are in the early logarithmic phase of growth. While there is evidence that signals are produced in biofilm settings (McClellan *et al.*, 1997b; Stickler *et al.*, 1998), there are few studies that have directly measured the concentration of AHLs or population density required to induce a phenotype in its natural setting and likewise, there are few studies that measure the induction of AHL mediated quorum sensing genes in biofilms. These concepts of cell-cell signalling, diffusion of signals, and activation at low cell densities in restricted environments are particularly relevant for marine bacteria, especially in the context of adhesion and colonisation phenotypes, where a single cell may interact with a surface.

Since bacteria use diffusible signalling molecules to regulate phenotypes in the environment, and these responses often involve a eukaryotic host, then it does not seem unlikely that eukaryotic organisms would have developed mechanisms to exploit those bacterial signals for their own benefit. There is evidence for such interactions between the light organs of squid and fish to attract and control their bioluminescent symbiont (see the review by Ruby in this issue) and there is evidence that plants may produce extra-cellular factors that induce AHL mediated phenotypes in *Agrobacterium* (Piper *et al.*, 1993; Zhang *et al.*, 1993). While the prokaryote-eukaryote interaction in these examples is beneficial to both, there are clearly instances where the prokaryote is damaging to the eukaryotic host. For example, there is evidence that OdDHL, produced by *P. aeruginosa*, can shift the host immune response from a Th1, cell mediated antibacterial response, to a Th2 response, which is less effective at clearing bacterial pathogens (Telford *et al.*, 1998). In this way, it is supposed that the pathogen modifies the host environment so that it might be better able to compete and survive. Therefore it would be advantageous to the eukaryote host to be able to inhibit bacterial signalling phenotypes which include the expression of virulence factors as well as colonisation traits.

Signals from *Delisea pulchra* Cross-talk with AHL Systems

An elegant example of eukaryotic interference with bacterial signalling processes comes from the control of bacterial fouling on the marine macro-algae, *Delisea pulchra*. This red alga originally came to our attention because it did not become fouled by other marine invertebrates or plants even though other plants in the same area were heavily fouled. Fouling is damaging to marine plants in that it decreases their photosynthetic ability, increases drag in flowing water, which leads to tearing of the plant, and fouling organisms

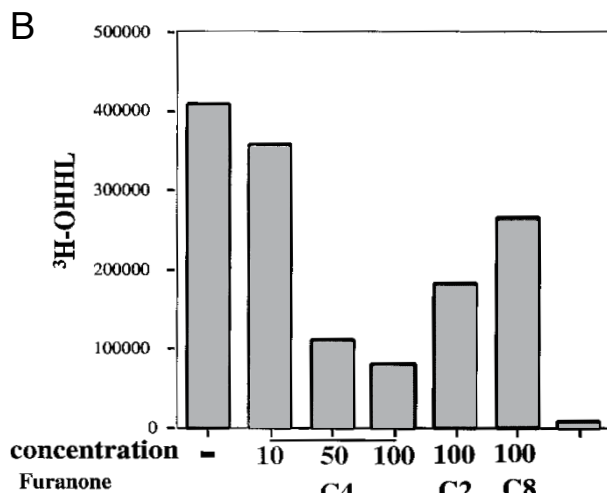
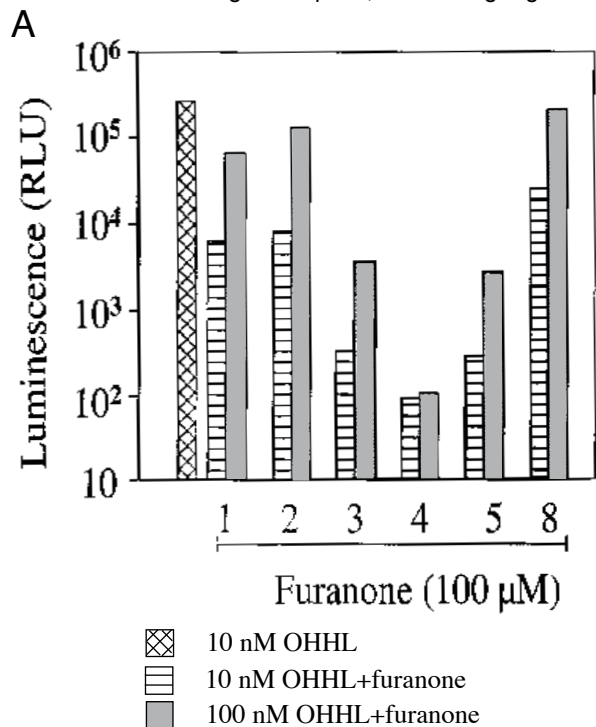


Figure 2. The Effect of Furanones in AHL Bioassays
 A. The effect of furanones on the bioluminescence of an *E. coli* strain carrying the bioluminescent monitor plasmid pSB403. Inhibition of bioluminescence in the presence of furanones is normalised against level of bioluminescence in the presence of 10 nM OHHL (100%). Two concentrations of OHHLs were used, 10 nM and 100 nM, while the furanone concentration was held constant at 100 μ M. B. Addition of furanones to a LuxR overproducing system displaces binding of the native signal, OHHL, in a concentration dependent manner. The degree of displacement is monitored by the amount of ³H-OHHL that binds to the LuxR producing strain in the presence of furanones. Note that the degree of displacement mirrors the degree of bioluminescence inhibition in that compound 4 is the most effective at displacement and is the most effective at inhibiting both

often parasitise the plant. Bacteria are important in the initial colonisation of surfaces by macroorganisms in that they provide a conditioning film which attracts other organisms to the site. When the surface of *D. pulchra* was examined more closely, it was observed that just as there were fewer fouling macroorganisms, there were also fewer bacteria on the surface of the plant with the tips of the plant having the fewest number of bacteria and the base or stem of the plant having the highest numbers (Maximilien *et al.*, 1998). Investigations into the strategy by which *D. pulchra* remains free of fouling organisms led to the discovery that *D. pulchra* produces a number of halogenated furanone compounds which are secreted onto the surface of the plant at concentrations ranging from 1-100 ng/cm² (deNys *et al.*, 1998; deNys *et al.*, 1993; Kazlauskas *et al.*, 1977). The variation of bacterial numbers inversely correlates with the surface concentration of furanones on the different parts of the plant with the tips having the highest furanone concentration and the lowest bacterial concentration (Maximilien *et al.*, 1998). Structurally, furanones are similar to AHLs which led to the hypothesis that furanones might specifically interfere with AHL mediated phenotypes. The alga primarily produces 4 furanones and approximately 10 others in minor quantities (Figure 1B). These halogenated furanones consist of a five carbon furan ring structure with an exocyclic double bond at the C-5 position and a substituted acyl chain at the C-3 position (Figure 1B) (deNys *et al.*, 1993). Molecular modeling of furanones and AHLs demonstrated the similarity of furanones and AHLs by comparing stick models of the most stable configurations for the molecules (not shown). The ring structures and the acylated side chain match quite closely and the substituted side-chains align and extend in similar fashion. As it is the acylated side chain of the AHL that gives it its specificity of interaction with a LuxR homologue, we anticipate that, if furanones interfere with AHL phenotypes, that furanones will show differential activity against AHL mediated phenotypes and that such differences will be reflected in the substitutions of the R groups.

When plastic coupons coated with furanones are incubated in the marine environment and compared with non-furanone controls, a concentration dependent inhibition of bacterial attachment to those coupons was observed (Kjelleberg *et al.*, 1997). Importantly, the furanone concentrations that inhibited attachment were similar to those presented on the surface of the plant in the natural environment. Similarly, when a number of marine isolates were assayed for their ability to swarm in the presence of furanones, swarming was inhibited in a concentration dependent manner. This data suggested that furanones were produced by the plant and secreted onto its surface to specifically inhibit the bacterial colonisation phenotypes of attachment and swarming. Importantly, swarming is a high cell density phenotype that is known to be regulated by AHLs (Eberl *et al.* 1996) and attachment and biofilm formation has recently been suggested to be controlled by AHL systems (Davies *et al.*, 1998). Therefore, based on the structural similarities of furanones with AHLs and the effect of furanones on controlling the AHL mediated colonisation phenotypes of attachment and swarming, it was hypothesised that furanones cause these effects by specifically inhibiting AHL mediated gene regulation.

To approach this hypothesis, the effect of furanones in a number of AHL bioassays was tested. Furanone mediated

inhibition of bioluminescence was monitored using either wild-type strains of *V. fischeri* and *V. harveyi* or by using a plasmid based reporter system (pSB403) in *E. coli* (Winson *et al.*, 1998). The plasmid reporter pSB403 lacks an AHL synthase gene, thus AHLs need to be added exogenously to induce bioluminescence. For all three systems, it was demonstrated that furanones dramatically inhibited bioluminescence (Figure 2A). Similarly, furanones inhibited swarming in *Serratia liquefaciens* at low concentrations and the degree of swarming inhibition was concentration dependent (Givskov *et al.*, 1996; Manefield *et al.*, 1999). Specifically, furanone compound 2 was demonstrated to reduce the swarming velocity of *S. liquefaciens* by >70% at 10 $\mu\text{g/ml}$ and completely inhibited swarming at 100 $\mu\text{g/ml}$ (Givskov *et al.* 1996). The concentrations used to inhibit bioluminescence and swarming in these assays were well within the range of concentrations presented at the surface of the plant (100 $\mu\text{g/ml}$ which is approximately equal to 100 ng/cm^2) (deNys *et al.*, 1998). Swarming of *S. liquefaciens* is particularly relevant in the marine setting as a number of bacteria isolated from the surface of *D. pulchra* have been demonstrated to exhibit a swarming phenotype and furanones were effective at inhibiting swarming of those marine isolates (Maximilien *et al.*, 1998). For both the swarming and the bioluminescence, different furanones had differential effects, with some furanones being strongly active and some having little or no effect (Givskov *et al.* 1996).

Some bacteria rely on proteases for the invasion and colonisation of a host; it has also been demonstrated that in *S. liquefaciens* the activity of two exoproteases is regulated in a density dependent fashion (Givskov *et al.* unpublished). Addition of furanones to cultures of *S. liquefaciens* also decreases the activity of these two exoproteases. The prawn pathogen *Vibrio harveyi*, which has two quorum sensing systems, regulates its toxicity in a density dependent manner and we have demonstrated that extra-cellular supernatants show reduced toxicity in prawns when *V. harveyi* was preincubated in the presence of furanones. This reduction in toxicity correlated to the reduction of two exoproducts as determined by PAGE analysis (Harris *et al.* unpublished). In all of the examples described here, the furanones were able to inhibit the AHL phenotypes in the presence of the native inducer, either produced naturally in the wild-type system or added exogenously to strains carrying the monitor plasmid or to genetically engineered strains which lack a functional AHL synthase gene (*luxI* homologue). This data would suggest that the furanones competitively inhibit AHLs from binding the LuxR receptor and thus prevent expression of the signalling phenotype.

To specifically address the mode of action, the effect of furanones on the global metabolism of the cells was assessed. At the concentrations tested, none of the furanones used were growth inhibitory to the bacteria in the bioassays. However, it was still possible that furanones effected the energy levels of the cells which might explain a decrease in bioluminescence, which is an energy intensive process. To test this, cells harboring a monitor plasmid which constitutively expresses the *lux* genes were incubated in the presence of furanones (Kjelleberg *et al.*, 1997). If furanones cause gross aberrations in energy metabolism, there should be a decrease in luminescence when these cells are grown in the presence of furanones, which was not observed. This observation would indicate

that the furanones do not cause a significant reduction in the cellular pool of ATP. The possibility still remained that inhibition is through some other non-specific mechanism such as protein regulation; for example, furanones could globally inhibit transcription or translation. Therefore, the effect of furanones on protein production was examined by looking at total protein production by 2 dimensional PAGE analysis. *E. coli* cells harboring *lux* monitor plasmids were incubated in the presence or absence of furanones as well as in the presence or absence of the autoinducer OHHL, the proteins were extracted and the protein patterns were compared for differences. Addition of OHHL resulted in an induction of three proteins which correspond to the pSB403 encoded proteins LuxA, LuxB, and LuxD (Manefield *et al.*, 1999). Incubation of the *E. coli* monitor strain in the presence of furanones resulted in the inhibition of six proteins, three of which corresponded to LuxA, LuxB, and LuxD, and six proteins were induced. Comparison with the *E. coli* proteome data base suggested that the other three repressed proteins were *OmpF*, *DnaK*, and glutamate ammonia ligase. Of the six induced, four are unidentified, while the remaining two were glucose-6-phosphate dehydrogenase, and alkylhydroperoxide reductase. This again indicated that furanones were not causing gross aberrations in cellular metabolism, but rather were having very specific effects on the AHL regulatory pathway. This hypothesis was supported by the molecular modeling data which shows that furanones and AHLs are very similar molecules. To rule out furanone mediated modification of the AHL, proton NMR spectroscopy of mixtures of furanones and AHLs was performed and no modification of either compound observed, which confirmed that there was no modification of AHLs by the furanones (Manefield *et al.*, 1999).

If the furanones do not effect global metabolism and do not alter the physical-chemical properties of the autoinducer, and if the furanones are structurally similar to AHLs then it is probable that they mediate their effects by competing for the same regulator, LuxR. This theory is supported by displacement experiments in which the binding of ^3H labeled OHHL to LuxR overproducing cells is reduced in a concentration dependent fashion by the presence of furanones (Figure 2B) (Manefield *et al.*, 1999). Similarly, the effects of the furanones could be overcome by adding back the OHHL in excess. The strength of displacement mirrored the degree of inhibition of bioluminescence by the furanone, indicating a correlation between furanone binding and inhibition of AHL phenotypes. Thus, it would appear that furanone mediated inhibition of AHL phenotypes occurs as a direct result of competition by the furanone for the AHL binding site on the LuxR receptor-transcriptional activator and leads to the model for inhibition described in Figure 3. In this model, furanones and AHLs compete for binding to the receptor site of the transcriptional activator, LuxR. When AHLs bind the receptor, the transcriptional activator can induce expression of *LuxI* and the phenotypic genes. In contrast, with furanones bound, LuxR is not able to activate transcription and the system remains in the off position.

Therefore, based on the available data, it appears that the marine red alga *D. pulchra* has evolved a mechanism whereby it can exploit quorum sensing regulation of bacteria to reduce the levels of bacteria on its surface which helps to alleviate problems associated with fouling. The alga does so through molecular mimicry by producing

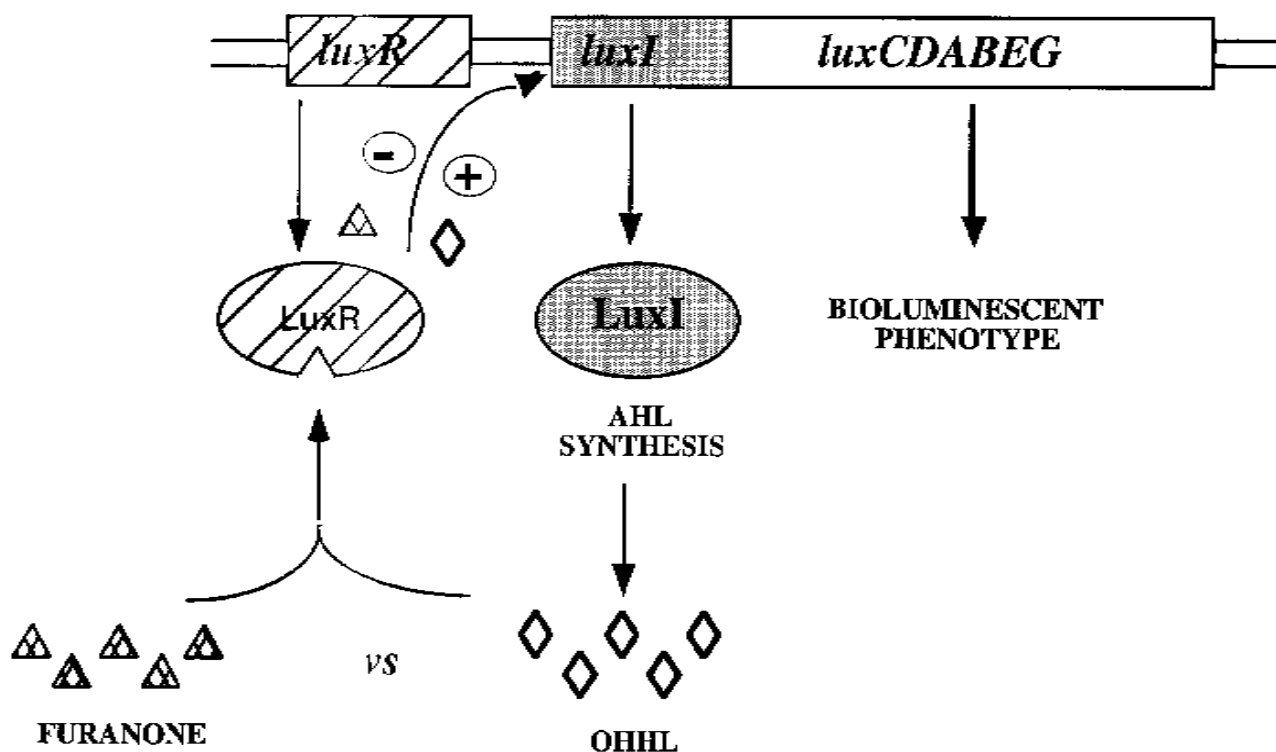


Figure 3. A Model for the Mechanism of Furanone Mediated Inhibition of AHL Phenotypes

In the absence of furanones, AHLs, produced by the LuxI protein, bind the receptor, LuxR, and induce transcription, noted in the figure as a "+", of *luxI* and the genes involved in development of the quorum sensing phenotype (e.g. bioluminescence). Based on the structural similarity of furanones and AHLs, their ability to inhibit AHL phenotypes, and their ability to displace the native AHL signal, we suggest that furanones competitively bind the LuxR receptor and prevent transcription of the phenotypic genes, noted in the figure as a "-".

signal analogues which competitively interfere with the bacterium's endogenous signal to bind its cognate receptor. This also provides the first example of inhibitory signal cross-talk in the marine environment. However, cross-talk is not likely to be limited solely to furanones, indeed, our laboratory has isolated several new classes of molecules from marine algae which are also capable of cross-talking with known bacterial signalling phenotypes (deNys *et al.* unpublished).

Other Novel Signals and Cross-talk

To identify novel bacterial signal systems which cross-talk with AHL quorum sensing systems, we have screened supernatants from a number of marine and non-marine bacteria using AHL based bioassays. One class of signalling molecules we have recently identified, diketopiperazines or cyclic dipeptides (CDPs), appear to be widely disseminated in biological systems. In collaboration with the University of Nottingham, we have identified CDPs as components of stationary phase supernatants from *P. aeruginosa* which demonstrate activity in AHL based bioassays (Chhabra *et al.*, unpublished). CDPs have been identified as being produced by a number of marine bacteria such as *V. parahaemolyticus* and *Micrococcus* (Bell *et al.*, 1994; Stierle *et al.*, 1988) and have also been identified in a number of other biological systems, including: mammalian systems as a neurotransmitter (Prasad, 1995), and in many food products including sake and beer (Gautschi and Schmid, 1997; Takahashi *et al.*, 1974).

A more detailed analysis of the cross-talk of CDPs

in other AHL bioassays indicates, that just as different furanones have different activities in AHL assays, different CDPs have different activities as well. For example, the CDPs L-proline-L-methionine, L-proline-L-valine, L-proline-L-tyrosine, D-alanine-L-valine, and L-proline-L-phenylalanine all induce bioluminescence in the μM range, while L-proline-L-alanine and L-proline-L-leucine have no effect on bioluminescence. However, the differences in activities do not follow a definable structure-function relationship with regard to which AHL phenotype a particular CDP will induce. For example, L-proline-L-tyrosine induces bioluminescence as well as pigment production in an *Agrobacterium* reporter yet L-proline-L-valine, which also induces bioluminescence is not active in the *Agrobacterium* assay. While AHLs are active at nM concentrations, CDPs are active in AHL system in the μM range. To determine if CDPs mediate their effects by binding to the LuxR protein, displacement experiments similar to those with AHLs and furanones were performed. However, unlike the furanones, no significant displacement of the labeled OHHL in the presence of CDP could be detected at the concentrations which induced bioluminescence (Manfield *et al.* unpublished). This would suggest that either CDPs have a binding affinity for the LuxR which is extremely low and they are therefore incapable of competing with AHLs for the binding site or that they act through another regulator or mechanism. It seems unlikely that CDPs are mediating their effects through some non-specific toxicity as to date there is no report that CDPs are toxic to either prokaryotes or eukaryotes; the food industry taste tests CDPs in mg quantities (Gautschi and Schmid, 1997), and

the presence of CDPs in mM concentrations does not effect the growth of either *E. coli* or *P. aeruginosa* (Labbate *et al.* unpublished).

The origin of CDPs in bacteria is also unknown. In the Cyanobacteria, cyclic peptides are produced by a series of non-ribosome peptide synthetases which are encoded by large operons consisting of conserved modules, each of which plays a particular role in cyclisation, recruiting, or modification of the various amino acids making up the cyclic peptide. Another possibility is that the CDPs are produced as degradative products of proteins. In Gram positive bacteria, the small peptide pheromones used to induce the density dependent phenotypes of competence, virulence expression, or antibiotic production are thought to be derived from limited proteolysis of a pre-peptide (Kleerebezem *et al.*, 1997). It is also suggested that a proline residue near the end of a peptide chain might spontaneously cyclize and self-cleave itself from the peptide, resulting in a cyclic dipeptide (Prasad, 1995). The mechanism of production of, and the genes controlled by, CDPs in bacteria is currently under investigation.

As yet, the biological role of CDPs in bacteria has not been elucidated. *P. aeruginosa* is an example of an organism with two functioning signalling systems, *i.e.* AHLs and CDPs, which may cross-talk with each other, although no specific CDP mediated regulation of *P. aeruginosa* genes has been observed. A homologue of the *V. fischeri luxR*, *sdiA*, has been identified in *E. coli*, and it is proposed to play a role in the regulation of the cell division genes *ftsQZA* (Wang *et al.*, 1991). Moreover, an as yet unidentified extracellular factor(s) produced by stationary phase *E. coli* cells (Garcia-Lara *et al.*, 1996; Sitnikov *et al.*, 1996; Surette and Basseler, 1998) is also involved in the regulation of these same genes and presumably mediate their effects through SdiA (Garcia-Lara *et al.*, 1996; Sitnikov *et al.*, 1996). Our laboratory has tested the effect of CDPs on the regulation of an *ftsQ-lacZ* fusion and found no difference in LacZ activity at the concentrations tested. However, incubation of a *bolA-lacZ* fusion (Bohannon *et al.*, 1991) in the presence of L-proline-L-leucine, L-proline-L-valine, L-proline-L-tyrosine, L-proline-L-alanine did reduce the LacZ activity of this fusion (deNys *et al.*, unpublished), indicating that CDPs may play a role in the regulation of cell morphogenesis through regulation of the stationary phase induced morphogene *bolA* (Aldea *et al.*, 1990). In addition, L-proline-L-tyrosine inhibited expression of the stationary phase gene *fic* (deNys *et al.*, unpublished) as determined by monitoring LacZ expression in a *fic-lacZ* fusion (Kawamukai *et al.*, 1989).

As described above, *V. harveyi* has two signalling systems, one that is AHL based and a second that is based on an as yet uncharacterised compound. The second signalling system has proven useful for the identification of novel signals in that it appears to be more promiscuous in its interactions with different signals than the AHL system. Bassler *et al.* (1997) have used this system to identify a number of different bacteria including the marine bacteria *V. cholerae*, *V. parahaemolyticus*, *V. anguillarum*, and *V. alginolyticus* which produce extra-cellular components that activate bioluminescence through the non-AHL pathway in *V. harveyi*. However, with the exception of *V. harveyi*, the phenotypes regulated by these extra-cellular signals are not known. In addition, two other marine vibrios, *V. vulnificus* and *V. angustum* also produce diffusible

signalling molecules which are active in the *V. harveyi* bioassay (deNys *et al.*, 1999; McDougald *et al.*, 1998; Srinivasan *et al.*, 1998). For these two bacteria, the signal extracts appear to be involved in the regulation of starvation induced phenotypes. However, the identities of the signal molecules are unknown.

Another useful method to identify bacteria with signalling systems is through gene homology either by genome analysis, hybridisation, or by PCR. Use of these methods has led to the identification of a number of homologues of known quorum sensing genes. Most notably, a homologue of the *V. fischeri luxR* gene has been identified, *sdiA*, in both *E. coli* and *S. typhimurium* (Ahmer *et al.*, 1998; Wang *et al.*, 1991). In *E. coli*, this gene is suspected of being involved in the regulation of cell division, while in *S. typhimurium*, the gene is involved in the regulation of genes encoded by the virulence plasmid (Ahmer *et al.*, 1998). Four marine vibrios, *V. vulnificus*, *V. angustum*, *V. cholerae*, and *V. parahaemolyticus* all encode homologues of the *V. harveyi luxR* gene. For the latter two species, this gene is involved in the regulation of proteases and capsule production respectively, while for the former two, the phenotype controlled by this gene has not been identified (Jobling and Holmes, 1997; McCarter, 1998; McDougald *et al.* unpublished).

It should be pointed out that the majority of bacteria discussed herein are Gram-negative and this is primarily because of the focus on AHL systems. However, signalling in Gram-positive bacteria is well established and there is every reason to believe that further studies into these bacteria will reveal many more signalling systems as well. In a continued effort to examine new species of marine bacteria that produce or respond to signal molecules and the phenotypes involved in those, examination of a range of marine isolates by our laboratory indicates that at least 10% of species produce AHLs, although the number and types of phenotypes regulated by those signalling molecules is more difficult to determine (Maximilien *et al.*, 1998; England *et al.* unpublished). This percentage of signal producing marine bacteria is probably an underestimate as it is clear that bacteria are not limited to AHLs as signals. Furthermore, the bioassays used and the signal extraction procedures also have their limitations in sensitivity and hence can also lead to an underestimation of the true number of bacteria that produce signalling molecules. There is also a whole other branch in the tree of life, the Archae, that has yet to be explored and we anticipate these organisms will also produce their own signals as well as possibly having or being able to respond to those systems described here. The work done with *D. pulchra* as an example of signals or signal antagonists derived from a eukaryote suggests there is some benefit to be gained in looking at organisms besides bacteria for signalling molecules or signal antagonists. As mentioned above, preliminary work with species of *Delisea* besides *D. pulchra* are also yielding novel compounds that appear to cross-talk with AHL based signal systems.

Acknowledgements

Research in the author's laboratory was funded by the Australian Research Council and the Centre for Marine Biofouling and Bio-Innovation. We would like to thank the many members of the research team who contributed information, ideas, and discussions for this manuscript including: Tim Charlton, Symon Dworjany, Rocky deNys, Dacre England, Maurice Labbate, Diane McDougald, Mike Manefield, and Sujatha Srinivasan. We

also wish to thank our collaborators for their input, Naresh Kumar and Roger Read (School of Chemistry, UNSW), Matt Holden (School of Pharmaceutical Sciences, The University of Nottingham), Lone Gram (Danish Fisheries Institute, Danish Technical Institute) and Patric Nilsson (Department of Theoretical Ecology, Lund University).

References

- Ahmer, B.M.M., van Reeuwijk, J., Timmers, C.D., Valentine, P.J., and Heffron, F. 1998. *Salmonella typhimurium* encodes an SdiA homolog, a putative quorum sensor of the LuxR family, that regulates genes on the virulence plasmid. *J. Bacteriol.* 180: 1185-1193.
- Albus, A.M., Pesci, E.C., Runyen-Janecky, L.J., West, S.E.H., and Iglewski, B.H. 1997. Vfr controls quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 179: 3928-3935.
- Aldea, M., Garrido, T., Pla, J., and Vicente, M. 1990. Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters. *EMBO J.* 9: 3787-3794.
- Bassler, B., Greenberg, A., and Stevens, A. 1997. Cross-species induction of luminescence in the quorum-sensing bacterium *Vibrio harveyi*. *J. Bacteriol.* 179: 4043-4045.
- Bassler, B., and Silverman, M.R. 1995. Intercellular communication in marine *Vibrio* species: Density-dependent regulation of the expression of bioluminescence. In: Two-component signal transduction, Hoch, J.A., and Silhavy, T.J., eds., ASM Press, Washington. p. 431-444.
- Bassler, B.L., Wright, M., and Silverman, M.R. 1994. Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi* sequence and function of genes encoding a second sensory pathway. *Mol. Microbiol.* 13: 273-286.
- Bell, R., Carmeli, S., and Sar, N. 1994. Vibrindole A, a metabolite of the marine bacterium, *V. parahaemolyticus*, isolated from the toxic mucus of the boxfish *Ostracion cubicus*. *J. Nat. Prod.* 57: 1587-1590.
- Bohannon, D.E., Connell, N., Kleener, J., Tormo, A., Espinosa-Urgel, M., Zambrano, M., and Kolter, R. 1991. Stationary-phase inducible "Gearbox" promoters: Differential effects of *katF* mutations and the role of sigma 70. *J. Bacteriol.* 173: 4482-4492.
- Cha, C., Gao, P., Chen, Y., Shaw, P., and Farrand, S. 1998. Production of acyl-homoserine lactone quorum-sensing signals by gram-negative plant-associated bacteria. *Mol. Plant Microbe Interactions.* 11: 1119-1129.
- Clewell, D.B., and Weaver, K.E. 1993. Sex pheromones and plasmid transfer in *Enterococcus faecalis*. *Plasmid.* 21: 175-184.
- Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., and Greenberg, E.P. 1998. The involvement of cell-cell signals in the development of a bacterial biofilm. *Science.* 280: 295-298.
- deNys, R., Dworjanyn, S.A., and Steinberg, P.D. 1998. A new method for determining surface concentrations of marine natural products on seaweeds. *Mar. Ecol. Prog. Ser.* 162: 79-87.
- deNys, R., Rice, S.A., Manefield, M., Srinivasan, S., McDougald, D., Loh, A., Ostling, J., Lindum, P., Givskov, M., Steinberg, P., and Kjelleberg, S. 1999. Cross-talk in bacterial extracellular signalling systems. In: Microbial Biosystems: New Frontiers. Proceedings of the 8th Intl. Symp. on Microbial Ecology., Bell, C. R., Brylinsky, M., and Johnson-Green, P., eds., Atlantic Canada Society for Microbial Ecology, Halifax. (In press).
- deNys, R., Wright, A.D., Konig, G.M., and Sticher, O. 1993. New halogenated furanones from the marine alga *Delisea pulchra* (cf. *fimbriata*). *Tetrahedron.* 49: 11213-11220.
- Eberhard, A.A., Burlingame, L., Eberhard, C., Kenyon, G.L., and Nealson, K.H. 1981. Structural identification of autoinducer of *Photobacterium fischeri* luciferase. *Biochem.* 20: 2444-2449.
- Eberl, L., Winson, M., Sternberg, C., Stewart, G., Christense, G., Chhabra, S., Bycroft, P., Williams, S., Molin, S., and Givskov, M. 1996. Involvement of N-acyl-homoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. *Mol. Microbiol.* 20: 1127-1136.
- Evans, K., Passador, L., Srikumar, R., Tsang, E., Nezezon, J., and Poole, K. 1998. Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* 180: 5443-5447.
- Flavier, A.B., Clough, S. J., Schell, M.A., and Denny, T.P. 1997. Identification of 3-Hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol. Microbiol.* 26: 251-259.
- Fuqua, C., Winans, S.C., and Greenberg, E.P. 1996. Census and Consensus in bacterial ecosystems: The LuxR-LuxI family of quorum-sensing transcriptional regulators. *Ann. Rev. Microbiol.* 50: 727-751.
- Fuqua, W.C., Winans, S.C., and Greenberg, E.P. 1994. Quorum sensing in bacteria: The LuxR-LuxI family of cell density-responsive transcriptoinal regulators. *J. Bacteriol.* 176: 269-275.
- Garcia-Lara, J., Shang, L.H., and Rothfield, L.I. 1996. An extracellular factor regulates expression of *sdiA*, a transcriptional activator of cell division genes in *Escherichia coli*. *J. Bacteriol.* 178: 2742-2748.
- Gautschi, M., and Schmid, J.P. 1997. Chemical characterization of diketopiperazines in beer. *J. Agric. Food Chem.* 45: 3183-3189.
- Givskov, M., deNys, R., Manefield, M., Gram, L., Maximilien, R., Eberl, L., Molin, S., Steinberg, P., Kjelleberg, S. 1996. Eukaryotic interference with homoserine lactone-mediated prokaryotic signalling. *J. Bacteriol.* 178:6618-6622.
- Huang, J., Yindeeoungyeon, W., Garg, R.P., Denny, T.P., and Schell, M. A. 1998. Joint transcriptional control of *xpsR*, the unusual signal integrator of the *Ralstonia solanacearum* virulence gene regulatory network, by a response regulator and a LysR-type transcriptional activator. *J. Bacteriol.* 180: 2736-2743.
- Jobling, M.G., and Holmes, R.K. 1997. Characterization of *hapR*, a positive regulator of the *Vibrio cholerae* HA/protease gene *hap*, and its identification as a functional homologue of the *Vibrio harveyi* luxR gene. *Mol. Microbiol.* 26: 1023-1034.
- Kaplan, H., and Greenberg, E.P. 1985. Diffusion of autoinducer is involved in regulation of the *Vibrio fischeri* luminescence system. *J. Bacteriol.* 163: 1210-1214.
- Kawamukai, M., Matsuda, H., Fujii, W., Utsumi, R., and Komano, T. 1989. Nucleotide sequences of *fic* and *fic-1* genes involved in cell filamentation induced by cyclic AMP in *Escherichia coli*. *J. Bacteriol.* 171: 4525-4529.
- Kazlauskas, R., Murphy, P.T., Quinn, R.J., and Wells, R.J. 1977. A new class of halogenated lactones from the red alga *Delisea fimbriata* (Bonnemaisoniaceae). *Tetrahedron Lett.* 1: 37-40.
- Kitten, T., Kinscherf, T.G., Mcevoy, J.L., and Willis, D.K. 1998. A newly identified regulator is required for virulence and toxin production in *Pseudomonas syringae*. *Mol. Microbiol.* 28: 917-929.
- Kjelleberg, S., Steinberg, P., Givskov, M., Gram, L., Manefield, M., and Denys, R. 1997. Do marine natural products interfere with prokaryotic AHL regulatory systems. *Aquatic Microbial Ecology.* 13: 85-93.
- Kleerebezem, M., Quadri, L.E.N., Kuipers, O.P., and deVos, W.M. 1997. Quorum sensing by peptide pheromones and two component signal transduction systems in Gram positive bacteria. *Mol. Microbiol.* 24: 895-904.
- Kuspa, A., Plamann, L., and Kaiser, D. 1992a. A-signaling and the cell density requirement for *Myxococcus xanthus* development. *J. Bacteriol.* 174: 7360-7369.
- Kuspa, A., Plamann, L., and Kaiser, D. 1992b. Identification of heat-stable A-factor from *Myxococcus xanthus*. *J. Bacteriol.* 174: 3319-3326.
- Latifi, A., Winson, M.K., Foglino, M., Bycroft, B.W., Stewart, G.S.A.B., Lazdunski, A., and Williams, P. 1995. Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PA01. *Mol. Microbiol.* 17: 333-343.
- Latifi, M.F., Tanaka, K., Williams, P., and Lazdunski, A. 1996. A hierarchical quorum-sensing cascade in *Pseudomonas aeruginosa* links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary phase sigma factor RpoS. *Mol. Microbiol.* 21: 1137-1146.
- Manefield, M., deNys, R., Kumar, N., Read, R., Givskov, M., Steinberg, P., and Kjelleberg, S. 1999. Evidence that halogenated furanones from *Delisea pulchra* inhibit AHL mediated gene expression by displacing the AHL signal from its receptor protein. *Microbiol.* 145:283-291.
- Maximilien, R., deNys, R., Holmstrom, C., Gram, L., Givskov, M., Crass, K., Kjelleberg, S., and Steinberg, P. 1998. Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga *Delisea pulchra*. *Aquatic Microb. Ecol.* 15: 233-246.
- McCarter, L.L. 1998. OpaR, a homolog of *Vibrio harveyi* LuxR, controls opacity of *Vibrio parahaemolyticus*. *J. Bacteriol.* 180: 3166-3173.
- McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S., Camara, M., Daykin, M., Lamb, J.H., Swift, S., Bycroft, B.W., Stewart, G.S.A.B., and Williams, P. 1997a. Quorum-sensing and *Chromobacterium violaceum*: Exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiol.* 143: 3703-3711.
- McClean, R.J.C., Whiteley, M., Stickler, D.J., and Fuqua, W.C. 1997b. Evidence of autoinducer activity in naturally occurring biofilms. *FEMS Microbiol. Lett.* 154: 259-263.
- McDougald, D., Rice, S.A., Weichert, D., and Kjelleberg, S. 1998. Non-culturability: Adaptation or debilitation. *FEMS Microbiol. Ecol.* 25: 1-9.
- Milton, D.L., Hardman, A., Camara, M., Chhabra, S.R., Bycroft, B.W., Stewart, G., and Williams, P. 1997. Quorum sensing in *Vibrio anguillarum* characterization of the *vanI/vanR* locus and identification of the autoinducer N (3-oxodecanoyl) homoserine lactone. *J. Bacteriol.* 179: 3004-3012.
- Nilsson, P., Ripa, J., Gardmark, A., Fagerstrom, T., Rice, S., Kjelleberg, S., and Steinberg, P. 1999. How many bacteria constitute a "quorum"? Kinetics of the AHL regulatory system in a bacterial biofilm model system. *Environ. Microbiol.* In press.
- Piper, K.R., Beck von Bodman, S., and Farrand, S.K. 1993. Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. *Nature.* 362: 448-450.
- Prasad, C. 1995. Bioactive cyclic peptides. *Peptides.* 16: 151-164.

- Shaw, P.D., Ping, G., Daly, S.L., Cha, C., Cronan, J.E., Rinehart, K.L., and Farrand, S.K. 1997. Detecting and characterizing *N*-acyl homoserine lactone signal molecules by thin layer chromatography. *Proc. Natl. Acad. Sci. USA*. 94: 6036-6041.
- Sitnikov, D.M., Schineller, J.B., and Baldwin, T.O. 1996. Control of cell division in *Escherichia coli*: Regulation of transcription of *ftsQA* involves both *rpoS* and SdiA-mediated autoinduction. *Proc. Natl. Acad. Sci. USA*. 93: 336-341.
- Srinivasan, S., Ostling, J., Charlton, T., deNys, R., Takayama, K., and Kjelleberg, S. 1998. Extra-cellular signal molecule(s) involved in the carbon starvation response of marine *Vibrio* sp. strain S14. *J. Bacteriol.* 180: 201-209.
- Stickler, D.J., Morris, N.S., McLean, R.J.C., and Fuqua, C. 1998. Biofilms on indwelling urethral catheters produce quorum-sensing signal molecules *in situ* and *in vitro*. *Appl. Environ. Microbiol.* 64: 3486-3490.
- Stierle, A.C., Cardellina, J.H., and Singleton, F.L. 1988. A marine *Micrococcus* produces metabolites ascribed to the sponge *Tedania ignis*. *Experimentia*. 44: 1021.
- Surrette, M., and Basseler, B. 1998. Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. USA*. 95: 7046-7050.
- Swift, S., Karlyshev, A.V., Fish, L., Durant, E.L., Winson, M.K., Chhabra, S. R., Williams, P., Macintyre, S., and Stewart, G. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida* - identification of the *luxRI* homologs *ahyRI* and *asaRI* and their cognate *N*-acylhomoserine lactone signal molecules. *J. Bacteriol.* 179: 5271-5281.
- Swift, S., Throup, J.P., Williams, P., and Stewart, G.S.A.B. 1996. Quorum sensing: a population-density component in the determination of bacterial phenotype. *Trends Biochem. Sci.* 21: 214-219.
- Takahashi, K., Tadenuma, M., Kitamoto, K., and Sato, S. 1974. L-Prolyl-L-leucine anhydride: A bitter compound formed in aged sake. *Agric. Biol. Chem.* 38: 927-938.
- Telford, G., Wheeler, D., Williams, P., Tomkins, P.T., Appleby, P., Sewell, H., Stewart, G.S.A.B., Bycroft, B.W., and Pritchard, D. 1998. The *Pseudomonas aeruginosa* quorum sensing signal molecule *N*-(3-Oxydodecanoyl)-L-homoserine lactone has immunomodulatory activity. *Infect. Immun.* 66: 36-42.
- Ulitzer, S., and Kuhn, J. 1988. The transcription of bacterial luminescence is regulated by sigma 32. *J. Biol. Chem.* 263: 81-93.
- Ulitzur, S., and Dunlap, P.V. 1995. Regulatory circuitry controlling luminescence autoinduction in *Vibrio fischeri*. *Photochem. Photobiol.* 62: 625-632.
- Ulitzur, S., Matin, A., Fraley, C., and Meighen, E. 1997. HNS protein represses transcription of the *lux* systems of *Vibrio fischeri* and other luminous bacteria cloned into *Escherichia coli*. *Curr. Microbiol.* 35: 336-342.
- Van Haastert, P.J.M. 1991. Transmembrane signal transduction pathways in *Dicyostelium*. In: *Advances in second messenger and phosphoprotein research*, Greengard, P., and Robinson, G. A., eds., Raven Press, Ltd., New York. p. 185-226.
- Wang, X., de Boer, P.A.J., and Rothfield, L.I. 1991. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. *EMBO J.* 10: 3363-3372.
- Winson, M.K., Swift, S., Fish, L., Throup, J.P., Jorgensen, F., Chhabra, S. R., Bycroft, B.W., Williams, P., and Stewart, G. 1998. Construction and analysis of *luxCDABE*-based plasmid sensors for investigating *N*-Acyl homoserine lactone-mediated quorum sensing. *FEMS Microbiol. Lett.* 163: 185-192.
- Yamada, Y., Sugamura, K., Kondo, K., Yanagimoto, M., and Okada, H. 1987. The structure of inducing factors for virginiamycin production in *Streptomyces virginiae*. *J. Antibiot.* 40: 496-504.
- Zhang, L.H., Murphy, P.J., Kerr, A., and Tate, M.E. 1993. *Agrobacterium* conjugation and gene regulation by *N*-acyl-L-homoserine lactones. *Nature*. 362: 446-448.

