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Chemical defenses of seaweeds against microbial colonization

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Abstract

Any living or non-living surface immersed in seawater rapidly acquires a bacterial biofilm. For living marine organisms, biofilm formation can result in the death of the host, and thus there is strong evolutionary pressure for marine eukaryotes to evolve mechanisms which inhibit or control the development of biofilms on their surfaces. Some marine eukaryotes are indeed successful in controlling biofilms on their surfaces, and in many instances this control is achieved by the production of inhibitory chemicals which act at or near the surface of the organism. In some cases these natural inhibitors are simply toxic to bacteria. However, increasingly it appears that at least some of these compounds act by interfering specifically with bacterial characteristics which effect the ability of bacteria to colonize their hosts, such as attachment, surface spreading, or the production of extracellular macromolecules. As an example, the Australian seaweed *Delisea pulchra* appears to control bacterial colonization by interfering with a bacterial regulatory system (the acylated homoserine lactone system) that regulates several colonization relevant bacterial traits. Understanding how marine organisms control specific bacterial colonization traits should provide us with insights into new technologies for the control of biofilms on artificial surfaces.

Introduction

Any undefended living or non-living surface immersed in seawater is rapidly colonized by bacteria, which then divide, accumulate, and spread over the surface to form biofilms. The consequences of biofilms for inanimate structures are numerous and include increased drag and changed hydrodynamics (e.g., Lethwaite et al. 1984), increased resistance to heat transfer, enhancement of subsequent colonisation by other fouling organisms, and corrosion and deterioration of the underlying surface (reviews by Alberte et al. 1992; Characklis & Marshall 1990; Dexter 1993; Wahl 1996). Thus not surprisingly, biofilms constitute a very costly problem for a wide diversity of marine industries (Clare 1996).

The effects of biofilms on submerged artificial surfaces have a close analogy with living marine systems, because biofilms are also present on living marine surfaces e.g., other marine organisms. Just like on inanimate surfaces, biofilms on living marine organisms have a variety of effects on their host organisms. For example, pathogenic bacteria in host-associated biofilms can cause significant mortality to their hosts (Littler & Littler 1995) or cause significant degradation of host tissue (Branch & Griffiths 1988; Correa & Sanchez 1996). Biofilms, like fouling by higher organisms, are likely to increase the drag on their hosts (D'Antonio 1985), and may also enhance subsequent settlement of higher organisms (Kirchman et al. 1982; Leitz & Wagner 1993; Holmström & Kjelleberg 1994), compounding the fouling problem. Bacteria may also compete for nutrients, inhibit gaseous exhange (Lewandowski 1994), or in the case of marine plants, block incident light, thereby decreasing photosynthesis (as for the effects of epiphytes on seagrasses; Sand-Jensen 1977).

One important difference, however, between biofilms on dead vs. living surfaces is that bacteria may also often have positive effects on living marine organisms, although direct demonstrations of the enhancement of host fitness by associated biofilms are rare. Gil-Turnes et al. (1989) showed that bacteria on the

surface of externally held eggs of the shrimp *Palaeman macrodactylus* produce a metabolite that inhibits fungal infections lethal to the eggs. More generally, the high biomass of bacteria associated with marine organisms such as sponges suggests that these bacteria have some positive effect on their hosts (up to 50% of the biomass of some 'sponges' is actually associated bacteria; Unson et al. 1994). Finally, biofilms on artificial surfaces (including those comprised of bacteria originally isolated from marine eukaryotes) can both strongly inhibit or retard subsequent settlement of macrofouling organisms (Maki et al. 1988; Holmström & Kjelleberg 1994; Neal & Yule 1994); analogous biofilms on eukaryotes may thus contribute to the inhibition of fouling of their hosts.

Bacterial biofilms on the surfaces of inanimate structures can cause their deterioration, leading to a loss of performance of that surface or even failure of the relevant equipment. However, the consequence of biofilms on living marine organisms can be the death of the host. Thus there is strong evolutionary pressure for marine organisms to evolve mechanisms which defend their surfaces against biofilms (Wahl 1989), or to at least control the composition and density of bacterial communities on their surfaces. Here we review some of these mechanisms of natural biofilm control, focussing in particular on the chemical inhibition and regulation of biofilms by their hosts. Our aims in this paper are:

(a) To understand how interactions between eukaryotes and prokaryotes in natural marine ecosytems are mediated, at both the ecological and molecular levels, and;

(b) To consider these interactions in the light of the applied problem of slowing or inhibiting bacterial degradation of artificial surfaces in seawater.

Bacteria associated with higher marine organisms

Bacteria are ubiquitous on the surfaces of higher marine organisms (e.g., Sieburth 1979). Cell densities can exceed 10^8 per cm^2 on some hosts (Sieburth 1979; Cundell et al. 1977; Novak 1984; Table 3 in Wahl 1995), and few if any host surfaces are truly axenic. However, the abundance of cells on different hosts or different parts of the same host often varies substantially. For example, colony counts of bacteria on different ascidian colonies studied by Wahl et al. (1994) varied between 500 CFUs (colony forming units) per cm^2 to $> 10^5$ CFU/cm^2, and abundances of bacteria on different parts of individual algae can vary by several orders of magnitude (Cundell et al. 1977). Similarly, although there is little relevant data, it is likely that there can be differences in the species composition between bacteria on different hosts, or between the surfaces of hosts and the water column. The rapid development of molecular technologies specific for certain species or groups of bacteria is now facilitating research into the species composition of biofilms associated with higher marine organisms.

Not surprisingly, bacteria in these abundances have strong effects on their hosts. For example, surface associated pathogens of marine eukaryotes are widespread. The coralline lethal orange disease, or 'CLOD', pathogen causes widespread mortality to coralline algae (Littler & Littler 1995). A marine *Vibrio* is a major cause of bleaching in corals through its effects on the corals' symbiotic zooxanthellae (Kushmaro et al. 1997). Surface associated pathogenic bacteria are a major threat to the aquaculture industry (Austin & Austin 1993; also see Correa & Sanchez 1996). In many cases virulence of bacterial pathogens is associated with the degradation of host tissue by exoenzymes (Atlas & Bartha 1993).

Perhaps of even more direct relevance to biodeterioration of artificial surfaces are the effects of epiphytic bacteria on degradation of marine organisms. For example, many of the larger species of brown algae which dominate temperate shorelines – such as *Laminaria* spp. – are essentially living conveyor belts in which new tissue is produced near the base of the plant while older (but still living) tissue at the distal end is simultaneously degraded (Mann 1973; Branch & Griffiths 1988).

Eukaryote defenses against bacterial fouling

Given the importance of bacteria to the ecology of higher organisms, it is not surprising that marine eukaryotes have evolved a variety of mechanisms which control bacterial biofilms on their surfaces. As with inhibition of fouling in general (reviews by Davis et al. 1989; Wahl 1989), defensive mechanisms against bacteria can be divided into non-chemical vs. chemical strategies. In this review we emphasize chemical inhibition or control of biofilms because we feel that non-chemical solutions such as burrowing or surface sloughing are less relevant to the applied context. However, we first briefly review non-chemically based inhibition of biofilms so as to provide an overview of

the diversity of control mechanisms used by marine organisms.

Inhibition by non-chemical means

Non-chemical inhibition of fouling by marine organisms falls into four main categories: (i) escape in space or time; (ii) behavioral cleaning; (iii) shedding of surface layers, and; (iv) non-stick surfaces. Escaping fouling in space or time is generally only an option for ephemeral organisms, or those which live in low fouling environments, and will not be further discussed. Active cleaning of surface fouling is restricted to those organisms with sufficient mobility or the appropriate anatomy, such as mutual grazing of conspecifics' shells by littorine gastropods (Wahl & Sonnichsen 1992). Shedding of surface layers of tissue or exudates (primarily mucous) is widespread among marine organisms, although in some cases it is clearly not an adaptation to fouling *per se*. For example, crustaceans (crabs, shrimps, etc.) moult as they grow, shedding their existing exoskeleton with each moult. Their new exoskeleton then remains clean of fouling for some period (Allen et al. 1993). For many crustaceans, however, the length between moults is long enough that moulting by itself is unlikely to be a sufficient defense against bacteria or other epibiota. Many algae and colonial invertebrates have a more effective shedding mechanisms, in wich outer layers of cells are sloughed more or less continuously (Filion-Myklebust & Norton 1986; Johnson & Mann 1986), removing any attached epibiota. Many marine invertebrates and algae are also slimy to the touch, the result of shedding often copious amounts of mucous from surface cells, which serves to clean newly settled epibiota off as the mucous sloughs away (Coll et al. 1987; Barthel & Wolfrath 1989).

Since the ability of bacteria to attach to a surface is fundamentally affected by physical properties of that surface (Van Loosdrecht et al. 1989), we might expect that host organisms would have evolved surfaces with physicochemical properties which minimize bacterial attachment. However, it is not clear that this is the case. Although little studied for bacterial attachment to marine organisms, evidence that marine organisms use particular physicochemical characteristics as antifouling strategies – against micro- or macrofouling – is equivocal at best. Schmitt et al. (1995) found no relationship between the wettability of different seaweeds and their susceptibility to being fouled by the bryozoan *Bugula neretina*. Steinberg et al. (in press)

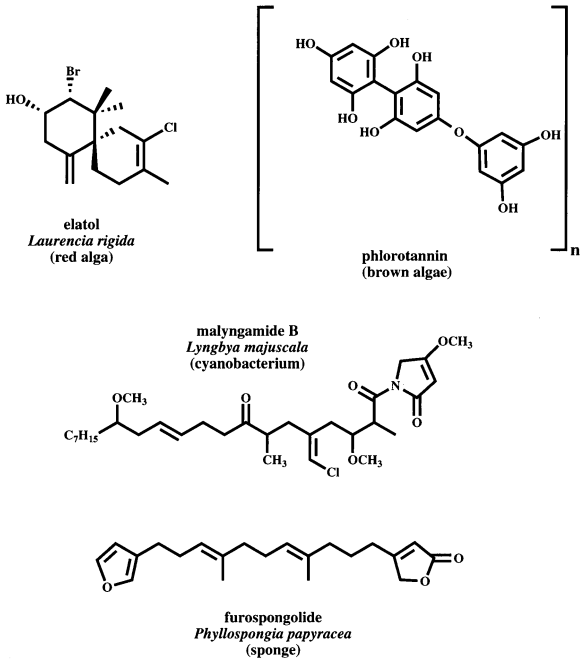


Figure 1. Representative natural products ('secondary metabolites') from marine benthic eukaryotes. In each case the trivial name of the metabolite, and the source organism, is given.

coated different seaweed metabolites or extracts onto artificial surfaces and measured both resulting wettabilities and the settlement success of several species of fouling organisms on these surfaces. They found no correlation between wettability of the coated extracts or metabolites and settlement success. Rittschof & Costlow (1989) found that the effect of variation in surface energies was species specific, affecting settlement success of some fouling organisms (barnacle cyprids), but not others (bryozoan larvae). More generally the physicochemical properties of an organism's surface *per se* is unlikely to be the determining characteristic for successful colonization by epibiota. This is because any substrata immersed in an aqueous environment rapidly acquires conditioning films, which creates a new interface with novel surface characteristics (Hunter & Liss 1979; Becker & Wahl 1991; Schneider 1996). Thus inhibition of biofilms by modification of the physical properties of surfaces may not be an effective strategy for marine organisms. However, physical characteristics of the surface may play a role in the specific sequence of biofilm formation, and its eventual composition.

Inhibition by the production of toxic or deterrent chemicals

Marine invertebrates and algae produce a remarkable array of unusual metabolites such as terpenoids, alkaloids, phenolics, etc. (Figure 1; reviewed by Faulkner 1996), which to date appear to have little or no obvious function in the internal physiology of the organisms. Rather, they primarily mediate external ecological interactions, including functioning as defenses against natural enemies such as predators, herbivores, epibiota (fouling), or pathogens (reviewed by Hay & Fenical 1988; Davis et al. 1989; Pawlik 1992) or as intraspecific attractants (Maier & Muller 1986). These metabolites are variously called 'secondary metabolites' or 'allomones' and are often very active deterrents or inhibitors of both macro- and microbiota. For the microbiota, there is an extensive literature demonstrating that many extracts or pure metabolites from marine benthic eukaryotes have bacteriocidal or bacteriostatic properties against a wide range of bacterial strains (Hornsey & Hilde 1974; Reichelt & Borowitzka 1984; McCaffrey & Endean 1985).

Many of these studies demonstrating antimicrobial effects, however, are limited in several regards. Firstly, the extracts or metabolites have often been tested against standard laboratory strains (e.g., Reichelt & Borowitzka 1984). While informative in the context of the search for new antibiotics, such studies tell us little about the effects of these metabolites on ecologically realistic strains of marine bacteria. Secondly, rarely is the concentration of the metabolites on or near the producing organisms measured (Steinberg et al. in press). This is very important in an ecological context, since metabolites sequestered inside a host may never reach the surface and be active against biofilms. Thirdly, the mechanism of action of the metabolites or extracts – either in terms of the effects on specific bacterial characteristics or in terms of specific physiological modes of action – have generally not been explored or understood.

Chemical interference with individual phenotypes or characteristics

Several more recent studies of chemical inhibition of biofilms associated with higher organisms have addressed some of these limitations. In particular, tests of the effects of metabolites or extracts have used ecologically relevant strains of bacteria (Slattery et al. 1995; Wahl et al. 1994; Maximilien 1995), and concentrations of extracts or metabolites that epiphytic bacteria actually encounter at or near the surface of the host organisms have been measured or estimated (Walker et al. 1985; Schmitt et al. 1995; Jennings & Steinberg 1997; De Nys et al. submitted). One important result of these studies is the demonstration that chemicals produced by higher organisms may not simply kill or inhibit growth of epiphytic bacteria, but in fact can act selectively against particular phenotypes that are expressed by the bacteria. Thus Slattery et al. (1995) and Wahl et al. (1994) showed that extracts of (respectively) Antarctic sponges and temperate ascidians ('sea squirts'), when coated onto artificial surfaces or incorporated into agar plates, strongly inhibited attachment of relevant bacteria with no or minimal affects on growth. Thus the inhibitory activity of these extracts did not necessarily correlate with their bacteriostatic or bacteriocidal effects, indicating selective activity against attachment.

One of the most detailed investigations to date of the non-toxic effects of secondary metabolites from higher marine organisms against bacteria is that of Maximilien (1995), who studied the effects of halogenated furanones from the Australian red alga *Delisea pulchra* on colonization phenotypes of epiphytic or epilithic marine bacteria. The abundance of bacteria on the surface of *D. pulchra* was low relative to other co-occurring algae, and the numbers of bacteria on different parts of the algae were strongly inversely correlated with variation in concentrations of furanones along the length of the plant (Figure 2; De Nys et al. 1996). However, these patterns of abundance were not due to any general bacteriocidal effects of the compounds. Rather, bioassays done against bacterial strains isolated from both *D. pulchra*, and from nearby submerged rock surfaces, indicated that the compounds inhibited expression of specific phenotypes representing different stages of the bacterial fouling process, in particular swimming, attachment, growth, and surface spreading *via* swarming (Maximilien 1995).

A model for the effects of furanones (or a non-polar extract containing furanones) from *Delisea pulchra* against these different phenotypes for isolates from both rocks and *D. pulchra* is shown in Figure 3. Both swarming and attachment of all strains tested was inhibited at (ecologically realistic) levels that had no effect on growth. However, attachment of bacterial strains isolated from rocks was inhibited at significantly lower concentrations than that of strains isolated from *Delisea*. This is consistent with specific adaptation of *Delisea* strains to the inhibitory effects of the

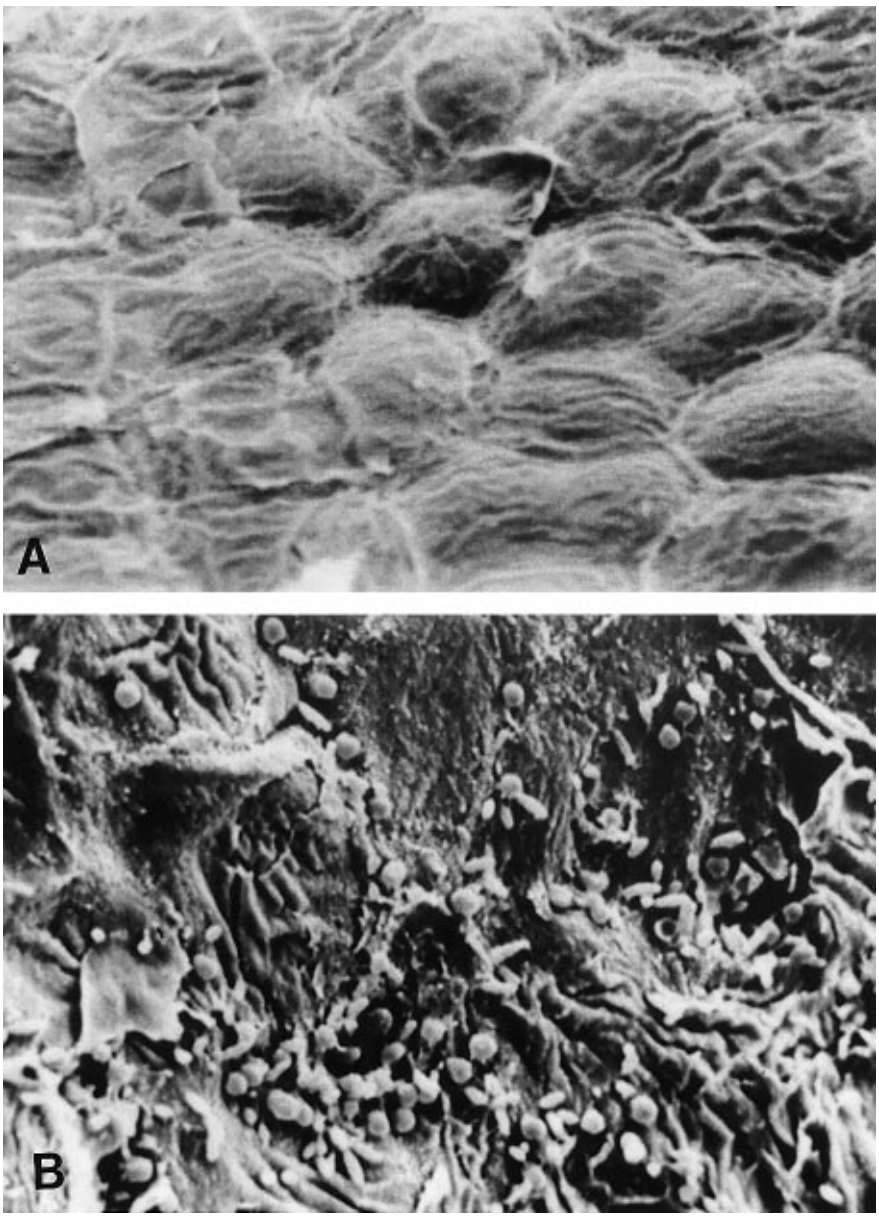


Figure 2. SEM of bacteria on the surface of *Delisea pulchra*. *(A)* Apical tip. *(B)* Base of the central axis near the holdfast. × 5000 magnification (from Maximilien 1995).

furanones. Surprisingly, however, furanones inhibited growth of *Delisea* strains more strongly than for rock strains. Thus even though bacteria isolated from the host plant could attach more readily to *D. pulchra*, their subsequent growth was strongly inhibited. As shown in the model in Figure 3, Maximilien (1995) suggested that by differentially affecting various characteristics of different bacterial strains, the alga could in effect specifically control the abundance and composition of its epiphytic bacterial community.

These studies (e.g., Wahl et al. 1994; Slattery et al. 1995; Maximilien 1995) demonstrate that the effects of metabolites from marine benthic eukaryotes can be considerably more subtle than simple toxicity or growth inhibition. However, these studies do not address the specific mechanisms by which these compounds inhibit particular traits or characteristics. In

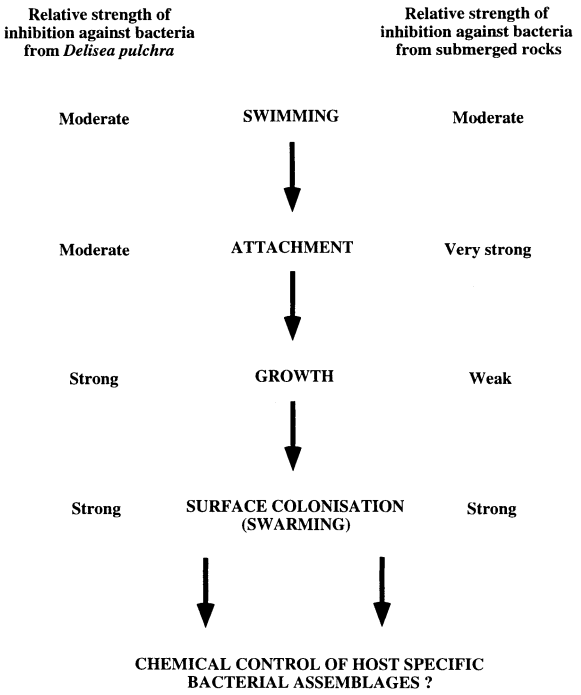


Figure 3. Model, adapted from Maximilien (1995) for the differential effects of furanones from *Delisea pulchra* on specific colonization phenotypes of bacteria isolated either from the surface of *Delisea pulchra* or from the surface of nearby submerged rocks. Maximilien (1995) tested the inhibitory effects of furanones (or, in the case of attachment, a non-polar extract of the alga containing furanones) against four phenotypes (swimming, attachment, etc.), for each of three bacterial strains isolated from rock surfaces, and three strains from *D. pulchra*. As shown, furanones on average inhibited all phenotypes against all strains, but the relative strength of the effects varied considerably as a function of the particular phenotypes tested and whether the strain was isolated from the alga or nearby rocks. Such specific effects may allow the alga (and, by implication, other eukayotes) to 'regulate' its associated bacterial community; that is, to allow for the development of a biofilm with a particular abundance and composition.

order to understand the specific mechanisms underlying these effects, some understanding of the means by which bacteria colonize surfaces – animate or inanimate – is required.

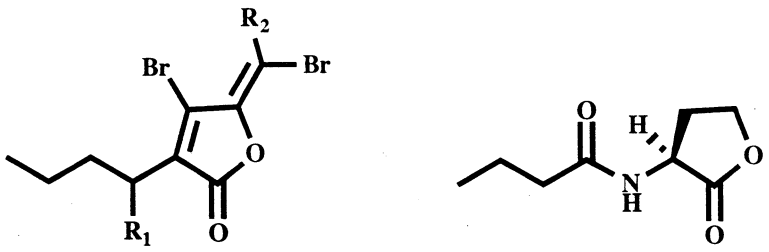
Bacterial adhesion and surface colonization

Bacteria colonize surfaces by a variety of mechanisms. Upon contacting a surface, a range of extracellular structures act as bacterial adhesins, allowing the bacteria to attach to the surface. Adhesins include, for example, proteinaceous fimbriae, which in many cases mediate specific adhesion by virtue of adhesin subunis on the fimbrial stalk (Jann & Hoschutzky 1990). Such adhesins mediate binding to carbohydrate receptors of the surface in a structure specific lock and key fashion. Other extracellular surface structures that regulate adhesion and biofilm processes include exopolysaccharides (e.g., Quintero & Weiner 1995), which can affect adhesion either directly or in conjunction with other molecules presented at the bacterial surface. Exopolysaccharides may also aid in detachment of attached bacteria from the substratum or the biofilm (Wrangstadh et al. 1989). However, exopolysaccharides are probably most important for biofilm growth and development, rather than as adhesins in the initial phase of colonization (Costerton & Irvin 1981).

As well as causing high affinity and irreversible binding of the bacterial cell to the substratum, adhesins may also mediate the induction of novel surface specific responses. For example, lateral flagella mediate irreversible adhesion to surfaces, but also serve as a prerequisite for surface motility, or swarming, which leads to a rapid further colonization of the substratum (Belas et al. 1986; McCarter et al. 1992). The formation of lateral flagella and subsequent swarming motility are surface induced effects, i.e. the bacteria respond to the presence of the solid/water interface, or conditions in the biofilm community, by induction of genes that are not expressed by non-surface associated cells. Several genes and protein responders are known to be induced at surfaces, and thus substratum induced responses and different strategies of genetic control adds to the complexity of the bacterial colonization process (Marshall & Goodman 1994; Dalton et al. 1994).

Is bacterial colonization and biofilm formation on marine eukaryotes mediated by acylated homoserine lactone (AHL) regulated systems?

Given that (a) higher organisms produce secondary metabolites which selectively inhibit specific bacterial phenotypes, and (b) these phenotypes are associated with both specific factors such as adhesins, exopolysaccharides, etc., we hypothesized that chemicals produced by some marine organisms might interfere in specific ways with the regulation or expression of colonization relevant traits. In particular, based on Maximilien (1995) observations of the inhibition of swarming by furanones from *Delisea pulchra*, and on the structural similarity between acylated homoserine lactones and furanones (Figure 4), we investigated



Furanone *N*-butanoyl-L-homoserine lactone (BHL)

Figure 4. Structures of selected furanones from the red alga *Delisea pulchra* and the bacterial signal molecule *N*-butanoyl-L-homoserine lactone (a representative AHL).

whether furanones could inhibit bacterial colonization by virtue of interference with acylated homoserine lactone (AHL) bacterial regulatory systems.

Acylated homoserine lactones (AHLs) are the signal molecules of a regulatory system that many bacteria use to coordinate their colonization and association with higher organisms (reviewed by Fuqua et al. 1994; Swift et al. 1994, 1996). AHLs produced by bacteria diffuse freely in and out of the cells, and upon reaching a sufficient concentration, bind to a protein that thereby becomes a transcriptional activator, inducing expression of different target genes. The gene which encodes the synthase protein responsible for AHL production is positively regulated by the activating protein, and thus the system functions as a positive feedback loop. This positive feedback can result in rapid induction of AHL regulated phenotypes. Moreover, because the organism can assess changes in its own density via changes in AHL concentration, the system has been termed 'quorum sensing' because it provides a mechanism for regulating density dependent multicellular behavior in bacteria. However, regulation by homoserine lactones may also occur, in a density independent fashion, at low cell densities (Ulitzur, submitted). This observation has important implications for colonization of surfaces by individual cells. Homologous AHL regulatory systems, in which the transcriptional activators and autoinducers are closely related but regulate different phenotypes, are known from a diverse range of Gram-negative bacteria. Phenotypes regulated by these systems include traits directly involved in colonization, such as conjugation in *Agrobacterium tumefaciens*, swarming, production of exoenzymes, and other traits such as bioluminescence (Fuqua et al. 1994; Swift et al. 1994, 1996). With regards to the corrosion of artificial surfaces, it is interesting to note that degradation of plant tissue by exoenzymes of the pathogen *Erwinia carotovora* is also under AHL regulation (Salmond et al. 1994; Chatterjee et al. 1995).

Because of the importance of AHL regulatory systems to bacterial colonization processes, such systems might be expected to be broadly important to bacterial/eukaryotic interactions. Marine or other aquatic habitats are of particular interest in this regard, as the aqueous medium is ideal for the dispersal of bacteria and chemical signals. Moreover, bacteria are common on the surface of marine eukaryotes, and can have a profound effect on their hosts. Thus from an evolutionary perspective, we would both expect marine eukaryotes to have evolved mechanisms that affect particular colonization traits in associated bacteria, and to have evolved the ability to interfere with specific regulatory processes of bacteria.

Furanones do specifically (e.g., with no other detectable phenotypic effects) inhibit a number of AHL regulated traits. These include swarming by *Serratia liquifaciens* (Givskov et al. 1996), exoenzyme production by *S. liquifaciens* (Gram et al. submitted), and bioluminescence in *S. liquifaciens* harboring the boluminescent monitor plasmid pSB403 (Givskov et al. 1996). For bioluminescence and swarming, there is structure/function specificity of different furanones (M. Manefield, unpublished). Evidence from our laboratories further indicates that furanones specifically interfere with other AHL regulated traits that are particularly relevant to attachment and subsequent degradation of surfaces, such as exoenzyme production in *Erwinia carotovora* and *Pseudomonas aeruginosa* (Kjelleberg, in press). Based on these and related experiments, we suggest that furanones may inhibit AHL processes by competitively binding to the AHL

receptor protein (the 'R' protein), thereby displacing AHLs.

Much less is known about the regulation of attachment, surface spreading, or degradation of surfaces by marine bacteria when compared to model strains such as *Serratia liquefaciens*. However, as well as inhibiting specific traits of marine bacteria that are AHL regulated in other bacteria (e.g., swarming), crude nonpolar extracts of *Delisea pulchra* (of which about 50% is furanones) also inhibit attachment of ecologically relevant bacteria when the bacteria are pre-incubated with the extracts, the cells washed, resuspended, and allowed to colonize on uncoated plastic surfaces (Maximilien, R., Crass, K., unpublished). These results suggest that furanones specifically interfere with metabolic processes that affect attachment.

From ecology to biotechnology

Most of the research on the attachment of bacteria to surfaces in marine environments has focused on artificial surfaces, and remarkably little is known about the specific mechanisms by which bacteria colonize marine eukaryotes, or whether bacterial attachment mechanisms on living vs. non-living surfaces are similar. Our research indicates that furanones inhibit bacterial colonization on both living and non-living surfaces, presumably by inhibiting the production of adhesins or other colonization relevant traits. However, the extent to which the concepts arising from our work can be used to develop novel technologies for inanimate surfaces depends on the specific means by which bacteria colonize and attach to living vs. non-living surfaces. For example, some bacteria have cell surface chemistry that can allow for attachment even if adhesins are removed from the cell envelope (Cowan & Fletcher 1987). It is not known if these cell surface biopolymers are regulated in the way we propose for adhesins, or whether attachment in this case is a purely physiochemical process. Thus an obvious area of future research stemming from our work is the analysis of adhesins or other cell surface polymers responsible for attachment. This could be done via investigations of bacterial 'footprints' (Neu & Marshall 1991) which remain on surfaces after attached bacteria are removed. For example, are such footprints the same on living vs. non-living surfaces? Do they change in composition when bacteria are preincubated with furanones? Preliminary investigations of 'footprints' (Neu & Marshall 1991) suggest that a range of cell surface materials are contained in these structure, but very little is known about the role of each component in atttachment, let alone their regulation.

Similarly, we know that furanones down regulate the production of various exoenzymes. Do they also inhibit the production of extracellular macromolecules that bacteria use to adsorb metal ions from metallic surfaces, and which leads to the corrosion of those surfaces? If these macromolecules are identified and quantifiable, then their specific inhibition by furanones or other signals could be studied.

The ability of eukaryotes to interfere with AHL regulated systems is only now being recognized. There is considerable potential for AHL regulation to play an important role in ecological interactions between eukaryotes and prokaryotes, as well as in prokaryote/prokaryote interactions (Kell et al. 1995). By excreting a range of metabolites, eukaryotes may be able to regulate phenotypic expression by a wide range of associated bacteria, as suggested by the model in Figure 3. We have begun to explore interference (inhibition) with these systems, but given the wide range of bacterial symbioses in the sea, facilitation of AHL regulation by eukaryotes is also obviously a possibility. Moreover, AHL signalling systems are by no means the only possible ways to 'talk' to bacteria on surfaces. Chemical signalling systems are widespread in nature, and it is likely that there are numerous such systems used by marine prokaryote/eukaryote associations which may also be relevant to the applied problem of inhibition of biofilms on artificial surfaces.

Given the diversity and potential for evolutionary change of micro-organisms, it is perhaps not surprising that living axenic surfaces are rare in the sea. However, there is enormous variation in the abundance, species composition, and activity of biofilms on the surface of eukaryotes, and we have suggested above that at least some organisms can specifically control a variety of characteristics of their associated epiphytic bacteria – to the considerable benefit of the host. Similarly, we suggest that an emphasis on the complete elimination of bacteria from artificial surfaces in aquatic systems may be inappropriate. Rather, perhaps we should be focussing on the selective regulation of bacterial communities and characteristics on synthetic surfaces. The ability to achieve such control would be of considerable benefit to marine (and other) industries.

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