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Emerging Strategies for the Chemical Treatment of Microbial Biofilms

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Introduction

Over the past decade, several strategies have been proposed for the control of surface-associated microbial populations. Physical methods, including electrification, ultrasonication, application of ablative laser light and mechanical cleaning or scraping are generally effective at removing surface growth. Chemical control methods, on the other hand, are often ineffective. This has led to a notorious association of biofilms with resistance towards antibiotics, biocides and disinfectants. In such respects, reaction-diffusion limitation of the passage of oxidizing biocides and antibiotics, across biofilms aided by the presence of extracellular enzymes often causes the failure of such agents to sanitize contaminated surfaces. Deep-lying cells within biofilms are often also severely nutrient- and oxygen-limited, causing the expression of starvation phenotypes, which include multi-drug efflux pumps and enhanced exopolymer synthesis. During exposure to antimicrobial agents, these slow growing organisms, being exposed to sub-lethal levels of agent, will generally out-survive their less nutrient-depleted congeners. This may well enrich the population for drug resistant phenotypes and genotypes during the post-treatment phase.

Emerging biofilm treatment methodologies are based on our knowledge of biofilm physiology and resistance mechanisms. For example, in an attempt to prevent early colonization of surfaces and in order to overcome reaction-diffusion limitation, treatment agents may be coated onto or incorporated into the substrate to be protected. More sophisticated approaches have been developed, with varying success, that deploy erodable, biocide-containing coatings. Erosion, in this instance being intended to purge the surface of attached bacteria and cellular debris. At the vanguard of emerging control strategies are surface-catalyzed hygiene and anti cell-cell signalling chemicals.

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Abbreviations: EPS, extracellular polysaccharide; CDFF, constant depth film fermentor; CIP, clean in place; SDS, sodium dodecyl sulphate.

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THE BIOFILM LIFESTYLE

Bacteria may colonize almost any surface in an aqueous or humid environment. Microbial biofilm phenotypes have probably evolved as a general survival strategy. Medical biofilms have been associated with a majority of chronic infection scenarios (Costerton *et al.*, 1987) and, being associated with fouling and corrosion of plant and pipework, are a significant problem in many industrial settings (Characklis, 1990; Little *et al.*, 1990). In food processing, biofilms formed on food contact surfaces contribute substantially both to the contamination and spoilage of product (Holah *et al.*, 1994; Eginton *et al.*, 1998). In all of these aspects, microbial biofilms exhibit a broad spectrum of resistance to antimicrobials and may be 100–1,000 times less susceptible than their free-living counterparts. Such resistance is demonstrated not only towards antibiotics and antiseptics, but also towards highly reactive chemical biocides. The latter include isothiazolones (Costerton and Lashen, 1984), halogens and halogen-release agents (Favero *et al.*, 1983), and quaternary ammonium compounds (Costerton and Lashen, 1984; Evans *et al.*, 1990).

Physiologically, biofilms are functional consortia of microbial cells enveloped within matrices of extracellular polymers (glycocalyx) and the concentrated products of their own metabolism. These include ions and nutrients sequestered from the general environment, together with extracellular enzymes such as lyases, proteases, and ß-lactamases. In the majority of natural habitats, the consortia comprise a variety of species and genera, whilst in biomedical situations, monocultures are more common.

A plethora of phenotypes is represented for each component biofilm species, the breadth of which reflects the extent of chemical and structural heterogeneity within the film (Gilbert *et al.*, 1990). The outcome of any antimicrobial treatment will, therefore, reflect the susceptibility of the most resistant phenotype within the consortium. As biofilms mature and exopolymer deposition increases, the magnitude of the nutrient and gaseous gradients within them will become increased, and the net growth rate of the community will become further reduced. This may possibly bring about the onset of dormancy in the cells and trigger the expression of stringent response genes (Zambrano and Kolter, 1995). The latter might, in turn, initiate escape mechanisms whereby the production of polymer-lyase enzymes is increased and the biofilm community is dispersed (Willcock *et al.*, 1997).

Current strategies for the control of microbial biofilms involve the design of antimicrobial agents that are specifically targeted towards the biofilm-specific phenotype or which chemically degrade the glycocalyx. Such approaches have, to date, met with limited success and the need to develop efficient, low cost, hygienic cleansing systems remains as urgent as ever. To aid the search for novel antimicrobial targets, there is a need not only to develop our knowledge of biofilm physiology, but also to examine the various mechanisms associated with resistance development within biofilm communities. In this article we evaluate emerging biofilm control strategies in the context of our current understanding of biofilm physiology and resistance mechanisms.

Why are biofilms so resistant to antimicrobial agents?

Explanation of the generalized recalcitrance of microbial biofilms towards antimicrobial agents centres on three distinct themes: (i) that the presence of an abundant

extracellular matrix hinders the access of antimicrobial agent to the deeper lying cells; (ii) that the close proximity of cells generates extreme nutrient limitation within the community and the expression of dormant, recalcitrant phenotypes; and (iii) that the cells within a biofilm alter their phenotype in response to the proximity of a surface or other cells to one that is less affected by conventional biocides and antibiotics. Whilst there is evidence in support of all three explanations, none of these can be the sole mediator of resistance. More likely, the recalcitrance of biofilm communities towards inimical treatments relates to a combination of all three.

ANTIMICROBIAL PENETRATION FAILURE

Electron microscopy shows that extracellular polysaccharide (EPS) forms a matrix in biofilms that is comprised of an ordered array of fine fibres. These arrays provide a relatively thick coating to the cells and are primarily composed of gelled, highly hydrated, extracellular polysaccharides (Sutherland, 1997). Up-regulation of extracellular polymer biosynthesis generally occurs within minutes of the irreversible attachment of a cell to a surface (Allison and Sutherland, 1987; Davies *et al.*, 1995). Deposition of EPS within the developing microcolony then proceeds over a period of hours. Such data suggest the existence of 'touch-sensors' in many bacterial species. Recent work (Davies *et al.*, 1998) utilizing strains of pseudomonas that were deficient in homoserine lactone-mediated quorum sensing suggested that up-regulation of EPS synthesis in a biofilm, and presumably on a surface, is mediated through chemical cell-cell signals. Mutant strains produced biofilms that were devoid of EPS and co-incidentally susceptible to dodecyl sulphate treatment.

With respect to biofilm resistance, EPS associated diffusion limitation has a profound influence on the penetration of strongly charged antimicrobial agents (ie tobramycin) or biocides (ie quaternary ammonium compounds) that bind strongly to anionic groupings within the EPS matrix. In order to access deeply embedded cells, either all of the binding sites within the matrix must be saturated or the affinity of cells for that antimicrobial agent must exceed that of the agent for the matrix. This intuitive explanation of biofilm resistance towards antimicrobials relates entirely to physical exclusion of the agents and is unlikely to account for resistance towards uncharged molecules (Costerton *et al.*, 1987; Slack and Nichols, 1981; Suci *et al.*, 1994). If, however, the treatment agent is chemically highly reactive, then it may covalently associate with the glycocalyx and be quenched. Such effects will be most pronounced for the oxidizing biocides that include iodine and iodine-polyvinylpyrollidone complexes (Favero *et al.*, 1983), chlorine and peroxygens (Huang *et al.*, 1995). All of these are notable for their lack of effect against thick, mature biofilms. Reaction-diffusion limitation such as this might also be brought about for less reactive molecules where inactivating enzymes, such as ß-lactamases (Giwercman *et al.*, 1991), formaldehyde lyase or formaldehyde dehydrogenase (Sondossi *et al.*, 1985), within the glycocalyx bring about the neutralization of the treatment agent. In these instances, enzyme degradation can lead to severe antibiotic penetration failure, provided that turnover is sufficiently rapid, (Stewart, 1996). In this respect, it is interesting that hydrolytic enzymes such as ß-lactamases are induced, or de-repressed, in adherent populations, and in populations exposed to sub-lethal concentrations of imipenem and/or piperacillin (Lambert *et al.*, 1993; Giwercman *et al.*, 1991). These

enzymes become trapped and concentrated within the biofilm matrix and further impede the action of susceptible antibiotics. Such effects would be additional to the loss, through irreversible binding to the matrix, of highly charged drug molecules such as the glycopeptides (Hoyle *et al.*, 1992).

In all of these instances, long-term resistance of the population will occur only if the bulk phase is depleted of antimicrobial. Whilst this might occur in single applications of antimicrobial to a surface, or where the exposure is transient (ie in clean in place (CIP)). It is unlikely to relate to antimicrobial therapy where the volume distribution of the drug (*ca.* 40 L) vastly exceeds the sink-capacity of the infection site. In such instances, the reaction limitation of access is likely only to delay the antibacterial effect for the deeper lying cells.

SLOW GROWTH RATE AND BIOFILM RESISTANCE

A major contributor towards biofilm drug resistance is associated with physiological gradients of growth rate and nutritional status within the biofilm. In much the same way as reaction-diffusion limits the access of biocides across the glycocalyx, the consumption of nutrients by the biofilm community will restrict their availability to the underlying cells. Thus, within the depths of a biofilm, growth rates will generally be suppressed relative to cells growing planktonically in the same medium or at the surface of the film. In this respect, workers have used perfused biofilm fermentors (Gilbert *et al.*, 1989), in conjunction with continuous culture, to evaluate the separate contributions towards resistance, of growth rate and the expression of biofilm-specific phenotypes. Using such techniques, much of the resistance of both Gram-positive and Gram-negative biofilm communities towards a wide variety of antibiotics and biocides can be attributed to the existence of physiological gradients of growth rate and the presence of a variety of nutrient-depleted phenotypes (recently reviewed by Gilbert and Allison, 1999). The advent of laser confocal microscopy has enabled the extent of these physiological gradients within biofilm communities to be elegantly visualized (Stewart *et al.*, 1993). Whilst the contribution of reduced growth rate within biofilm communities cannot be denied, as with diffusion-limitation it cannot be the sole explanation of resistance. Cells on the periphery of the biofilm will be exposed to similar nutrient fluxes as the planktonic cells. If growth rate were the sole determinant of susceptibility, then these cells would be readily killed by the application of a treatment agent. Co-incident with their death would be an increased flux of nutrients to the underlying cells. These cells would grow faster, adopt a more susceptible phenotype and die. The lysed products of the killed cells would, in turn, feed the cells within the depths of the biofilm. Whilst such processes might delay the onset of killing in the recesses of the film, they could not confer resistance to a sustained exposure to antimicrobial.

BIOFILM-SPECIFIC, RESISTANT PHENOTYPES

Not only can bacteria sense the proximity of a surface and up-regulate production of extracellular polymers, they can also alter in their susceptibility towards antibiotics (Ashby *et al.*, 1994) and biocides (Das *et al.*, 1998) shortly after binding to a surface (indicated by a loss of Brownian motion). Such changes are relatively minor in their

extent and are associated with only small changes in growth inhibitory concentration (2–4-fold increase). As such, these changes are insufficient to account for the reported levels of resistance in biofilm communities. The possibility exists, however, that the changes are mediated through the accumulation of signal substances, such as homoserine lactone, at the occluded surface (Davies *et al.*, 1998).

Antibiotic resistance in biofilms: phenotypic and genotypic selection

The explanations of biofilm recalcitrance discussed above are either insufficient in the extent of resistance conferred or serve only to delay the effects of the treatment agents to the underlying cells. In order to demonstrate resistance to sustained exposure to antimicrobials, the biofilms must adapt their phenotype during the early phases of treatment. This might involve the expression of multi-drug efflux pumps or the selection and propagation of particular clones or species within the community. A major consequence of the resistance of biofilms, however mediated, is that some cells within the community will be exposed to the antimicrobial over a prolonged time scale. During exposure to biocides and antibiotics, the selection pressures on the community will shift from cells and cellular associations that are most competitive in their handling and acquisition of the depleted nutrients to those that are least affected by sub-inhibitory levels of the treatment agent. Such exposure might cause the induction or enrichment of more tolerant phenotypes. The expression of multi-drug resistance operons, (ie *mar)* and efflux pumps (ie AcrAB) are up-regulated during the exposure of cells to sub-effective concentrations of antibiotics, such as tetracycline and chloramphenicol, and to xenobiotics, such as salicylate, chlorinated phenols, etc. (George and Levy, 1983; Levy, 1992; Ma *et al.*, 1993). Under such circumstances, cells that are constitutive for the expression of such genes can be isolated at a relative high frequency. Such selection pressures would occur within a treated biofilm during the period when reaction-diffusion limitation and growth rate conferred a survival advantage on the underlying cells. The induction, of *mar* or its equivalents, during the delayed onset of the action of inducer-antibiotics is, therefore, a plausible explanation of the long-term resistance of biofilms. The importance of *mar* would be far greater, however, if it were induced by growth as a biofilm *per se,* and conferred a more resistant phenotype upon the cells prior to exposure. Ciprofloxacin exposure does not induce the expression of *mar* or AcrAB in *Escherichia coli* but such expression confers a limited degree of protection against the agent. Exposure to ciprofloxacin of biofilms comprised of wild-type, constitutive and *mar*-deleted strains ought to evaluate whether or not such genes were up-regulated in unexposed biofilm communities. Maira-Litran *et al.* (1998) perfused biofilms of such *E. coli* strains for 48 h with various concentrations of ciprofloxacin. Whilst these studies confirmed a reduced susceptibility of biofilms of the *mar* constitutive strain, they also demonstrated little difference between wild-type and *mar*-deleted strains. Similar experiments, using biofilms constructed from strains in which the efflux pump AcrAB was either deleted or constitutively expressed (Maira-Litran, 1998; Maira-Litran *et al.*, 1999), showed the AcrAB deletion did not significantly affect susceptibility. Clearly, neither *mar* nor AcrAB are induced by sub-lethal treatment of biofilms with anything other than the appropriate inducers. Further studies have demonstrated that constitutive expression of AcrAB protects the biofilm against low

concentrations of ciprofloxacin and that the expression of *mar* and its target genes is inversely related to specific growth rate (Maira-Litran *et al.*, 1999). Hence, following exposure of biofilms to sub-lethal levels of ß-lactams, tetracyclines, salicylates or other inducer substances, *mar* expression will be at its greatest within the depths of the biofilm, where growth rates are most suppressed. This will provide an additional survival advantage to these cells. Other multi-drug efflux pumps, under the regulation of different inducing agents, might extend this explanation of biofilm tolerance to include other treatment agents.

Strategies for the control and treatment of biofilms on surfaces

From the foregoing, it would appear that established biofilms are particularly recalcitrant to chemical hygienic measures. Therefore, there has been considerable interest in the development of strategies that may prevent the initial attachment process and therefore arrest biofilm formation at an early stage. Since a major contributor to the resistance of treated biofilm is an inability of agents to traverse the exopolymer matrix in sufficient concentration as to affect the deeper-lying, slow-growing cells, the majority of approaches to the control of biofilms has been to deliver the agent from the colonized surface. Alternative approaches aim to prevent the formation of the biofilm, either by modifying those surfaces to be intrinsically biocidal, or by the development of anti-signalling molecules that interfere with induction of the biofilm phenotype.

BIOCIDE-IMPREGNATED SURFACES AND SURFACE COATINGS

Incorporation of biocides into various substrata has been attempted in the hope of developing intrinsically colonization-resistant materials. In industrial applications, such approaches have been associated with varying degrees of failure. Important limitations are that such approaches provide for a reservoir of biocide within the surface material that will become depleted during its use, and that leaching of the agent to the general environment might provide for a selection pressure towards resistant bacteria. Furthermore, the surface killing effect may be overcome when conditioning films coat the surface and when drug resistant or killed cells occlude the surface and may themselves be colonized. Losses of biocide from such surfaces relate to their solubility and effective concentration. Organo-silver complexes and silver ions have therefore been variously investigated as anti-biofilm coatings since they are highly active and possess very low water solubilities. In this respect, Rogers *et al.* (1995) studied the efficacy of silver-coated surfaces to resist colonization by a consortium of organisms, which included *Legionella pneumophila.* Although the biocide slowed down the initial colonization, it failed to prevent biofilm formation. One explanation for this was that pioneering organisms, such as *Methylobacterium* spp. and *Pseudomonas* spp., had formed a protective layer of cells on to which less resistant species could establish. It seems likely that, in situations where there is a constant challenge with microorganisms, simple biocidal surfaces are unlikely to prove effective.

From a biomedical perspective, the performance of silver-coated urethral catheters has also been disappointing. A large clinical trial of a silver-coated indwelling device

(Riley *et al.*, 1995) failed to demonstrate efficacy in preventing urinary tract infection, and vascular catheters impregnated with silver sulphadiazine and chlorhexidine completely lost their antibacterial activity after 10 days of use (Schmitt *et al.*, 1995). The evidence that these catheters resist bacterial colonization is therefore suspect (Stickler and Winters, 1994; Stickler, 1999). Such catheters are challenged with microorganisms throughout their implantation. Indwelling medical devices, on the other hand, are only at risk from microorganisms during, and immediately after, implantation. There will, therefore, only be a minimal opportunity for early colonizers to deplete the antibacterials. Subsequent diffusion and loss of the agents will be inconsequential. It is therefore conceivable that impregnation with the correct antibiotics may prove efficacious. Bayston (1995) impregnated silicone shunts with a range of antibiotics, and investigated their ability to resist bacterial colonization *in vitro*. The catheters were challenged with single doses of *ca.* 10^7 sensitive staphylococci and coryneforms and were perfused with liquid culture medium for 14 days before being examined for colonization. Whilst trimethoprim, clindamycin, spiromycin, and sodium fusidate-impregnated catheters did not resist colonization over this time period, those treated with rifampicin or combinations of rifampicin with either trimethoprim or clindamycin, did. These catheters were re-challenged and by day 28, only those catheters that had been impregnated with clindamycin and rifampicin in combination were able to resist colonization. These also went on to resist colonization by a third challenge. Such a period of protection was suggested to be sufficient to eliminate nosocomial infections associated with the implantation (Stanton and Bayston, 1999).

Such approaches are unlikely to be appropriate for all but short-term use of indwelling urinary catheters, where the likely contaminants will be Gram-negative. In this respect, other workers have reported that silicone catheters treated with ciprofloxacin failed to resist colonization by sensitive strains of *Proteus mirabilis, P. aeruginosa, Escherichia coli* and *Providencia stuartii* over 48 h exposure periods (Stickler *et al.*, 1994). It seems likely that, in clinical situations, the efficacy of surface-coated devices may be compromised by antibiotic-resistant bacteria, together with the barrier effect of conditioning films that rapidly coat the biomaterials *in vivo* (Stickler, 1995).

BIOCIDE-CONTAINING ERODABLE SURFACE COATINGS

Since many of the attempts to provide for colonization-resistant surfaces through the incorporation of leachable biocides within the materials that comprise or coat them have failed because of the formation of protective conditioning films and cellular debris, self-cleaning biocidal materials were an attractive proposition. One way of achieving a self-cleaning surface is to make it ablate during exposure to fluid dynamic forces. Such ablation might not only remove residual attached cells, but it might also release further biocide.

Cooksey *et al.* (1992) reported some success in preventing biofouling of marine surfaces using ablative, biocide-containing surface coatings where the biocide was incorporated into a soluble matrix-coat and was released by dissolution into the biofilm. The fluid dynamic forces on the surface to which the coat matrix was exposed dictated the extent of biocide dissolution. Self-polishing polymers, such as organo-tin

acrylates, will slowly hydrolyse in water at a rate that is independent of fluid dynamics and, in doing so, will release the incorporated biocide at the rate of hydrolysis (Holmstrom and Kjelleberg, 1994).

A further patented (patent WO 97/05182; PCT/GB96/01617) development of hydrolysis driven ablation provides for a novel biocide-containing coating material where the release of the hydrophobic, quaternary biocide is driven by the attachment of bacterial cells. Release of the biocide exposes sites within the polymer matrix which may then be hydrolysed, causing the depleted matrix to ablate. This was intended not only to release the attached cellular debris, but also to expose new biocide reservoirs within the coating. Ablation in this instance was therefore driven indirectly by the attachment of bacteria. Suzanger *et al.* (1999) studied a number of these novel polymer coatings, containing various levels of three quaternary ammonium biocides. These workers found that there was an initial rapid release of biocide from the coatings over the first few days of submersion in water. Sadly, this related not only to losses of poorly incorporated biocide and monomer, but also to much of the bound material. This rapid loss was complete within *ca.* 5 days. It was concluded that, although the technique had much potential, more development work is necessary in order to refine the release properties of the coating materials.

TURNING THE TABLES ON BIOFILMS: SURFACE CATALYZED HYGIENE

Wood *et al.* (1996) have developed an alternative approach to the delivery of active biocide to the colonized surface whereby biocide is generated *in-situ* by catalysis from a relatively innocuous treatment agent at the colonized surface. Importantly, surface catalyzed hygiene, as it has been termed, effectively overcomes the reaction-diffusion limitation imposed on oxidizing biocides by biofilm physiology. Since the catalysts are not consumed during the generation of active agent, the addition of further treatment agent to the exterior of the biofilm will replenish the biocidal action at the substratum. Further, with peroxides and persulphates as the treatment agents, the treatments resulted in a significant weakening of the attachment of the biofilm and promoted its release from the surface.

One application of this strategy incorporates transition metal catalysts such as cobalt phthalocyanine and copper phthalocyanine into the material that comprises the target surface. Catalysts such as these break down peroxides and persulphates to liberate active oxygen species. Susceptibility of *P. aeruginosa* biofilms towards potassium monopersulphate and hydrogen peroxide was enhanced significantly in all cases where such catalysts were incorporated in a trylon resin coupon. In all instances, catalyzed killing of biofilm bacteria occurred at lower concentrations of treatment agent than were required for killing planktonic bacteria. Cobalt sulphonated phthalocyanine was more effective both as a catalyst for the decomposition of peroxide and as a hygiene enhancer than was copper sulphonated phthalocyanine. Significant improvements in delivered hygiene were apparent even with the relatively thick biofilms (100 μm) (Wood *et al.*, 1998). Improved hygiene occurred not only through a concentration of active species at the biofilm-substratum interface but also through the creation of a catalysis-driven diffusion pump. The latter increased the flux of treatment agent across the biofilm and provided enhanced penetration and killing throughout the depths of the biofilm. Provided that catalytic activity is maintained and

fresh treatment agent is available, such an approach ought to continually provide active biocide to the interactive surface between the biofilm and the substratum. Importantly, this technique overcomes the problems of biocide depletion since the catalyst is not depleted. It was noted that the formation of oxygen radicals at the surface interface had a direct effect upon the visco-elastic properties of the exopolymer matrix and actively facilitated its removal (Wood *et al.*, 1998).

Suggested developments of this approach have been to utilize enzymes or enzyme combinations rather than inorganic catalysts. For example, if enzyme combinations such as glucose oxidase and haloperoxidase were coated onto tooth surfaces or upon oral prostheses, then hygiene might be delivered by the body's own supply of treatment agent in the form of glucose and chloride. Significant enhancements of this form of oral hygiene might then be obtained by sucking a boiled sweet.

Conclusions

Microbial biofilms are simultaneously fascinating to the microbial physiologist, yet troublesome to industry and medicine. Much of this fascination and concern relates to their recalcitrance towards even the most aggressive antimicrobial treatments. Considerable research effort has gone into the development of new strategies for prevention and control of such biofilms in a wide variety of situations. Physical control methods, whilst effective, are frequently impracticable and consequently, antimicrobial agents, preservatives and antibiotics have remained a primary treatment tool. Most antimicrobial agents have however, been developed for their activity against fast-growing planktonic cultures and are only poorly active against slow-growing sessile phenotypes. Biofilms are especially drug resistant for a variety of reasons, each of which have become targets for the development of novel treatment agents/strategies.

To date, there have been only limited developments that may specifically address the problem of biofilm formation and control. Biocide-containing surfaces have demonstrated some success in controlling the formation of biofilms in limited situations. A necessary part of their effectiveness is however, that they release biocide locally. Whilst this local concentration of biocide might protect the surface, it is only short-lived and inevitably leads to the exposure of microorganisms remote from the surface, or deep within the biofilm, to a continuous, sub-lethal level of agent. There is currently much concern that such exposures may lead not only to losses in the effectiveness of the incorporated agents, but also to indirect effects upon the activity of third-party therapeutic agents (McMurry *et al.*, 1998a; McMurry *et al.*, 1998b). Whilst ablating, biocide-containing surfaces can to some extent, overcome the problems associated with fouling of the substratum, they require further development and will still suffer from associated problems of chronic biocide release at low levels to the environment. Surface catalyzed hygiene, on the other hand, is particularly promising since it enables relatively innocuous treatment agents to be deployed, and does not challenge the environment with potential inducers of drug and biocide resistance.

Another promising strategy is the use of anti-signalling chemicals to interfere with biofilm quorum sensing mechanisms. Davies *et al.* (1998) showed that signalling mutants of *P. aeruginosa*, deficient to varying extents in homoserine lactone, produced biofilms that were devoid of extracellular polymeric materials. In contrast to wild-

type biofilms, these were sensitive to sodium dodecyl sulphate (SDS). This inferred that homoserine lactones are involved not only in the adoption of attachment phenotypes but that they are also involved in regulation of biofilm exopolymers. Previously, Gram *et al.* (1996) had demonstrated the use of naturally occurring anti-signals to interfere with the normal swarming motility of *Pseudomonas mirabilis,* also regulated through homoserine lactone. This discovery of anti-quorum-sensing chemicals such as the furanones (de Nys *et al.*, 1995; Givskov *et al.*, 1996) heralds the possibility of preventing adoption by bacteria of the biofilm phenotype, thereby preventing resistance expression, regardless of its cause. Such bacteria ought then to succumb to old-established antibiotics and biocides.

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