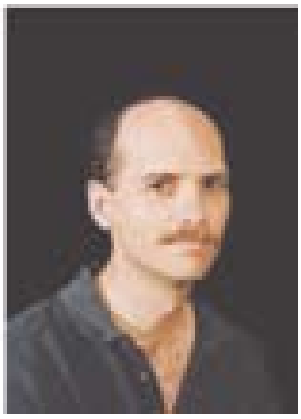
The slippery business of slime control



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What do that scummy feeling on your teeth in the morning, corrosion of oil and gas pipelines, and the slick film building up on the mystery food in the back of your refrigerator have in common? They are all manifestations of microbial slime, which is the result of bacteria growing as a community on a surface held together in an extracellular matrix. These are more commonly referred to as biofilms, which we constantly encounter in everyday life. Bacteria seem to favour growing as biofilms because this provides a range of distinct advantages, including better access to nutrients that partition to surfaces, protection from external stresses such as UV and inhibitors produced by competing microorganisms, and resistance to protozoan grazing that is otherwise one of the main mortality factors for bacteria.

Biofilms are essential for the wellbeing of the planet by driving biogeochemical cycles and other biotransformation processes. They are also important for a range of industrial processes, such as wastewater remediation and bioconversions, and in medical settings they are estimated to cause more than 70% of all infections [1]. Biofilms on implants such as artificial joints, catheters, and even contact lenses are of particular concern. Because of the ubiquity of biofilms and their impact, both positive or negative, there is a real need to be able to manipulate this behaviour of bacteria to our advantage. This is perhaps most evident when infections cannot be cleared by current practices or when significant damage to infrastructure is caused, such as microbially induced corrosion of oil pipelines as highlighted by the 2006 shutdown of the Alaskan pipeline from Prudhoe Bay. However, we now know that such problems are not effectively

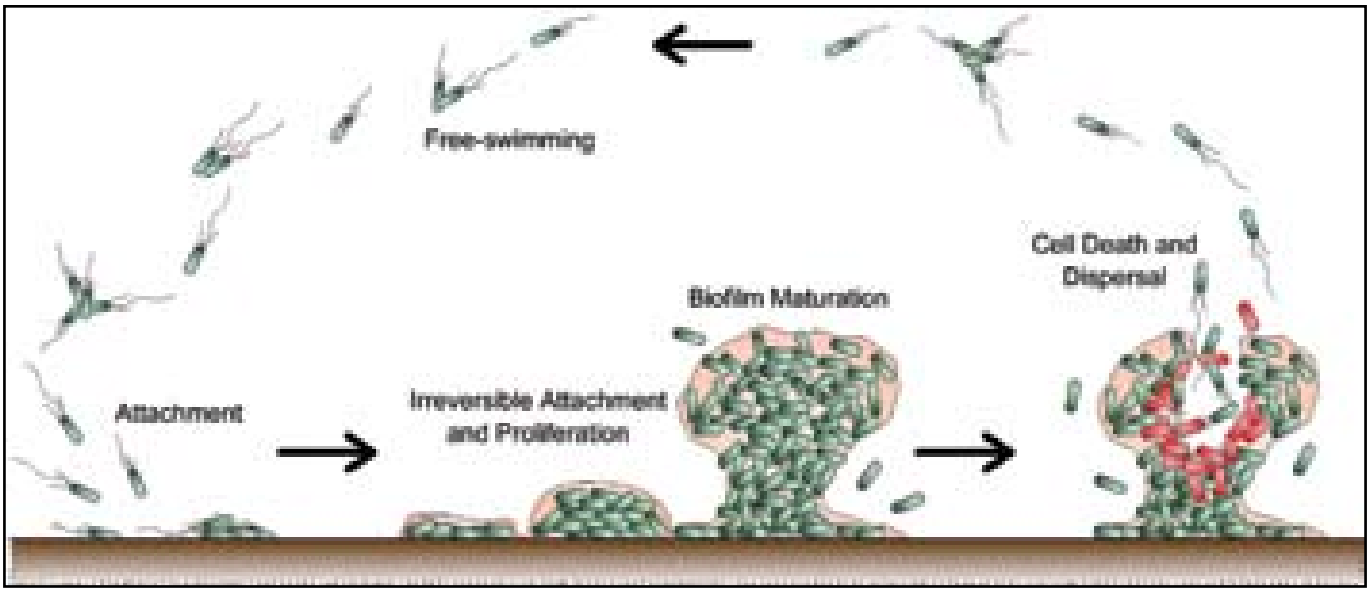

Figure 1. Biofilm lifecycle. Bacteria begin as planktonic cells and the initial attachment is reversible. Irreversible attachment is followed by the maturation stage, where the biofilm develops into a three-dimensional structure (microcolony), often taking on a mushroom- like appearance complete with cells making up the cap of the microcolony. As the biofilm ages, some cells within the centre of the microcolony begin to die and others become motile and disperse as planktonic cells to seek out and colonise new sites, completing the cycle (drawing courtesy of Dr Nicolas Barraud).

dealt with by treating the infection or a pipeline with antibiotics or biocides, because bacterial biofilms can be up to 1000 fold more resistant to such treatments than their free living brethren [2].

The life cycle of biofilms has now been explored in some detail by many laboratories and shown to progress through a defined series of steps (Figure 1), beginning with attachment and ending with release of the bacteria back into the free living phase. This life cycle is regulated and there are specific genes involved in biofilm formation, suggesting that bacteria respond to environmental cues and engage in intercellular communication to accomplish these various stages of multicellular living. It is an emerging realisation that effective biofilm control measures most likely will be derived from understanding the genes and physiological processes that underpin biofilm formation and persistence.

The biofilm life cycle

Biofilms may be composed of single species, or more commonly, multiple species living together in a high density environments. During the maturation phase, where the biofilms can develop into dense, organised three-dimensional structures (Figure 2), the community may experience diverse physiological conditions depending where they are located within the biofilm. For example, steep oxygen gradients have been demonstrated where the inner regions are anoxic compared to the oxygen rich exterior surface of the biofilm [3]. We have observed that, during the dispersal stage of the *Pseudomonas aeruginosa* biofilm life cycle that takes place at the time mature microcolonies have been formed, reactive nitrogen species (RNS) accumulate in the centre (low oxygen region) of the microcolonies and this corresponds to the regions from which dispersal begins [4]. Furthermore, the exogenous addition of RNS generators to an established *P. aeruginosa* biofilm can induce dispersal. DNA microarray studies have shown that the addition of RNS generators to biofilms affects the expression of genes that are involved in the control of cyclic-di-GMP levels in the cells (Barraud *et al;* submitted). It has been shown that proteins that respond to or control cyclic-di-GMP concentrations also regulate dispersal in *Pseudomonas putida* [5]. These data suggest that RNS act as a metabolic signal to regulate whether the bacteria should adopt a surface associated or planktonic lifestyle. Indeed, we have shown that RNS can induce dispersal in a wide range of bacteria [15] suggesting that it may be a more general signal regulating this lifestyle switch.

It has been estimated that nutrients diffusing into and waste products diffusing out of the biofilm, establish strong gradients, which could lead to a situation where the interior of the biofilm is nutrient depleted. Based on this, researchers have sought to test the effect on dispersal by manipulating nutrient concentrations. There are now several reports on the effect of nutrient conditions on biofilm formation, especially the three-dimensional structure formation. It has been reported that nutrient upshift [6] or depletion [7] can lead to biofilm dissolution via the induction of master regulators, highlighting that biofilm formation is a dynamic process where the bacterial cells continually survey their environment to optimise their chances of survival. Micronutrients are also important, and it has been shown that low iron levels discourage biofilm formation or induce bacteria to revert to the planktonic mode of growth [8]. An interesting development from the latter work is the use of gallium, which is not a biologically available metal because it can not be reduced, but which apparently can block iron pathways due to its similarity to iron. The addition of gallium was shown to prevent biofilm formation as well as to reduce bacterial growth [9].

Regulation of biofilm formation and dispersal

If the biofilm regulatory network can be elucidated, it would provide new targets for the control of biofilms. Approaches to identify such networks are facilitated by global analysis studies of either protein (proteomic) and/or DNA (genomic) expression and by transposon mutagenesis screens. While there are too many such studies to review here, there are some trends emerging that are of interest.

The first is that a large number of genes that appear to be biofilm specific appear to be those with no known function. This disproportionate number of genes with unknown function may reflect our historic bias of studying planktonic bacteria. A second trend is the identification of biofilm regulated genes with GGDEF and EAL domains, which are involved in the production and turnover of cyclic-di-GMP. Such proteins are implicated in being environmental sensors and as such would be involved in monitoring conditions that dictate whether the bacteria will be sessile or planktonic. Implicit in the function of such gene products as sensors is their association with signal transduction pathways, such as two component regulatory proteins so that the perceived signal can direct gene expression and bacterial behaviour. Indeed, there are several two component regulatory genes or proteins that have been associated with biofilm formation, development or maintenance. For example, previous studies [10, 11] show that mutants of specific two component regulatory systems form biofilms that stall early in the development process. Such systems would be ideally suited as targets for biofilm control, where the immature biofilms should be more easily removed or eradicated by the addition of traditional biocides or the host immune system.

A third theme, now established in the literature, derives from studies on quorum sensing systems in bacteria as key to biofilm formation and dispersal. These systems rely on the self-production of small, diffusible compounds that can be perceived by the binding of the signal to a specific receptor. Intricate and interconnected signalling controlled networks have been unravelled in many bacteria. Thus, if parts of or whole signalling systems can be disrupted, it should be possible, in a non-bactericidal fashion, to control biofilm formation. Work from our laboratory [12] and others [13] have shown that such signal antagonists can be isolated from organisms that might naturally interact with biofilms, e.g. for the host to prevent colonisation by bacteria.

Furthermore, such compounds have been shown to block signal mediated biofilm formation, biofilm mediated antibiotic resistance and virulence factor production [14]. Both naturally produced signal antagonists and an understanding of signal-receptor interactions form a strong basis for synthetic structure function based development of novel biofilm control agents.

In summary, biofilm formation by bacteria represents a new challenge for the control of infections and damage to industrial infrastructure because of their recalcitrance to eradication by traditional antibiotics or antimicrobials. This is a fact that is worsened by the development of multi-drug resistance in bacteria and the lack of current drugs that can be used to remove such biofilms. The search for new targets and for biofilm control strategies that do not impose the high level of selective pressure (i.e. life or death choices) as current drugs and biocidal agents do, but rather simply manipulate the lifestyle of bacteria, represent new and exciting targets for biological control. The search for these targets is dependent on our continued investigation and further understanding of the basic processes that underpin the biofilm mode of growth.

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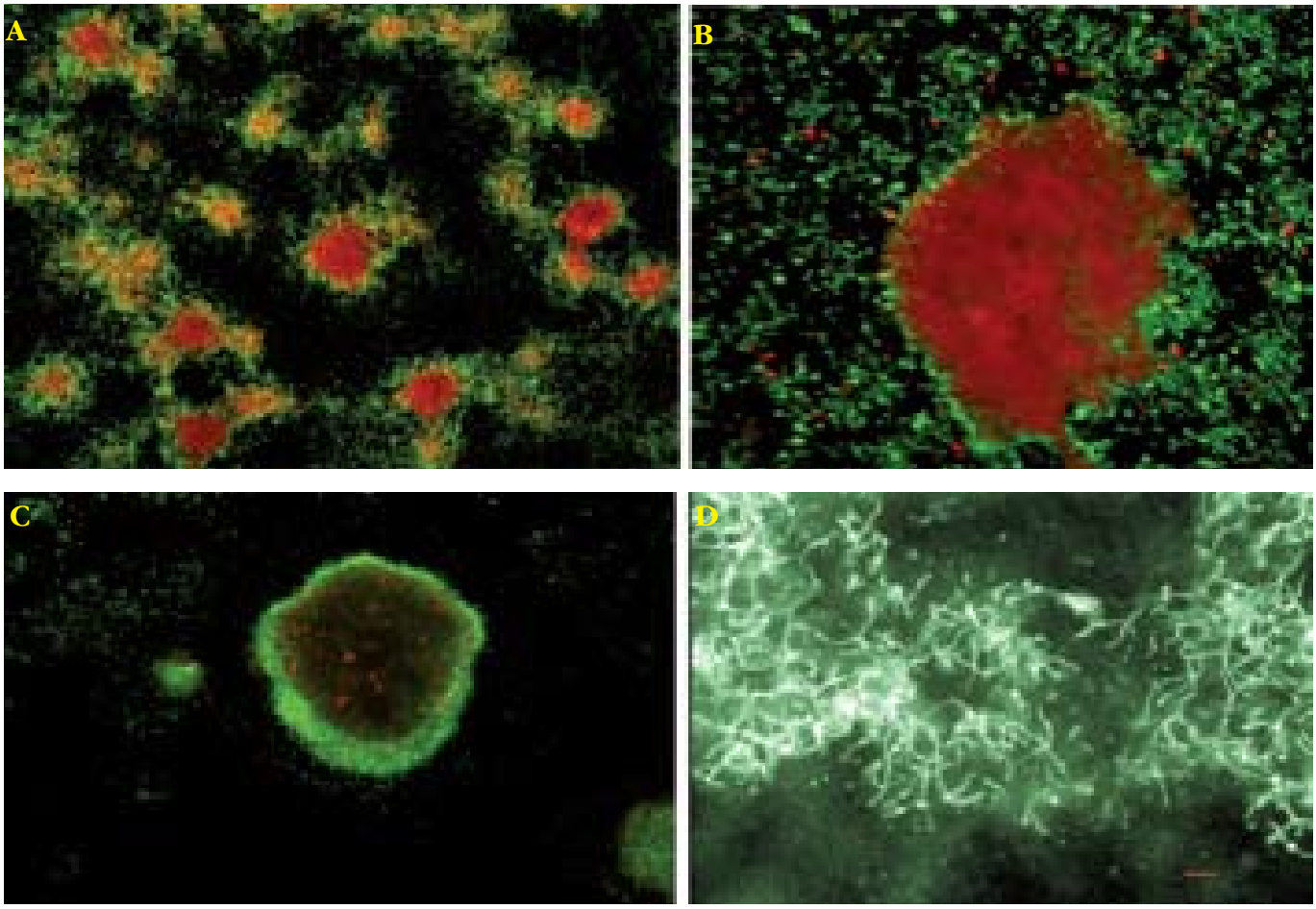

Figure 2. *In vitro* biofilm formation observed by confocal microscopy of BacLight Live-Dead stained cells. Live cells appear green and dead cells appear red. Bacterial biofilms can take on microcolony structures, A-C or be filamentous, D. Maturation of the biofilm can also result in localised cell death, as shown by the regions of red stained cells, typically precedes dispersal. The bacteria are *Pseudoaltermonas tunicata* (A), *Vibrio cholerae* (B), *Pseudomonas aeruginosa* (C), and *Serratia marcescens* (D).