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The use of signal molecules to manipulate the behavior of biofilm bacteria

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Recent examinations of the architecture of bacterial biofilms, using techniques developed by the engineers at the Center for Biofilm Engineering, have shown that these sessile communities are very complex [1] and that their functional subunits are individual microcolonies (Figure 1). As this perception began to emerge, from the examination of living biofilms by confocal microscopy, we reasoned that some kinds of chemical signal molecules must be present to control the structural development and the programmed detachment of biofilms. We reasoned that these attached communities, which comprise the majority of bacteria in most ecosystems, might use the well-known acyl homoserine lactones (AHLs) to control sessile populations, much as they control the activities of planktonic cells by quorum sensing [2].

Accordingly, we monitored the formation of biofilms by wild-type cells of the PAO 1 strain of *Pseudomonas aeruginosa*, and compared these biofilms with those made by PAO 1 mutants unable to synthesize that organism's predominant quorum signals—ODdHL and BHL. Normal biofilms were formed in the absence of BHL (the *rhlI* minus mutant), but cells of the *lasI* mutant (JP 1) that are unable to make ODdHL [3] were unable to develop biofilms when they were grown in the surface-rich environment of the flow cell. The ODdHL minus mutant adhered to surfaces in large numbers but, once they were attached, the sessile cells failed to synthesize matrix material (exopolysaccharides) and were readily removed by small pressure shifts or by treatment with weak surfactants. It is clear, from these data, that this AHL signal exerts a

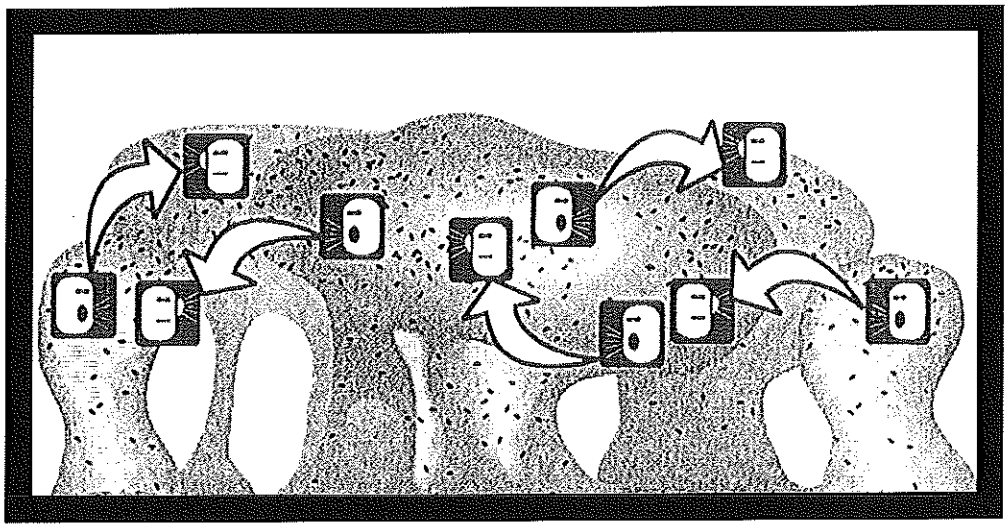

Figure 1 Diagrammatic representation of the basic architecture of bacterial biofilms, showing the maintenance of water channels between the component microcolonies, and suggesting that a signal mechanism may be involved in the development and maintenance of these complex structures.

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large measure of control over biofilm formation by this organism.

The possibility that the formation, and even the detachment, of biofilms might be controlled by simple signaling molecules has caused some excitement because of the possibility of the manipulation of this process by natural signals and chemical analogs. Kolter and Losick [4] have suggested that we may be entering a new era in which we will seek to manipulate bacterial behavior, instead of simply killing these organisms when their activities cause us problems. The possibility of preventing biofilm formation, or of removing preformed biofilms from colonized surfaces, would be very attractive in several industrial areas and in the prevention and treatment of chronic biofilm infections [5], like pneumonia in patients with cystic fibrosis.

References

1. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol 1995; 49: 711–45.
2. Fuqua WC, Winans EP, Greenberg EP. Quorum sensing in bacteria: the Lux R–Lux I family of cell density-responsive transcriptional regulators. J Bacteriol 1994; 176: 269–75.
3. Pearson JP, Gray KM, Passador L, et al. Structure of the autoinducer required for the expression of *Pseudomonas aeruginosa* virulence genes. Proc Natl Acad Sci USA 1994; 91: 197–201.
4. Kolter R, Losick R. All for one and one for all. Science 1998; 280: 226–7.
5. Khoury AE, Lam K, Ellis B, Costerton JW. Prevention and control of bacterial infections associated with medical devices. ASAIO Trans 1992; 38(3): M174–8.

Reactive oxygen species-mediated induction of *Pseudomonas aeruginosa* alginate production: a possible induction mechanism in cystic fibrosis lungs

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The leading pathogen responsible for morbidity and mortality in patients with cystic fibrosis (CF) is *Pseudomonas aeruginosa*. The initial and intermittent colonization of CF lungs by non-mucoid *P. aeruginosa* can be eradicated by early aggressive antibiotic therapy, but such treatment usually fails if the colony morphology of bacteria isolated from sputum samples is mucoid [1] due to overproduction of a capsule-like polysaccharide called alginate. The potential roles of this exopolysaccharide in pathogenesis include a mechanism for bacterial adherence, a barrier to phagocytosis and a mechanism to neutralize oxygen radicals [2]. The inflammatory defense mechanisms in the CF lung against mucoid *P. aeruginosa* are dominated by PMNs and antibodies [3]. *P. aeruginosa* grown as a biofilm (as in the CF lung) activates the oxidative burst of PMNs and the complement system [3]. Thus, free oxygen radicals produced by PMNs generate oxidative stress and lead to further inflammation. Although the environment of dehydration and high osmolarity of the CF lung may be contributing factors, we have investigated the possibility that an important effector in generating mucoid variants may be the patient's inflammatory response to the infection.

We were able to mimic the biofilm mode of growth seen in vivo by growing a prototrophic non-mucoid *P. aeruginosa* PAO1 in continuous-flow culture chambers (i.e. flow cell). Mucoid variants of PAO1 were isolated from a biofilm that was repeatedly exposed to sublethal concentrations of hydrogen peroxide [4].

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