Fossil and Recent Biofilms

A Natural History of Life on Earth

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PREFACE

MICROBIAL BIOFILMS: PROTECTIVE NICHES IN ANCIENT AND MODERN GEOMICROBIOLOGY

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As this book is published based on discussions of a conference that was held in 2001, it may be useful to provide an update on the most recent revelations about biofilms, so that this excellent exposition of the contribution of microbial biofilms to geological processes may be placed in a modern context. The importance of the contribution of microbial biofilms to global processes is only now being appreciated as it is revealed that all terrestrial surfaces are teeming with microbial life in the form of biofilm communities. These communities live on soil particles, in rock fissures, marine and river sediments and at the very extremes of terrestrial habitats from inside Antarctic ice to the walls of deep sea hydrothermal vents. The contribution of these biofilm communities generally went unrecognized because it was the water that was where microbiologists looked for life, not the surfaces, although, evidence of the early association of microbes with surfaces was in fact present in the fossil record (Rasmussen, 2000; Reysenbach, and Cady, 2001). It is also revealing that biofilm formation is found in prokaryotes from the most deeply rooted branches of the phylogenetic tree in both the *Archaea* and *Bacteria* kingdoms, the *Korarchaeota* and *Aquificales* respectively (Jahnke *et al.* 2001; Reysenbach *et al.* 2000). There are also striking similarities in the biofilm morphology from biofilms grown in widely different environments suggesting adaptive convergence of forms. For example, in high shear flows biofilms tend to form filamentous streamers whether in acid mine drainage (Edwards *et al.* 2000), hydrothermal vents (Reysenbach and Shock 2002), surface hot springs (Reysenbach, and Cady, 2001), or human pathogens in laboratory flow cells (Stoodley *et al.* 2000). Molecular techniques using knock out mutants have also demonstrated that a wide range of surface adhesion proteins and appendages, often in the same organism (one of the best studied being *Pseudomonas aeruginosa*) play a role in initial attachment and subsequent biofilm formation (Hall-Stoodley and Stoodley 2002). However, closer inspection of the papers reporting the knockout of genes "required" for biofilm formation actually show that biofilms still form, but to a reduced degree compared to the wild type. Also models based on *P. aeruginosa*

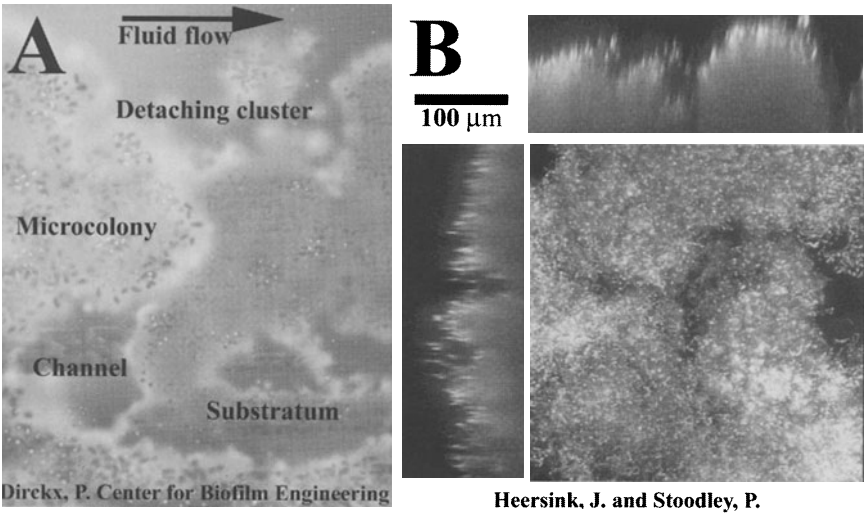
generalize motility as being integral for the biofilm life cycle of 1) attachment, 2) the formation of complex structures and 3) detachment, although the same complex processes also occur in non-motile species such as *Staphylococcus aureus* and *Streptococcus mutans* (Stoodley, P. unpublished result). This redundancy is further evidence of the importance of biofilm formation to many prokaryotes and taken together these data suggest that biofilm formation is an ancient and integral component of prokaryotic life.

Further mysteries concerning biofilms were revealed when it was discovered (Prigent-Combaret *et al.*, 1999; Sauer *et al.*, 2001) that bacterial cells, up regulate large numbers of their genes when they form these sessile communities. Modern proteomic techniques have shown that cells in the biofilm phenotype vary from their planktonic counterparts, in the expression of as many as 70% of the genes in their genomes. Analysis of gene expression in biofilms, using m RNA-based methods (Wagner *et al.*, 2003), tends to confirm this conclusion that biofilm cells are as different from planktonic cells of their own species, as planktonic cells of a given species are from those of a completely different species (Stoodley *et al.*, 2002). The continuing examination of bacterial and fungal biofilms shows that there is no single biofilm phenotype, but that sessile cells vary in gene expression in different locations within biofilms, and that these communities follow a developmental cycle not unlike that of other complex multi-cellular organisms. The bacteria and fungi that formed the biofilm communities that are described in this book grew in phenotypes that bear little or no relationship to planktonic cells in lab cultures, and the best modern models we have for them are the biofilms that we now study in lab reactors and in nature.

Because we are considering microbial biofilms that grew millions of years ago, and have become important parts of the fossil record, it may be useful to ponder the advantages of biofilm over planktonic modes-of-growth in the conditions that existed in the primitive earth. In a volcanic environment, without the "buffering" effects of large amounts of biomass, aquatic environments may have had localized niches that permitted microbial growth while in other areas growth was precluded by extreme temperatures, pressures and/or incompatible chemistry. Microbial life in these permissive/non-permissive streams would favor the formation of stationary sessile communities, because planktonic cells would be swept to their deaths in downstream maelstroms of hostile environments. However, the viscoelastic nature of all of the biofilms we have yet tested (*Desulfovibrio* spp, *P. aeruginosa*, *S. mutans*, *S. aureus*, tap water, pond water, and hot spring biofilms) may also be an adapted and adaptive strategy for survival in flowing environments. Viscoelasticity allows biofilm to

absorb some energy elastically but under sustained elevated shear can allow them to flow along surfaces thus remaining attached (Stoodley *et al.* 1999). Even when shear induces the detachment of parts of the biofilm the crude homeostatic environment provided by the remaining attached "mother colony" allows survival and the production of further "seeding" events.

One of the most successful strategies for growth in primitive ecosystems may have been the combination of heterotrophic bacteria with photosynthetic and chemoautotrophic bacteria, in biofilms on sun-lit surfaces. In these metabolically integrated communities, the symbionts would flourish, but they would be subjected to periodic drying and exposure to intense ultraviolet irradiation, when the water splashed and receded in the normal diurnal rhythms. While bacteria in the planktonic phenotype are exquisitely sensitive to drying and UV light, sessile microbes in biofilms are very well protected by their enveloping matrix material, which retains water and blocks UV irradiation (Costerton *et al.*, 1995). It seems, that even multiple species symbiotic spherical films may develop in such biofilm systems (Brehm et al. 2003). These properties of stationary growth in well-protected biofilms have allowed modern organisms to thrive in intermittently moist environments, like tidal zones, and many of the large biofilms that have been described in various geological contexts may have had similar origins. As life on earth developed, in a progressive manner, microbial populations would have been challenged, at some time in their evolution, by both bacteriophage and free-living amoebae. While planktonic bacteria are sensitive to bacteriophage, and are readily taken up and digested by amoebae, sessile bacteria are protected from both of these biological agents and this characteristic of biofilms will have enhanced their predominance in the primitive earth.



In recent publications (Stoodley *et al.*, 2002) many authors have drawn attention to the fact that biofilms provide a stable platform for genetic interchange, and for metabolic interactions, that are really not possible between planktonic cells as they "wheel and dance" in fluid space. The structure of biofilms, which are composed of matrix-enclosed bacterial micro-colonies separated by open water channels (Figure 1), allows the sessile organisms access to nutrients in the bulk fluid. Within the matrix-enclosed micro-colonies, bacterial cells comprise approximately 15% of the volume, while the matrix occupies the remaining 85% of the space, and cells are apposed to each other in spatial relationships that facilitate genetic exchange and metabolic interaction. We have observed that rates of "horizontal gene transfer" are very high in biofilms (Ghigo, 2001). Shen *et al.* (2003) have suggested that bacteria may even divide up the genomic elements that comprise pathogenicity islands, between planktonic cells of different strains, and re-assemble these elements to make functional genomic structures, when the strains are again apposed in biofilms. Morphological examinations of biofilm communities that carry out complex integrated metabolic activities, like cellulose digestion (Kudo *et al.*, 1987) and methane generation (MacLeod *et al.* 1990), have shown that cells of species with complementary activities are organized in ways that appose co-operative species. Bacteria that remove critical end products of a particular metabolic process often "drive" the overall activity, by being adjacent to primary digestive organisms, and scavenging the end products so efficiently that very high turnover rates can be maintained. In methanogenic biofilms, the methane-producing archaea are directly apposed to concentric rings of heterotrophic organisms that produce the substrates (acetate and hydrogen) for methane production, and many similar metabolic co-operations are reflected by cellular apposition in other sessile communities. If the metabolic activities in a biofilm generate salts that tend to crystallize, like the magnesium ammonium phosphate (struvite) produced by many strains of *Proteus*, the biofilm matrix is gradually filled up and mineralized. This process appears to have produced many of the stromatolites, and other large heavily mineralized structures, that have entered many geological structures as a result of the predominance of biofilms in the primitive earth (Brehm et al., 2003).

In our recent review of biofilms as multi-cellular "organisms" (Stoodley *et al.*, 2002) we considered the probable sequence of the evolution of these complex multi-species communities. Taking the development of plants as an approximate model, we suggested that microbial biofilms may have evolved early, and reached a relatively high level of structural and functional sophistication, before the planktonic phenotype emerged. In the case of plants, the earliest forms occupied the habitats within which they evolved

with considerable success, and only developed elaborate means of dissemination much later in their developmental sequence. Flowering plants, with seed dispersal strategies, developed relatively late in evolution, presumably because these characteristics allowed them to colonize favorable distant habitats. Similar variation in dispersal strategies, although more subtle, may be found in prokaryotes. For example, motile species such as *P. aeruginosa* tend to "seed" individual cells, which although unprotected, through chemotaxis may swim to favorable environments while non-motile species such as *S. aureus* tend to detach in clumps, maintaining the protective environment seen in attached biofilms. Spore formation is another dispersal strategy. As we gradually identify the genes that are expressed in the biofilm phenotype, we perceive that biofilms predominate in all natural ecosystems, because they are protected, metabolically integrated, and highly adaptive both morphologically and phenotypically. These sessile communities continuously shed matrix-enclosed "clumps", in which the cells are still in the biofilm phenotype, and these potential propagules drift downstream and may set up new biofilm communities in distant favorable locations. However, modern biofilm communities have much more elaborate dissemination strategies. In any mature biofilm a proportion of the cells assume the planktonic phenotype, digest the matrix material in their immediate location, and leave the community in a regular "programmed detachment" pattern. This programmed detachment is accelerated by certain environmental factors, like stagnation due to low rates of bulk fluid flow, so it appears to be a dissemination strategy. These detaching cells may be individually motile, if the species is capable of flagellar motility or pilus-mediated twitching motility, and the planktonic phenotypes of many species have evolved very elaborate mechanisms of chemotaxis, that help them in their search for a perfect remote habitat. To carry the analogy with plant evolution to its logical conclusion, we suggest that the biofilm phenotype may not only be the predominant form of growth of bacteria in the biosphere, but it may also be the form that evolved first, to exploit scattered favorable niches in the primitive earth. We may look at the large bacterial residues in geological formations as representing very ancient remnants of an earlier and more primitive stage in the evolution of the multi-cellular multi-species biofilm communities that are so successful in the contemporary biosphere.

FIGURES

Figure 1. 3D imaging by confocal microscopy has revealed that biofilms consist of many different types of complex architectures. Panel A) is a schematic highlighting some of the common features such as microcolonies separated by water channels. Schematic provided by Peg Dirckx, Center for Biofilm Engineering. Panel B) Confocal image of a biofilm formed from Streptococcus mutans, an early dental plaque colonizer. The square panel is a plan view and the top and side bars show the structure in cross-section. Image provided by Joanna Heersink and Paul Stoodley, Center for Biofilm Engineering, funding by Philips Oral Health Care.

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