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Biofilm Differentiation and Dispersal

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The bacterial biofilm is a multicellular organism that undergoes a complex set of events during its lifecycle of formation and dissolution. For example, many bacteria undergo cell death and differentiation of subpopulations of cells within mature microcolonies at a specific stage of biofilm formation. Biofilm cell death normally precedes an active dispersal event, where the dispersing cells display a broad spectrum of phenotypic variants, such as substrate utilization as well as fitness traits important for recolonisation, competitiveness and resistance. These variants do not occur in the biofilm prior to the differentiation event, nor for planktonic cells; apparently biofilm unique gradients are required to induce the differentiation response.

The outcome of such biofilm specific differentiation has profound implications for the ecology of surface associated bacteria, whether for pathogens in medical settings, biofilms in water distribution systems, or sessile microbial communities in natural environments.

Mechanisms of dispersal and differentiation

Some of the mechanisms that drive the development of autolysis and dispersal are now understood in several bacterial species. For example, several pathways exist for inducing autolysis and seeding dispersal in *Pseudomonas aeruginosa.* Physiologically, two signals, carbon starvation and nitric oxide production, have been shown to separately induce biofilm dispersal. The latter binds to downstream targets that alter

the c-di-CMP levels and modulate the expression of genes involved in mediating the sessile vs the free living mode of life. NO mediated dispersal appears to be conserved across prokaryotes. In contrast, a number of surface dwelling marine bacteria, such as members of *Pseudoalteromonas*, *Marinomonas*, *Chromobacterium* and *Caulobacter*, rely on the expression of an autotoxic lysine oxidase protein (AlpP or LodA) to induce dispersal.

The dispersal event coincides with the release of genetic variants from the biofilm. In the case of the marine strains, AlpP or LodA homologues generate H_2O_2 which is proposed to have a dual function, depending on the concentration of H_2O_2 generated in the microcolony. High concentrations of H_2O_2 may lead to killing of cells and subinhibitory concentrations induce mutations, driving the range of variation observed in the dispersal population. In an analogous fashion, the mature microcolony in *P. aeruginosa* contains appreciable levels of reactive oxygen and nitrogen species at the time of differentiation, which may induce mutations, as reflected in the high percentage of dispersal mutants in this organism. Additionally, the formation of small colony variants in *Pseudomonas* has been linked to the conversion of an endogenous phage into a superinfective form, capable of mediating both cell death and genetic variation.

Type of mutations

The consistent loss or gain in utilization of specific carbon sources by different dispersal variants suggests that genes involved in the metabolism or transport of those carbon sources could contain mutation hot spots, providing selective advantages to persist in the environment. There are similar observations for variant isolates from biofilm associated chronic infections. These have typically been show to be the result of specific point mutations rather than general large scale genomic rearrangements, indicating a possible loss of function in DNA repair mechanisms is involved in this process. This is supported by recent data from our laboratory, where we have sequenced the dispersal population of *P. aeruginosa* PAO1 and have established that there are no apparent recombinations, no large indels, but only single nucleotide mutations (SNPs), several of which reside in genes encoding regulatory proteins. SNPs drive the formation also of stable colony variants, with different phenotypic profiles across different colonization traits, that occur in the seeding dispersal population of *Serratia marcescens*, a ubiquitous environmental bacterium. The mutations have occurred in genes that alter surface colonisation phenotypes and these are overrepresented against the general mutational background, suggestive that these genes contain hotspots for mutations.

Ecological models for biofilm dispersal

Several ecological models can be tested using the biofilm dispersal system. We have addressed two

models: 1) Adaptive maternal effects, i.e. the environmental quality effects dispersal and the promotion of variable dispersal phenotypes and 2) Increased functional diversity increases resilience to disturbance.

In testing the first model, nutrient down shift during biofilm growth of marine epiphytic bacterial species was found to lead to a shift in the dispersal cell population from relatively low activity, prior to the nutrient downshift, to cells with high activity and motility. Also, relatively more inhibitory dispersal cells, against other co-occurring colonising epiphytic bacteria, appear after the down shift induced response, i.e. poor quality environments produce propagules with better dispersive properties. Experiments with low and high quality environments for biofilms were also conducted across other species, telling a similar story of environmental quality effects.

We addressed whether the second model can be applied to stable variants observed in the dispersal population. In one such experiment, biofilms of dispersal colony variants generated by *S. marcescens* were assessed for resistance to grazing by protozoans, one of the main mortality factors of bacteria. Such mono-variant biofilms exhibited a diverse pattern of resistance, or susceptibility, against biofilm feeding protozoans. In contrast, mixed variant biofilms were established and found to be protected. Analysis of the biofilm community in the mixed variant biofilm after grazing showed that they were all present and hence variants that were susceptible to grazing as monovariant biofilms were protected in the mixed community. Cross-protection between the variants as observed for the biofilm did not occur in the planktonic phase.

Summary

Bacterial biofilms are analogous to multi-cellular organisms or to clonal communities of higher organisms. In this respect, it can be demonstrated that biofilms display the type of genetic variation associated with macroorganisms. The formation of genetic variants from biofilms is the result of internally produced and regulated signals and the appearance of these variants coincides with dispersal from the biofilm. Moreover, the generation of such variation, has similar outcomes for the bacterial community, where diversification of phenotypic traits ensures that the bacterial community optimizes its chances of success when dispersing or surviving when challenged with environmental stress. These observations increase the complexity with which we view bacteria and also suggest that microbial systems can serve as models for the testing of eukaryotic ecological theories.