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**Purevdorj-Gage, B., Costerton, W. J. & Stoodley, P. (2005).** Phenotypic differentiation and seeding dispersal in non-mucoid and mucoid *Pseudomonas aeruginosa* biofilms. *Microbiology* **145**, 1569–1576.

**Webb, J. S., Thompson, L. S., James, S., Charlton, T., Tolker-Nielsen, T., Koch, B., Givskov, M. & Kjelleberg, S. (2003).** Cell death in *Pseudomonas aeruginosa* biofilm development. *J Bacteriol* **185**, 4585–4592.

**Webb, J. S., Lau, M. & Kjelleberg, S. (2004).** Bacteriophage and phenotypic variation in *Pseudomonas aeruginosa* biofilm development. *J Bacteriol* **186**, 8066–8073.

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## Clinical significance of seeding dispersal in biofilms: a response

We welcome the dialogue concerning the potential clinical significance of seeding dispersal (Purevdorj-Gage *et al.*, 2005) in the life cycle of mucoid *Pseudomonas aeruginosa* biofilms. We had based our hypothesis that the seeding dispersal phenomenon may be more relevant for non-mucoid, environmental strains on (1) the propensity of diseases associated with reduced mucociliary clearance in the lung, such as cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD), to select for mucoid *P. aeruginosa* phenotypes, and (2) that conversion to mucoidy is usually associated with a concomitant down-regulation of flagella production and loss of swimming motility (see Garrett *et al.*, 1999). However, as Kirov *et al.* discuss above, there may be greater diversity in mucoid CF isolates than generally acknowledged, and the perceived dichotomy between mucoidy and swimming phenotypes should be a topic for debate. More recently it has been shown that expression of flagellum genes in

response to oxygen limitation precedes loss of mucoidy and is reversible during this state of transition (Wyckoff *et al.*, 2002). Further, in an ongoing screen of mucoid CF isolates it was found that 6 out of 20 were motile (D. J. Wozniak, personal communication). However, it was suspected that growth medium may also play a role in the outcome of the motility assay and exact proportions may vary depending on culture conditions. The growth-condition-dependent transient switching between mucoid and swimming phenotypes is problematic when relating a particular biofilm behaviour with phenotype and stresses the importance of attempting to characterize the phenotypic state at various time points during biofilm development. Broadly though, the finding of Dr Wozniak is in agreement with that of Kirov *et al.* We agree that to fully assess the role of seeding dispersal (and other yet unidentified behavioural developmental phenotypes) in the context of lung infections, biofilm studies should include greater diversity in strains, growth conditions and be conducted over longer time scales. The interesting observation that seeding motility occurred in a mucoid CF isolate clearly demonstrates that the phenomenon may have clinical relevance.

Another point we would like to briefly address is the difference in terminology used by our two groups in reference to the hollowing out of clusters. Kirov *et al.* refer to hollowing resulting from ‘bacteriophage-mediated lysis’ as ‘death-associated dispersal’. We use the term ‘seeding dispersal’ in a more general sense to describe sequential steps from the formation of microcolonies culminating in the release of live cells from the centres of the colonies. Our working hypothesis for seeding dispersal which integrates observations from our two groups is thus: (1) the formation of cell clusters; (2) the differentiation of the cell cluster into two or possibly three distinct phenotypes consisting of the cells in the stationary cluster ‘wall’, the highly motile seeding subpopulation in the interior and the lysing interior subpopulation (Webb *et al.*, 2003) which allows the interior to liquefy; (3) highly agitated ‘seeding motility’ in the interior; and finally (4) the swimming, or swarming, of cells out of the microcolony. In the case of PAO1 the release appears to

occur through directed motility, but we recognize that hollowing in non-motile species such as *Staphylococcus epidermidis* (P. Stewart & B. Pitts, personal communication) and *Actinobacillus actinomycetemcomitans* (Kaplan *et al.*, 2003) may be driven by water currents. It is interesting to speculate that the recent increase in reports of hollow clusters occurring in a diversity of bacterial biofilms is evidence of convergent evolution in biofilm development.

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