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Adaptive evolution of phages towards *Pseudomonas aeruginosa* biofilm control

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Pseudomonas aeruginosa is the leading cause of chronic lung infection in patients with Cystic Fibrosis (CF). The main reason for the persistence of *P. aeruginosa* in CF lung environment is its biofilm mode of growth, associated with increased tolerance to antibiotics and host immune defenses. Phage therapy is a promising approach to treat biofilm-related infections such as CF. However, the complete eradication of biofilms is almost impossible. Given the natural ability of (bacterio)phages to evolve and counterattack the bacterial defense mechanisms, the aim here was to improve the anti-biofilm activity of phages through adaptive evolution.

The phage evolution was stimulated for 8 days in 24h-old biofilms formed by a *P. aeruginosa* clinical isolate recovered from a CF patient. The biofilms were treated with phage PE1, a Pseudomonas PB1-like phage. After 24h of infection, the phages were recovered from the wells and added to a fresh 24h-biofilm. This procedure was repeated daily in 24-well plates and the final biofilm-adapted phages were recovered for phage production and characterization.

The evolution process resulted in an increased anti-biofilm activity of the adapted phages compared to the wildtype phage, leading to a greater biofilm reduction. The two adapted-phages with the best anti-biofilm activity revealed an increased efficiency-of-plating against several *P. aeruginosa* clinical strains and *P. aeruginosa* colonies isolated from the biofilm. When comparing the phage genomes, it was possible to identify two SNPs in genes encoding a tail-fiber and a baseplate.

The emergence of mutations in genes involved in bacterial recognition and binding, together with the increased efficiency-of-plating, indicate that the biofilm evolution process improves phage host range and infectivity efficiency. Given the common heterogenicity of biofilms, the enhancement of bacterial recognition may be the key for the increased anti-biofilm activity of the evolved phages.