Directed in vitro evolution of virulent bacteriophages against Enterococcus faecium following the Appelmans Protocol

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Résumé

Enterococcus faecium is a ubiquitous Gram-positive bacterium that commensally colonizes the human intestinal tract. It is also an opportunistic pathogen that has become a major cause of nosocomial infections worldwide. This is related to the emergence of clinical isolates belonging to the clonal complex 17 (CC17), which are multiply resistant to antibiotics and particularly vancomycin (the so-called vancomycin-resistant enterococci (VRE)). Phage therapy is a way to combat multidrug-resistant bacteria, including VRE. For that, the productive host spectrum needs to be broad enough. However, we and others found that this was not the case for phages infecting *E. faecium*. We therefore attempted to generate " generalist phages " active on a wide panel of CC17 clinical isolates.
To this end, we used a protocol recently developed by Burrowes and his colleagues, inspired by Georgian researchers and designated the Appelmans protocol. It consists in the iterative growth of a serially-diluted phage cocktail on a set of naive related strains, most of which were initially refractory. Phages from the evolved cocktail were then isolated and their individual host spectrum evaluated to find " generalist candidates ". The experiment was conducted with a phage cocktail composed of four well-characterized CC17 bacteriophages from our collection: one myophage Porthos belonging to *Shiekvirus* genus and *Herelleviridae* family, one siphophage Planchet from the *Denvervirus* genus and two related siphophages dArtagnan and Aramis representing a new genus. Fifteen successive passages of the cocktail were performed using eight VRE strains, of which five were initially refractory to at least one of the phage from the cocktail. Eighteen phages were isolated and purified, and their host ranges were analyzed on a total of 14 CC17 isolates. Genomes of four evolved phages, with a more extended host spectrum, were sequenced, assembled and analyzed. Three phages corresponded to Porthos mutants that were active against 10 of the 14 strains, representing a spectrum twice as large as the WT Porthos. Three point mutations in the tail module of the evolved Porthos could at least partly explain their extended host ranges. The fourth mutant was an Aramis recombinant, in which a DNA region of the tail module had been acquired from dArtagnan. This mutant was active against five of the 14 strains, which represented two additional targets compared to the WT Aramis. These results are encouraging and illustrate that the Appelmans approach is an elegant way to enlarge host spectrum of virulent phages.

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Mots-Clés: Enterococcus faecium, virulent bacteriophage in vitro directed evolution, extended host spectrum