Directed Evolution Improves Infection Dynamics of *Mycobacteroides abscessus* Phages

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Background: *Mycobacteroides abscessus* (MAB) infections are increasingly recognized as a global clinical challenge because of extensive resistance to conventional antibiotics. This has renewed interest in phage therapy as a potential treatment strategy.

Methods: Two parental MAB phages (NP5 and NP15) underwent 20 serial rounds of directed evolution using the Appelmans protocol. Evolved plaques were purified and characterized by host range and efficiency of plating (EOP), adsorption kinetics, and one-step growth assays. Whole-genome sequencing and comparative genomic analysis were performed to identify genetic changes associated with the observed phenotypic differences.

Results: Four genetically distinct derivatives (Nep7, Nep8, Nep12, Nep13) were recovered. Compared with their parental phages, Nep7 and Nep12 adsorbed more efficiently, leaving 37% and 55% of input phage unabsorbed at the end of the adsorption interval, and produced markedly larger bursts (183 and 409 PFU/cell). In contrast, Nep8 showed reduced infectivity. Comparative genomics identified recombination tracts and point mutations concentrated in structural loci, including a minor tail protein gene, consistent with altered host interaction and infection dynamics.

Conclusion: Iterative in vitro selection can rapidly generate MAB-infecting phages with enhanced adsorption and replication properties. These findings provide mechanistic clues to phage adaptation and support directed evolution as a strategy to improve therapeutic phage candidates.