Elucidating key steps of *Pseudomonas aeruginosa* infection by DEV lytic phage for phage therapy.

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Phage therapy is a promising strategy to combat infections caused by antibiotic-resistant bacteria, including *Pseudomonas aeruginosa (Pa*). Understanding the biology of therapeutic phages is essential to ensure their safety and efficacy. I am investigating the adsorption mechanism of DEV, a lytic phage killing *Pa*. DEV gp53 fibers are required to infect Pa strains exposing on their outer membrane (OM) a lipopolysaccharide (LPS) capped with the O-antigen moiety, which is DEV primary receptor, but are dispensable in *galU* mutants lacking the O-antigen, suggesting a secondary receptor and a distinct receptor-binding protein.[1,2] To identify DEV secondary receptor, we isolated a DEV-resistant *galU* double mutant. The mutant carried a 33-bp deletion in *lptD*, an essential gene encoding an OM protein. Complementation assays demonstrated that *lptD* expression restored phage adsorption, suggesting that LptD may be the secondary receptor. In agreement with this hypothesis, the expression of *P. aeruginosa lptD* in *E. coli*, normally not susceptible to DEV, enabled adsorption.To identify LptD receptor binding protein, UV mutagenesis of wt DEV or DEV Δ*gp53* mutant was performed. The phages were plated on the DEV resistant *galU lptD* strain and mutant phages forming plaques were genome sequenced. In both cases, mutations were identified only in the *gp54* gene, identifying gp54 as the LptD-specific receptor-binding protein. In collaboration with G. Cingolani group (UAB, USA), we demonstrated that gp54 forms a complex with gp56 and gp55 that regulates DEV tail unplugging.[3]Given the high conservation of gp54 among *Litunaviruses*, other phages in this genus may also exploit LptD as their receptor.

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2) Lokareddy RK, Hou C-FD, Forti F, Iglesias SM, Li F, Pavlenok M, Horner DS, 553 Niederweis M, Briani F, Cingolani G. 2024. Integrative structural analysis of 554 Pseudomonas phage DEV reveals a genome ejection motor. Nat Commun 15:8482.

3) Nieto Noblecia, J., Bellis, N. F., Antichi, C. A., Aminian, S., Forti, F., Falchi, F. A., Sposato, D., Imperi, F., Cingolani, G., & Briani, F. (2026). Pseudomonas aeruginosa DEV phage exploits the essential LptD outer membrane protein as receptor for adsorption. mBio, 17(2), e0356125. https://doi.org/10.1128/mbio.03561-25