Pisa4: a potential candidate for *Mycobacterium tuberculosis* treatment

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Pisa4 is a recently identified cluster K1 mycobacteriophage that lacks integrase and encodes a partial immunity repressor. We aimed to evaluate its antibacterial activity against *Mycobacterium tuberculosis* (MTB) following complete deletion of the repressor gene, to prevent reversion to a temperate phenotype, while also exploring its host range in *Mycobacterium abscessus* (MAB) another clinically relevant species. Pisa4 was engineered using BRED, generating the Pisa4Δ_IR mutant, which was tested in time-kill assays against MTB H37Rv. Sera from TB patients in Cape Town were used to assess phage neutralization, and their MTB isolates were evaluated for susceptibility. In parallel, host range was further explored in 48 clinical isolates of MAB, collected in Pisa, Rome and Palermo. The repressor gene of Pisa4 was successfully deleted. Pisa4Δ_IR infected MTB H37Rv, showing an increase in plaque-forming units (~3.5 $\log_{10}$). From day 5 post-infection, it suppressed bacterial growth, maintaining colony-forming units close to baseline, while untreated controls increased (~2 $\log_{10}$). Overall, 30/34 (88%) clinical MTB isolates were susceptible with an EOP ≥0.01. In contrast, a limited activity was revealed against MAB, with lytic infection observed in only 8/48 isolates. No serum neutralization was detected. Deletion of the partial repressor ensures stable lytic activity. Pisa4Δ_IR effectively controls MTB growth *in vitro* and shows a broad host range among the MTB isolates, while displaying limited activity against MAB. These findings support its potential as a targeted anti-MTB therapeutic and warrant further evaluation across genetically diverse isolates, as well as in intracellular and low-metabolic conditions.