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Poster #62: Phage P2-encoded Orf30 protein as a novel regulator of virion formation

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Phage P2 is a temperate bacteriophage infecting Escherichia coli and the helper phage of the satellite phage P4. Despite decades of research, several highly conserved P2 genes remain uncharacterized. Among these, orf30 is constitutively expressed, has its own promoter, and is highly conserved among P2-like phages, yet its biological role has remained unknown. This study aimed to generate, for the first time, a P2 Δorf30 mutant and investigate its function.

The lambda-red recombination system was optimised to overcome P2 Old protein interference, previously reported to hinder P2 engineering. An E. coli lysogen carrying a P2Δorf30 mutant was generated, and its plaque-forming ability was compared with the WT strain. Comparative genomic analyses assessed the conservation of orf30 and its distribution among E. coli genomes carrying P2- and P4-like phages.

The modified lambda-red system enabled construction of the mutant, which remained viable but exhibited a fourfold reduction in plaque-forming efficiency compared with the WT. Comparative genomic analysis showed that orf30 is highly conserved among P2-like prophages and present in 96% of genomes harbouring both P2- and P4-like phages, suggesting that P4 satellites are predominantly associated with P2 phages carrying the orf30 region.

This work establishes an effective strategy for engineering P2 phages and identifies Orf30 as a conserved factor contributing to efficient phage propagation. The conservation of orf30, together with prevalence in P2/P4 helper-satellite systems and the reduced plaque-forming efficiency of the Δorf30 mutant, identifies Orf30 as a promising candidate for understanding the molecular mechanisms regulating P2 propagation and helper-satellite interactions.

Reference #1

Reference #2

Reference #3

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