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Successful Phage Isolation for *Pseudomonas putida* S12

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ABSTRACT

Pseudomonas putida is a saprophytic organism that has metabolic versatility and tolerance to many organic compounds. These factors have allowed *P. putida* to become a widely used biomanufacturing strain.

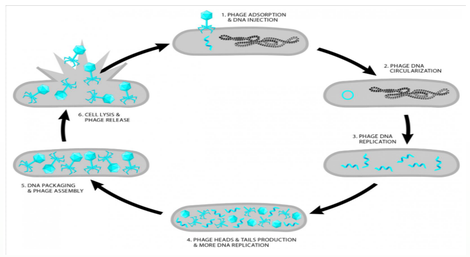
We sought to identify phages that could be useful for *P. putida* to further understand its viral interactions and vulnerabilities, and to facilitate development of phage vectors for delivery of large genetic cassettes to *P. putida* in culture or within microbiomes. We computationally predicted 237 prophages from 116 *P. putida* genomes.

We down selected to six *P. putida* strains of interest with 14 prophages. We verified nine of these prophages are active after mitomycin C induction by applying next generation sequencing and our Juxtaposer software [4]; however, no induced phages produced plaques or clearings.

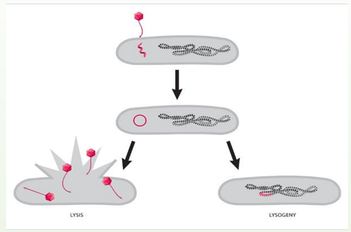
As an alternative route to identify phages, we then did traditional phage hunting from soil samples. We isolated the first known phage against *P. putida* S12, MiCath, from an enrichment culture. MiCath has a dsDNA genome of 60,958bp and is a new phage species (based on ICTV rules). It has 98 ORFS with 50% hypothetical. MiCath is a lytic phage with no evidence of an integrase gene. It has a broad host range infecting six *P. putida* strains, including KT2440. It has a siphoviridae morphology.

INTRODUCTION

Bacteriophages or phages are viruses that infect bacterial hosts and have properties shared by all viruses. Due to bacteriophage inability to replicate by themselves, they take advantage of the host cellular (bacteria) machinery by hijacking the host cell and using it for viral production. Like all viruses, bacteriophages are specific to a particular bacteria host of interest. The host range can be restricted to a single bacterial strain, or have a larger host range. Bacteriophages are found everywhere; soil, water and even air. There are an estimate of 10^{31} phages/particles population; however these phages are very old, genetically diverse and dynamic. Bacteriophages can be virulent or temperate. Virulent phages use the lytic cycle as a source of replication within the host cell. The phages attach and absorb to the host cell by using it tail fibers and inject their DNA into the bacterium and the injected DNA is circularized. This allowed the replication process to begin and the phages DNA is replicated. The head and tails are produced, virions are assembled, the host cell is lysed and new virions released.



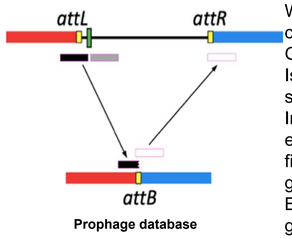
Temperate phages use the lysogen cycle where the phages genes required for the lytic cycle are repressed and the genome referred to as prophage integrates into the host bacteria chromosome at specific nucleotide sequence using an integrase gene.



Images from:
https://seaphagesphagediscoveryguide.com

METHODS and RESULTS

Identification of Bacteria-Phage Pairs



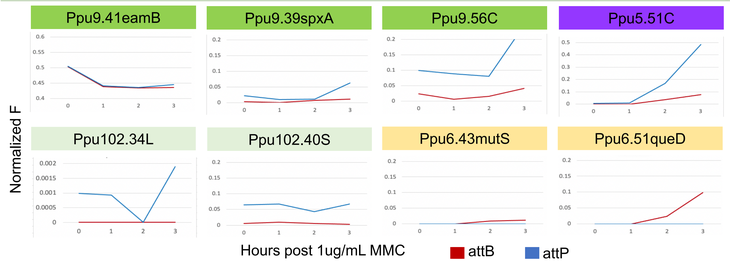
Prophage database

We have created a prophage database containing all sequences from GenBank using our TIGER [1, 2] and Islander [3] programs. We seed our searches with manually-curated Integrase HMMs and find the precise endpoints of our prophages. Islander find prophages in tRNA and tmRNA genes, while TIGER uses ping-pong BLAST to find prophages in any genomic loci. This database can be mined to find prophages for bacteria of interest.

P. putida Strain	GIs	Prophages (Cat. 1 and 2)	Cat. 1 Phage
KT2440	11	4	39.spxA, 40.L, 41.eamB, 56.C
S12	8	1	None
F1	5	2	51.queD, 43mutS
DOT-T1E	6	2	51.C, 61.C
NCTC10936	7	0	None
JUB85	7	4	53.DUF1654|pbpG, 40.L, 34.L, 40.S

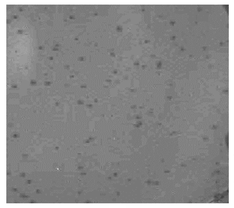
We down selected six strains of *Pseudomonas putida*. Prophages are categorized in two groups: **Cat. 1 -** prophages which contain structural and functional phage proteins, hypothesized to be functional phages; **Cat. 2** - prophages with either structural or functional phage proteins.

Induction of *P. putida* Prophages



attCt [4] uses att site probes for each prophage to count the number of corresponding reads. We induced all six of our strains with 1ug/mL of mitomycin C (MMC) and used attCt to map the normalized F values for each prophage. Each strain is listed in a different color and each category 1 phage is mapped. 8 prophages are active showing measurable excision (attB) and replication (attP). Although phage induction is occurring, no phage infection of any strains were observed.

Isolation of Environmental Phage

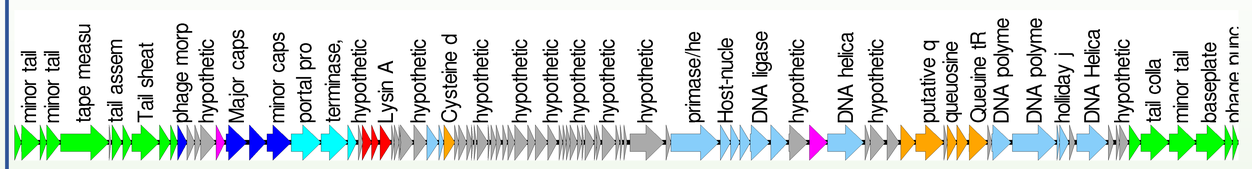


Phage MiCath Plaques

MiCath was isolated from a backyard garden soil using the enrichment plating method with host bacteria *Pseudomonas putida* S12. After isolation, phage purification, amplification and DNA extraction were performed. MiCath forms 1mm wide clear plaques. The phage DNA was then sequenced to determine it genomic characteristics and functions. Electron Microscopy (TEM) imaging was performed to observe the siphoviridae morphology.

We tested MiCath on a collection of *P. putida* strains to determine the host range. The stains are shown in order of phylogenetic distance from S12.

Host Bacteria	Infectivity	EOP
Pseudomonas putida S12	Yes	1
Pseudomonas putida KT2440	Yes	10^{-2}
Pseudomonas putida EM383	Yes	10^{-4}
Pseudomonas putida F1	Yes	10^{-2}
Pseudomonas putida DOT-T1E	No	0
Pseudomonas putida ATCC12633	Yes	10^{-3}
Pseudomonas putida JUB85	No	0
Pseudomonas putida p106	No	0
Pseudomonas putida M2	No	0
Pseudomonas putida M5	No	0



MiCath is dsDNA genome of 60,958bp and has 98 ORFS with 50% of ORFs annotated as hypothetical. It does not show evidence of integrase gene, therefore it is a lytic phage. Moreover, it was observed that the genome has an entire cassette dedicated to queuosine biosynthesis which could enable the phage to encode non-cannonical DNA bases. Furthermore, there is a putative endosialidase, which may allow for increased host range.

CONCLUSIONS

- *P. putida* strains (2.1) have higher than average(1.1) prophages predicted
- *P. putida* prophages can be induced but do not infect hosts >99% average nucleotide distance from host strain
- MiCath is a newly isolated lytic phage against *P. putida* S12 using the enrichment platting method.
- MiCath has a broad host range infecting 5 phylogenetically distant *P. putida* strains.
- MiCath has a 60,958bp dsDNA genome with 98 ORFs predicted
- MiCath has a few predicted functions of interest:
  - cas4 protein – host immune evasion
  - Endosialidase – contributes to increased host range
  - Queuosine biosynthesis cassette – encodes alternative nucleotide base

Future Directions

- Determine the limits for prophage-host targets in *P. putida*
- Explore the role of cas4 in MiCath infection cycle
- Determine if MiCath has alternative nucleotide bases
- Determine essential genes for MiCath infection

Acknowledgements

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