Sandia National Laboratories

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DISCOVERY AND CHARACTERIZATION OF *PSEUDOMONAS PUTIDA* PHAGES

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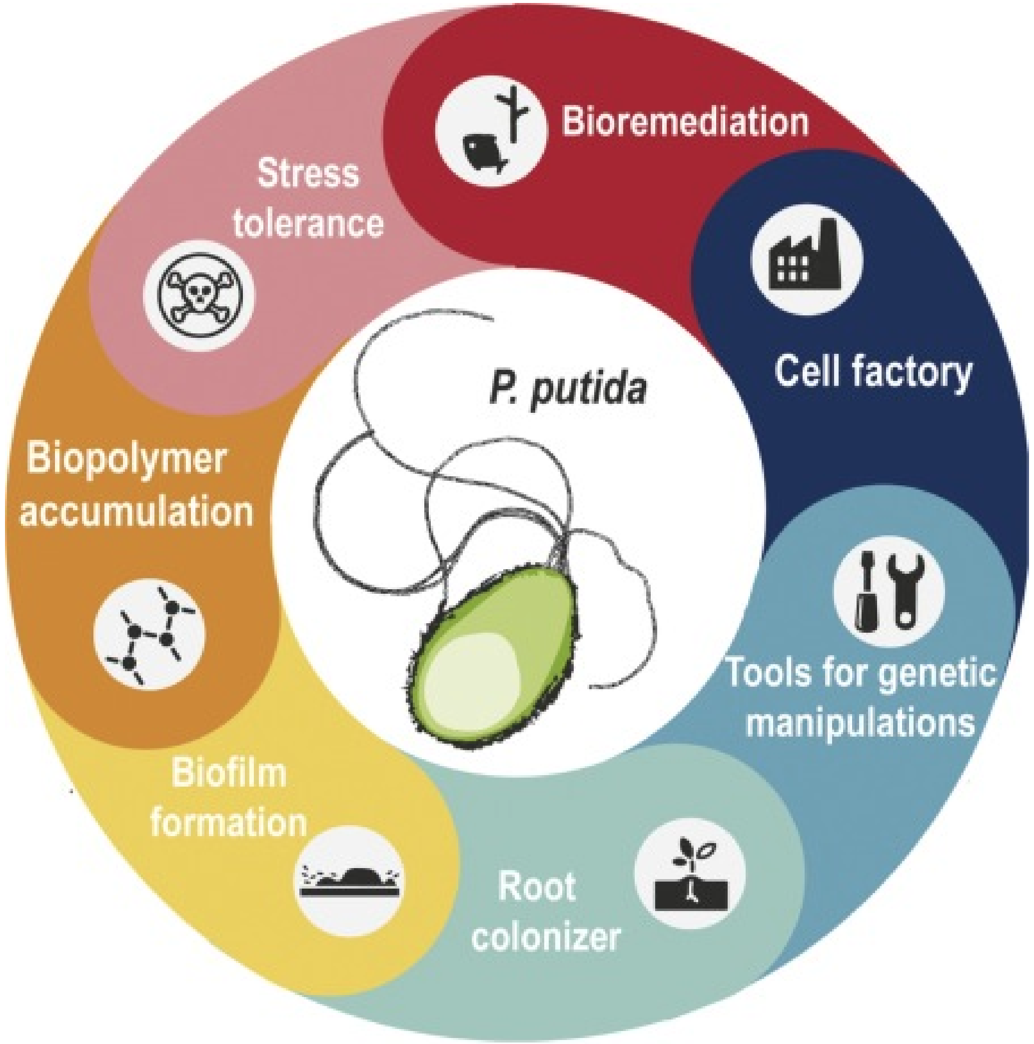
PSEUDOMONAS PUTIDA HAS IMPORTANT ROLES IN THE SOIL



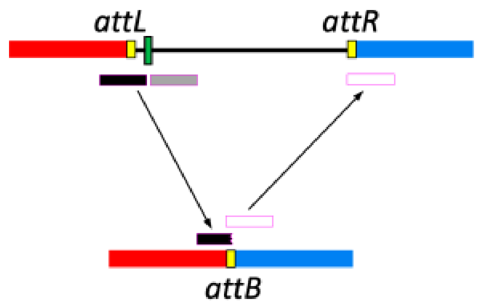
Volke et al., 2020 Trends in Microbiology

- Ubiquitous and metabolically diverse and versatile
- Bioremediation potential
  - Degrades aromatic and xenobiotic compounds
- Root colonization and plant health
  - Can use carbon, nitrogen, iron, phosphorous, or sulfur from soil
  - Establish and migrate in Rhizosphere
  - Avoid competition of other microbes
- Genetic manipulation tools exist

PSEUDOMONAS PUTIDA PHAGE LANDSCAPE

- Few isolated:
  - 23 as of Jan. 2024
  - 67 in recent report from Estonia (CEPEST collection) at low temperatures (20°C)
- Biofilm degrading activity
- Diversity of phage related to hosts
- AMGs

PHAGE FACTORY FOR PROPHAGE DISCOVERY AND ENGINEERING



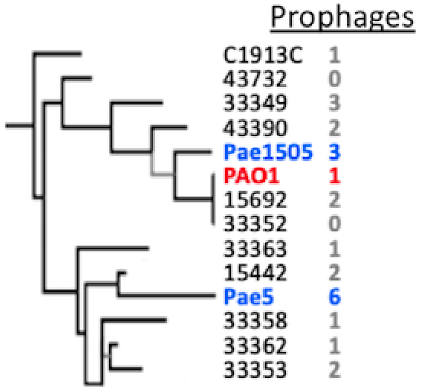
Prophage database →

Tiger and Islander Prophage Predictions

Islander: Hudson et al., 2015 NAR

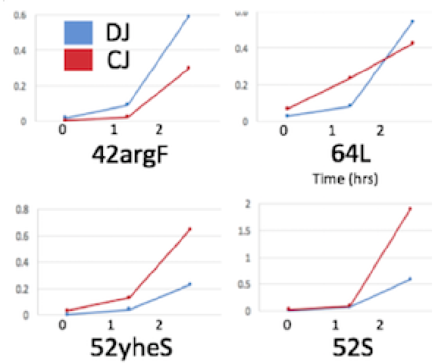
TIGER v1: Mageeney et al., 2020 NAR

TIGER v2: Mageeney, Trubl, Williams, 2022 Frontiers in Bioinformatics



Identify prophage-rich close relatives of target →

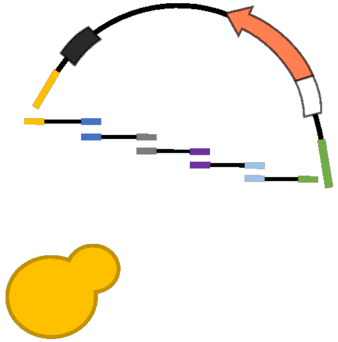
Tree of strains. Cluster phages to find non-duplicated prophages for engineering purposes



Validate phages

Validate excision by PCR and understand prophage excision biology with juxtaposer

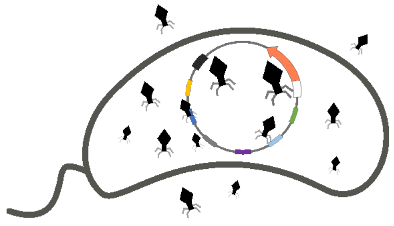
Phage Annotation →



Engineer phages →

Remove integrase, att site, repressor toxins

- PCR/GA
- YAC assembly

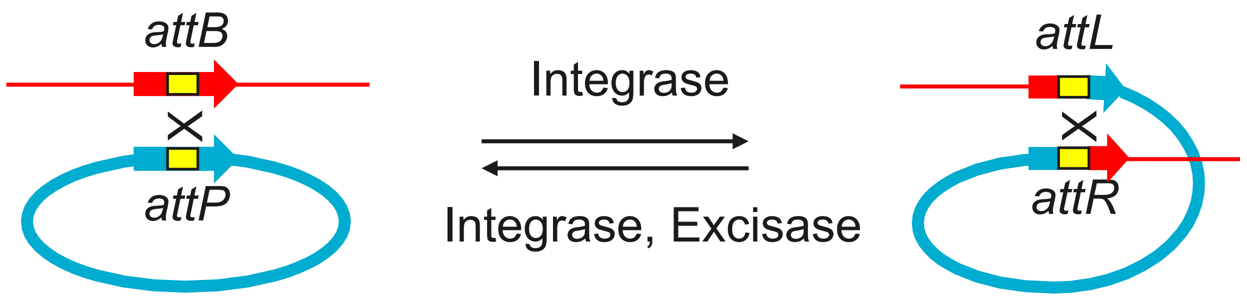


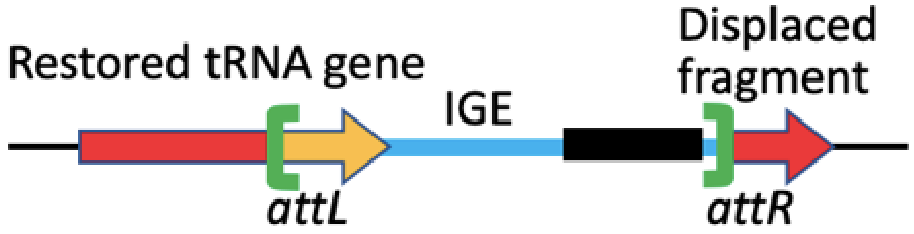
Reboot

- Cell-based systems (E. coli / phage host)
- Cell free

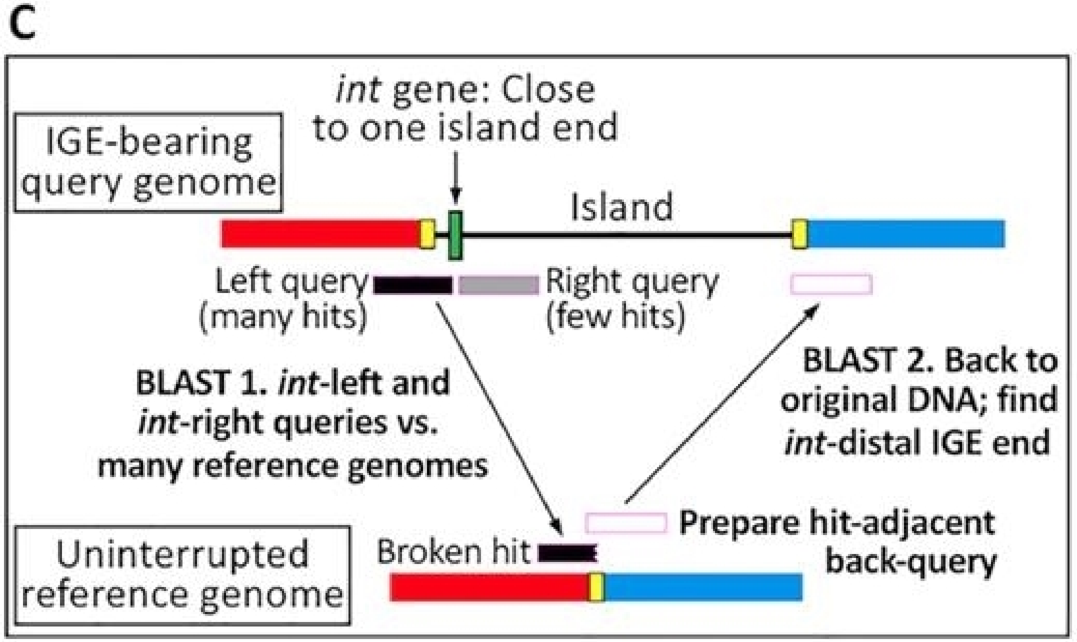
Adapted from Mageeney et al., 2020 mSystems

TIGER AND ISLANDER DETECT MOBILE GENETIC ELEMENTS INCLUDING PROPHAGE

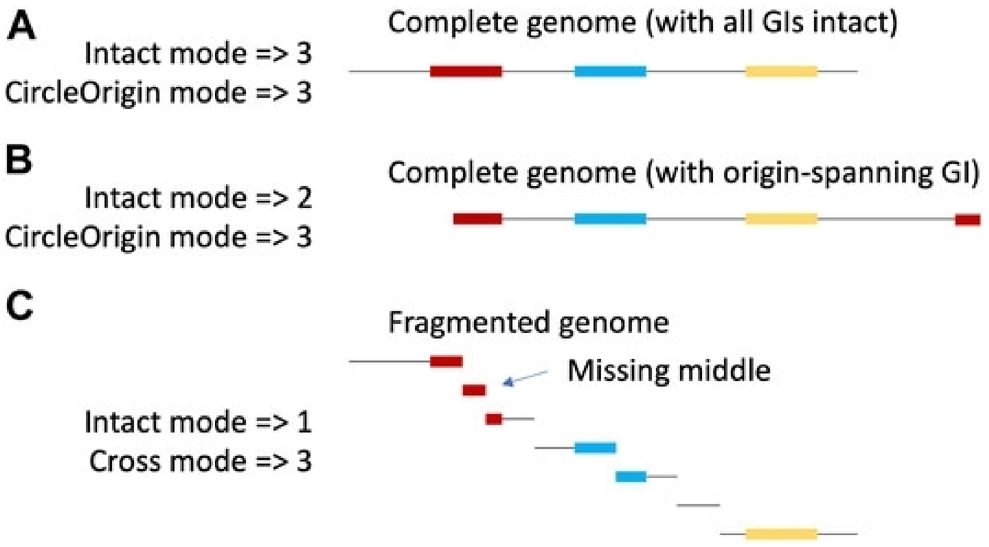




Islander: Hudson et al., 2015 NAR



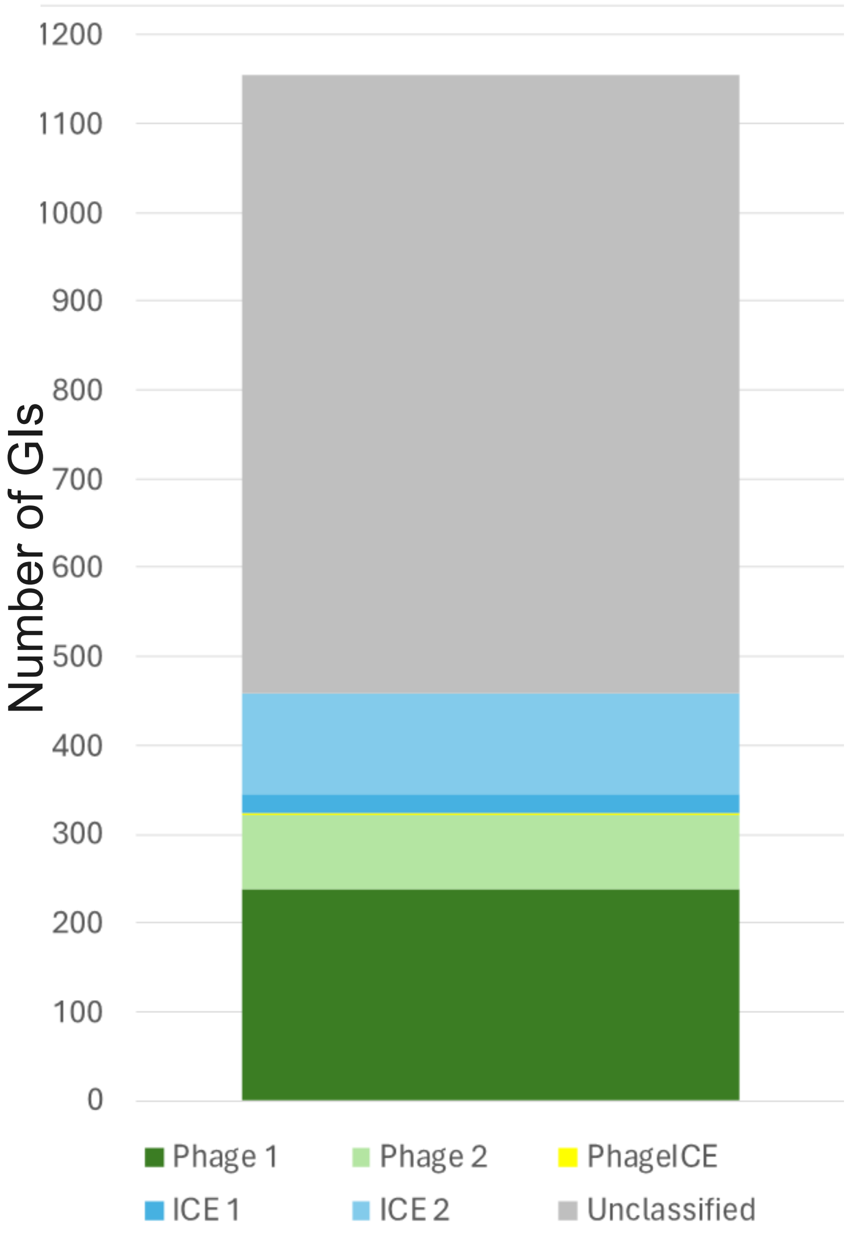
TIGER v1: Mageeney et al., 2020 NAR



TIGER v2: Mageeney, Trubl, Williams, 2022 Frontiers in Bioinformatics

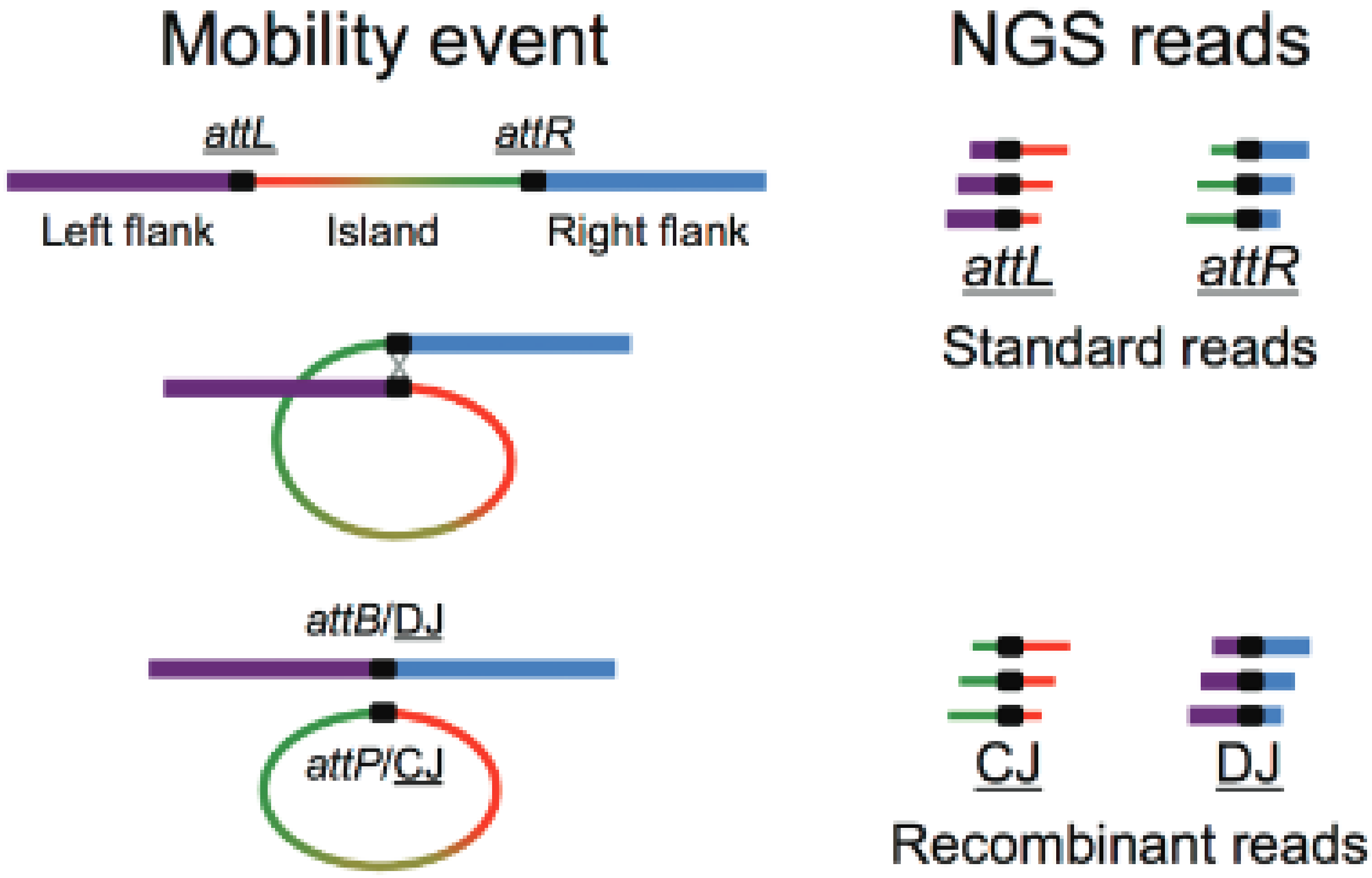
PROPHAGES ARE COMPUTATIONALLY PREDICTED IN *P. PUTIDA* STRAINS

- *P. putida* KT2440
  - 134 bacterial genomes in *Pseudomonas__E_alloputida* at GTDB (r214)
- 1191 genomic islands
  - 27% are Prophages (2.4/genome)
  - 11.4% are Integrative Conjugative Elements (ICEs)
  - 0.2% are Phage/ICE tandems
  - 58.4% are Unclassified
    - These have yet to be functionally determined



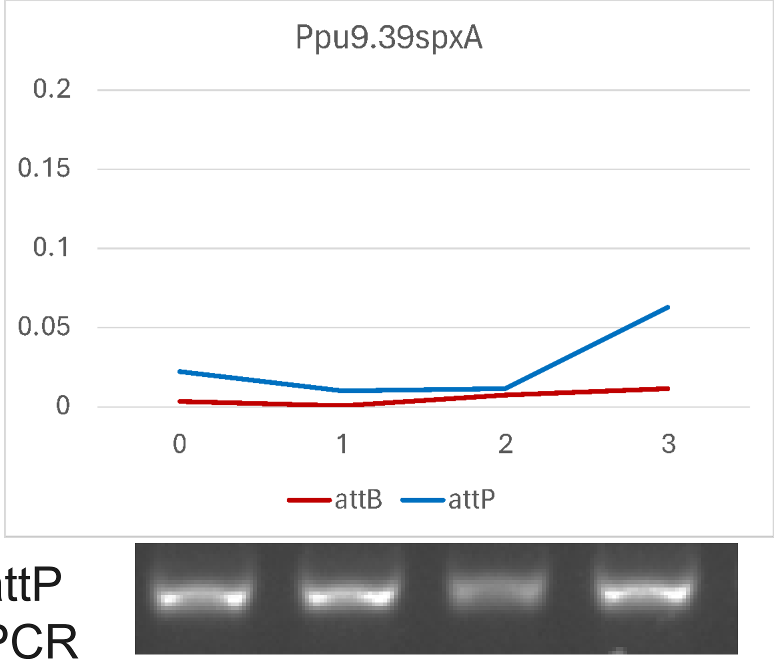
Kelly Williams and Ellis Torrance

WE HAVE DEVELOPED SOFTWARE THAT CAN DETECT AND CHARACTERIZE PROPHAGE ACTIVATION THROUGH DEEP SEQUENCING

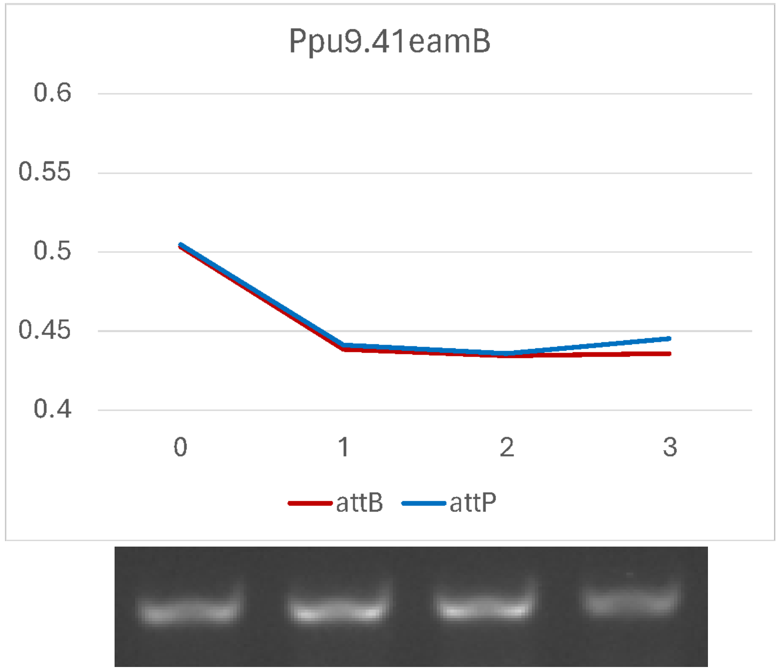


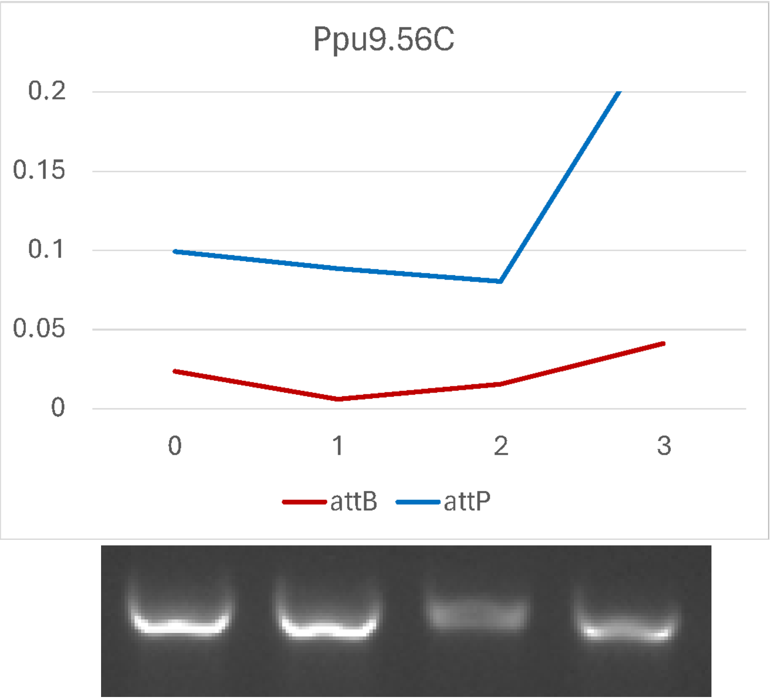
Schoeniger et al., 2016 NAR

MITOMYCIN C INDUCTION OF *P. PUTIDA* KT2440 REVEALS 3 ACTIVE PROPHAGES WITH DIFFERENT INDUCTION KINETICS

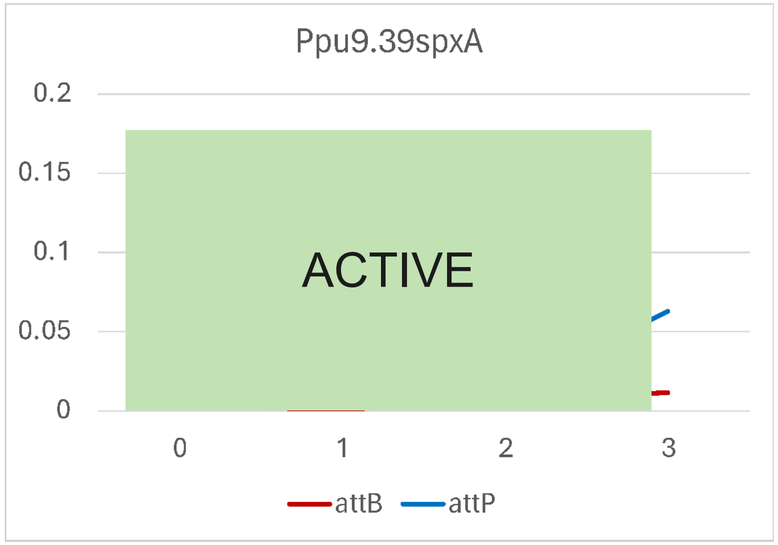


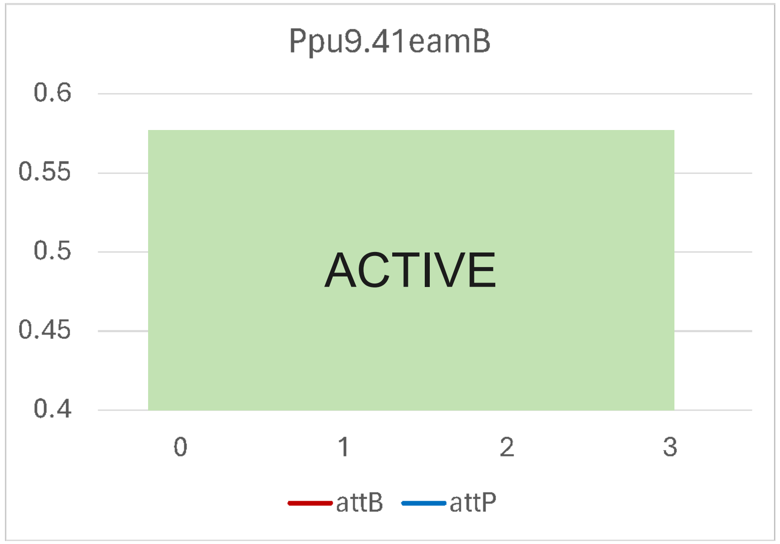
attP
PCR

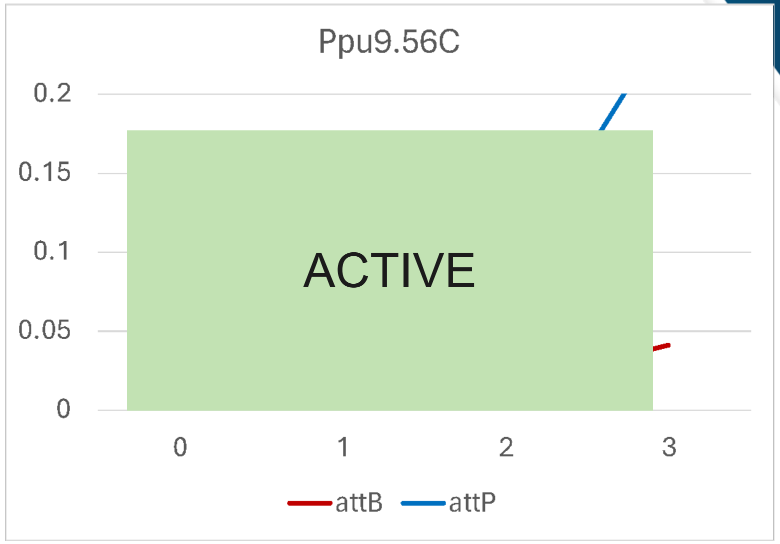




MITOMYCIN C INDUCTION OF P. PUTIDA KT2440 REVEALS 3 ACTIVE PROPHAGES WITH DIFFERENT INDUCTION KINETICS







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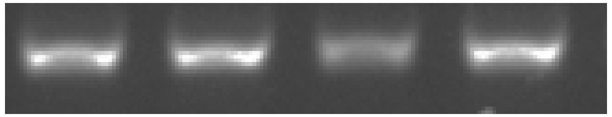
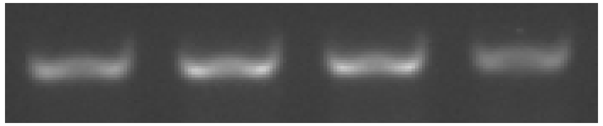
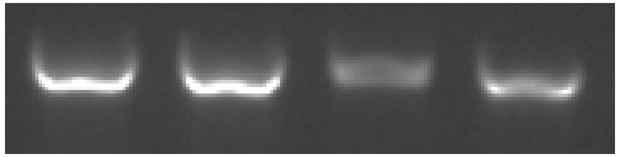
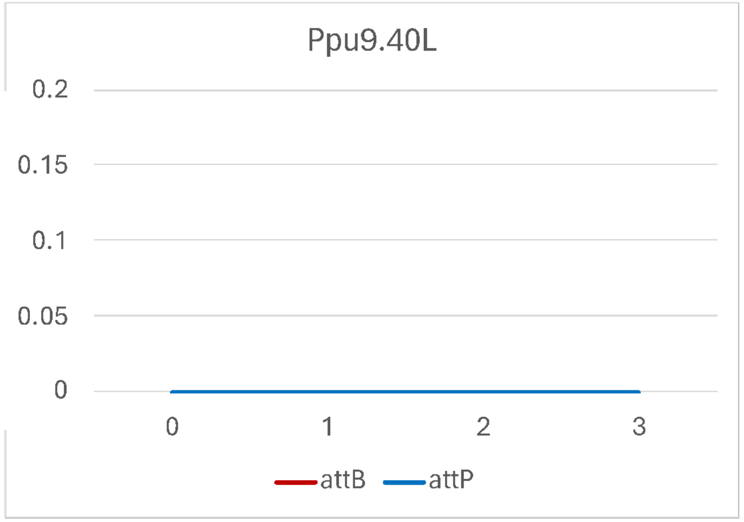


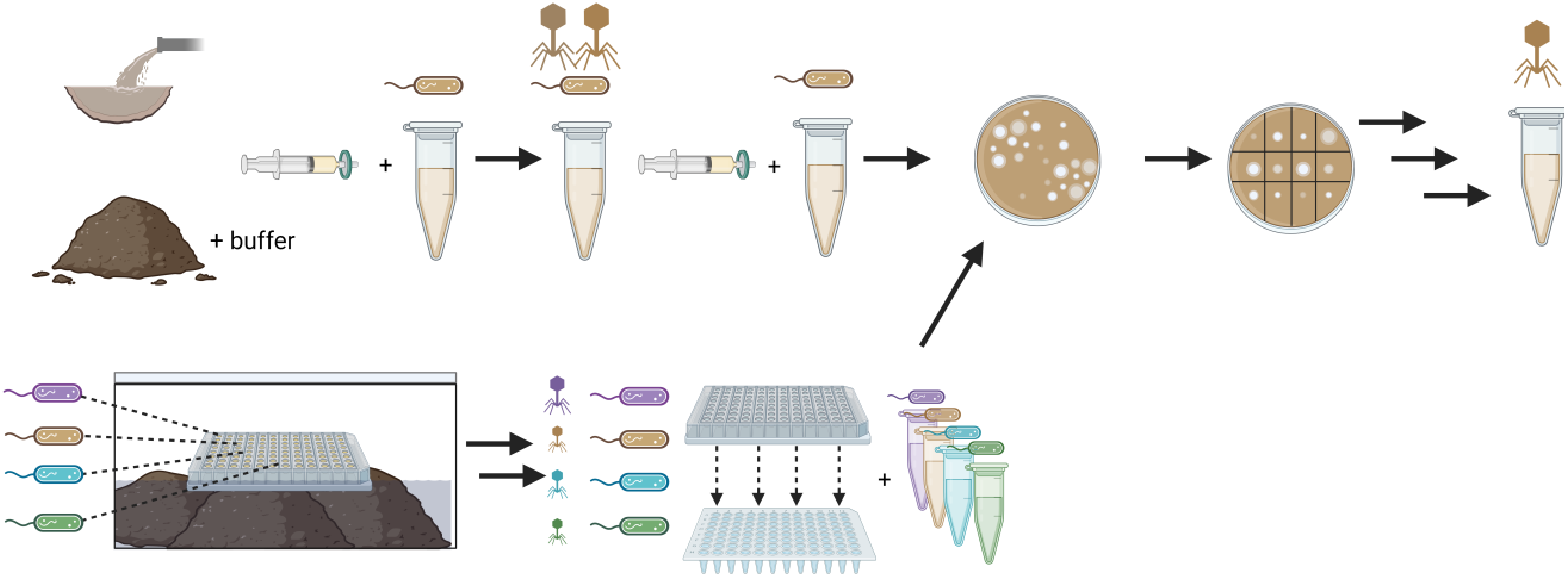




Not induced by MMC

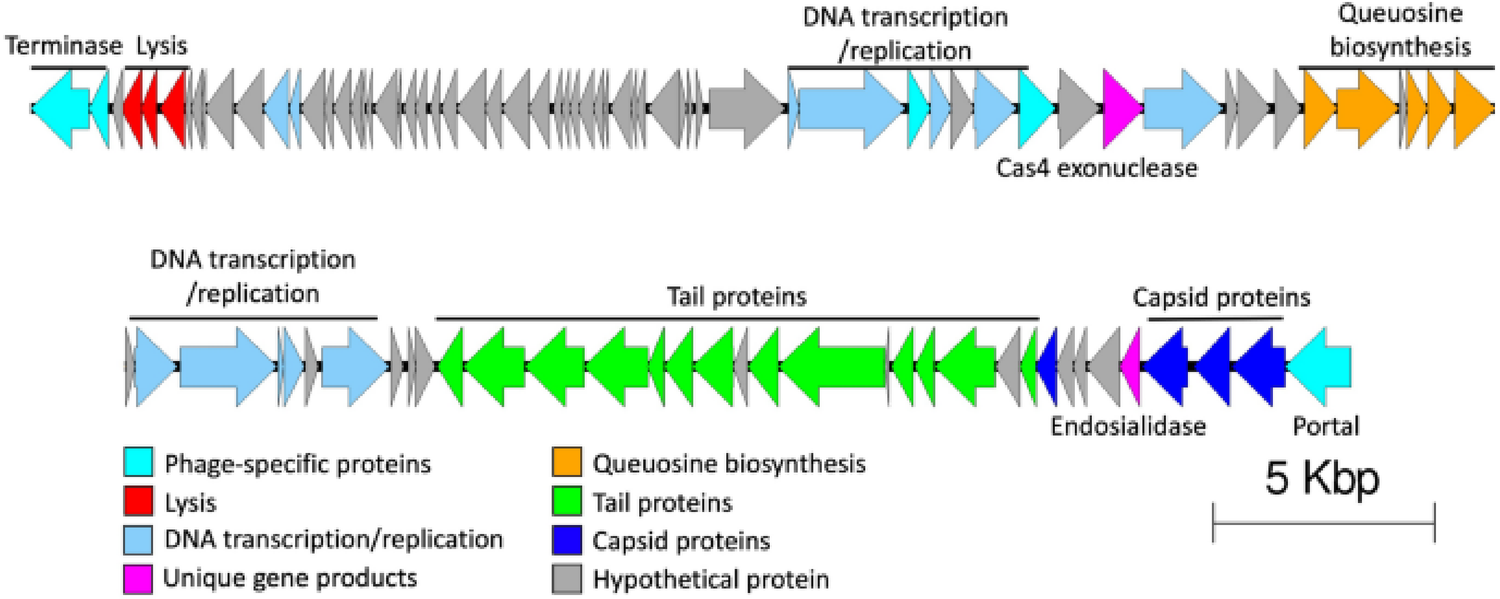


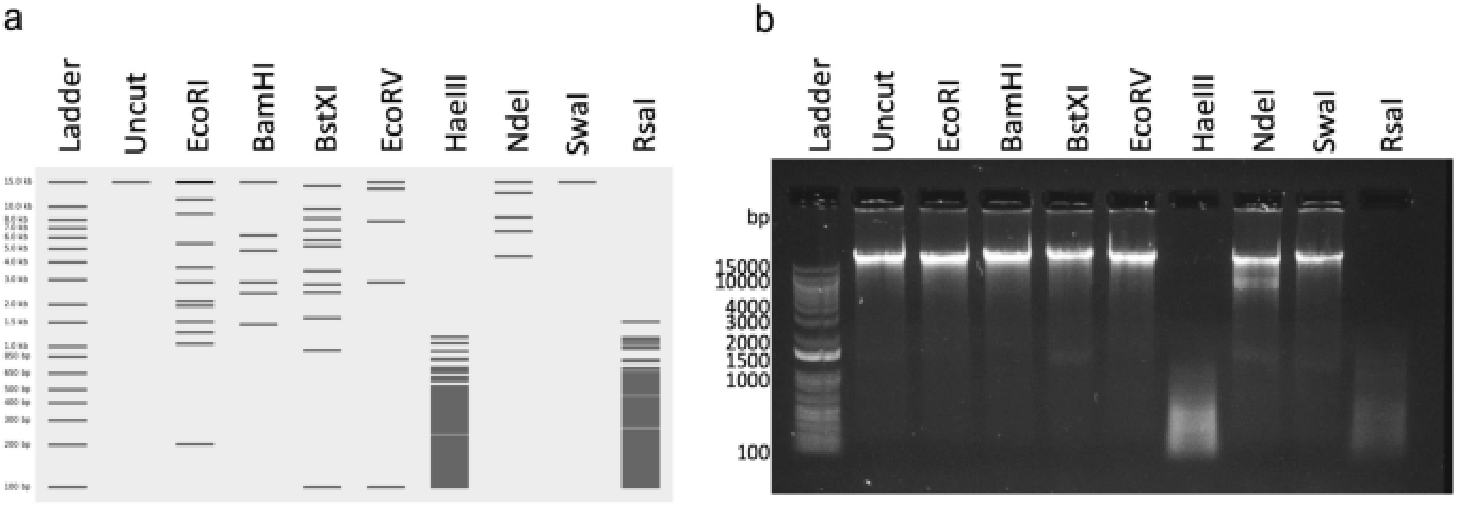
WE HAVE PERFORMED ENVIRONMENTAL PHAGE HUNTS IN HIGH AND LOW THROUGHPUT



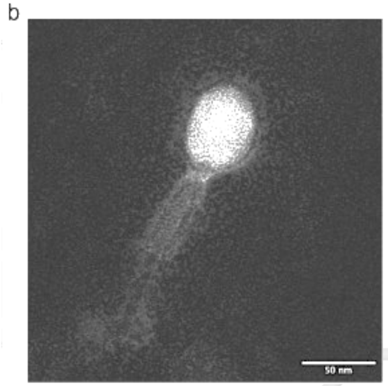
Ben Diaz

P. PUTIDA PHAGE MICATH HAS MODIFIED DNA AND A LARGE HOST RANGE





- MiCath was isolated from garden soil on P. putida S12
  - Can infect strains from two GTDB species
- Genome encodes a queuosine biosynthesis pathway that protects MiCath DNA from restriction enzymes
  - Hypothesized due to hyper modified guanine, 2′-deoxyarcheosine (dG+) based on reports from other phages



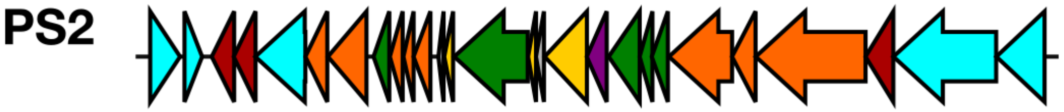
Jaryenneh, Schoeniger, Mageeney 2023 Scientific Reports

TWO ADDITIONAL NOVEL PHAGES FOR *P. PUTIDA* KT2440 HAVE BEEN ISOLATED

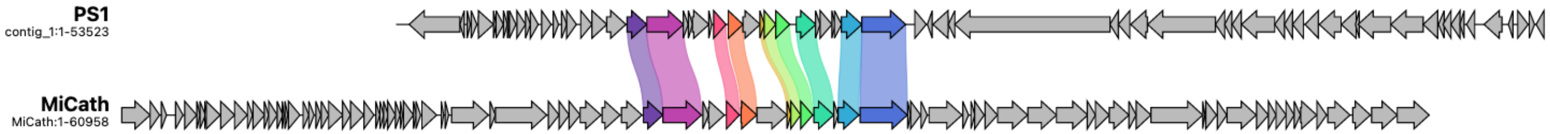
- PS1: 53.5kbp dsDNA phage; little similarity to known phages

- PS2: 14.9kbp dsDNA phage and resembles coli phage PRD1

■ DNA interacting
■ Lysis
■ Tail
■ Hypothetical
■ Capsid
■ Phage-specific
■ Assembly
■ Queuosine Biosynthesis



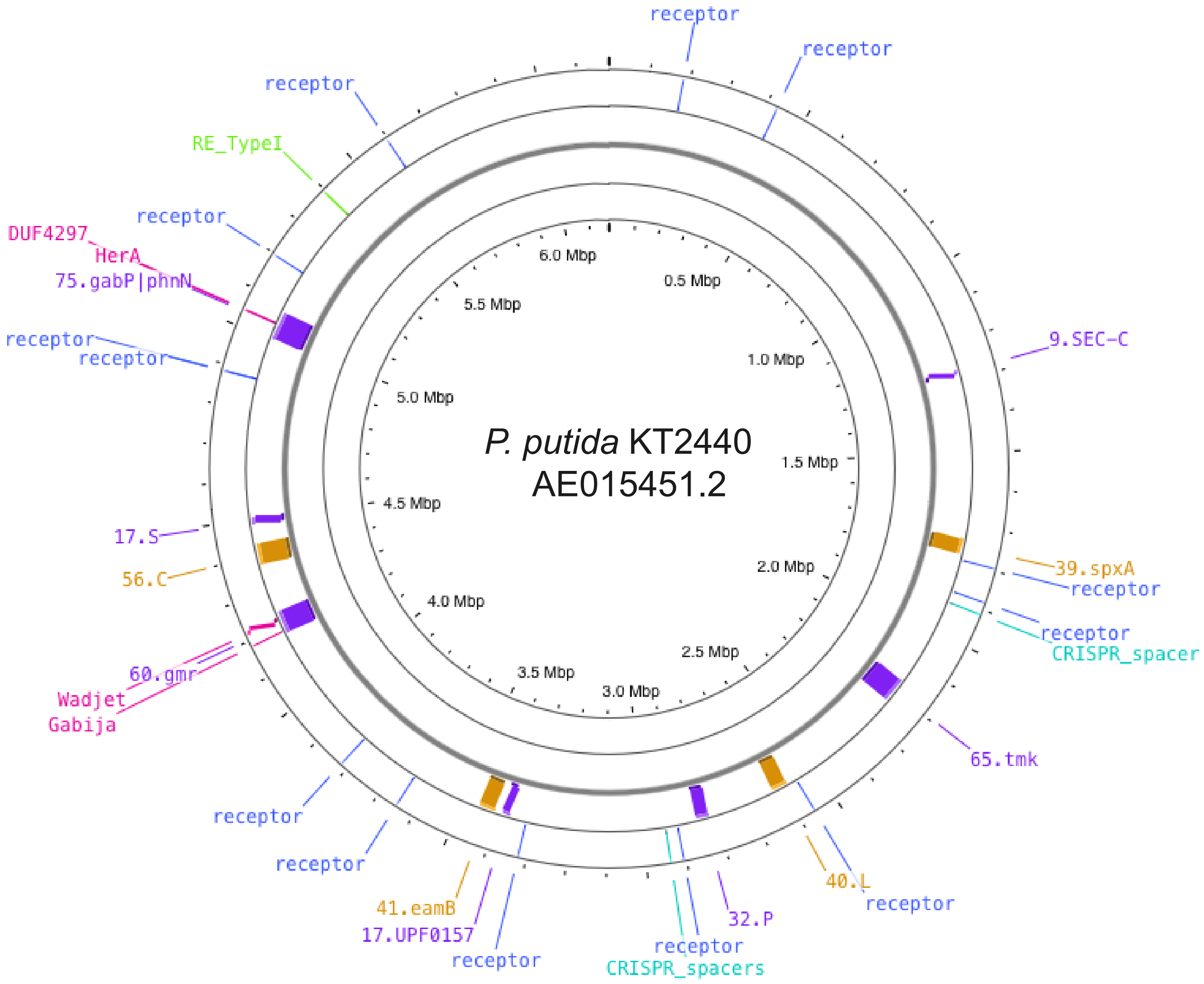
- PS1 and MiCath share the same queuosine biosynthesis pathway



Ben Diaz

UNDERSTANDING DEFENSE SYSTEMS AND PHAGE DYNAMICS IN KT2440

- BADIS software tool combines GI searching with padloc defense finding and custom receptor HMMs for a comprehensive look at phage defense
- 4 systems called in KT2440
  - 3 located on MGEs
  - 8 putative defense proteins called



Rohan Krishna

CONCLUSION

- Prophages and genomic islands are abundant in Pseudomonas putida genomes
- Although prophages are active traditional strategies for isolation were unsuccessful
- We have discovered three novels phages
  - MiCath
    - Modified DNA genome
    - Large host range
  - PS1
    - Hypothesized modified genome
  - PS2
    - Small genome (<15kbp)
    - Similar to tectiviridae PRD1
- Defense systems are found and may play a role in low phage numbers

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