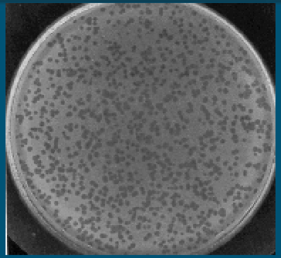
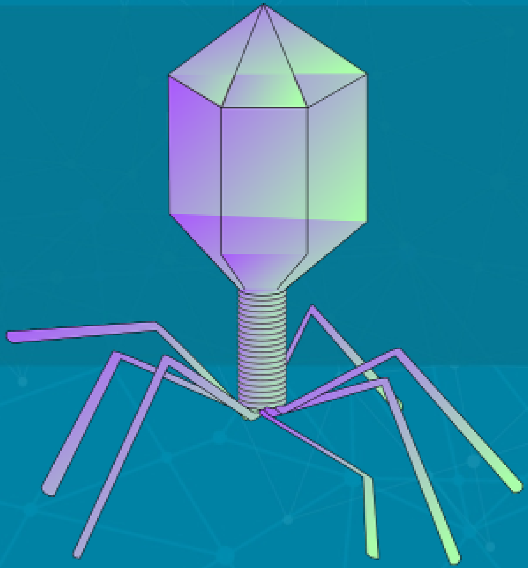
SAND2021-11585C

Session 1: Phage Ecology





Thursday September 23, 2021 AM Session

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Phage Ecology

September 23, 2021

All times in MDT.

9:00-9:10am Opening Remarks

9:10-9:40am Britt Koskella, UC Berkeley

Phages as key members of their ecosystem

9:40-9:50 Gareth Trubl, Lawrence Livermore National Laboratory

Unraveling the hidden potential of active microbial and viral communities in soil ecosystems

9:50-10:00 Joanne Emerson, UC Davis

Exploring the spatiotemporal patterns of soil viral communities in tomato fields

10:00-10:10 Kim Seed, UC Berkeley –

Fighting with phages: how epidemic *Vibrio cholerae* defends against viral attack

10:10-10:20 BREAK

10:20-10:30 Basem Al-Shayeb, UC Berkeley

10:30- 10:40 Karthik Anantharaman, UW Madison.

Computational approaches to advance phage ecology in microbiomes

10:40- 11:10 Ruonan Wu, Pacific Northwest National Lab -

Unearthing climate impacts on soil viruses.

11:10- 11:40 Catherine Mageeney, Sandia National Lab -

Phage Factory: A phage for any bacteria

BREAK

RETURN BY:

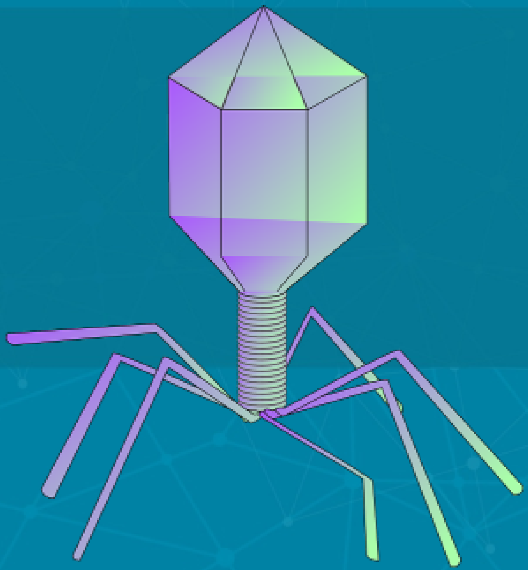
9:20AM PDT

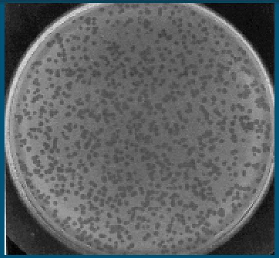
10:20 AM MDT

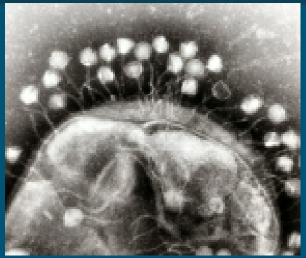
11:20 AM CDT

12:20 PM EDT

Phage Factory: A phage for any bacteria







Catherine M. Mageeney, Ph.D.

Senior Member of Technical Staff

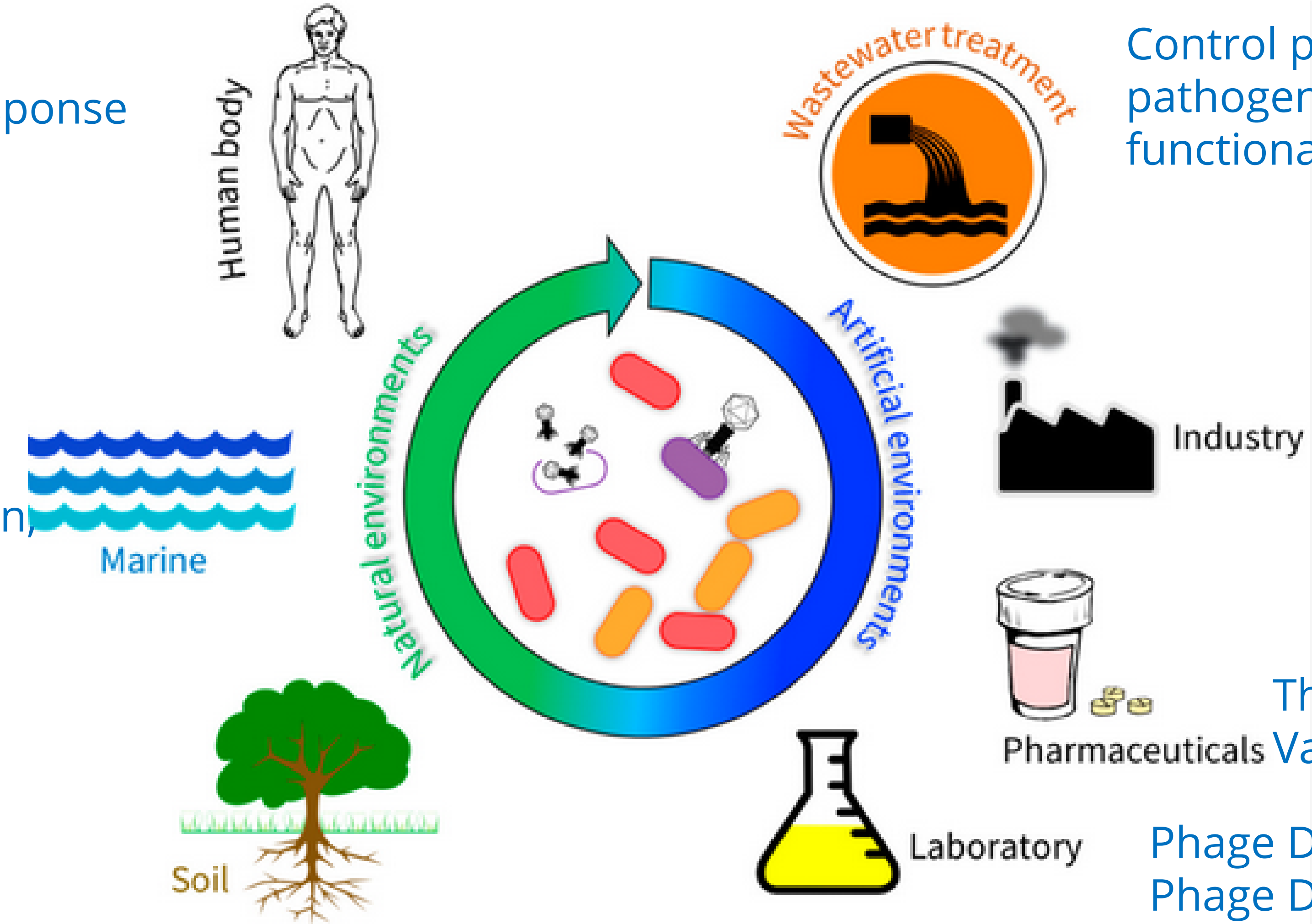
Sandia National Laboratories, Systems Biology

Phages for Health & Energy
SYMPOSIUM
Bridging National Lab & Academic Research Capabilities

Outline

- Phage Application Spaces
- Phage Discovery and Phage Factory
- Phage Factory for Therapeutics
  - Application to *Pseudomonas aeruginosa*
- Phage Factory for Energy
  - Phage Landscape for organisms of DOE relevance
  - Application to *Burkholderia cepacia* complex and *Streptomyces venezulae*

Phages are found in nearly every environment with broad impacts and applications



Microbiome
Immune response

Biogeochemical processing (turn over of carbon, nitrogen, and phosphorus)

Biogeochemical processing

Control problems such as pathogenic bacteria, maintain functional microbiomes

Bacterial control in algal ponds
Fermenter bacterial crashes
Pathogen in food processing
Microbiological induced corrosion

Therapeutics
Vaccines

Phage Display
Phage Directed Evolution

Batinovic et al., 2019

Prophages mined from bacterial genomes yield far more phages

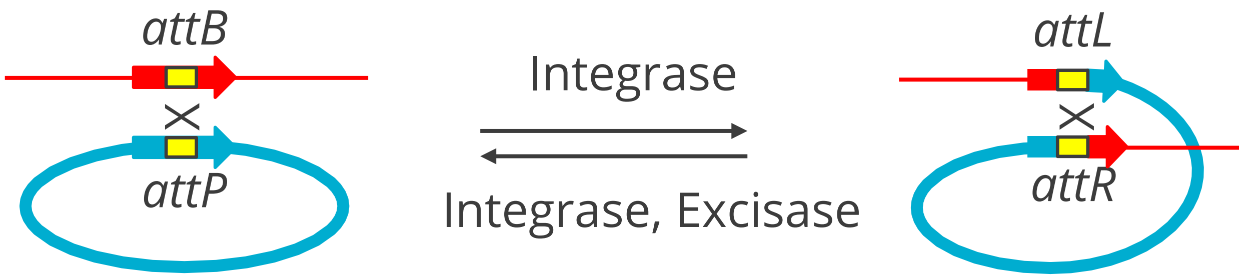
Current WAY

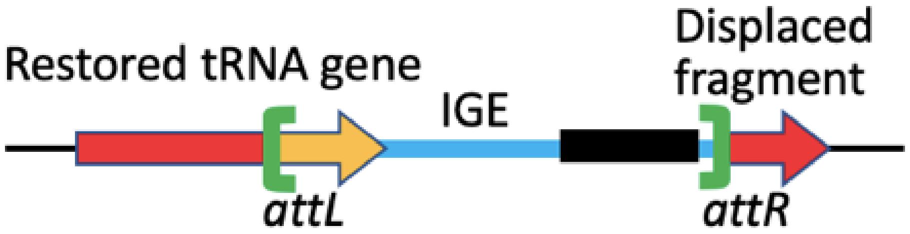
- Fishing approach can have low yield
- Not necessarily host-adapted (may be better adapted to other host bacteria)
- ~15,000 unique genomes in GenBank

Our WAY

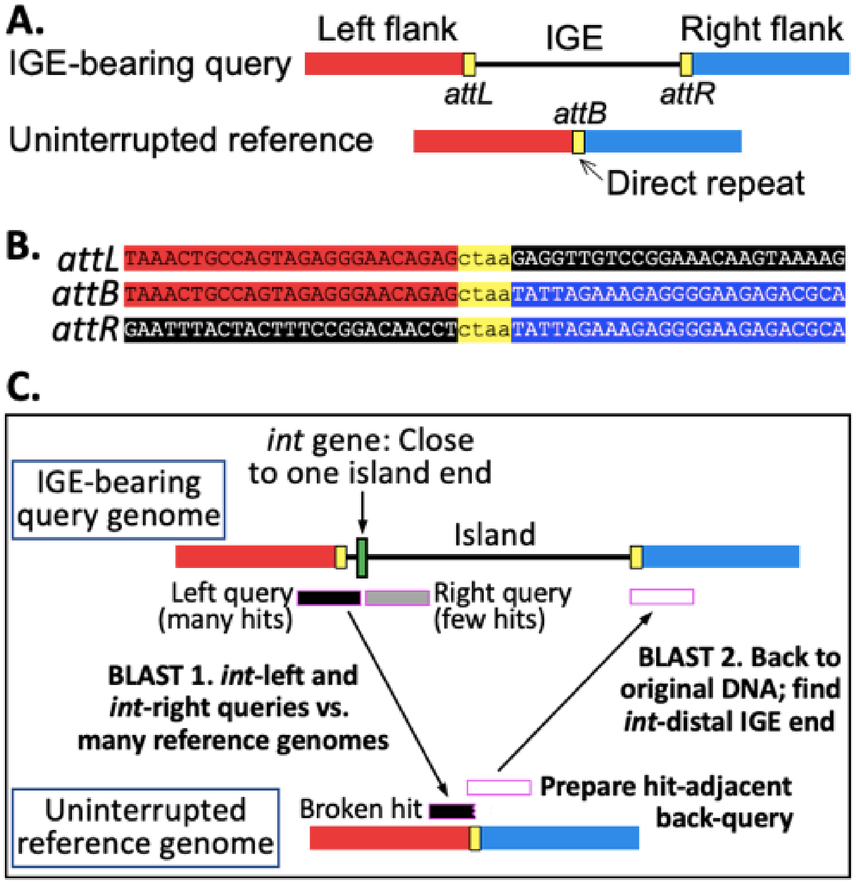
- Bacterial genomes are nets that catch phages
- Phages are host-adapted because we choose them from close relatives
- ~1.2 prophage/genome x 343,000 genomes in GenBank

Our software discovers genomic islands precisely





Islander (Hudson et al., 2015, NAR)

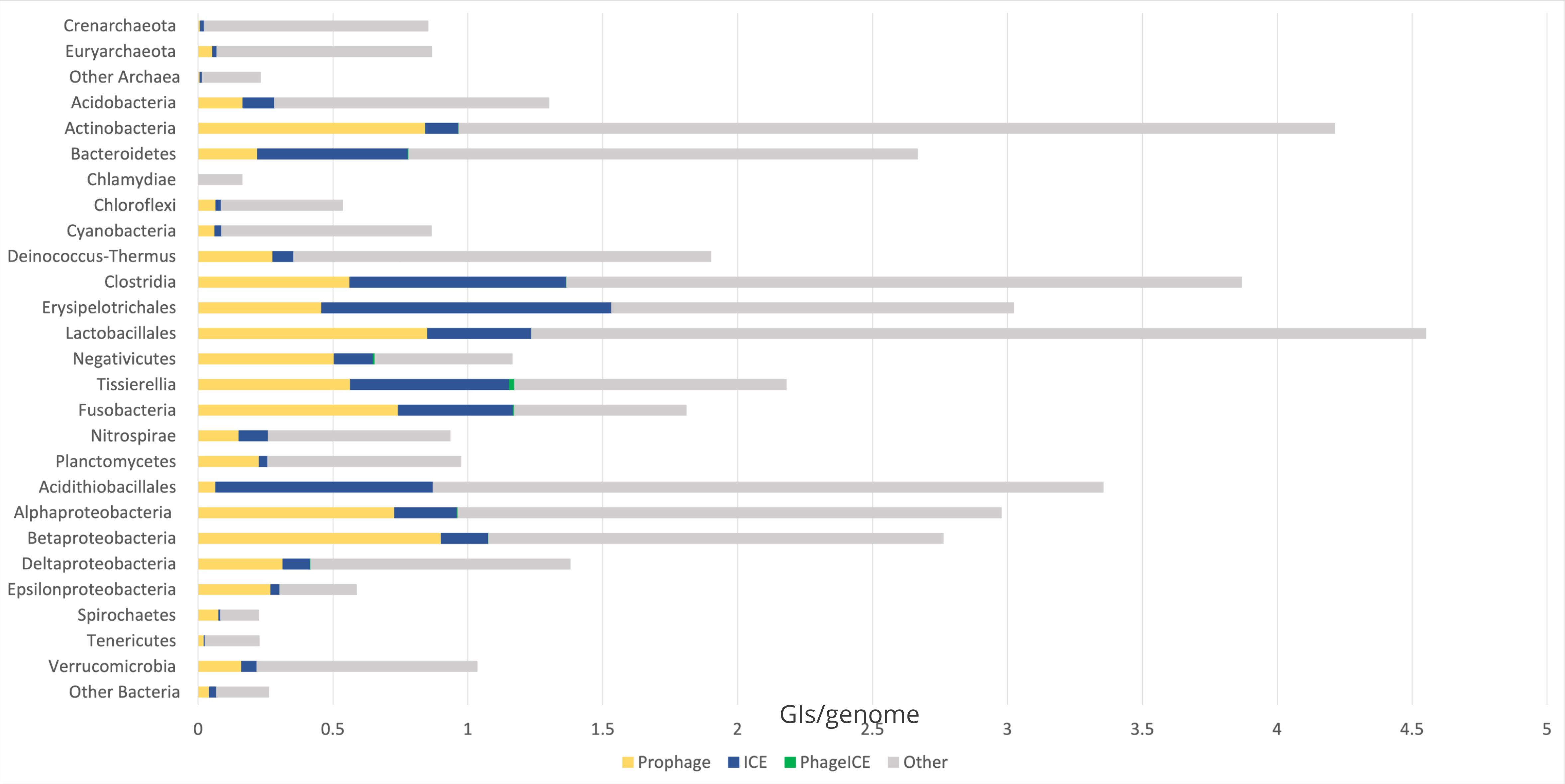


TIGER (Mageeney et al., 2020, NAR)

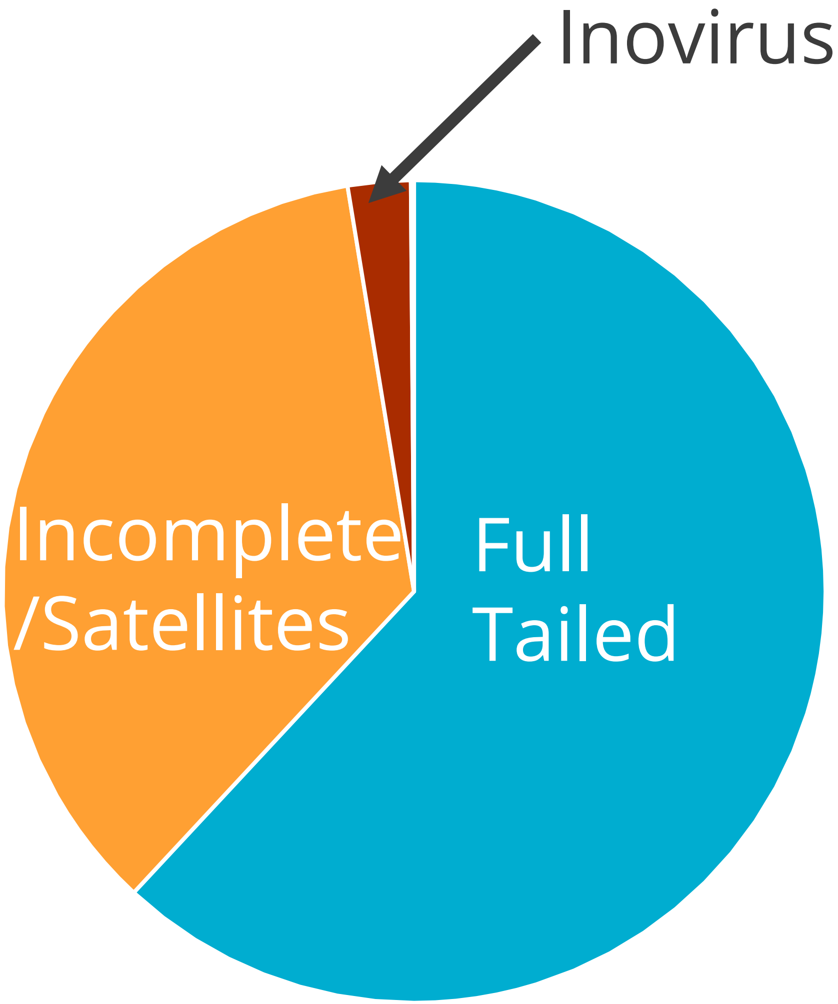
Prophages are found in many bacterial and archaeal genomes

- Applied software to 134,378 bacterial and archaeal genomes
  - Currently working through >300,000 genomes at Genbank
- Precisely identified 358,555 islands = 2.7 islands/genome
  - 74,876 called as prophages

Prophages are found for every phylogenetic group of bacteria and archaea



Most prophages appear to contain a full gene complement

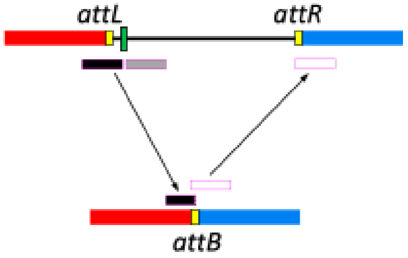


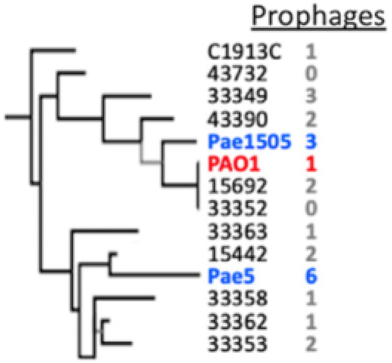
Full Tailed Gene Complement– greater than 25kbp, contains structural and functional phage proteins

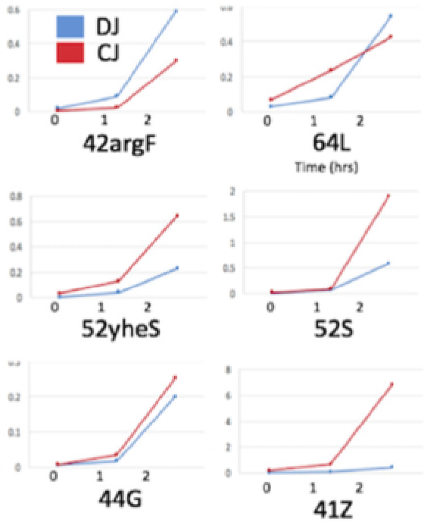
Incomplete Gene Complement/Satellites – contains only one class of phage proteins (structural/functional)

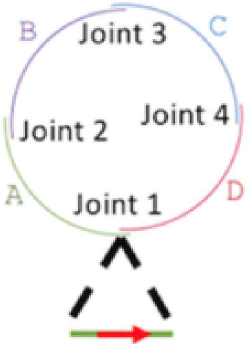
Inovirus - < 20kb, contains pfam zot and/or coat proteins

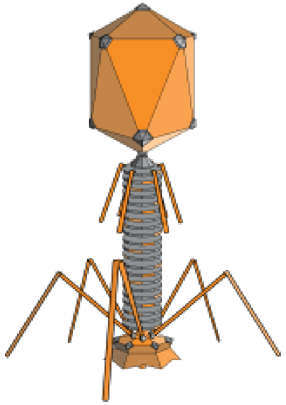
Phage Factory: Bacteria-Agnostic Phage Discovery and Engineering Platform











Prophage database → Identify prophage-rich close relatives of target → Validate phages → [Engineer phages → Reboot]

Mageeney *et al*., 2020, mSystems

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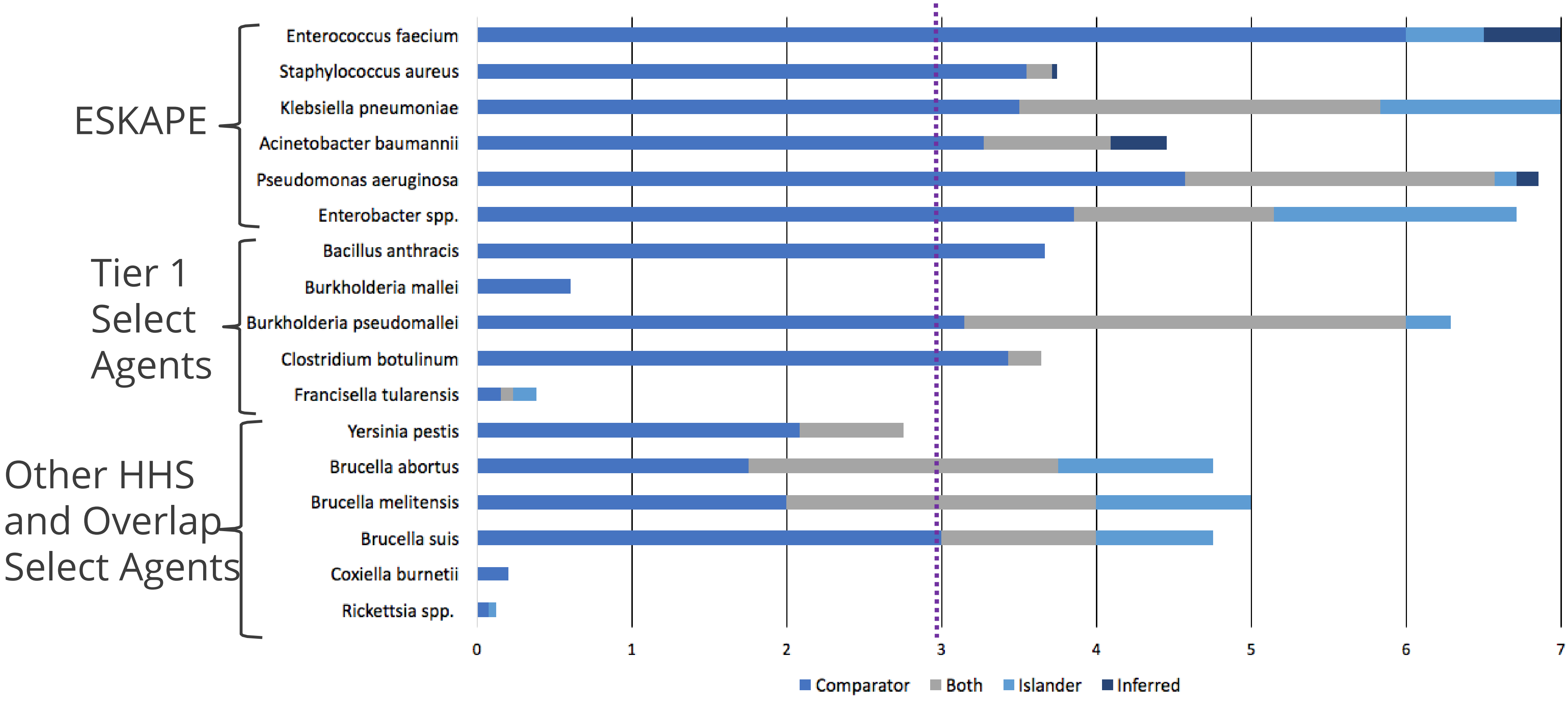
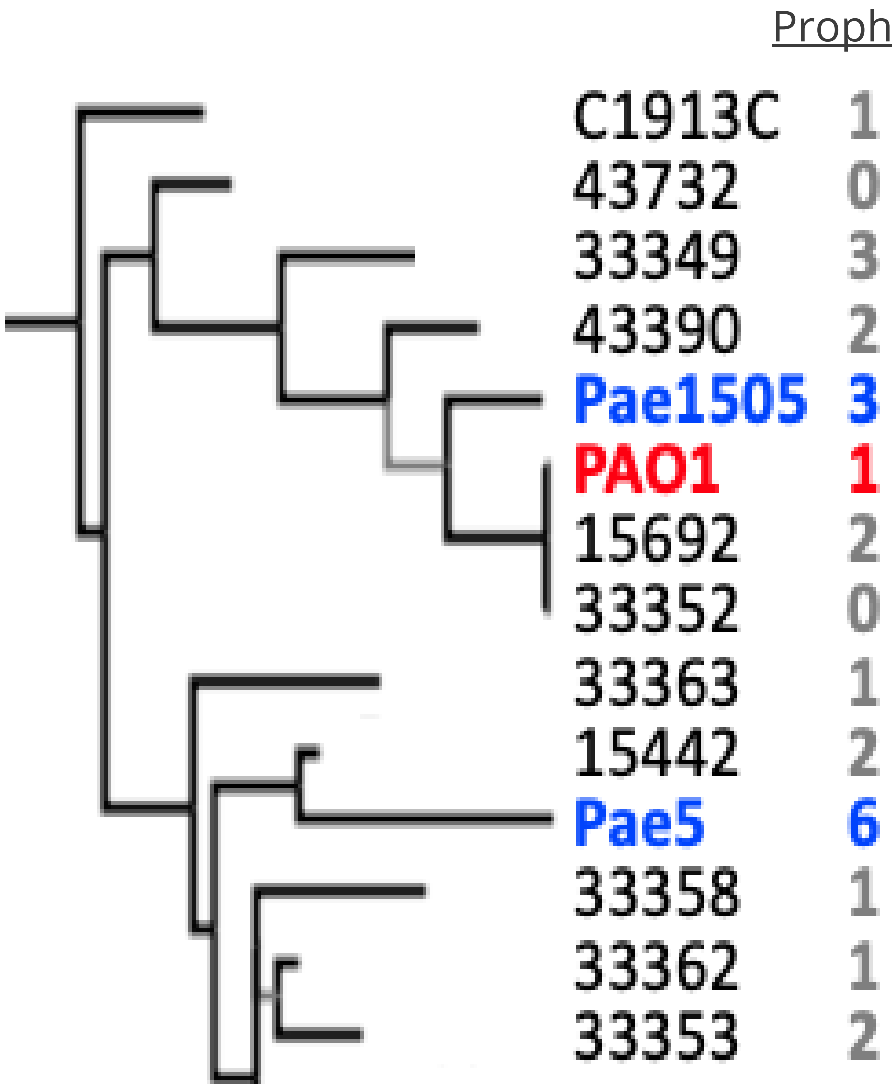
Prophage Yields are Higher than Average for Pathogens



Mageeney et al., 2020 mSystems

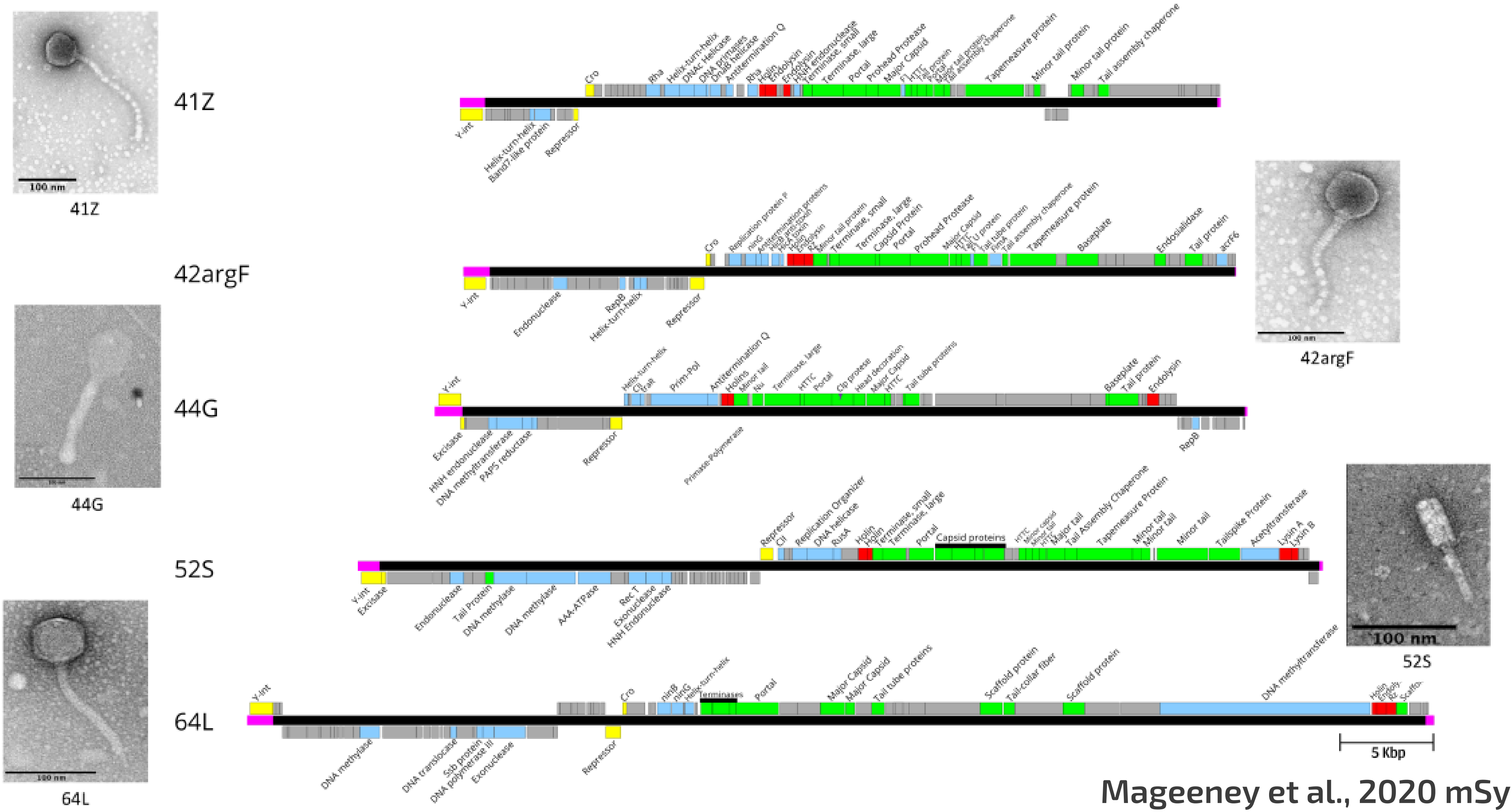
Phages were sourced from prophage-laden close relatives of *P. aeruginosa* PAO1



- Analyzed all *P. aeruginosa* strains available at ATCC
- Chose Pae1505 (ATCC 39324) and Pae5 (ATCC 27853) as prophage sources

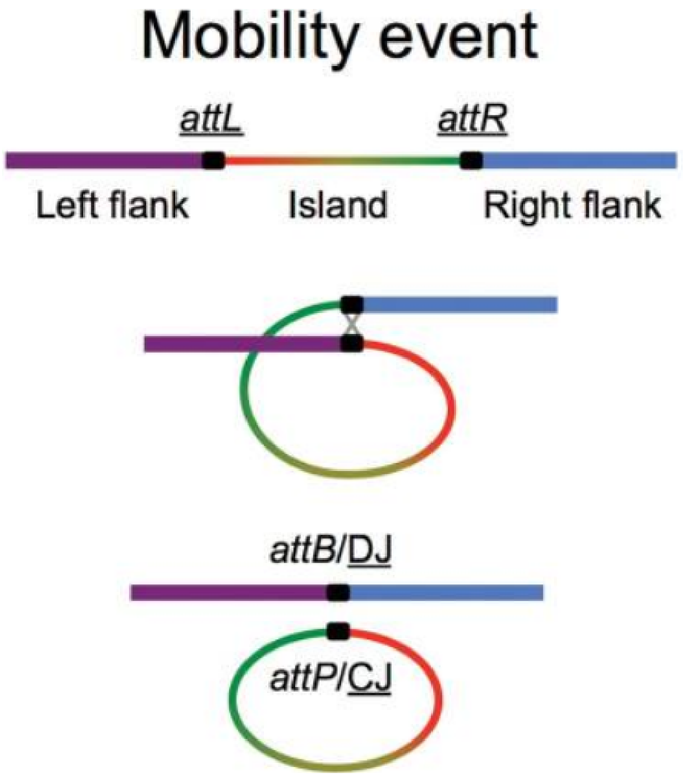
Mageeney et al., 2020 mSystems

5 Prophages were Identified for Engineering

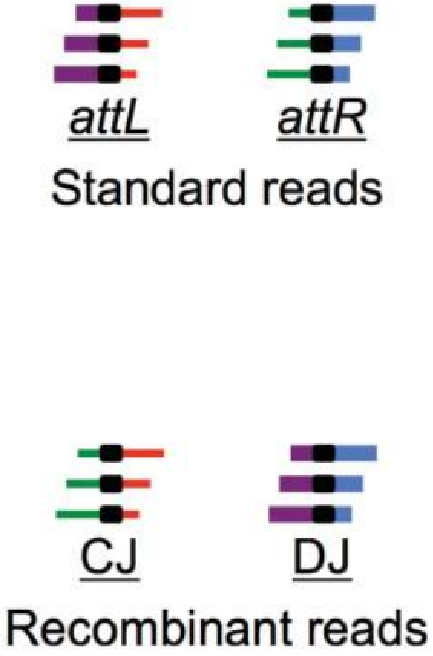


Mageeney et al., 2020 mSystems

Deep Sequencing can detect active prophages

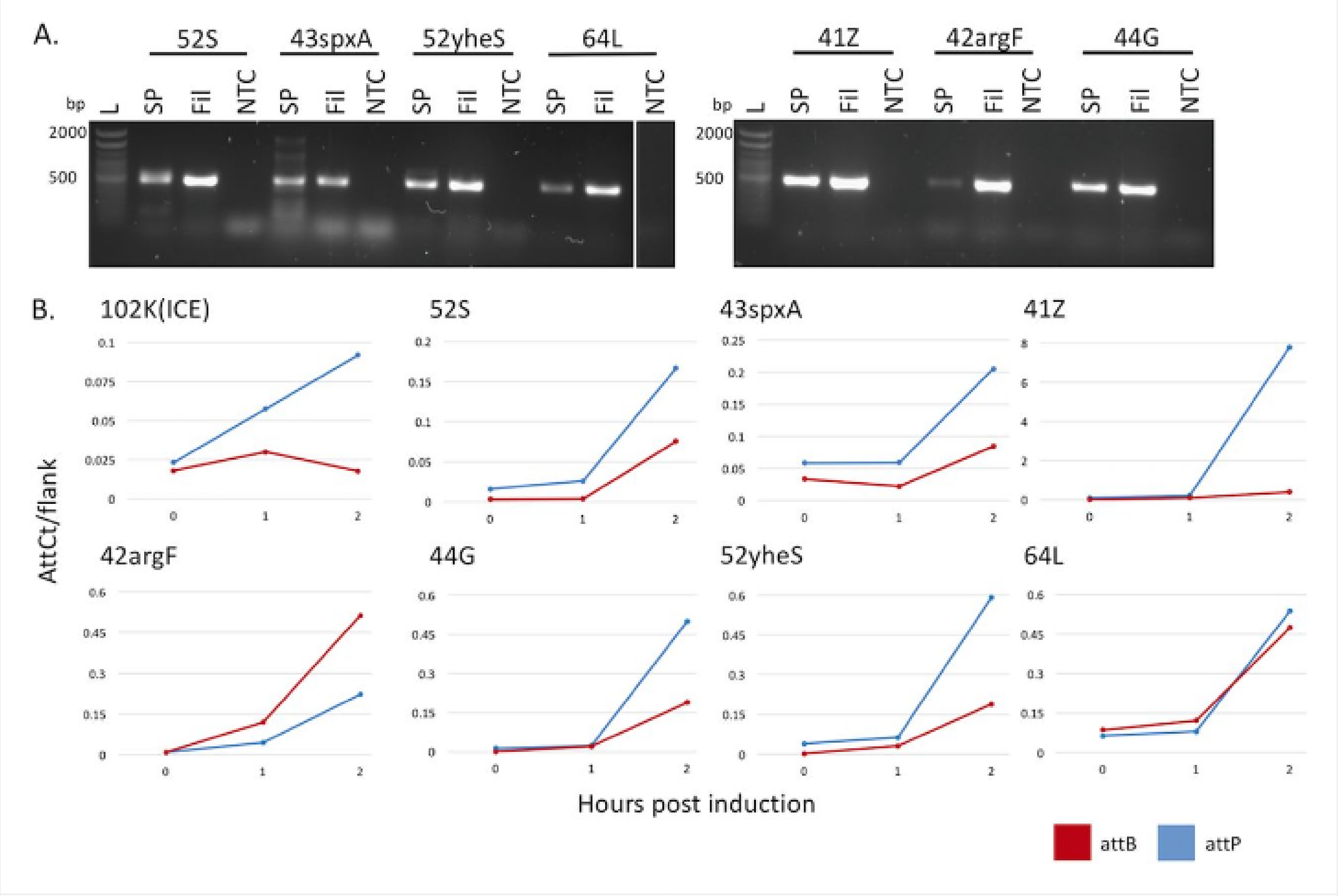


Deep Sequencing



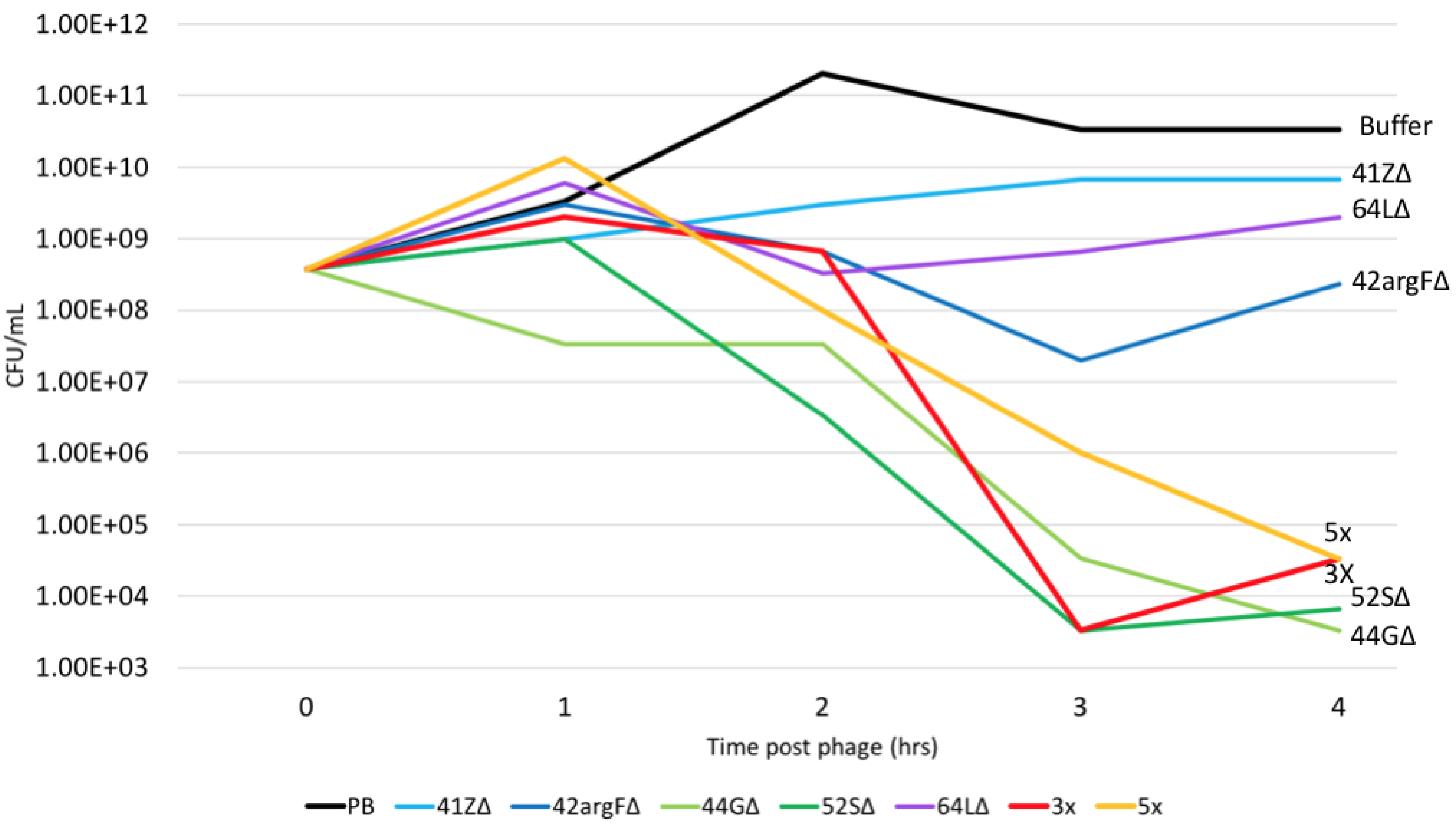
Juxtaposer and attCt - Schoeniger et al., 2016 NAR

Active Prophages can be identified by PCR and deep sequencing



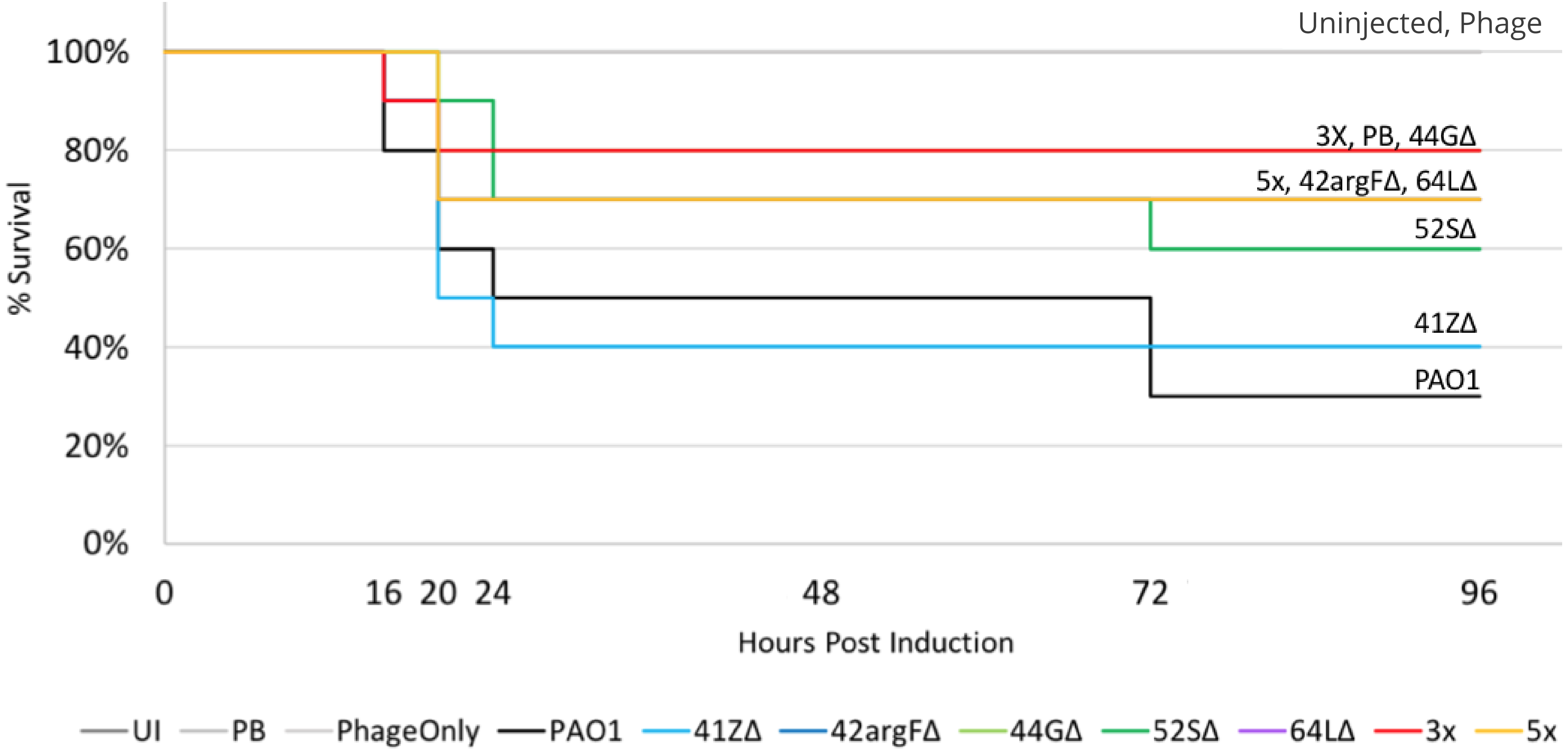
Mageeney et al., 2020 mSystems

Engineered Phages Kill *Pseudomonas aeruginosa* in liquid culture



Mageeney et al., 2020 mSystems

Engineered phages save waxworms in phage therapy trials



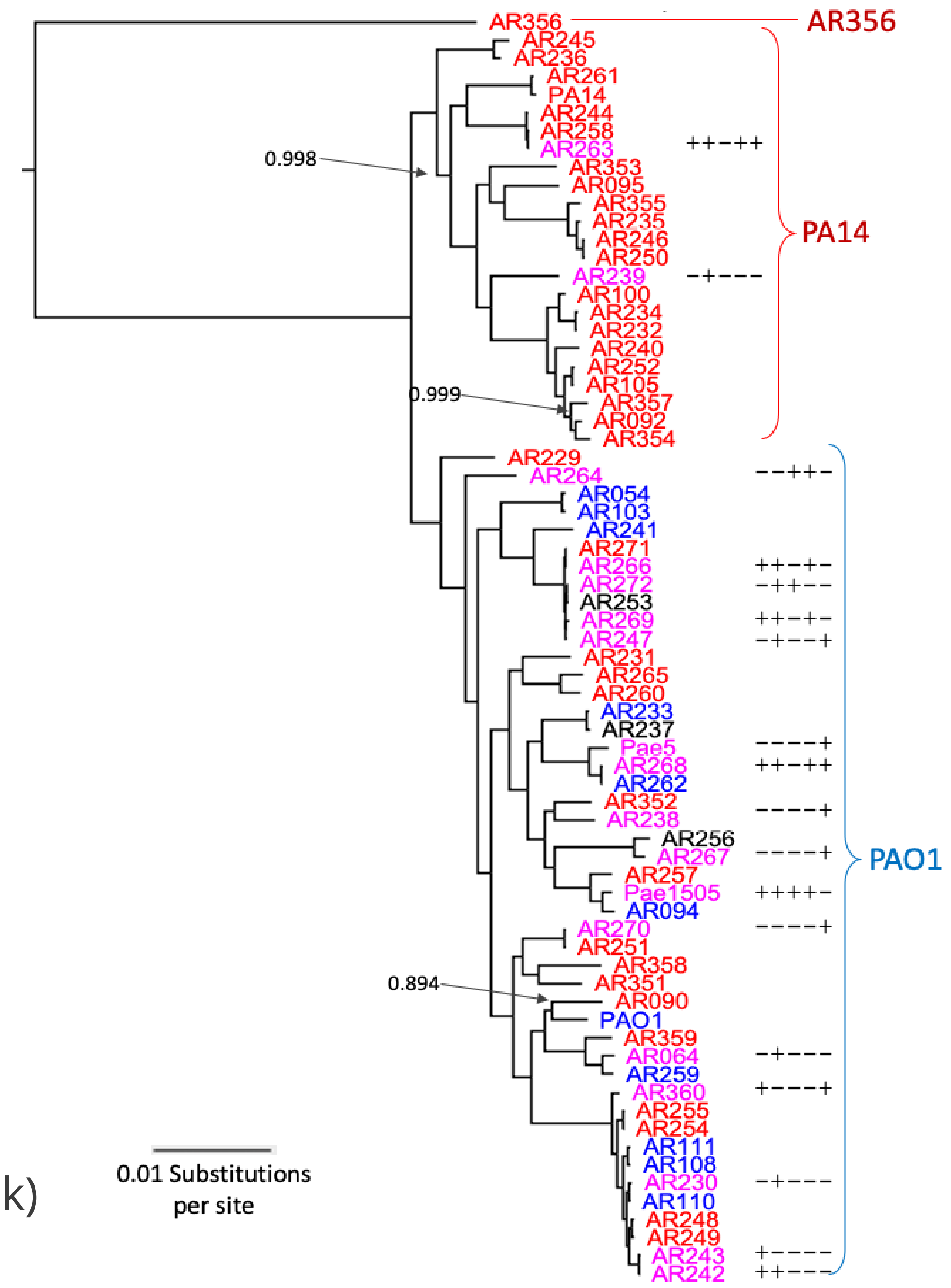
Mageeney et al., 2020 mSystems

Mixed host ranges are seen for engineered phages against antibiotic resistant P. aeruginosa strains

- Obtained collections of AR isolates from CDC collection
- Tested WT and Δintegrase phages in spot tests
- Distance from host strain is the strong correlation for phage infectivity

Work done in collaboration with Linda DeVeaux's Lab at New Mexico Tech

- 11 Fully Sensitive (Blue)
- 18 Mixed Sensitivity (Pink)
- 38 Fully Resistant (Red)



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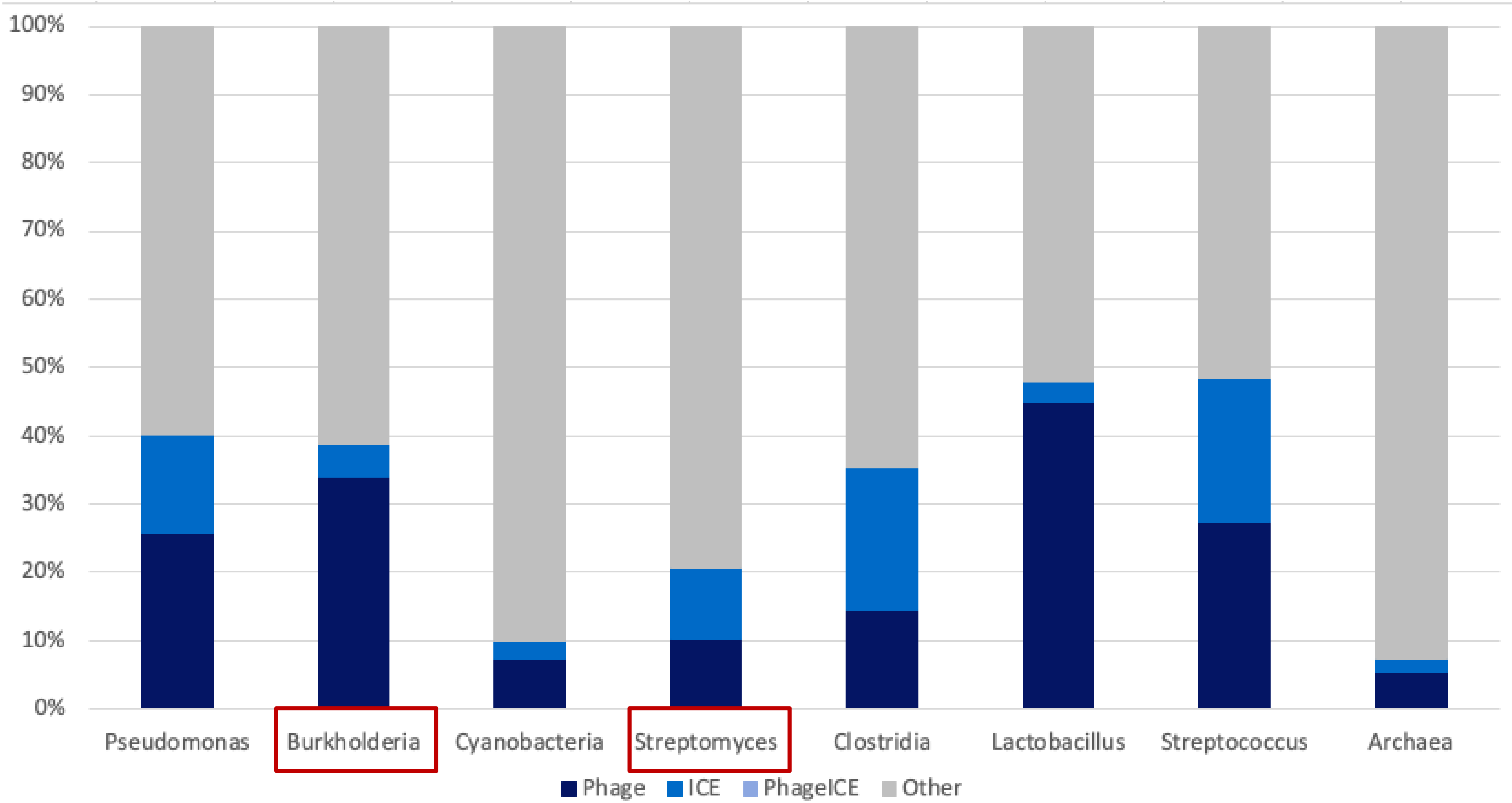
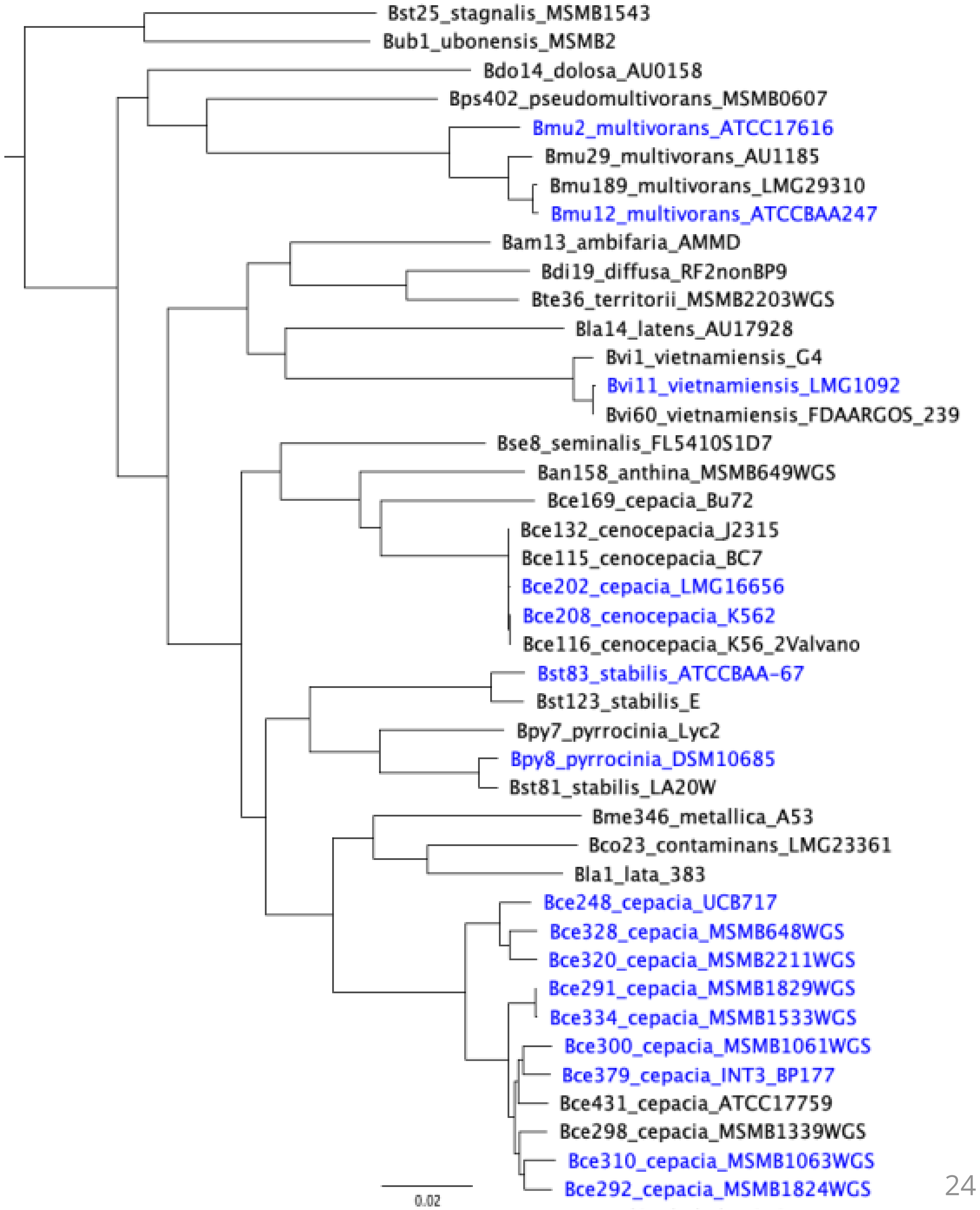
Prophages can be detected for all soil bacteria of interest



B. cepacia complex strains harbor many prophages

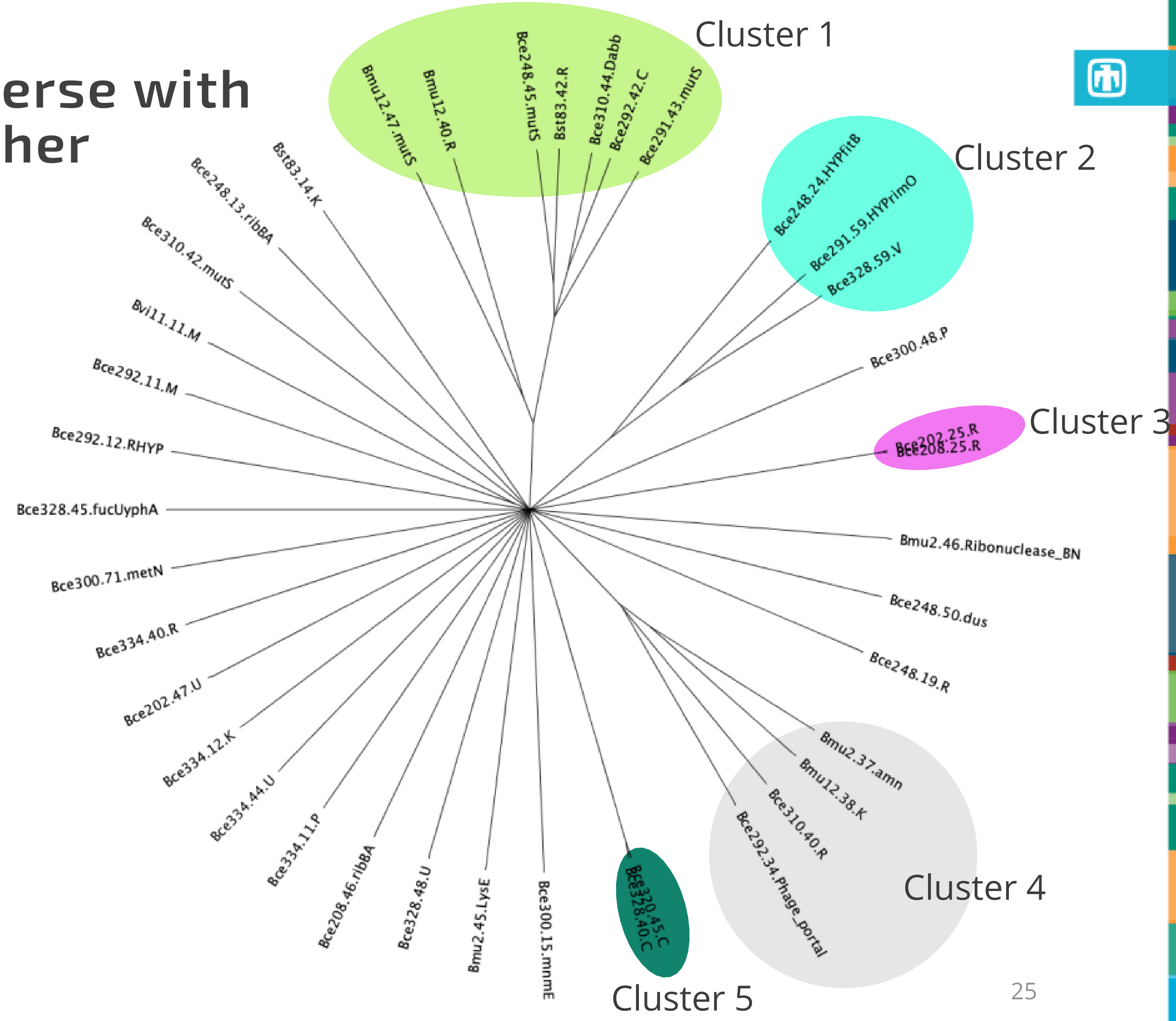
- 16 strains obtained and tested
  - 9 *cepacia*, 2 *cenocepacia*, 2 *multivornas*, 1 *vietnamiensis*, 1 *stabilis*, 1 *pyrrocinia*
- 123 Islands Discovered
- 39 Prophages
  - 30 Full Tailed Gene Complement
  - 6 Incomplete/Satellite
  - 3 Inovirus



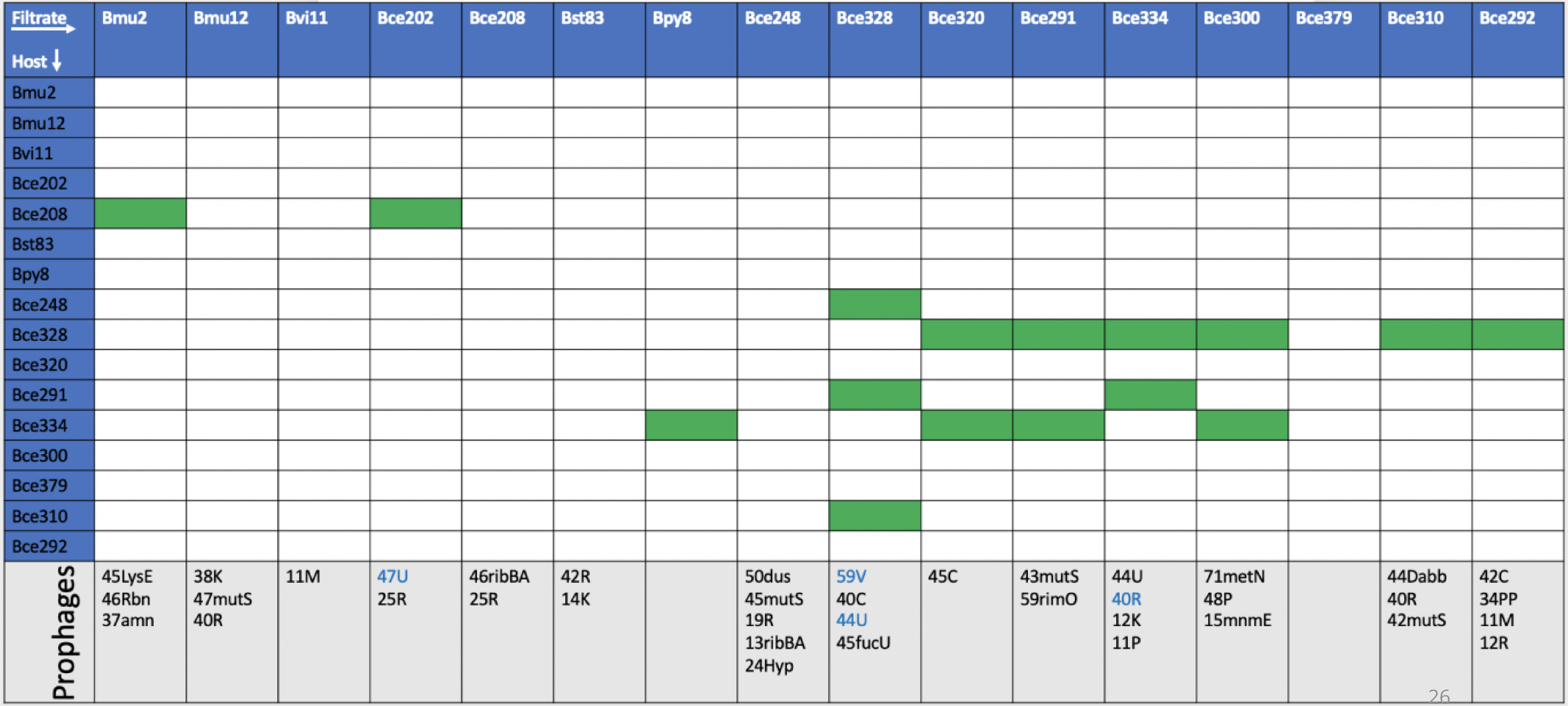
Work Done with Summer Intern
Shawn Barman

Prophages from 16 *Burkholderia* are diverse with few clustering together

- Prophages form 5 cluster with a stringent criteria of 80% ANI spanning >50% of the genome
- There are 21 Singletons not clustered with any other prophages
- Validated 20 prophages through PCR and Juxtaposer analysis



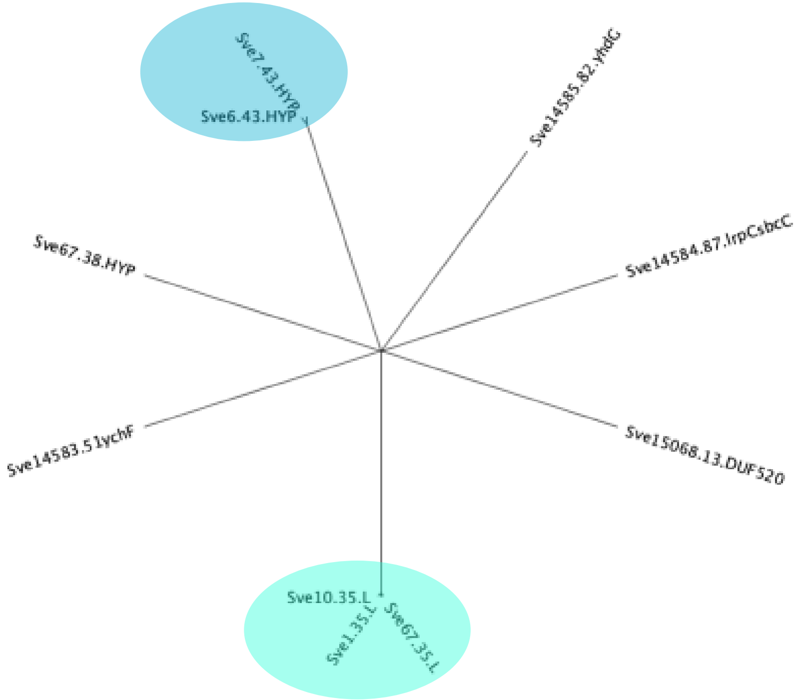
Burkholderia prophages have narrow host ranges



Filtrate → Host ↓	Bmu2	Bmu12	Bvi11	Bce202	Bce208	Bst83	Bpy8	Bce248	Bce328	Bce320	Bce291	Bce334	Bce300	Bce379	Bce310	Bce292
Bmu2																
Bmu12																
Bvi11																
Bce202																
Bce208	🟩			🟩												
Bst83																
Bpy8																
Bce248									🟩							
Bce328										🟩	🟩	🟩	🟩		🟩	🟩
Bce320																
Bce291									🟩			🟩				
Bce334							🟩			🟩	🟩		🟩			
Bce300																
Bce379																
Bce310									🟩							
Bce292																
Prophages	45LysE 46Rbn 37amn	38K 47mutS 40R	11M	47U 25R	46ribBA 25R	42R 14K		50dus 45mutS 19R 13ribBA 24Hyp	59V 40C 44U 45fucU	45C	43mutS 59rimO	44U 40R 12K 11P	71metN 48P 15mnmE		44Dabb 40R 42mutS	42C 34PP 11M 12R

Streptomyces venezuelae

- Filamentous soil dwelling bacterial with uses in natural product and found in many soil consortia
- Produces many antibiotic compounds which may have unknown impacts on GI mobility and community structure
- Few bacterial strains sequenced (13 found in NCBI)
- Few phages identified (19 sequenced)



Identified 10 prophages and validated 1 which has 3 members in cluster

Work Done with DOE SULI
Summer Intern Catherine Ly

Conclusions

- Bacterial genomes are a rich source of prophages
- We have developed a large prophage database
- Our phage platform is agnostic and can be rapidly implemented to make phages that kill or phages with cargo
- Engineered prophages are capable of killing *P. aeruginosa in vivo* and *in vitro*
- We have discovered many phages for energy applications in *Burkholderia* and *Streptomyces*
  - Currently working to convert into phages for cargo delivery

Acknowledgements

Sandia National Laboratories

- Kelly P. Williams
- Raga Krishnakumar
- Joseph Schoeniger
- Steve Branda
- Anupama Sinha
- Shawn Barman – Summer Intern
- Catherine Ly – DOE SULI Summer Intern
- Alicia Rokes – Summer Intern
- Britney Lau – Summer Intern

New Mexico Tech

- Linda DeVeaux
- Ashley Chavez
- Angelica Cave
- Katie Persinger

Michael Jewett's Lab (Northwestern Univeristy) - *Streptomyces* Strains
David Wagner (Northern Arizona University) – *Burkholderia* Strains

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THANK YOU

Questions?

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