The toxin-antitoxin module PemIK may allow bladder isolates of *Escherichia coli* to resist lysis by bladder phage

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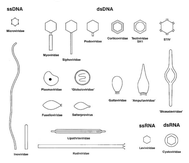
Abstract

Bacteria populations in the bladder microbiome can fluctuate drastically from day to day, which may have implications for bladder health. Bacterial population boom-and-bust patterns resemble the dynamics of bacteria and its predator viruses, bacteriophage (phage). The bacterial population grows until lysed by a phage, whereupon the bacterial population declines; the phage population follows, first increasing and then decreasing. As the phage population decreases, the bacteria population rebounds and the cycle repeats. Phage can interact with bacteria in complex ways: they can eradicate bacterial populations via cell lysis, provide genetic traits to bacteria via transduction and chromosome integration, and block infection by other phages. Phage are highly abundant in the bladder, they rival bladder bacteria in terms of diversity, and their presence correlates with horizontal acquisition of genetic content by bladder bacteria. To assess the effect of phage on the bladder microbiota, we must determine the host range of bladder phage and understand genetic determinants associated with that host range. Here, we utilize *E. coli* phages Greed and Lust as a model system to identify genetic content differences between *E. coli* strains susceptible and resistant to infection by these bladder coliphages. Using a standard phage lysis assay, we screened *E. coli* urine bladder isolates (UMB), standard lab strains (B, C, K-12), and uropathogenic *E. coli* (UPEC). All lab *E. coli* strains (K-12, B, C) and three UMB strains were susceptible to both Greed and Lust. All three UPEC and 47 UMB strains were resistant to both Greed and Lust. We then analyzed the genomes of the screened *E. coli* strains using a novel algorithm that identifies genetic content associated with a difference in a binary phenotype (e.g. phage susceptible and resistant). The core of the algorithm matches a query sequence to genomes of the susceptible and resistant strains. If a hit is shared between susceptible and resistant strains, it is eliminated from further consideration; if a hit is not shared, then its open reading frame (ORF) is outputted. This ORF is then analyzed for sequence homology to known genes to assess if it is relevant to the phenotype of interest (e.g., phage host range). Using this approach, we identified PemIK, a toxin-antitoxin (TA) module, that is present in some resistant strains but not in any of the susceptible strains. TA modules consist of a toxin that arrests cell growth under stress conditions and eventually kill the cell. Under unstressful conditions, the antitoxin binds and neutralizes the toxin; under stressful conditions, the antitoxin is degraded by proteases. the toxin is released and the cell dies. Little is known about PemIK in terms of phage infection; however, its homologue MazEF has been associated with phage cycle abortion. We hypothesize that cloning of the PemIK module into the susceptible *E. coli* strain K-12 will result in a K-12 strain resistant to Greed and Lust infections. We further hypothesize that bladder bacteria could use TA systems to resist phage infection and thus modulate predator-prey dynamics.

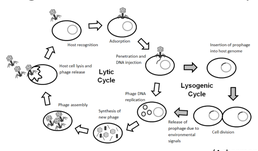
Phage, bacteria, and the bladder

1. Phage are viruses of bacteria



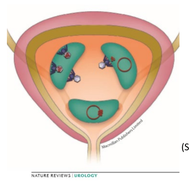
(Doss et al. 2017)

2. Phage can infect via different pathways



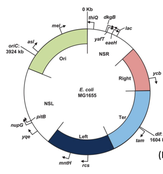
(Ackerman 2009)

3. Phage infect bladder bacteria



(Stone 2018)

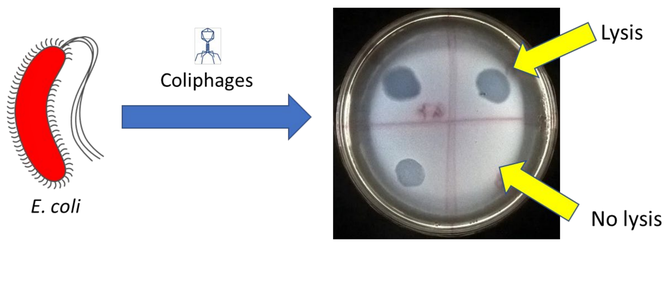
4. Can we use genomics to determine how phage infect bladder bacteria?



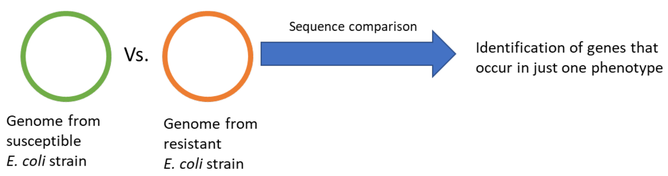
(Pósfai et al. 2006)

Methods

1) Phenotype screen of phage lysis in bladder *E. coli* strains



2) Identification of genes differentially present in strains that are lysed and those that are not



Results

Table 1. Bladder *E. coli* can be resistant or susceptible to coliphage lysis

E. coli strain	Strain type	Greed coliphage infection	Lust coliphage infection
B	Asymptomatic	Susceptible	Susceptible
C	Asymptomatic	Susceptible	Susceptible
K-12	Asymptomatic	Susceptible	Susceptible
CFT073	Uropathogenic	Resistant	Resistant
NU14	Uropathogenic	Resistant	Resistant
UTI89	Uropathogenic	Resistant	Resistant
UMB0149	Bladder isolate	Susceptible	Susceptible
UMB2019	Bladder isolate	Susceptible	Susceptible
UMB6655	Bladder isolate	Susceptible	Susceptible
UMBs (n=44)	Bladder isolate	Resistant	Resistant

Figure 1. The Toxin-Antitoxin *mazEF* is differentially present in resistant and susceptible strains

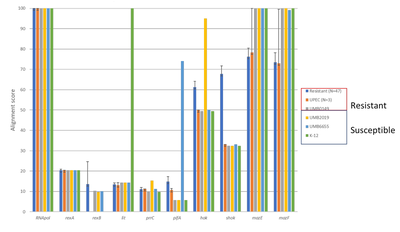
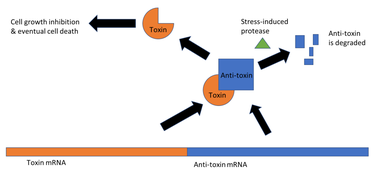


Figure 2. A Toxin-Antitoxin may modulate cell death when infected with phage



(Yamaguchi & Inouye 2011)

Figure 3. *pemIK is* not present in the phage susceptible strain *E. coli* K-12

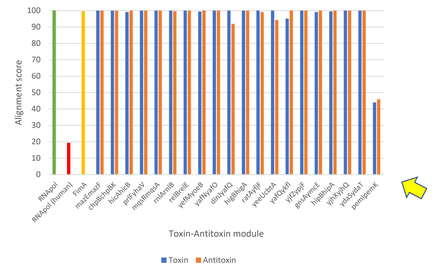


Figure 4. *pemI* and *pemK* are not present in susceptible strains but are present in some resistant strains

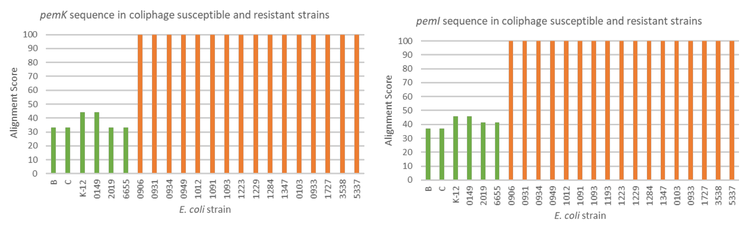


Figure 5. Phage-antagonism genes are differentially present in *pemIK*$^-$ and *pemIK*$^+$ strains

pemIK$^-$ pemIK$^+$ →

UMB /Gene	UMB0149	UMB6655	UMB2019	K-12	B	C	UMB5337	UMB1347	UMB1229	UMB9066	UMB3538	UMB0933	UMB1284	UMB1012	UMB1091	UMB0949	UMB0934	UMB1221	UMB1093	UMB1727	UMB0931	UMB1229
RNApol (+control)	100	100	100	100	100	100	100	100	100	100	100	100	100	99.8	100	100	100	100	100	100	100	100
RexA	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4	20.4
RexB	10.4	10.1	9.7	0	9.7	9.7	0	0	0	0	0	0	0	0	0	6.9	6.9	6.9	6.9	6.9	0	0
Lit	14.3	14.3	14.3	100	14.3	14.3	14.3	14.3	14.3	14.8	14.8	14.3	14.8	14.8	14.8	14.8	10.8	10.8	10.8	10.8	10.8	12.1
PrrC	9.7	11.2	15.1	9.7	9.7	9.7	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2
PfA	5.8	24.1	5.8	5.8	5.8	5.8	5.8	5.8	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
Hok	49.5	50	19.1	49.5	50	49.5	50	50	50	50.5	50.5	99	50.5	50.5	50.5	50	50	50	100	100	50	50.5
Shok	32.4	33.1	32.4	32.4	33.1	32.4	49.8	49.8	98	91	91	98	96.5	67.7	83	99.3	100	100	83	99.3	100	79.1
MazE	100	100	100	100	100	100	100	100	49.4	47.6	47.6	39	47.6	39	39	39.6	39.6	39.6	39.6	39.6	39.6	100
MazF	100	99.1	100	100	100	100	99.5	99.5	77.2	45	45	37.4	45	37.4	37.4	37.4	37.4	37.4	37.4	37.4	37.4	99.5
MrcA	15.5	15.5	15.5	100	11.9	14.6	13.2	11.9	11.9	23.3	23.3	13.2	21.5	30	30.1	30.1	30.1	30.1	21.5	13	30.1	30
MrcB	20.1	20.1	18.6	100	98.3	20.1	20.1	98.3	100	49	49	20.1	49	49	49	96.6	100	100	100	100	100	20.1
MrcC	9.2	9.2	10.1	99	99.5	10.1	9.2	99.9	99	14.2	14.2	9.2	14.2	14.2	14.2	99	99	99	99	99	9.2	9.2
Mrr	19.8	10.7	16.5	14.7	14.7	14.4	10.7	99.8	86.3	99.1	99.8	10.7	16.7	16.7	16.7	14.7	14.7	24.1	14.7	14.7	71.5	10.7
DarA	8.3	6.6	71.5	8.8	8.8	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	71.5	99.3
DarB	99.5	3.9	99.5	3.7	3	3.7	4	4	4	3.4	5.4	5.4	4.7	4.7	4.7	3.3	4	4	3.4	42.7	67.5	99.3
HsdR	99.5	11	83	8.2	7.8	8.3	53.2	8.2	8.2	99.1	99.1	94.7	58.5	53.7	53.7	54	8.2	8.2	8.2	8.2	8.2	55.2
HsdM	99.5	36	99.3	22.3	22.3	15.7	74.8	22.3	22.3	99.9	99.9	58.3	69.8	57.5	57.5	73.3	79.9	79.9	28.9	22.3	79.9	74
HsdS	75	19.7	68.8	18.4	9.4	75	9.4	11.4	18.4	99.6	99.8	58.5	11.4	11.4	9.4	18.4	18.4	18.4	18.4	18.4	18.4	11.4
EcorIR	18.6	18.6	18.6	16.6	16.6	18.6	18.2	16.6	18.6	18.6	18.6	18.6	18.6	18.6	18.6	17.1	17.1	17.1	17.1	17.1	17.1	18.6
EcorIM	11.2	29.4	11.2	5.3	14.6	5.8	15.8	6.2	29.4	19.5	19.5	29.4	19.5	19.5	19.5	14.1	14.1	14.1	14.1	14.1	14.1	6.2
Cas2	98.9	31.9	98.9	100	31.9	98.9	34	34	31.9	31.9	31.9	31.9	31.9	31.5	26.6	92.6	92.6	92.6	92.6	92.6	92.6	97.9
Cas1	99.8	13.8	99.8	99.7	13.8	99.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8	13.8	92.1	92.1	92.1	92.1	92.1	92.1	99.6
Cse3	16	20.6	16	49.1	18.3	49.1	16.9	16.9	14.8	14.8	14.8	14.8	14.8	10	10	99.1	99.1	99.1	99.1	99.1	99.1	49.1
Cas5e	14.7	11.9	14.7	30.8	14.7	30.8	14.7	14.7	11.9	14.7	14.7	11.9	11.9	11.9	11.9	97.2	97.2	97.2	97.2	97.2	97.2	30.8
Cse4	99.99	99	99.99	99	55	99.4	10.7	10.7	11	11	11	11	11	11	11	66	66	66	66	66	66	99.2
Cse2	100	20.9	100	99.4	19.7	99.7	19.7	19.7	21.6	19.7	19.7	21.6	19.7	19.7	19.7	20.9	20.9	20.9	20.9	20.9	20.9	100
Cse1	29.2	10.4	10.4	29.2	10.4	29.2	10.4	10.4	10.4	12.4	12.4	10.4	12.4	12.4	12.4	97	97	97	97	97	97	29.2
Cas3	99.99	8.1	55.5	99.3	8.1	99.8	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	8.1	68.4	68.4	68.4	68.4	68.4	68.4	98.4
Imm	30.2	31.5	99.3	30.2	30.2	30.2	30.2	30.2	81.7	99.1	15.8	81.7	30.2	99.1	99.1	99.1	30.2	99.1	99.1	30.2	30.2	32
Sp	26.3	23.7	26.3	24.7	25.8	25.8	23.2	23.2	23.2	29.4	29.4	23.2	29.4	28.4	29.4	23.2	23.2	23.2	23.2	23.2	23.2	23.2
Up	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7	48.7
Mcc J25	41.4	41.4	41.4	41.4	38.8	41.4	38.8	38.8	41.4	41.4	41.4	41.4	41.4	41.4	41.4	41.4	41.4	41.4	41.4	41.4	41.4	38.8
TraT	11.4	7.8	10.1	10.1	10.1	10.1	98.4	98.4	98.6	98.2	98.2	98.6	93.9	98.2	93.9	94.2	98.2	98.2	93.9	94	98.2	98.6

Homology
90-100%
70-89%
50-69%
30-49%
10-29%
0-9%

Figure 6. The genes *pemIK* and *traT* are present in resistant strains but not in strains susceptible to Greed/Lust lysis

Greed & Lust resistant

Greed & Lust resistant →

Results

Figure 7. Strains with the genes *pemIK traT* are linked with coliphage lysogeny rather than lysis

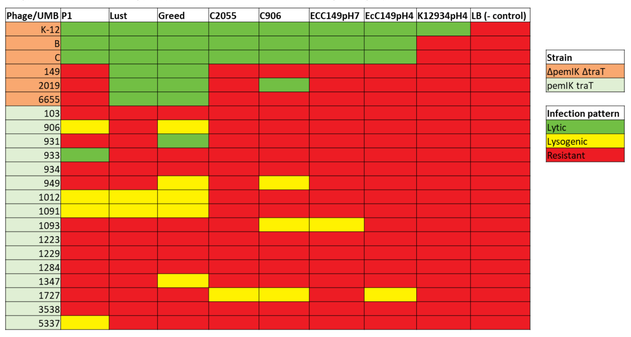
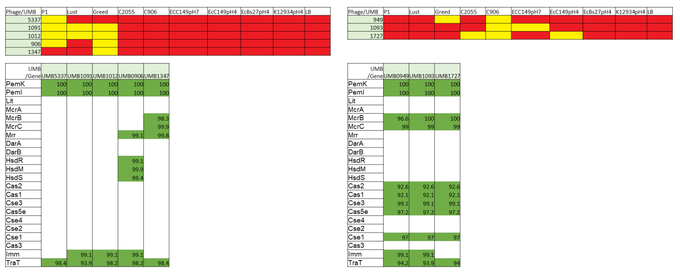
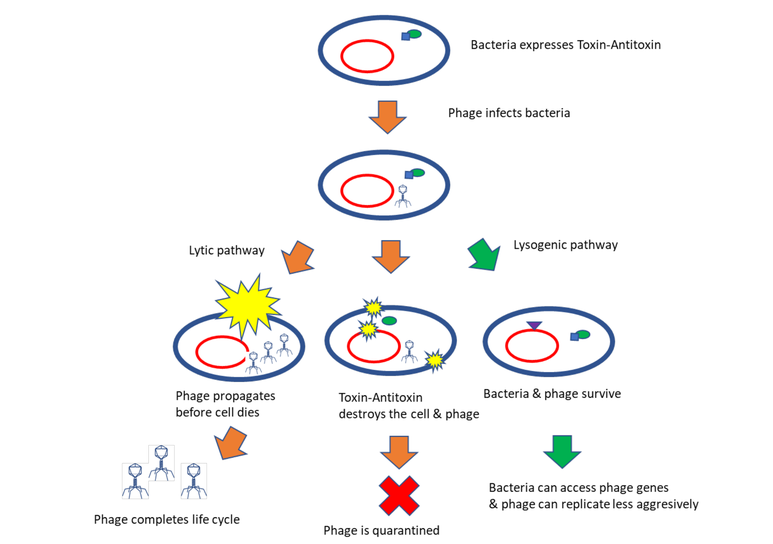


Figure 8. Specific gene profiles are linked with lysogeny of specific phages



Toxin-Antitoxin modules may promote phage lysogeny



Future Directions

- Test if overexpression or deletion of *pemIK* and/or *traT* in bladder *E. coli* changes the infection pattern of coliphage
- Profile the prophage content in bladder *E. coli* strains that are *ΔpemIK ΔtraT* and those that have *pemIK* and *traT*
- Catalogue the plasmids in bladder *E. coli* strains and their content of prophage and phage-antagonism genes

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