



New Views of Biocontrol:
Alternative Methods for Phage Detection
in Activated Sludge

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Beyond Indicators: Alternative Methods for Phage Detection in Activated Sludge



Beyond Indicators

- Background
- Problem statement and hypothesis
- Materials and Methods
- Results
- Conclusions



Beyond Indicators

- What is a phage?...and other background
- Problem statement and hypothesis
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Beyond Indicators

- What is a phage?...and other background
- Challenges to examining phage in “messy” environments
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- How we looked for evidence of phage
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- ...found evidence of phage
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Beyond Indicators

- What is a phage?...and other background
- Challenges to examining phage in “messy” environments
- How we looked for evidence of phage
- ...found evidence of phage
-plan to continue



Bacteriophage

- Viruses of bacteria
- Discovered in 1917
- Selective or generalist?
- Lytic or latent



Lytic or latent cycles

Surface attachment

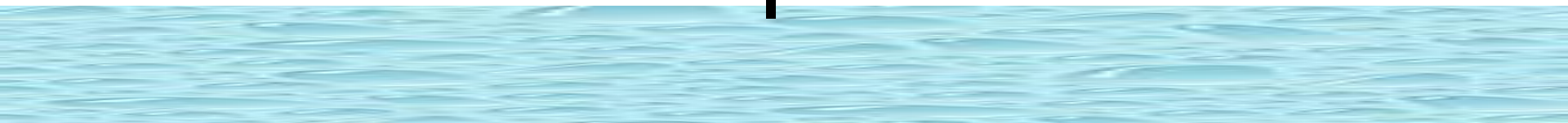
Transfer of genetic material

Viral DNA/RNA directs
bacterial host
processes

- Manufacturing viral components
- Assembly of components
- Host cell bursts to release new virus particles

Viral DNA/RNA joins
bacterial host DNA

- Quiet, “latent” replication in cell replication
- Trigger?



Phage in Wastewater

- As treatment indicators
- As filtration indicators



Phage in Biocontrol

- Phage to reduce bacterial pathogens of plants
- Phage to prevent bacterial growth on produce, reduce rate of spoilage



Biocontrol in Wastewater?

- Synnott et al. 2009
 - Phage of *Staphylococcus aureus*

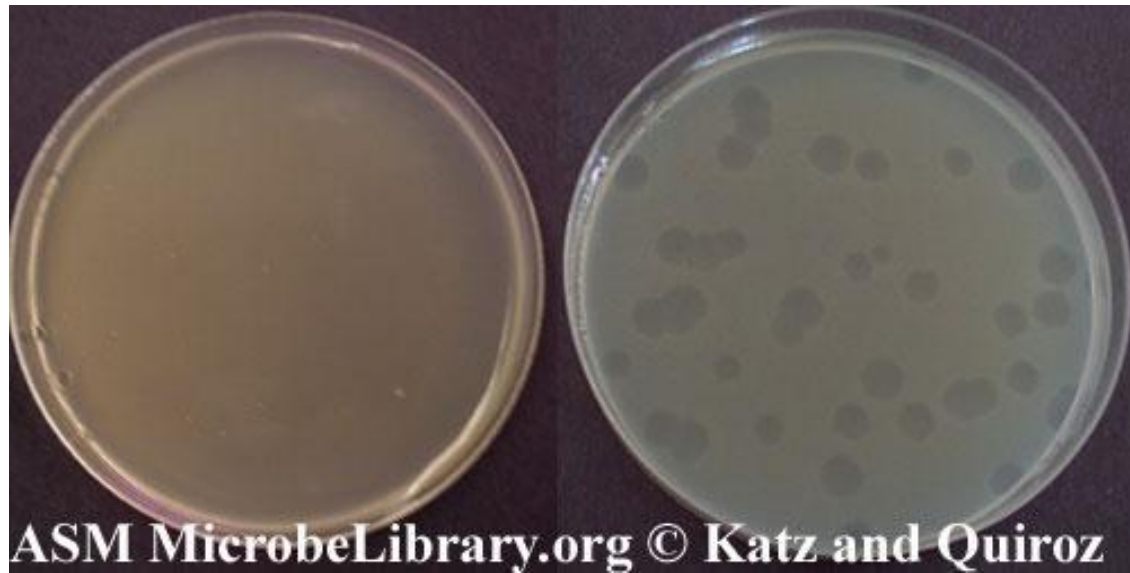
- Kotay, Datta, Choi, and Goel (2011)
 - Phage control of biomass bulking by a phage of *Haliscomenobacter hydrossis*.

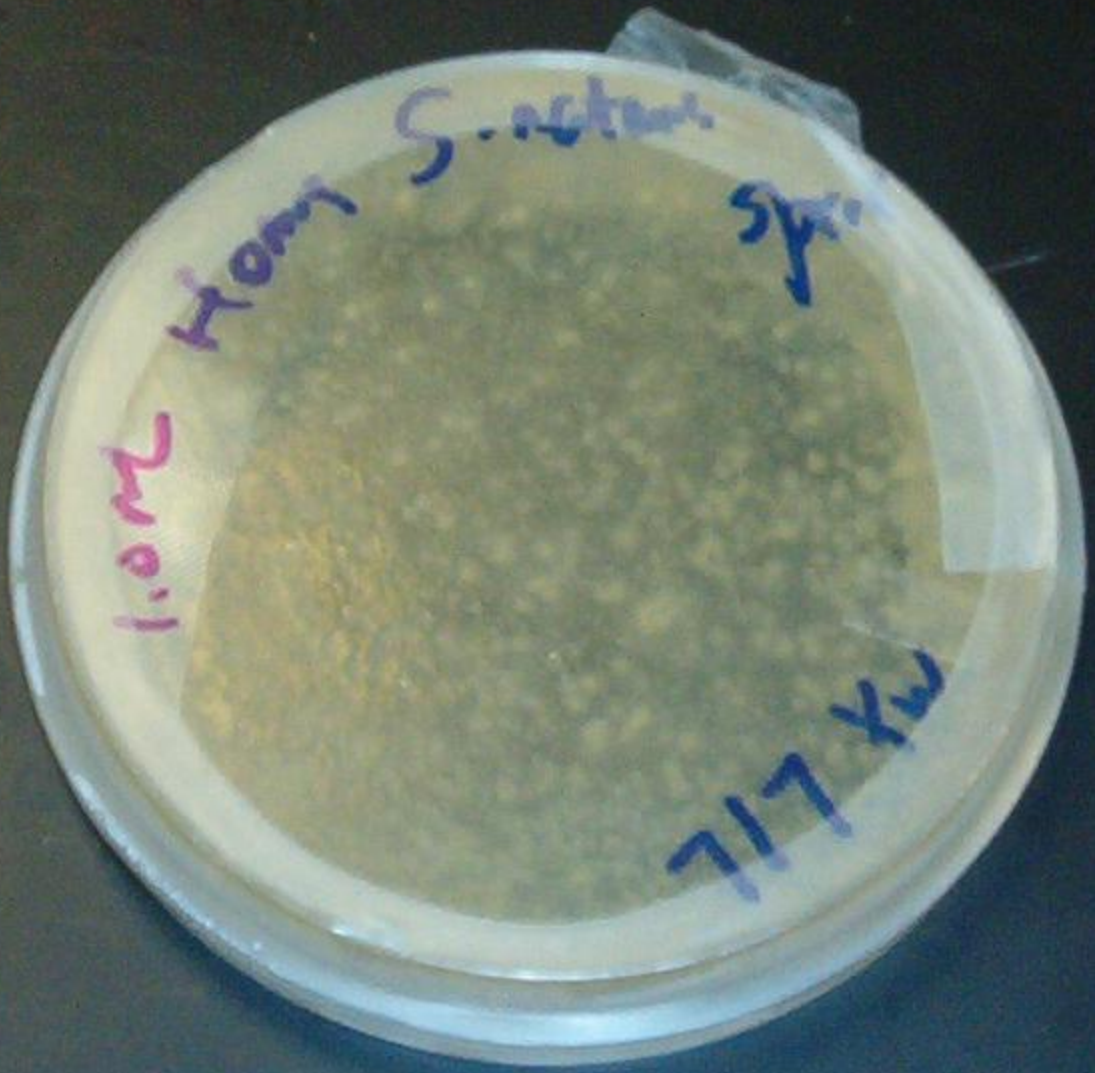


What's the problem?

- Bacterial hosts are microscopic
 - Traditional methods dependent on growth.
 - Estimates say 1 – 10% can be lab-grown.

- Phage are smaller
 - Traditional methods dependent on absence of host growth.





1.0ml

KOH

S. rectus

sp

717 KW

Solution

➤ Hypothesis:

Broth based bacterial assays can show evidence of phage infection.



Materials ...

- Model system (ATCC)
 - Bacterial host
 - *Escherichia coli* 11303
 - Bacteriophage
 - T2 phage



... and Methods

- Biomass by absorbance
 - Measurements at 630 nm
- Activity by ATP
 - Promega BacTiter-Glo™
- Cell integrity
 - Invitrogen™ LIVE/DEAD® *BacLight*™
Stain



	empty	10^3 pfu	10^2 pfu	10^1 pfu	no phage
empty					
10^5 <i>E. coli</i>					
10^6 <i>E. coli</i>					
10^7 <i>E. coli</i>					



	empty	10^3 pfu	10^2 pfu	10^1 pfu	no phage
empty					
10^5 <i>E. coli</i>		10^5 host 10^3 phage	10^5 host 10^2 phage	10^5 host 10^1 phage	10^5 host
10^6 <i>E. coli</i>					
10^7 <i>E. coli</i>					



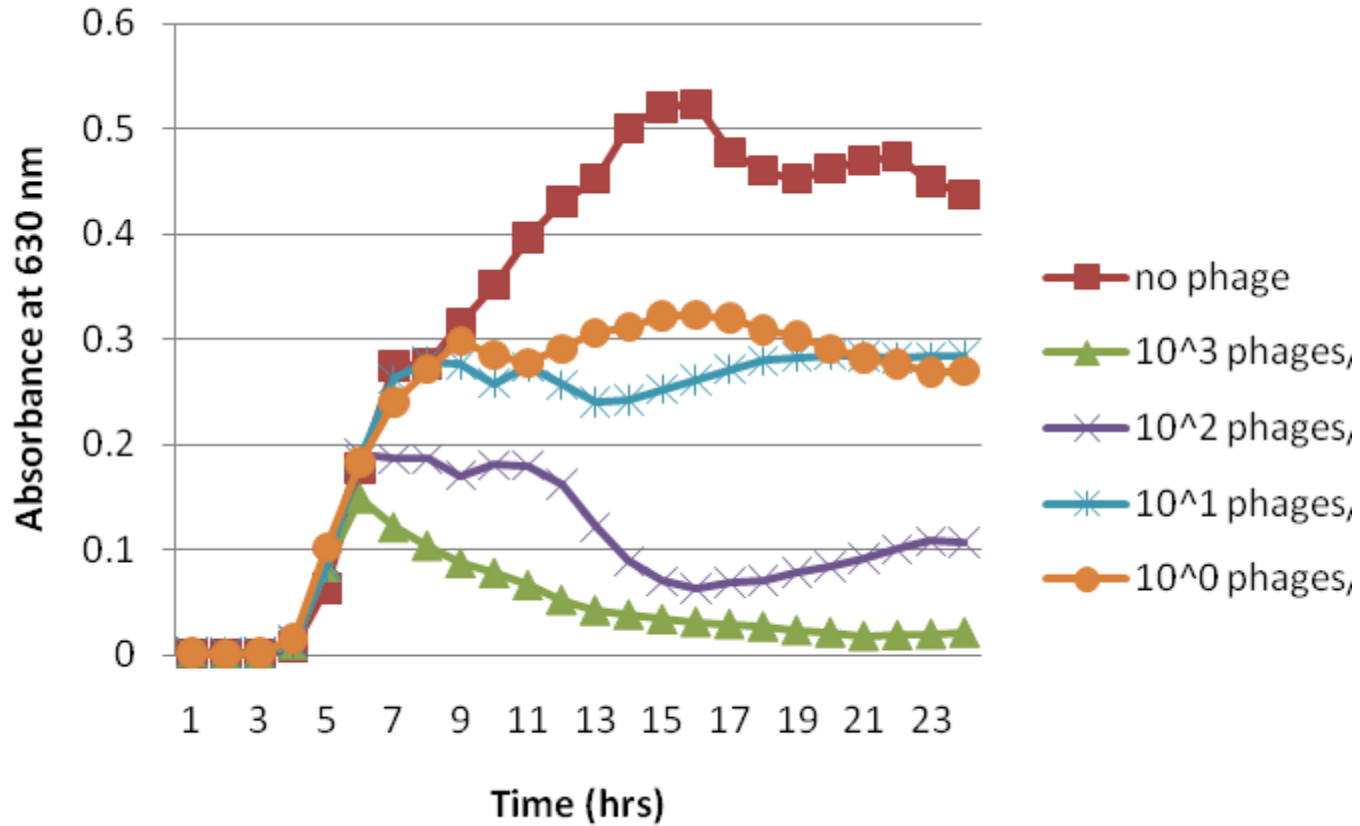
	empty	10^3 pfu	10^2 pfu	10^1 pfu	no phage
empty					
10^5 <i>E. coli</i>		10^5 host 10^3 phage	10^5 host 10^2 phage	10^5 host 10^1 phage	10^5 host
10^6 <i>E. coli</i>			10^6 host 10^2 phage		
10^7 <i>E. coli</i>			10^7 host 10^2 phage		



	empty	10^3 pfu	10^2 pfu	10^1 pfu	no phage
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10^6 <i>E. coli</i>			10^6 host 10^2 phage		10^6 host
10^7 <i>E. coli</i>			10^7 host 10^2 phage		

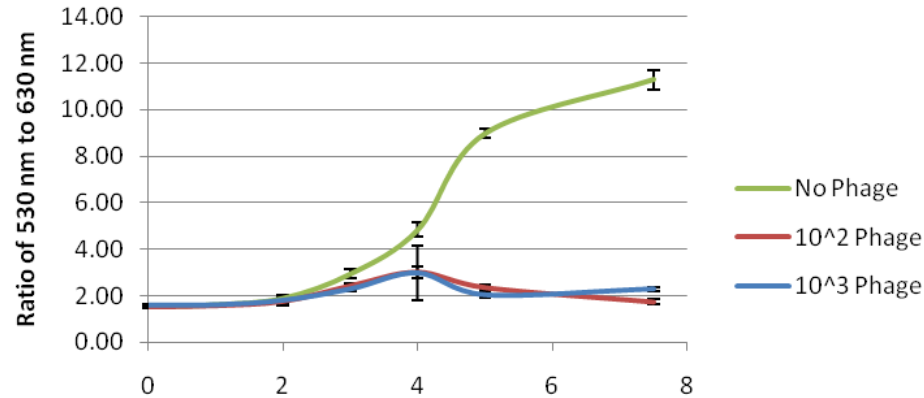


10^5 *E. coli* /100 μ l, Abs at 630 nm

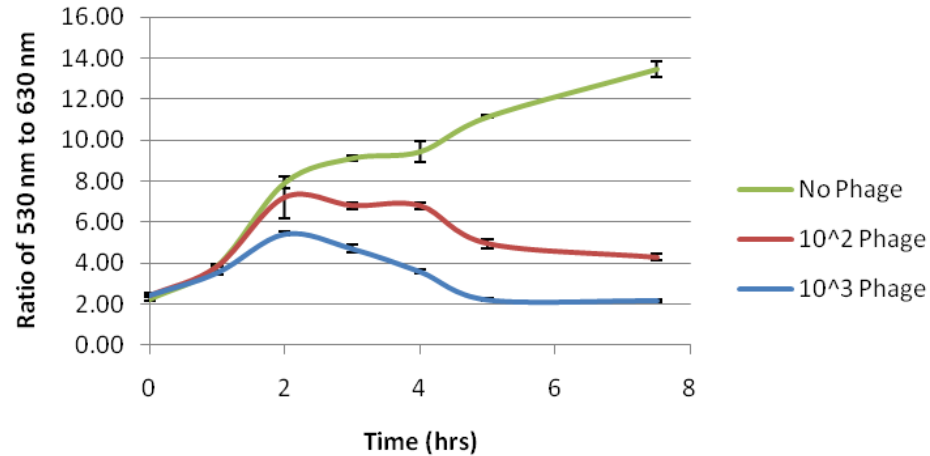


Cell membrane integrity (Live/Dead stain)

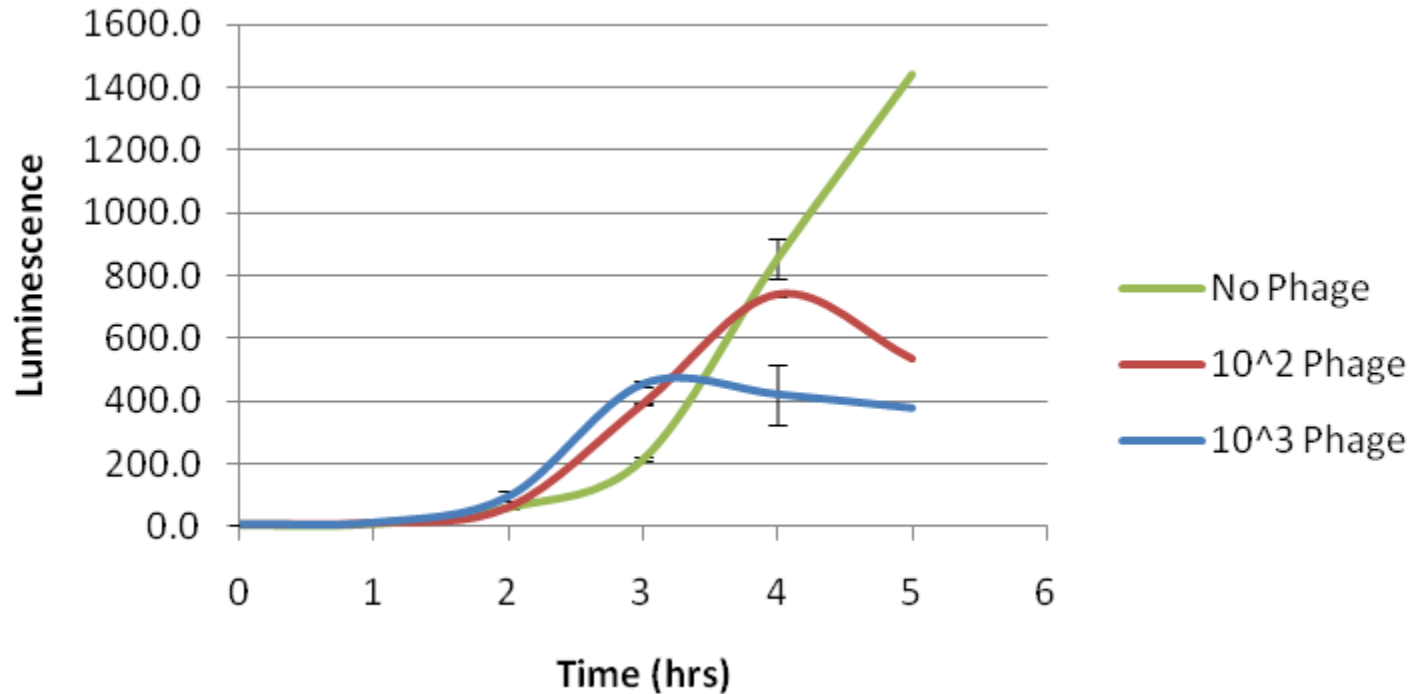
10^5 *E. coli* /
100 μ l



10^7 *E. coli* /
100 μ l

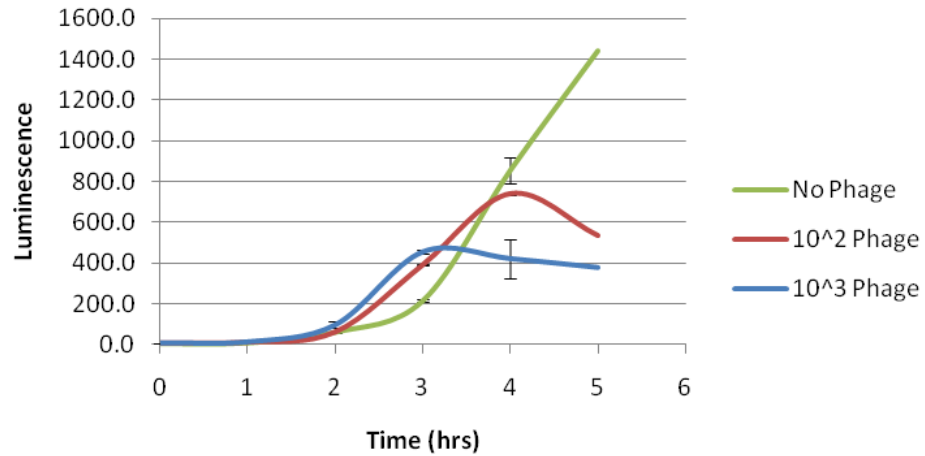


10^5 *E. coli* /100 μ l, ATP assay

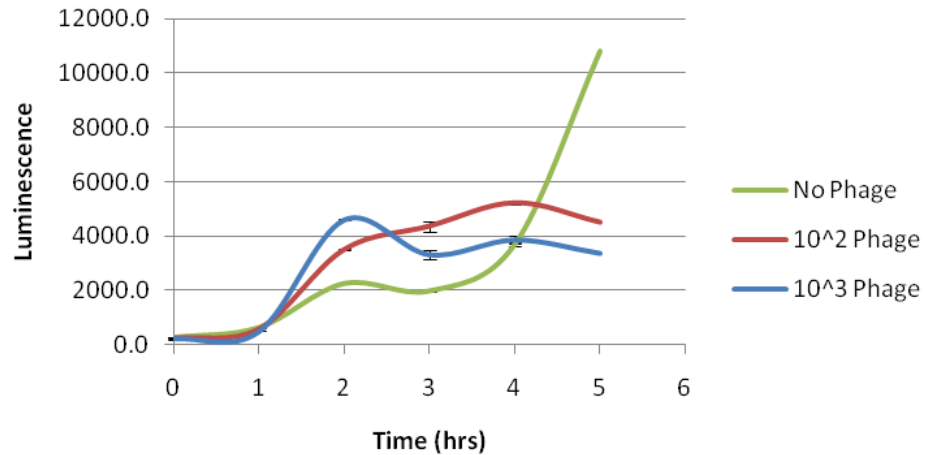


ATP Assay

10^5 *E. coli* /
100 μ l



10^7 *E. coli* /
100 μ l



Conclusions

- All three broth-based methods provide evidence of phage infection
- Microwell format provides results even in presence of low phage concentration.



Conclusions

- Cell integrity results show phage infection more rapidly than absorbance measurements.
- Inexpensive reagents.



Conclusions

- ATP and cell membrane assays show phage infection more rapidly than the absorbance measurements.
- ATP assays show two forms of evidence of phage infection.



Acknowledgements



Serving Ohio Since 1968...



Lindsey Staley
Yuxing Li

Thank you !