

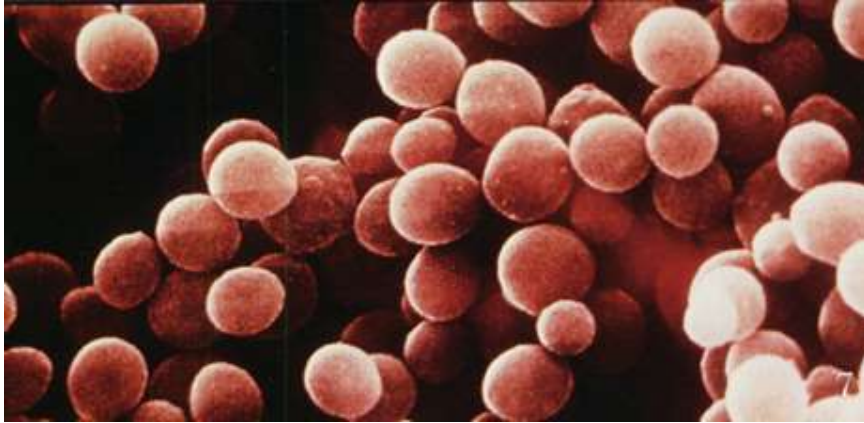
# Isolation and Characterization of *Staphylococcus aureus* Bacteriophages

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April, 2011

# Introduction to *Staphylococcus aureus*

- Gram Positive coccus
- Common (Skin, Throat, Nasal cavity)
  - About 30% of the human population are carriers  
(CDC <http://www.cdc.gov/HAI/organisms/staph.html>)
- Generally a commensal
  - Can easily become pathogenic
- Commonly known as a 'Staph infection'
- Easily transmitted through direct contact

# Pictures of *S. aureus*



<http://www.textbookofbacteriology.net/staph.html>



Illustration: Don Smith



ASM MicrobeLibrary.org © Hedetniemi and Liao

# *S. aureus* Microbiology

- Catalase Positive
  - Resistant to reactive oxygen species
- $\beta$ -hemolytic
  - Destroys/lyses RBC's
- Staphyloxanthin Pigment
  - Root of 'aureus' species name, golden, antioxidant
- Coagulase positive
  - Causes blood to clot (Fibrin  $\rightarrow$  Fibrinogen)

# *S. aureus* in disease/infection

- Furuncles and Carbuncles
  - Boils, abscesses, pimples
- Bacteremia
  - Septicemia and possible septic shock
- Toxic Shock Syndrome
  - TSST-1 exotoxin (mitogen)
- Scalded Skin Syndrome
  - Mostly in infants, can be severe necrosis of skin
- Food poisoning
  - Exotoxins (heat stable) secreted into food and can then be ingested

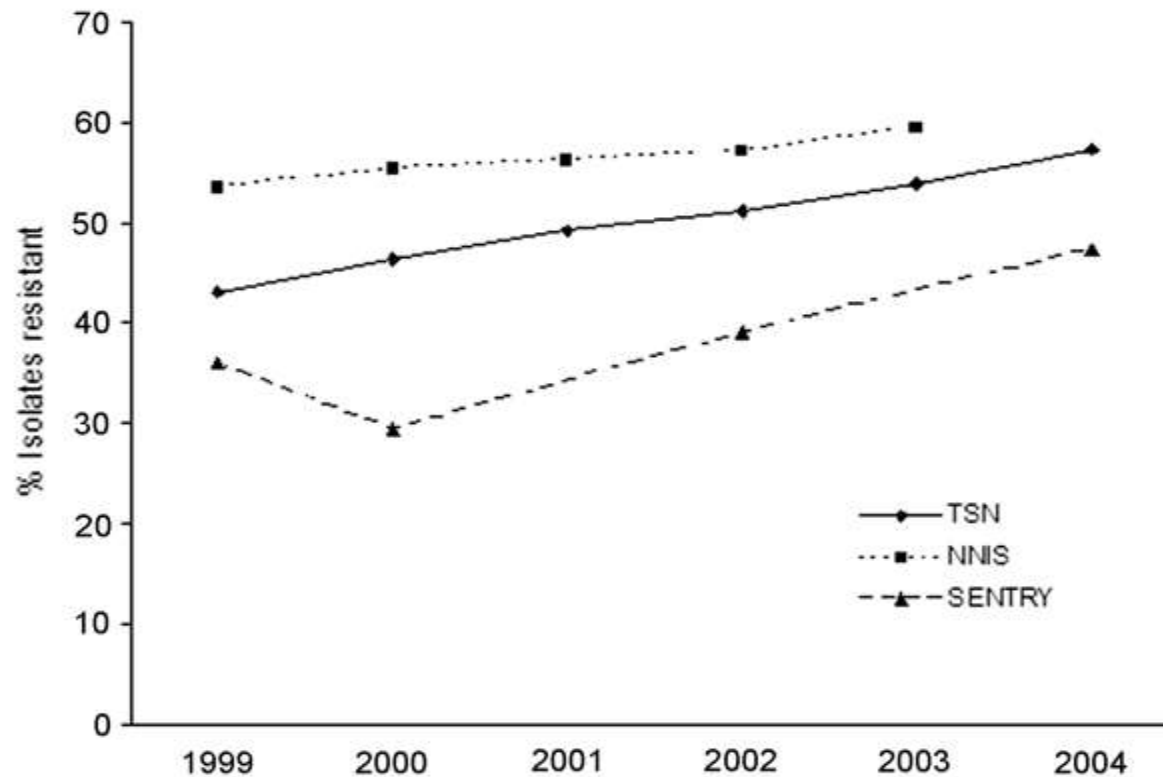
▪ Information taken from: *Sherris Medical Microbiology: An introduction to Infectious Diseases*

# Treatment of *S. aureus*

- Penicillin
  - 1<sup>st</sup> effective treatment, 1950's
  - High resistance found by 1960
- Beta-lactams effective in many cases
  - Methicillin for example
- *S. aureus* strains became resistant to many beta-lactams
  - MRSA (Methicillin Resistant *S. aureus*)
  - Treated with Vancomycin
- Recently, some VRSA strains have become apparent
  - Mostly nosocomial in nature
    - Information taken from *Sherris Medical Microbiology: An introduction to Infectious Diseases*

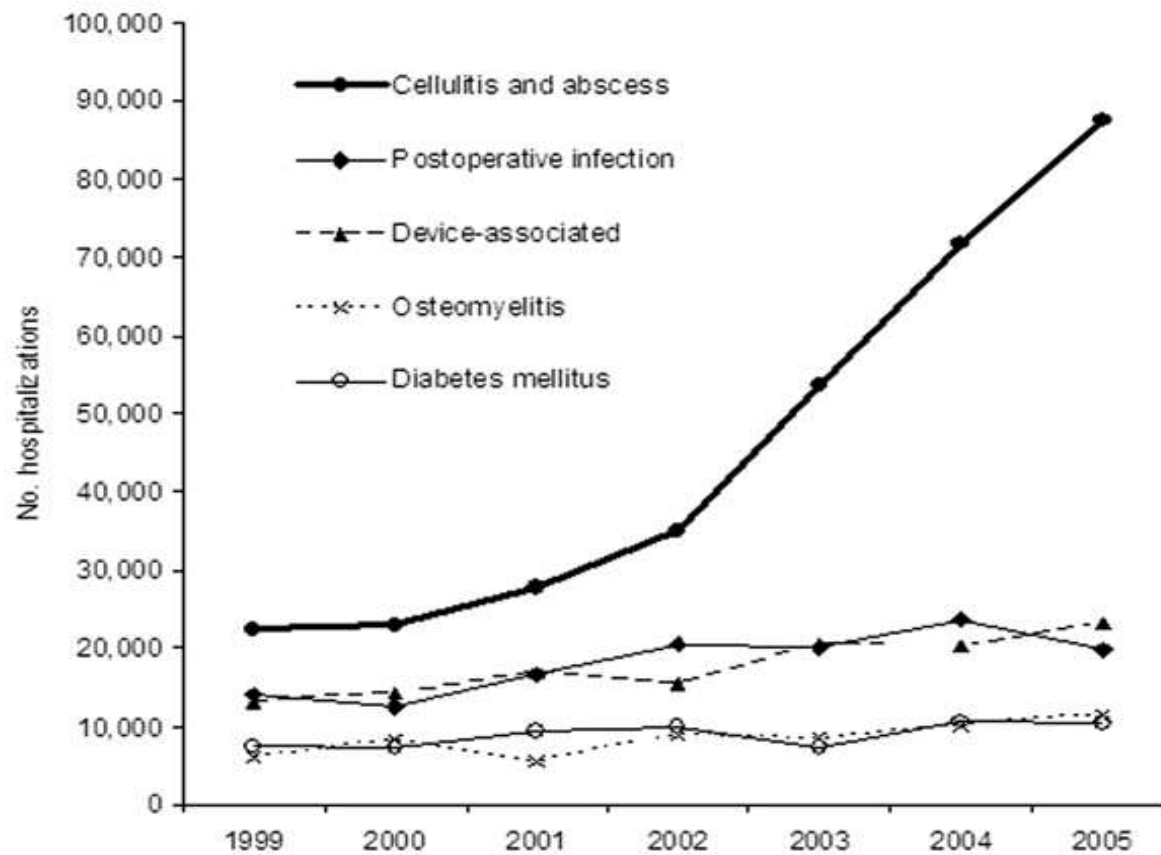
# MRSA on the rise

Amount of resistance to methicillin in *S. aureus* according to the CDC



# Cases of MRSA

Hospitalizations due to MRSA in the US on the rise; CDC

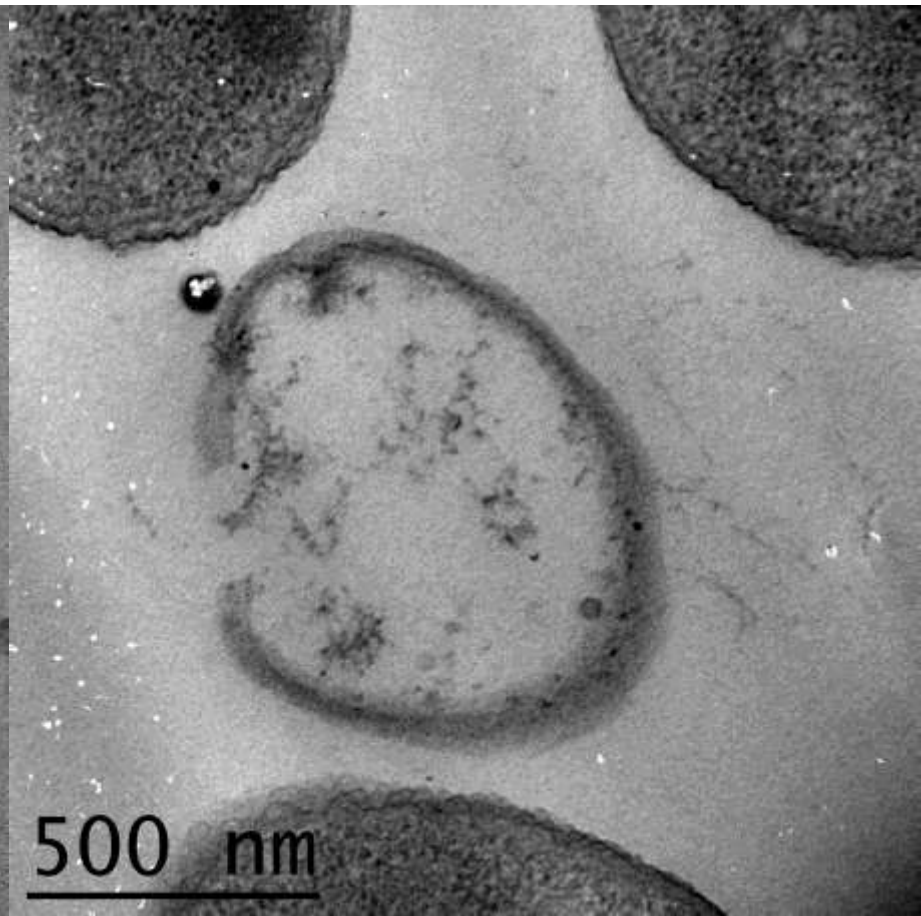
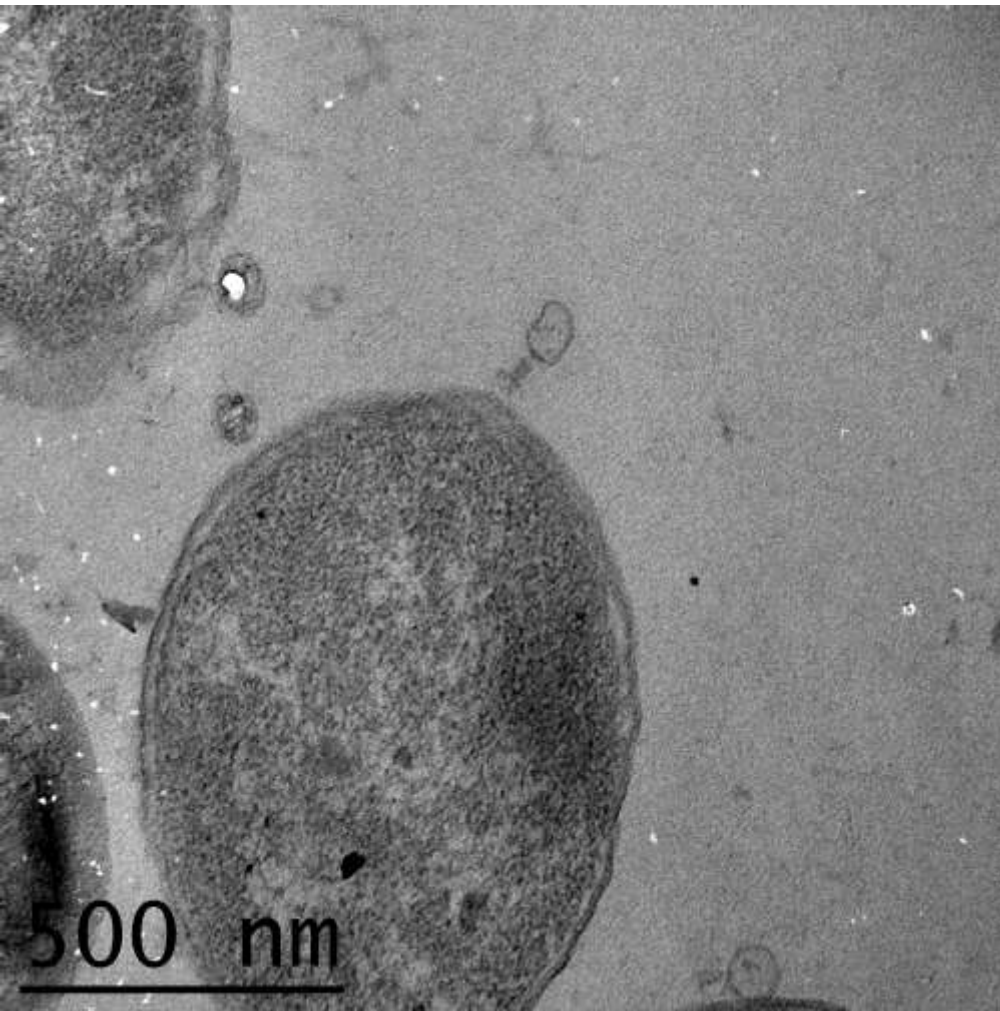




# Bacteriophage

- Phage
  - Viruses that infect specific bacterial hosts
  - Non-invasive to eukaryotic (human type) cells
  - Naturally occurring
    - Found in sewage, on towels, other unsavory places (needs bacterial host to persist)
  - Filterable agents
- Possible alternative treatment against *S. aureus* infections
  - In cases such as multi-drug resistance (MRSA and VRSA)
    - Information taken from:
      - [Microbiology: An Evolving Science](#)
      - Zimecki, Michal. BMC Microbiology 2009; 9: 169.

# Bacteriophage pictures



# Research objectives

- Isolate bacteriophage and additional host *S. aureus* strains from environmental samples
- Characterize bacteriophage
- Develop a phage cocktail effective at eliminating *S. aureus*

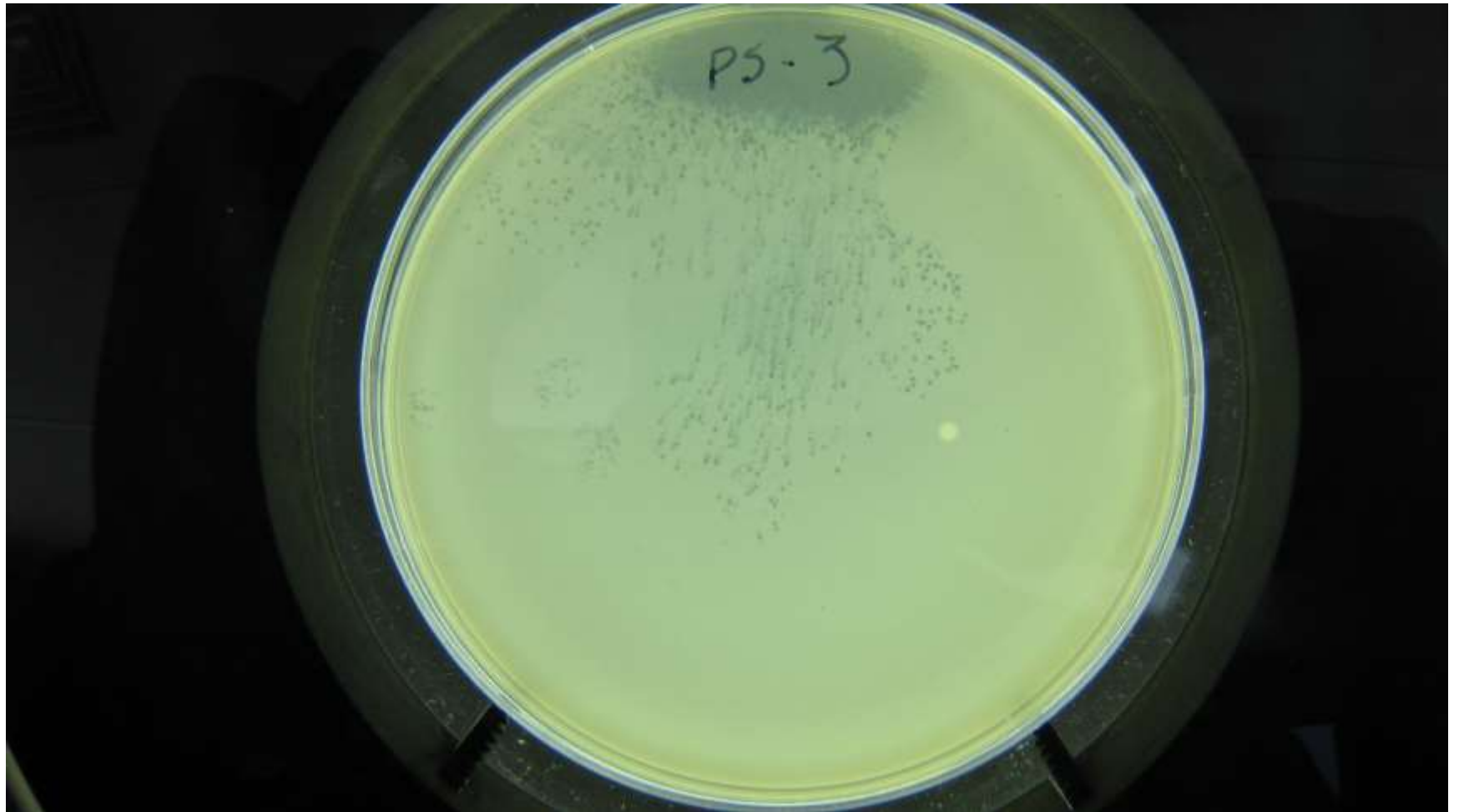
# Methods

- Isolated bacteriophage from raw sewage
  - Waste Water Treatment Influent sample
- Amplified phage in presence of *S. aureus* host
  - 37 C in Tryptic Soy Broth (TSB) for 24 hours
- Filtered enriched TSB for phage particles
  - .22µm syringe filter
- Drop plated on specific hosts
  - 200µL drops of filtrate on 6 strains of *S. aureus*
    - 5 lab strains of *S. aureus*, one strain isolated from human host

# Methods cont...

- Cut plaques from drop plates
  - Resuspended clearance for phage amplification
- Amplified phage isolates on *S. aureus* host strains
  - To gain a larger concentration of the phage isolates

# Phage Isolation/Amplification



# Results

- PHAGE FOUND – 38 isolates to date
  - Host Range of *Staphylococcus aureus* strains:
    - S95
    - NC
    - 78I
    - PS
- Stability Issue
  - Heat, Chloroform, or Buffer problem

# Conclusion

- Larger host range of *S. aureus* strains
  - In order to ensure better performance as a possible treatment option
- Many phage isolates recorded and characterization is under way
- Stability of phage in solution
  - Possible buffer solution optimization
- A Good Start
  - Further work needed



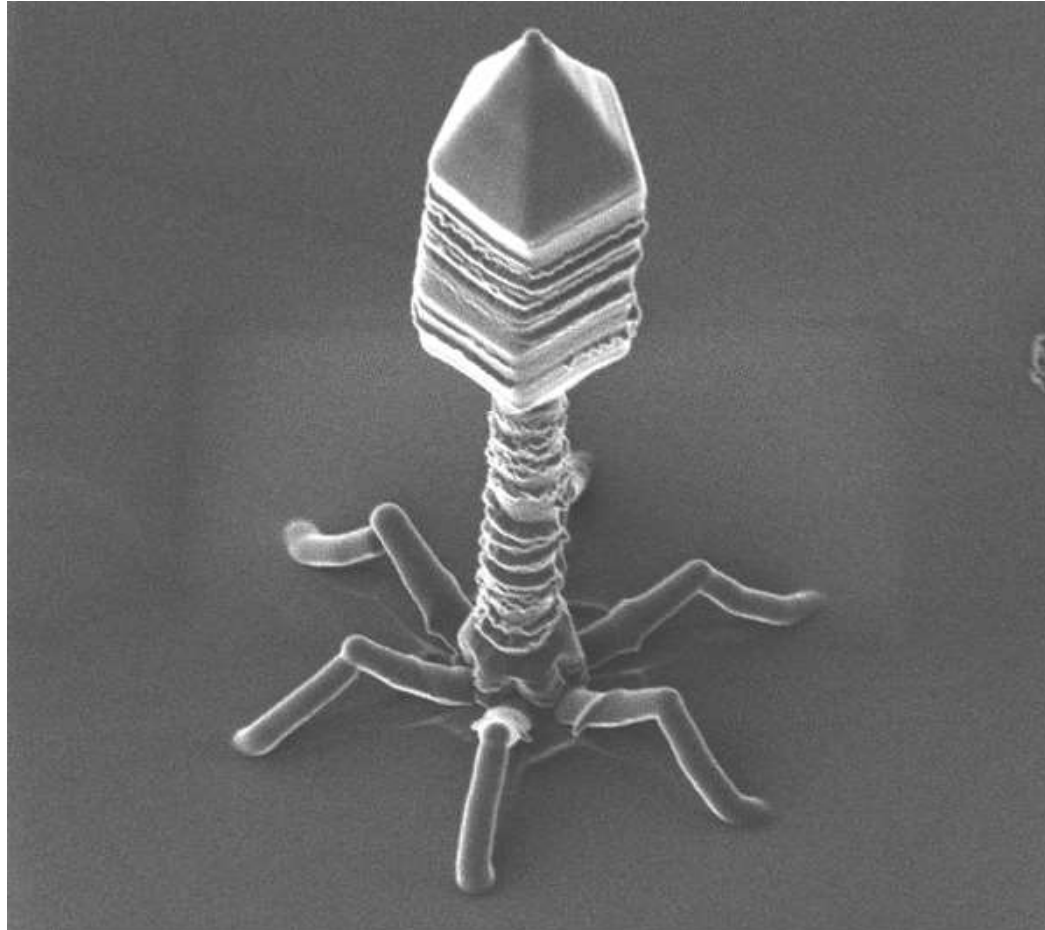
# Future Work

- Characterization of isolated phage
  - Thorough host range
  - Genome sequencing
- Stability studies
  - Buffer and environmental exploration
- Electron Microscopy
  - View and characterize the phage types
    - Make available the phage to other research and use

# Acknowledgements

- INBRE
- MOLB Department
  - Karen White
- Rachel Watson
- Dr. G. Andrews
- Dr. John Willford
- Laramie Waste Water Treatment Plant

# Questions?



Picture: Zyvex Labs