Breaking the Bacterial Barrier: Enhanced Targeted Therapy on the Lipopolysaccharide of *Pseudomonas aeruginosa* Using a Phage-Antibiotic Cocktail

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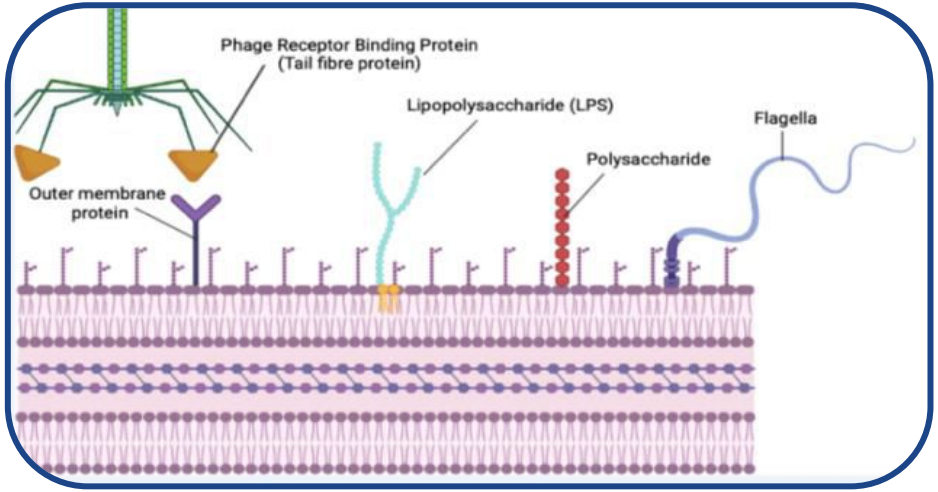
Pseudomonas aeruginosa biofilm

Extracellular matrix of secreted proteins, sugars, and DNA are stained in red. *P.aeruginosa*'s natural pigments are in the darkened center.

https://www.rcsb.org/structure/5BX9



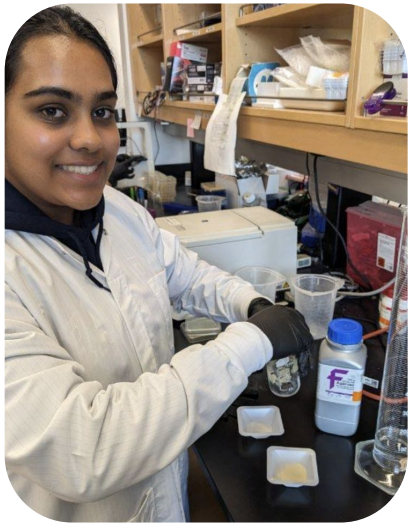
Personal Information

PORTOLA BULLDOGS

Future aspirations:

- **Receive a Bachelors in Science and eventual PhD.**
- **Work in R&D.**

AbbVie Internship



Prof. Eric Pearlman's Lab, UCI Institute for Immunology, working under Dr. Karl Liboro

1st place in Microbiology & Cell Biology at OCSEF and CSEF Finalist

Background

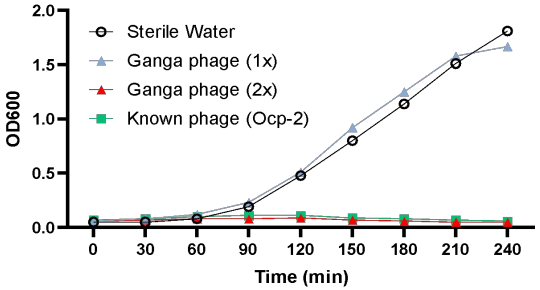
- Multidrug resistant bacterial infections are classified as a top ten global threats by WHO, causing over a million deaths each year and estimated to surpass 10 million by 2050, exceeding even cancer-related deaths.
- *Pseudomonas aeruginosa* (PA) is one of the ESKAPE pathogens causing severe hospital-acquired and chronic infections. It is also treatment resistant to antibiotics due to its biofilm forming ability.
- Alternative therapy is phage based; phages are viruses that infect bacteria to inhibit infections.
  - Phages exhibit high specificity by binding to specific receptors on the bacterial cell surface, which is the first step towards their invasion.
  - Phages also self-amplify, help with biofilm degradation, and exhibit low toxicity
  - Phages are currently in clinical trials and compassionate use; data from clinical studies strongly support that phage therapy is safe and viable.

Bacteriophages can be isolated from natural sources like rivers and sewage. In hospital settings, they are especially useful for targeting ESKAPE pathogens. Sequencing and engineering these phages can improve their specificity and therapeutic power, either alone or in combination with antibiotics, a strategy known as phage–antibiotic synergy (PAS) therapy.

In vitro
Ganga Water bacteriophage is EFFECTIVE in preventing *Pseudomonas aeruginosa* (PA) bacterial growth

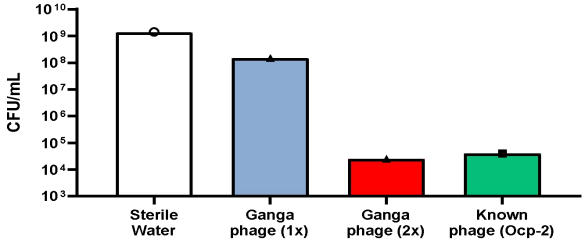
Bacterial Growth Kinetics

2X Phage Enriched Ganga Water Effectively Prevented Bacterial Growth



Anti-bacterial Activity

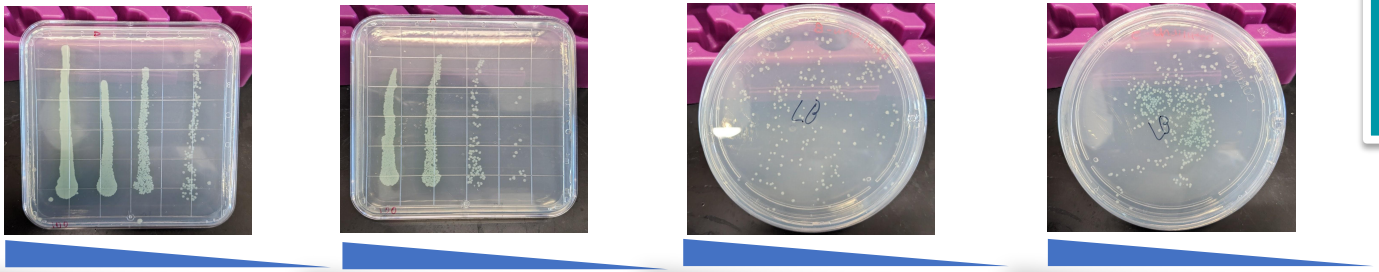
2X Phage Enriched Ganga Water Significantly Reduced Number of Bacterial Colonies



2X phage enriched Ganga water (Source: Rishikesh, India) is effective in preventing *Pseudomonas aeruginosa* (PA01) bacterial growth as evident from:

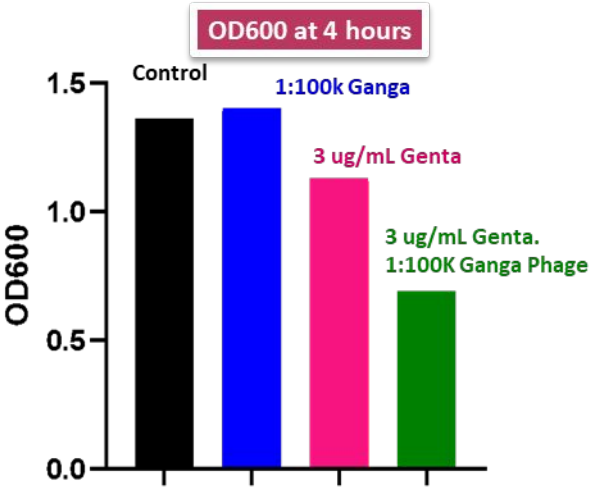
- OD600
- CFU/mL

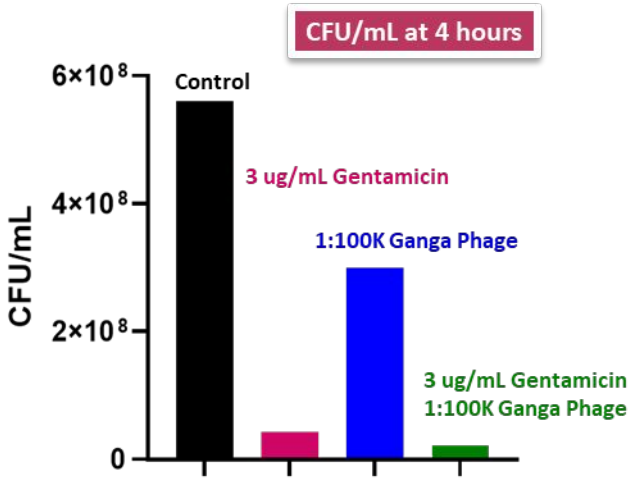
Effect on Bacterial Growth

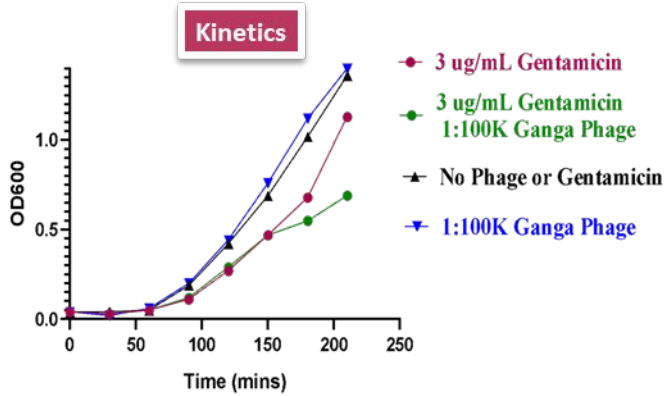


diluted: **sterile water** | diluted: **1x enriched Ganga** | undiluted: **2x enriched Ganga** | undiluted: **filtered Ocp-2**

In vitro
Phage Antibiotic Synergy (Ganga Phage + Gentamicin) in Preventing PA Growth





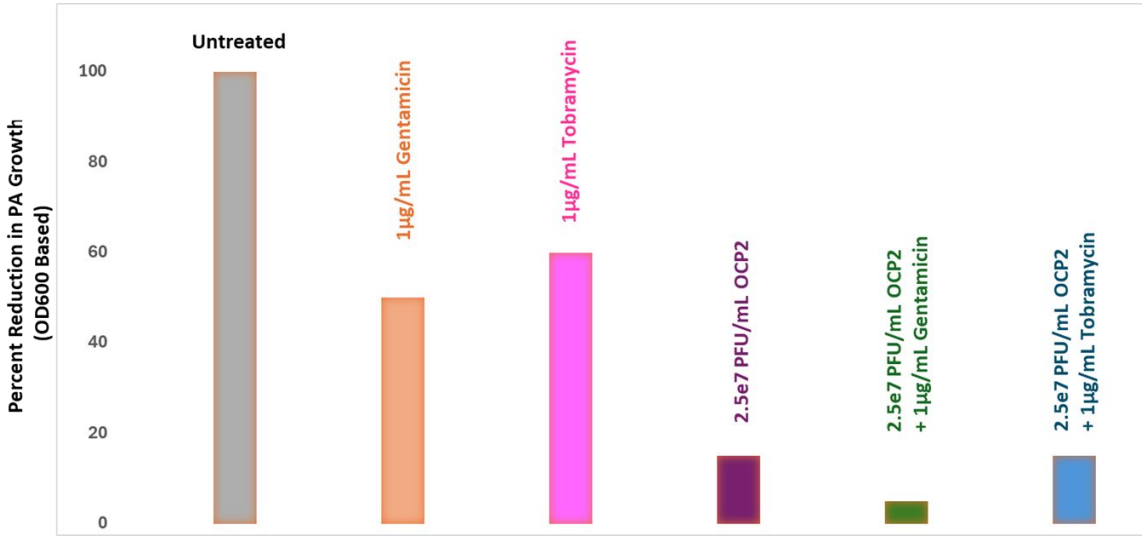


Different concentration combinations of Gentamicin and 4X enriched Ganga water were used in this experiment. As evident from both OD600 and CFU/mL data, 3 µg/mL Gentamicin combined with 1:100K Ganga Phage offers superior inhibitory effect under the tested experimental conditions. This concentration is 50% less compared to the required concentration of Gentamicin to achieve peak level therapeutic effect in serum in humans.

In vitro
Phage Antibiotic Synergy (OCP2 Phage + Gentamicin/Tobramycin) in Preventing PA Growth

OCP2 Phage is extracted from Ohio sewage water. It is O-antigen specific phage that uses LPS as an essential receptor for entering PA cell wall

Percent Reduction of PA Growth at 12 hours Time Point Based on OD600



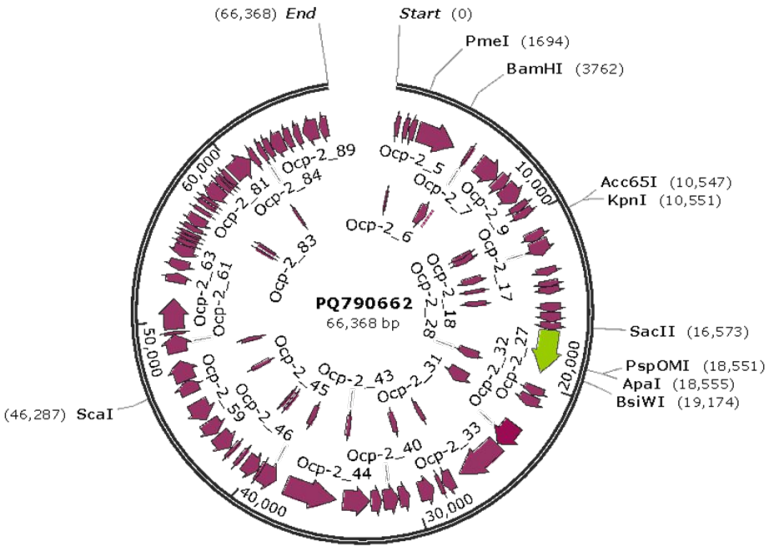
Gentamicin and tobramycin are aminoglycosides.

The first stage of aminoglycoside entry into bacterial cells involves electrostatic binding of polycationic aminoglycoside to the negatively charged LPS followed by displacement of magnesium ions. These magnesium ions stabilize the lipid components and responsible for cross bridging. Removing these ions leads to disruption of outer membrane and initiation of antibiotic uptake. This phenomenon triggers the entry into the cytoplasm, initiates the inhibition of protein synthesis and accelerates cell death.

1 µg/mL of Gentamicin combined with 2.5e7 PFU/mL of OCP2 has reduced ~95% of the *Pseudomonas aeruginosa* growth at 12 hours time point (OD600 Based)

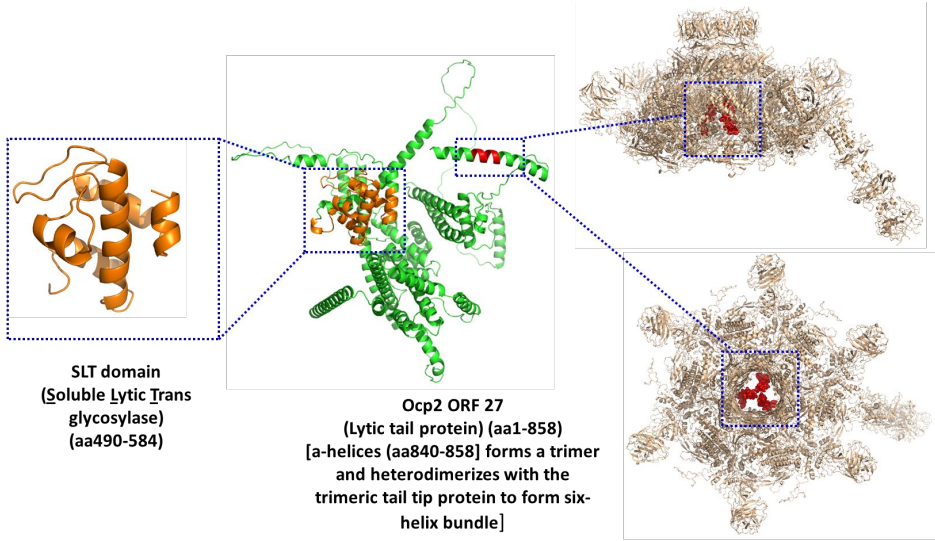
Mechanism of Action
Insights from Phage Genomic Analysis of OCP2

Ocp2_27_lytic_tail_protein_transglycosylase (858 aa)



OCP2_27 has sequence homology (**99.77%**) to PA193 phage [PDB 9B45]. Closest match was A0A5P1KVB9 (Chain S) **gp41** tape measure protein (TMP) [one of the 11 baseplate proteins].

Structural Analysis of OCP2 (ORF27)



SLT domain (Soluble Lytic Trans glycosylase) (aa490-584)

Ocp2 ORF 27 (Lytic tail protein) (aa1-858) [a-helices (aa840-858] forms a trimer and heterodimerizes with the trimeric tail tip protein to form six-helix bundle]

Crystal structure of Base plate proteins (PDB 9B45)

ORF27 sequence suggests a potential SLT domain structure which might play a role in binding to LPS of the bacterial cell wall

Future Outlook

- Strength of PAS (Phage Antibiotic Synergy) is proven through this project by using lower concentrations of antibiotics along with phages for effective bacterial growth inhibition
- My data correlates strongly with the current clinical progress including dozens of ongoing trials showing improved biofilm disruption when phages and antibiotics are used
- My future research as an undergrad will focus on isolating phages from natural environments to enhance treatment effectiveness and reduce healthcare costs. This direction offers a promising path toward addressing the growing challenge of antibiotic resistance, often referred to as the silent pandemic

Closing

Special thanks to:

1. The SCPDG Committee
2. Dr. Karl Liboro
3. Prof. Arne Rietsch and Prof. Eric Pearlman
4. My science advisors at PHS

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