# Burkholderia Growth Curve and Susceptibility to Phage

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## Introduction

- Burkholderia is a gram-negative aerobic bacteria exhibiting exemplary antibiotic resistance. Research on Burkholderia growth treatments are rare, limiting the understanding of this bacteria.
- Bacteriophages are viruses that infect bacterial cells, disrupting normal cell metabolism and causing lysis (Alavadize, Sulakvelidze, Morris Jr. 2001) . It uses a separate mechanism than those of antibiotics to lyse bacterial cells.
- Due to its incredible antibiotic resistance, Burkholderia can and has been used as a biological warfare agent, primarily towards animals (CDC, 2017). It is important to study alternatives to antibiotics, such as bacteriophage, to tackle this threat if aimed towards organisms in the future.

## Approach

- The goal of the project was to understand the Burkholderia bacteria growth process and its response to specific bacteriophage found in the Gainesville water supply.
- The Burkholderia growth curve was identified using optical density readings. Dilutions of the phage with the bacteria were incubated on hard agar plates that resulted in countable plaques.
- We ran into resistance issues twice during the project, which required us to isolate susceptible colonies from the bacterial culture.

## Methodology

**Bacterial Growth Curve**

- Measured optical density (OD) readings of a bacteria culture over 10 hours.

**Isolating Susceptible Colonies**

- 4 isolated colonies selected from a streak of bacterial culture and grown into separate stocks.
- Used a 96-well plate to analyze the different stocks' susceptibility to phage.
  - Used combinations of "log phase" bacteria and phage in test wells (layout of plate in results).

**Plating**

- Inoculated serial dilutions of bacteriophage with bacterial culture in "log" phase.
- Plated and counted plaques.

**CFU Count**

- To determine the number of bacterial cells in a culture at a specific OD.
- Plated bacterial culture at target ODs and counted resulting isolated colonies.

## Results

**Bacterial Growth Curve**

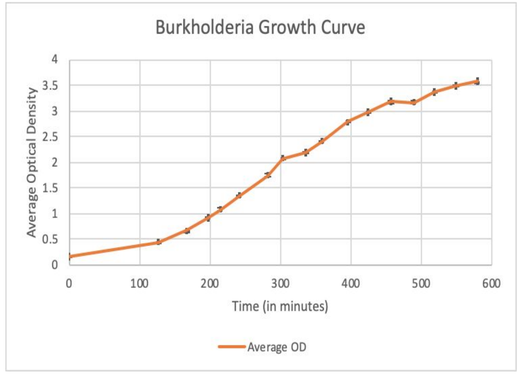



**Figure 1 (above) - Growth Curve.** The growth curve, constructed using OD readings, does not exhibit an exact logarithmic phase as seen with other species of bacteria. This growth curve shows more of a linear trend with sigmoidal characteristics.

**Plating**

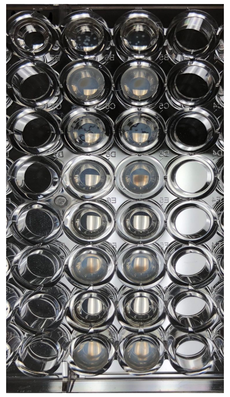


**Figures 4 & 5 (above) - Countable Plaques on Plates.** Figure 4 (left) shows the $10^{-7}$ plate plaques and Figure 5 (right) shows the $10^{-8}$ plate plaques.

**Figure 6 (below) - Plaque Counts.** This chart quantifies our plating results. The $10^{-7}$ and $10^{-8}$ plates were used for CFU calculations.

| Dilution Factor | Plaque Count |
|---|---|
| $10^{-6}$ | Complete clearing (TMTC) |
| $10^{-7}$ | >100 |
| $10^{-8}$ | 23 |
| $10^{-9}$ | 2 |
| control | 0 |

**Isolating Susceptible Colonies**



| Row # | Strain | Treatment | Result |
|---|---|---|---|
| 1 (top) | D1 | w/ phage | Partial clearing |
| 2 | D1 | No phage | cloudy |
| 3 | D2 | w/ phage | Partial clearing |
| 4 | D2 | No phage | cloudy |
| 5 | D3 | w/ phage | Completely clear |
| 6 | D3 | No phage | cloudy |
| 7 | D4 | w/ phage | Completely clear |
| 8 | D4 | No phage | cloudy |

**Figures 2 and 3 (above) - Susceptibility Experiment.** Figure 2 (left) shows the 96-well plate results. Figure 3 (right) shows the setup of the plate and characterizes the appearance of the test wells.

In each well, NB media was included as well as $CaCl_2$ which is necessary in order for the phage to bind to the bacteria. Based on the results, we determined that both strains D3 and D4 were entirely susceptible to our Burkholderia phage stock. For continuity purposes, we strictly used D4 stock as we continued along.

**CFU Experiment**

| Target Optical Density | Dilution Plate | | | | |
|---|---|---|---|---|---|
| | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | control |
| 0.71 | 59 | TFTC | TFTC | 0 | 0 |
| 0.887 | 84 | 33 | 34 | TFTC | 0 |
| 1.032 | 50 | TFTC | 0 | 0 | 0 |

**Figure 7 (above) - Colony Counts Per Plate.** This chart specifies the number of colonies counted on each plate corresponding to its dilution factor of bacterial culture.

Using our plaque counts and our CFU counts we were able to calculate the titer of our Burkholderia phage stock.

## Literature cited

Sulakvelidze A, Alavidze Z, Morris Jr., JG. 2001. Bacteriophage Therapy. Antimicrobial Agents of Chemotherapy doi: 10.1128/AAC.45.3.649-659.2001

Centers for Disease Control and Prevention. 2017. Bioterrorism. online. Available at: https://jmbesubmissions.asm.org/asm/pages/files/asmstyleguidesreferences.pdf.

## Conclusions

**Growth curve:**

- The data shows that the Burkholderia bacteria enter the unique "logarithmic" phase approximately 4 - 5 hours after the 24-hour inoculation period. The bacterial growth curve is unique in that it follows a linear trend rather than a logarithmic trend. This made it difficult to pinpoint exact O.D values for plating; instead, we followed a time-based pattern.

**Plating and Calculations:**

- Plating showed that Burkholderia bacteria is indeed susceptible to phage, and that phage is a beneficial way to initiate antibiotic effects. The results of the plating trials indicate that our phage was able to successfully bind and lyse the bacterial cells. The counted plaques highlight the antibacterial properties of phage and its implications for future research in antibiotic alternatives.
- The titer of our Burkholderia phage stock was calculated to be $2.3x10^{10}$ PFU/mL.

## Future Work

- **Antibiotic alternatives:** A future aspect of this study could be phage alteration to optimize its lysing of Burkholderia. Studies can also seek ways to make viral phage safe for human consumption, while retaining its antibiotic properties.
- **Genome sequencing:** Future studies could analyze the phage and sequence it, and then compare its genome against other known phages to view similarities and differences.

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