PSEUDOMONAS AERUGINOSA

OLIVIA BOYKIN

SUMMER INTERNSHIP 2019

INTERNSHIP CHRONOLOGY

FIRST WEEK- ONLINE TRAINING AND LEARNING LAB PROCEDURES

WEEKS 2-3- EXCLUSIVELY GROWTH CURVES

WEEKS 4-5- ALTERNATING GROWTH CURVES AND BIOFILMS

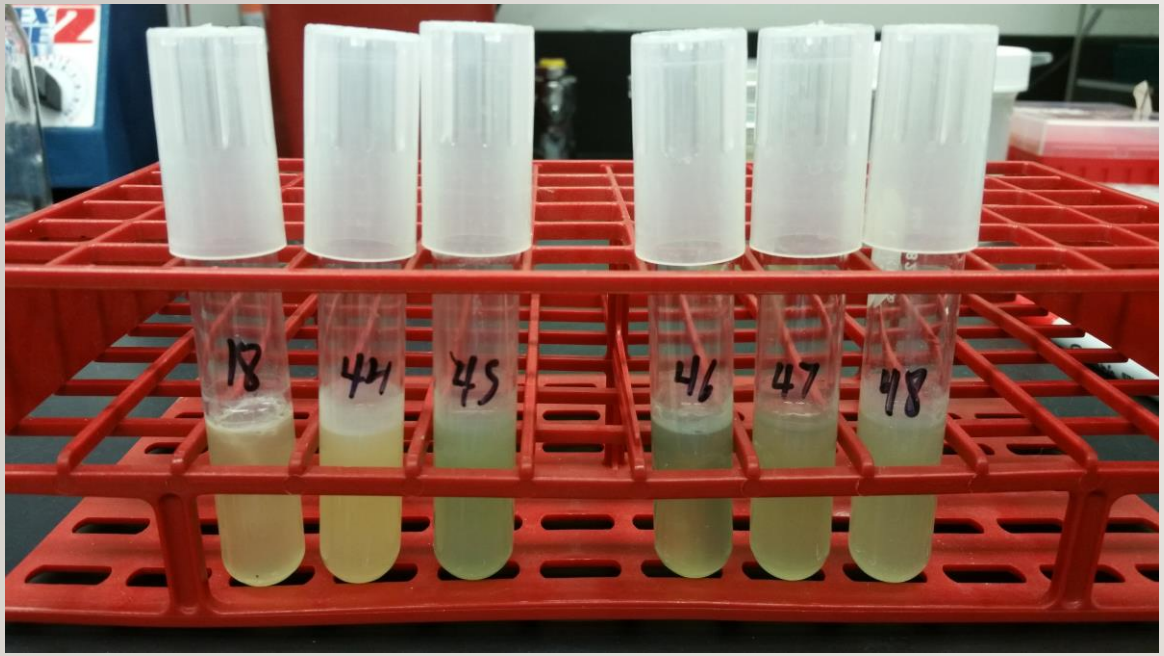
WEEK 6- FINAL BIOFILMS, DATA COLLECTIONS

WEEK 7- COMPILING DATA, LAB PRESENTATION, WRAP-UP

WHAT IS PSEUDOMONAS?

- Opportunistic Gram-negative bacterium; bacillus shape
- Facultative anaerobe
- Some strains produce high levels of pyocyanin and pyoverdine→ greenish blue color
- Lives in soil
- Mostly affects people with large open wounds (example: soldiers with shrapnel) and compromised immune systems

WHAT IS A BIOFILM?



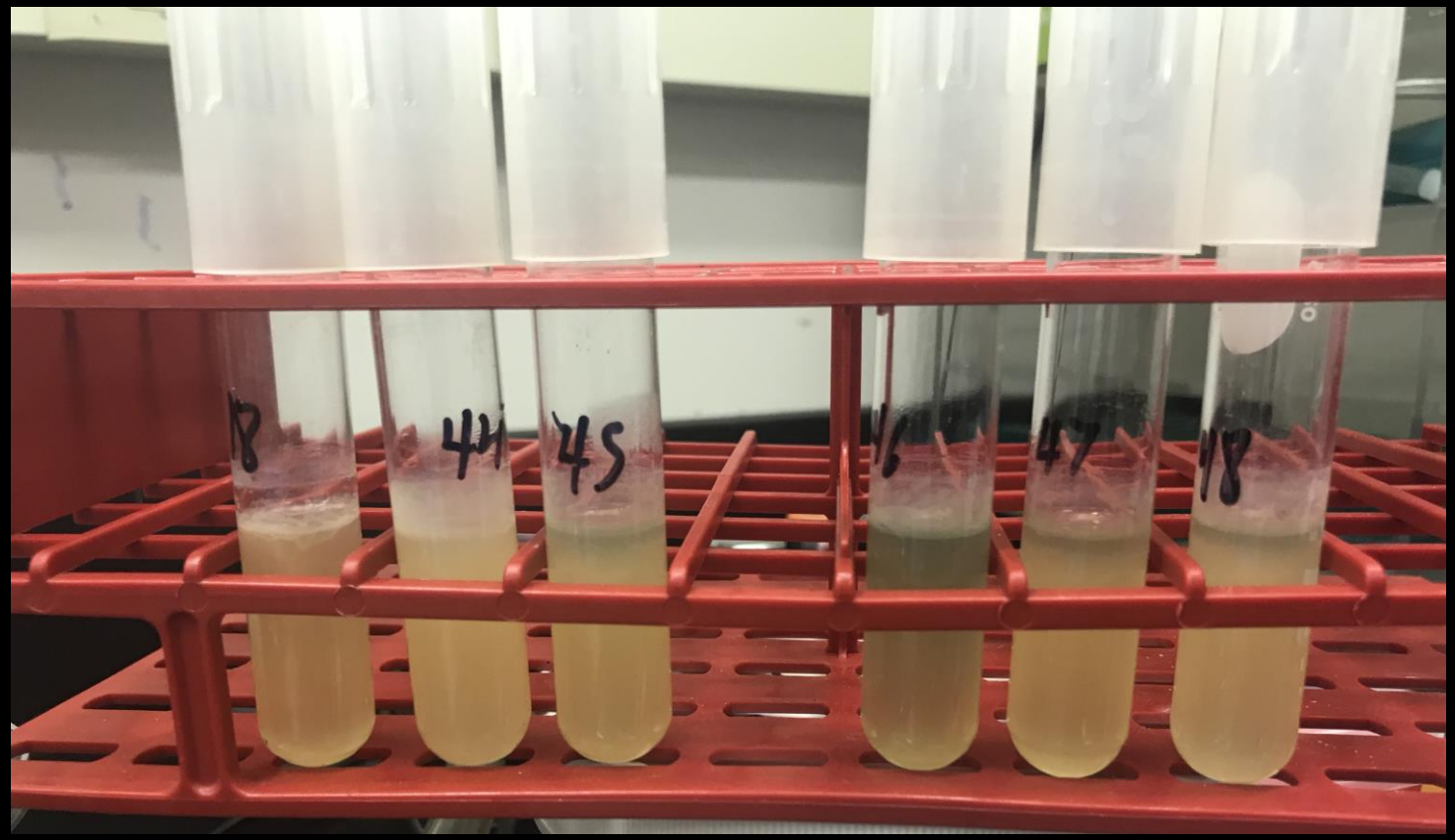
- "Community of microbes that inhabit various surfaces and are typically surrounded by extracellular matrix [ECM]"[1]
- Survives in nutrient poor environments
- Heterogeneous- almost every cell in a different state
- Resistant to many antibiotics and adaptable to many unique environments
  - Difficult to treat
- Responsible for many chronic/reoccurring infections (many nosocomial)
  - Cystic fibrosis, UTIs, tonsillitis, chronic wounds, acne

1. Lee, Keehoon, and Sang Sun Yoon. "*Pseudomonas Aeruginosa* Biofilm, a Programmed Bacterial Life for Fitness." *Journal of Microbiology and Biotechnology*, U.S. National Library of Medicine, 28 June 2017, www.ncbi.nlm.nih.gov/pubmed/28301918.

GOAL:
TO CHARACTERIZE THE ABILITY OF CLINICAL ISOLATES TO FORM BIOFILMS

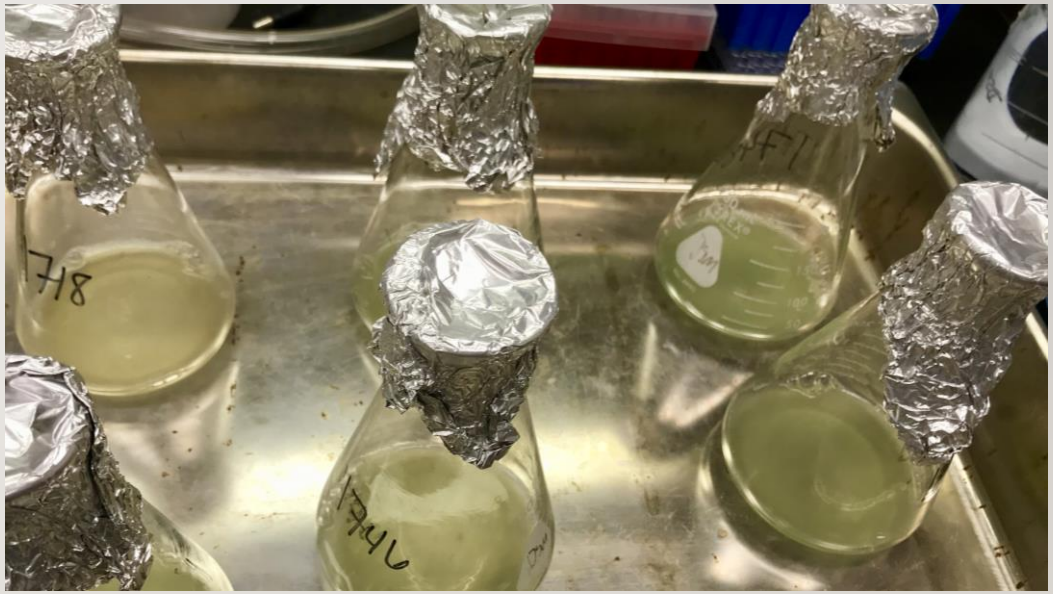
How?

- Growth Curves
  - Analyzing OD_{600} and CFU
- Biofilms



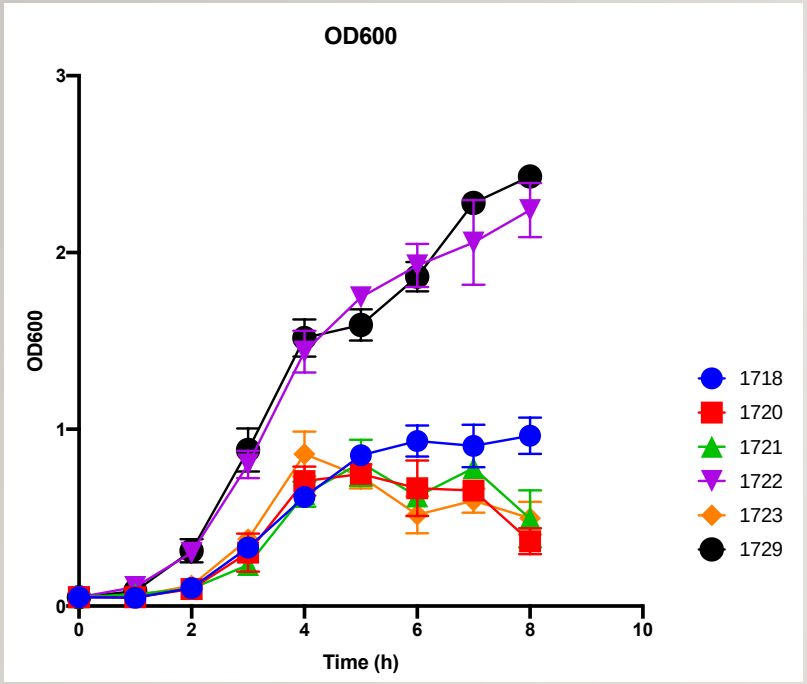
GROWTH CURVE PURPOSE

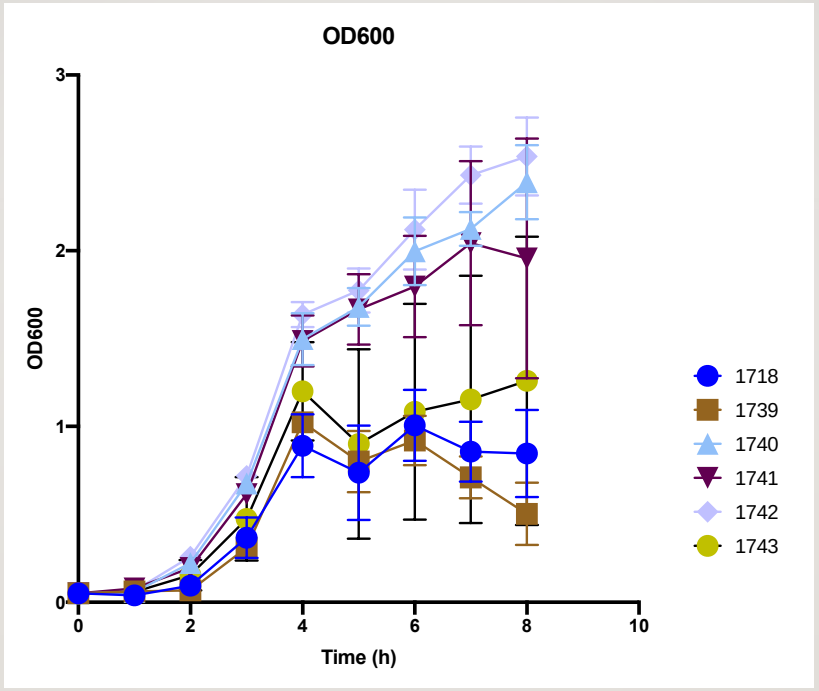
- Use the information from 8 hour growth curves to:
  - Estimate the start of each of the 4 growth phases: Lag, exponential, post-exponential, stationary
  - Look for middle of exponential phase (varied with each set of strains)
    - Used to start biofilm

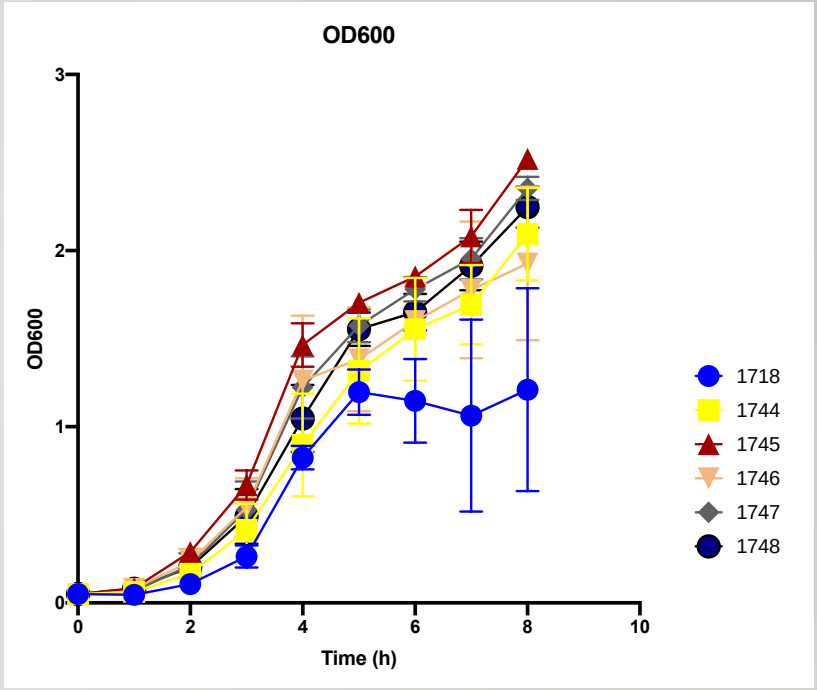


PROCEDURE

- At each time point:
  - Take OD_{600} of flasks (1 mL)
    - Dilute after hour 4
  - At every other timepoint plate CFU using serial dilutions



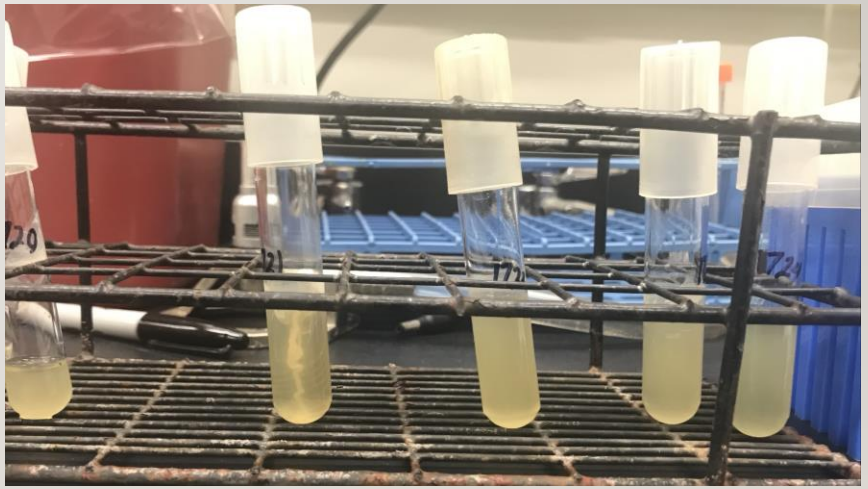


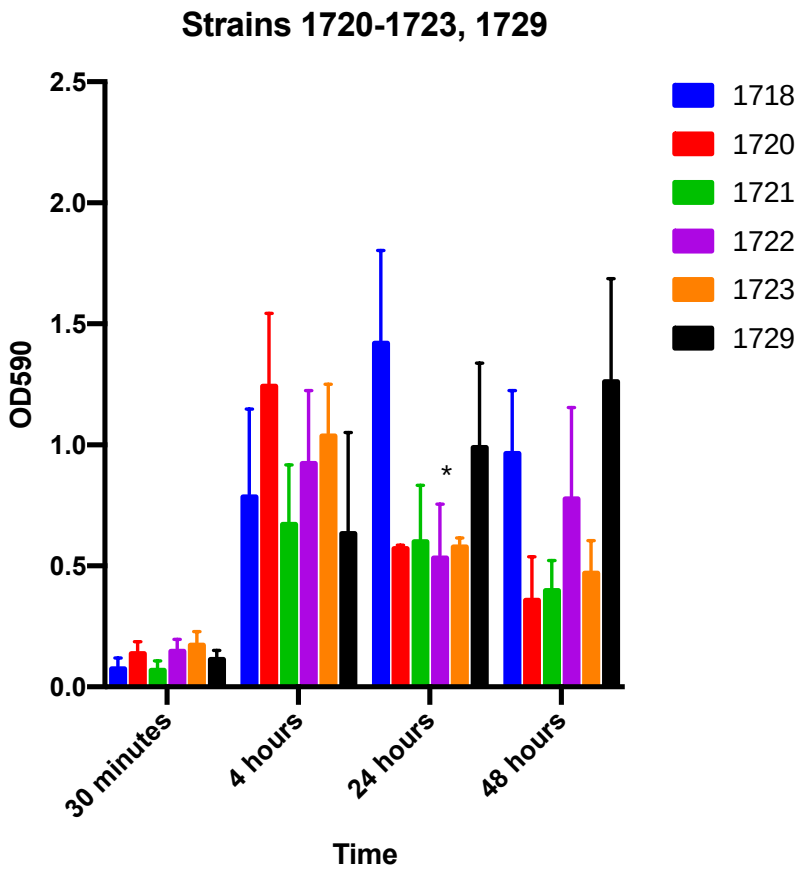


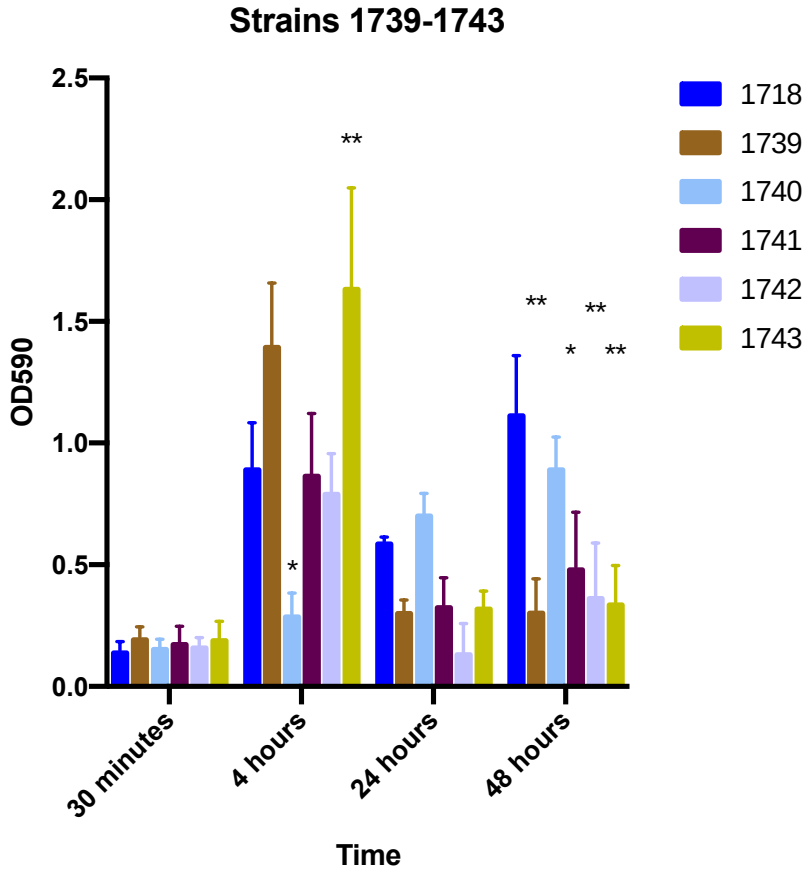
GROWTH CURVE DATA (OPTICAL DENSITIES)

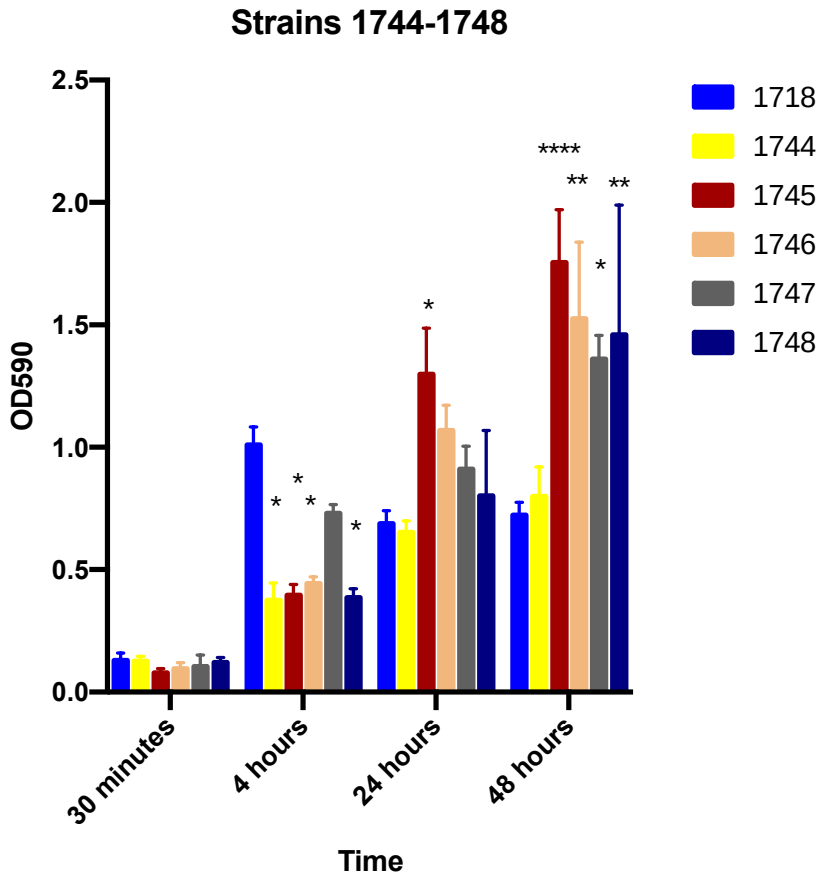
PLATING BIOFILMS

- Use 96-well tissue-culture treated plates (4 time points per biological replicate- separate plate for each).
- Incubate 100 µL of correct sample in each well.
- Fill the surrounding wells with 200 µL water.
- Perform 3 biological replicates with 6 technical replicates each.









BIOFILM DATA

GRAM STAINING

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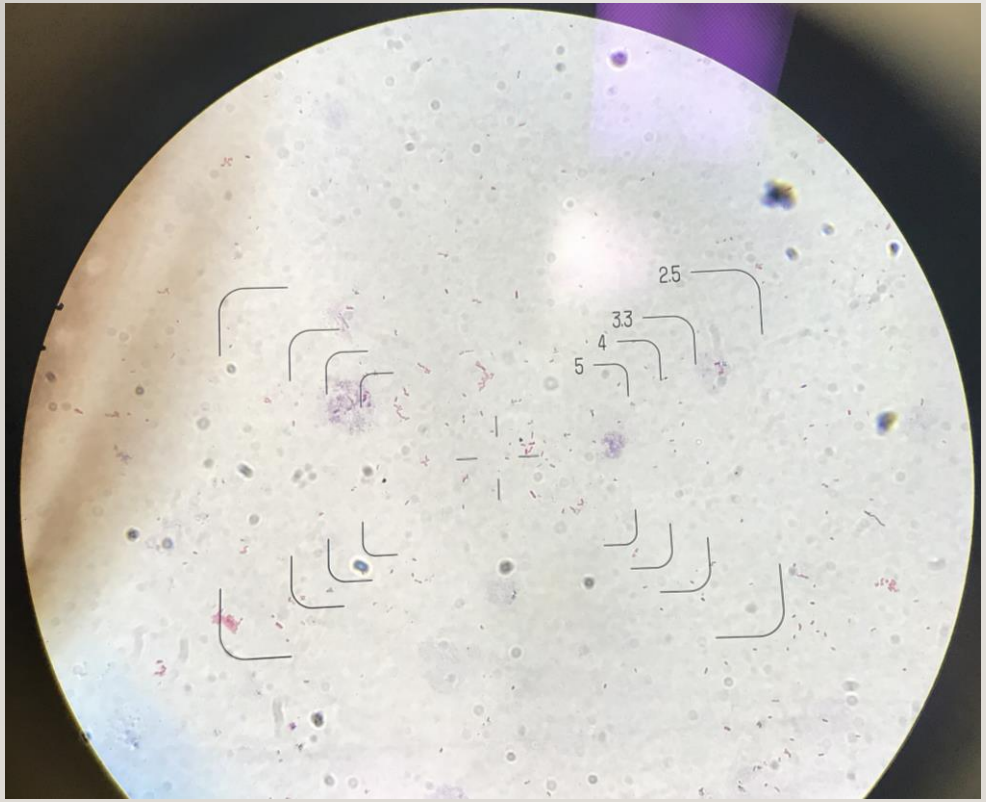

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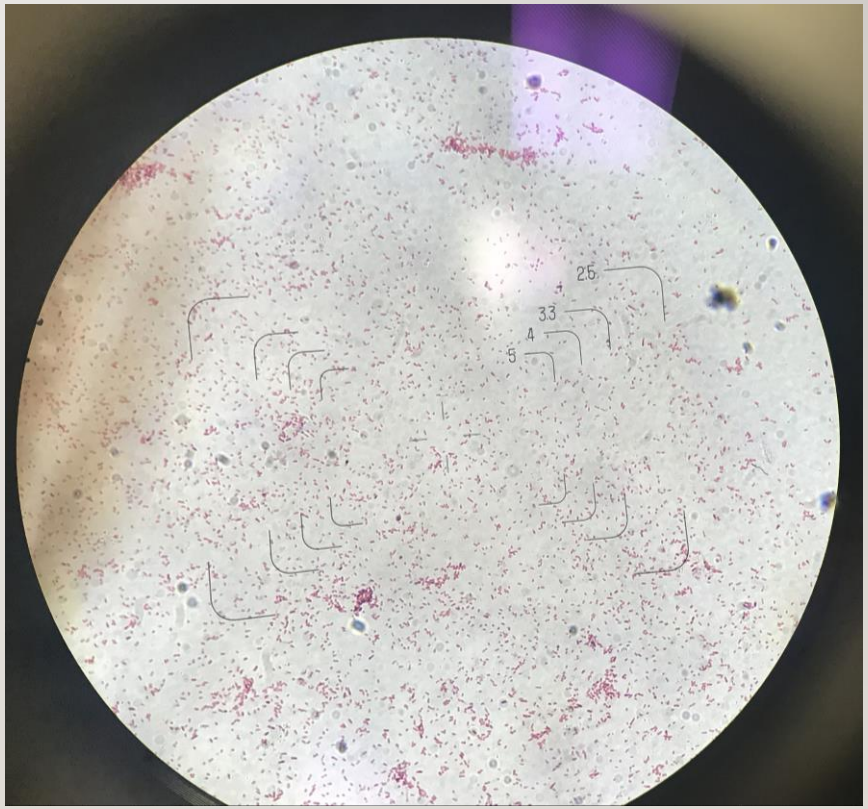
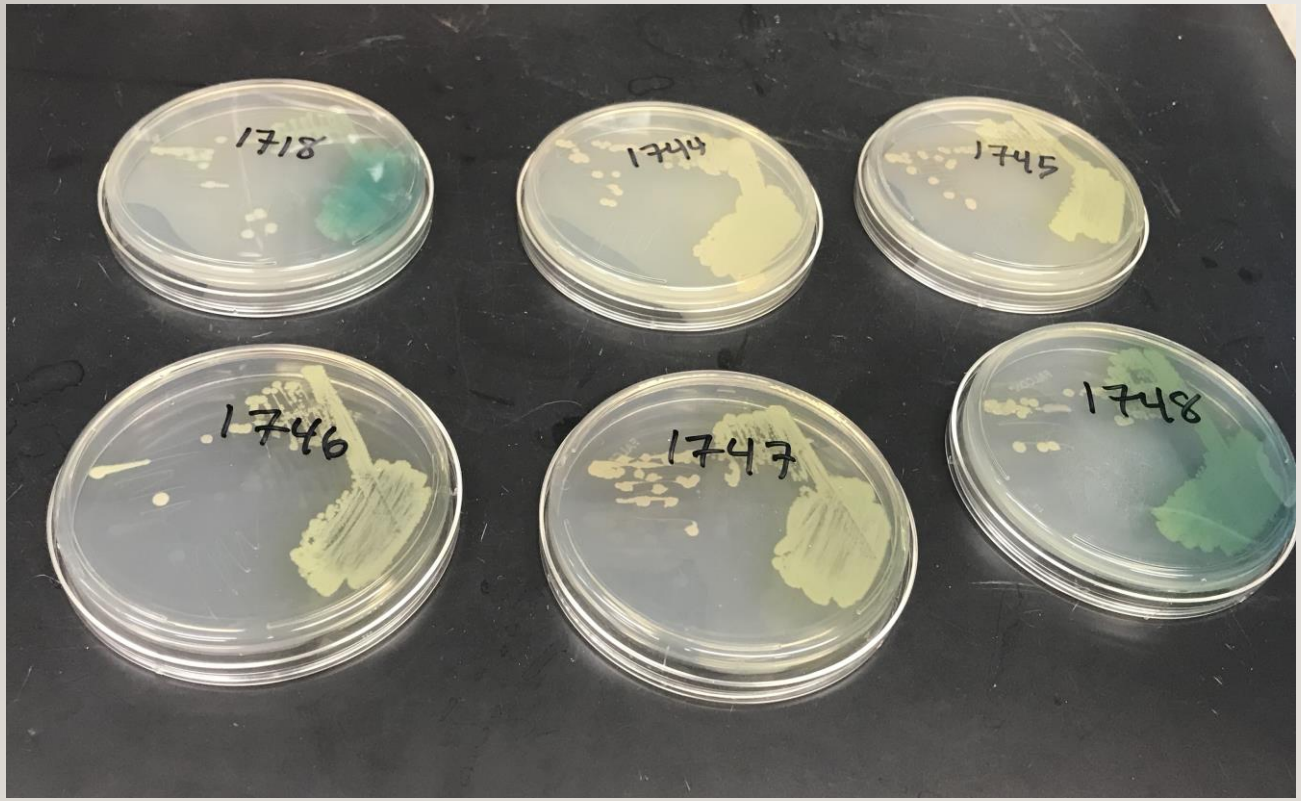


OBSERVATIONS



- Correlation between aggregation and color
  - Strains that turned green did not experience much aggregation and if they did, the chunks were thin and brown (easy to break up)
- Strains that aggregated a lot had extremely low OD (sometimes lower than initial) by hour 6-7, but still maintained increasing CFUs
- Lab strain aggregated a lot and never turned green in flasks (very mucoid)
- Strains that aggregated a lot did not necessarily form strongest biofilms
  - Cell ability to stick together differs from ability to stick to surfaces
- Most biofilms formed within 4 hours

OBSTACLES/ POSSIBLE ERRORS

- Breaking up aggregates to attempt to get accurate OD readings
- Wide variability between replicates
  - Growth curves
    - Slightly different incubation times for overnight cultures
    - Starting Tween treatment too late (one time point might dip)
  - Biofilms
    - Pipetting up biofilm when washing wells (esp. 48 hour)
- My amount of experience
  - Earlier data less consistent

WHAT I LEARNED

- Lab Techniques
  - Pouring plates
  - Streaking for single colonies
  - Inoculating overnight cultures
  - Measuring OD and plating CFU
  - Developing biofilms
  - Gram Staining

WHAT I LEARNED (CONTINUED)

- First real work experience
- Positive growth mindset and work ethic
  - Errors are expected- just learn what needs to be corrected for next time
- Responsibility and independence

THANK YOU…

- Dr. Douglas Merrell
- Dr. Ian Windham
- Dr. Hannah Krug
- Ms. Cheryl Hansen