Spatiotemporal Modeling of Bacterial Co-culture using Partial Differential Equations

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Abstract

Bacterial co-cultures are complex systems that exhibit spatiotemporal dynamics that are difficult to observe experimentally. In this study, we developed a mathematical model using partial differential equations to predict the behavior of two strains of E. coli in co-culture. Our model was able to effectively capture the dynamics of bacterial growth, considering their interactions and the effects of glucose and antibiotic diffusion. We observed interesting phenomena such as the formation of the "coffee ring" pattern and differences in fluorescence intensity between the two strains. Our results demonstrate the potential of mathematical modeling to provide insights into bacterial co-cultures and highlight the importance of considering the spatiotemporal dynamics in such systems. Future work involves improving the accuracy of the model predictions by calculating exact parameters for each experiment and investigating the kinetics of antibiotic inhibition for both strains.

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1.0 Introduction

The field of synthetic biology has gained a lot of attention in recent years due to its potential applications in various fields such as bioremediation, agriculture, biotech, biofuel, wastewater treatment, medicine, food, and more [1,2]. Synthetic ecology, which involves the design and manipulation of microbial communities, has emerged as a key area of research in synthetic biology [1]. Understanding the dynamics of microbial communities is crucial for developing strategies to engineer them for desired functions [2]. Bacterial co-cultures have been a subject of interest in recent years. One of the challenges in studying bacterial co-cultures is understanding the spatiotemporal dynamics of different bacterial populations in the same environment.

In this study, we investigate the spatiotemporal modeling of bacterial co-culture using partial differential equations (PDEs) by performing co-cultures of two strains of Escherichia coli (E. coli). Our aim is to understand the dynamics of bacterial populations in co-culture and to develop a mathematical model that can predict the behavior of such systems.

The general goal of this project is as follows: incorporate experimental data on monoculture growth and parameter estimates into the model, use the model to investigate the growth and spatial distribution of the first strain in the presence of a second strain, compare the model predictions with experimental observations of co-cultured bacteria, explore the impact of different growth conditions and initial conditions on the dynamics of the bacterial co-culture, and develop a quantitative understanding of the factors that influence the spatial organization of bacterial co-cultures.

While the overall goal of this project is to contribute to the development of strategies for engineering microbial communities, the focus of this study is more specifically on comparing model predictions with experimental observations of co-cultured bacteria growing on agar pads and exploring the impact of different growth conditions and initial conditions on the dynamics of the bacterial co-culture.

2.0 Background

2.1 Escherichia Coli

Escherichia coli (E. coli) is a gram-negative, rod-shaped bacterium commonly found in the lower intestine of warm-blooded organisms, including humans. It is a widely used model organism in

microbiology and biotechnology due to its ease of cultivation and manipulation in the laboratory [3,4].

2.2 Biomarkers

Biomarkers are measurable biological indicators that are used to identify or characterize a biological state or condition [5]. Fluorescent protein tags are a type of biomarker that allows researchers to visualize and track specific proteins in cells or tissues [5]. The most used fluorescent protein is green fluorescent protein (GFP), which was first isolated from the jellyfish Aequorea victoria in the 1960s [5,6].

Fluorescent protein tags have revolutionized the field of molecular biology by enabling real-time visualization of protein expression and localization in living cells [6]. By fusing GFP or other fluorescent proteins to specific target proteins, researchers can study the behavior and interactions of these proteins in vivo [5]. This method has been widely applied to various areas of research, including cell biology, developmental biology, and biotechnology [5,6]. In this study, fluorescent protein tags are used as biomarkers to distinguish between different bacterial strains in co-culture experiments. In this experiment Orange-yellow fluorescent protein or Cyanine3 (Cy3) was used for the bacteria that has Kanamycin resistance and cyan fluorescent protein (CFP) was used for chloramphenicol resistant strain.

2.3 Co-cultures: Cheaters and co-operators

Bacterial co-cultures often involve the interaction between two or more strains of bacteria. These interactions can be classified into two broad categories: cheaters and co-operators. Cheaters are strains that benefit from the co-culture without contributing to it, while co-operators are strains that contribute to the co-culture for the benefit of the group [7,8,9]. Cheaters often exploit public goods, such as nutrients or signaling molecules, which are produced by co-operators [7,9]. This exploitation can lead to the collapse of the co-culture if there are too many cheaters present [8].

In this study, both strains can be cheaters. The antibiotic resistance for kanamycin and chloramphenicol both works internally. Since the protein responsible for depletion of the antibiotic is not secreted, they are not co-operators, but they gain dependency on each other to deplete the necessary antibiotic for survival. They may outcompete each other once enough antibiotics are depleted.

2.4 PDE Modeling

Partial differential equation (PDE) modeling has been widely used in the field of mathematical biology to describe the spatiotemporal dynamics of bacterial populations [2]. PDE models provide a powerful tool for predicting how bacterial populations will grow and interact with each other under different conditions. By incorporating relevant parameters such as growth rates, diffusion coefficients, and spatial heterogeneities, PDE models can capture the essential features of bacterial population dynamics [2,11].

One of the advantages of PDE models is that they can capture the effects of spatial heterogeneity on bacterial growth and population dynamics [11]. This is particularly important in the context of bacterial co-cultures, where different strains may occupy different regions of the same environment [8]. PDE models can be used to predict how these strains will interact with each other and how their spatial distribution will change over time [12].

Reaction-diffusion models are the most used PDE models in bacterial population studies [2,11,12,13]. These models describe the diffusion of bacteria in a medium and their interactions with other bacteria through reaction terms that capture the effect of growth, death, and other biological processes [11,12].

In our study, we use a reaction-diffusion model to describe the spatiotemporal dynamics of bacterial co-cultures. The model incorporates parameters such as bacterial growth rates, diffusion coefficients, and interactions between different bacterial strains. We use Python to solve the PDEs numerically and compare the model predictions with experimental data.

2.5 Historic Work

This study builds upon previous research conducted by Sultan Nazir for their master's thesis. He worked on monoculture growth and parameter estimation of the two strains of E. coli. In his work, he conducted monoculture growth curve experiments to obtain growth curve parameters and monoculture time-lapse experiments to determine diffusion and nutrient uptake parameters for each strain [14]. Using simulated annealing, they obtained the initial parameters for the yellow fluorescent protein strain.

In this study, we continue the investigation of the two E. coli strains in co-culture and focus on the antibiotic inactivation parameters. By incorporating the previously obtained parameters into a partial differential equation (PDE) model, we aim to understand the spatiotemporal dynamics of the co-cultured bacteria and develop a mathematical model that can predict their behavior.

3.0 Method

The primary experimental method follows a modified version of protocol outlined in Young et al (2012) [15].

Bacterial Strains and Growth Conditions:

Preparation of overnight culture

1. In two autoclaved test tube inside the Biosafety Cabinet transfer 3mL of LB media each to the test tubes
2. Add 3.0µL of Chloramphenicol 25mg/ml (Cm25) to one of the test tubes and 3.0 µL Kanamycin 50mg/ml (Km50). Label both test tubes.
3. With a clean pipette tip transfer some Cm resistant E. coli strain labelled AY43 to Cm test tube and AY41 to Km test tube.
4. Grow overnight while stirring at 37ºC.

Growth to exponential phase

5. Perform a 100x dilution of the overnight culture (50µL) into 5mL of Sterile M9 media.
    a. Note: M9 media is 0.4% glucose (w/v), hence the only source of carbon.
6. Grow for 5-6 hours until OD600 is around 0.5. (OD600 refers to optical density at 600nm based on absorbance)

Preparation of Gel Pads

1. Transfer 0.150 g of Low melt agarose into a falcon tube.
2. Add 10mL of Sterile M9 media, stir gently.
3. Heat up the falcon tube in the microwave for few seconds in pulses, just until it looks like bubbling. Keep stirring between each pulse. Once done the solution should look clear.
4. Let it cool, once around 50ºC or easy to hold in hand add 1µL Km50 and 1.5µL Cm25 to the low melt agar solution and mix well on vortex.
5. Get a few 22x22 coverslips and add 1 mL of the Agarose mixture onto a coverslip, and quickly add a second coverslip on top of the agar pad.
6. Let the agar solidify.

Spotting the cells

1. Transfer 1mL of exponential culture into a microfuge
2. Centrifuge the exponential culture at 6800 g for 3min.
3. Resuspend in M9 media. Combining this with the previous step is called washing.
4. Do another washing (steps 2-3).
5. Accounting for differences in OD600 of the two cultures appropriately take the right amounts of culture to get a homogenous mixture in a new microfuge tube and mix well.
6. Carefully slide the top coverslip away from the agar pad.
7. Take 1µL of the mixed culture and spot carefully spot it onto the agar pad.
8. Cut 1cm x 1cm square around the circular spot using a scalpel.
9. Get a clean petri dish that can be viewed with the microscope.
10. With a flat surgical spatula and scalpel, first carefully slide the flat spatula underneath the 1x1 cm pad. Once on it, carefully slide it onto the petri dish using the blunt end of scalpel. Make sure that the spotted cell region is the side touching the glass of petri dish, avoid formation of air bubble when transferring the pad.
11. Cover the petri dish and seal it with parafilm.

Co-culture Time-Lapse Microscopy:

1. The microscope was equipped with an environmental chamber that maintained a constant temperature of 37°C.
2. A small bottle cap was filled with water and placed in a corner for some humidity control.
3. The light source intensity was set at 50% with appropriate fluorescent channels turned on (CFP and Cy3)
4. Cells were found and regions of interest were chosen on the various pads in the petri dish.
5. Once the tiles were mapped, the microscope was set to image for every hour for 48 hours.

Image Processing and Data Analysis:

Post imaging, sometimes background subtraction or deconvolution was used to make images clearer, this is not a crucial step. Each image was saved with the phase channel, CFP channel, and Cy3 channels. The images obtained from the microscope were analyzed using custom scripts written in Python. The scripts extracted the location and fluorescence intensity of each bacterial

cell in each frame of the time-lapse series. The data were then used to track the growth and movement of each bacterial population and to generate spatiotemporal plots of bacterial co-culture growth.

Partial Differential Equation (PDE) Modeling:

To model our system, we can start with a single-species model (1) which would model presented by Khassehkhan et. al (2009) [11]. Where C is the cell density as a function of space and time [11]. α_0 is a constant and M is the reaction term. And D is a density dependent diffusion term [11,13,14].

$$\frac{\partial C}{\partial \epsilon} = \alpha_0(D(C)\nabla c) + M \tag{1}$$

The density dependent diffusion term can further be further be classified as (2), which is equivalent to (3) by chain rule. Here, p is a decreasing function and q is an increasing function [11,14]

$$D(C) = pq + C\left(p\frac{\partial q}{\partial C} - q\frac{\partial p}{\partial C}\right) \tag{2}$$

$$\frac{\partial C}{\partial t} = \alpha_0\left(p\nabla_2\left(C_q\right) - Cq\nabla^2 p\right) + M \tag{3}$$

Since in our model the bacteria are not motile and we are not interested in the formation of a biofilm, we can remove the max capacity and our model becomes the following, where δ_0 is a dispersal constant.

$$\frac{\partial C}{\partial t} = \delta_0(p\nabla^2(Mq) - Mq\nabla^2 p) + Mp \tag{4}$$

Now let us consider the role of antibiotics and how it affects our model. As briefly mentioned earlier both strains are dependent on each other to grow since they are only resistant to one of the antibiotics. Once the antibiotic diffuses into the cell, it slows down the growth of the cell or makes it dormant [16,17]. If the cell is resistant to the antibiotic, let us say Km, it eventually regains function by breaking it down, as a result for the Km sensitive strain bacteria to survive it needs Km resistant strain to break down Km. This is known as antibiotic mediated cross-protection mutualism [14].

In our study, we employ Michaelis-Menten kinetics (Enzyme kinetics) to estimate glucose uptake and the Hill function to penalize the growth rate of the sensitive strain in the presence of antibiotics [11,13,14]. We assume simple Fickian diffusion for the dispersal of glucose and antibiotics, without any density dependence [14]. Hence, we get the following:

$$\frac{\partial c_1}{\partial t} = \delta_0 \left(p\nabla^2 \frac{Mqc_1}{c_1 + c_2} - \frac{Mqc_2}{c_1 + c_2} \nabla^2 p \right) + M_1 c_1 p \tag{5}$$

$$\frac{\partial c_2}{\partial t} = \delta_0 \left(p\nabla^2 \frac{Mqc_2}{c_1 + c_2} - \frac{Mqc_2}{c_1 + c_2} \nabla^2 p \right) + M_2 c_2 p \tag{6}$$

$$\frac{\partial S}{\partial t} = \delta_s \nabla^2 S - \frac{M}{Y} \tag{7}$$

$$\frac{\partial A_1}{\partial t} = \delta_{A_1} \nabla^2 A_1 - V_{max,1} \frac{A_1 c_1}{k_{m,1} + A_1} \tag{8}$$

$$\frac{\partial A_2}{\partial t} = \delta_{A_2} \nabla^2 A_2 - V_{max,2} \frac{A_2 c_2}{k_{m,2} + A_2} \tag{9}$$

where,

$$M = M_1 c_1 + M_2 c_2 \tag{10}$$

$$M_1 = \mu_1 \left(\frac{S}{\mathrm{K}_s + S} \right) \left(\frac{1}{1 + \left(\frac{A_2}{IC_{50,2}} \right)^{n_2}} \right) \tag{11}$$

$$M_2 = \mu_2 \left(\frac{S}{\mathrm{K}_s + S}\right)\left(\frac{1}{1 + \left(\frac{A_1}{IC_{50,1}}\right)^{n_1}}\right) \quad (12)$$

(5) and (6) are changes in Km resistant strain and Cm resistant strain, respectively. (7) is Fickian diffusion model for glucose, here Y is the biomass yield, and S is the substrate concentration [14]. δ_{A_1} refers to diffusion constant for Km and δ_{A_2} refers to diffusion constant for Cm. (8) and (9) are using Michelis-Menten and diffusion models for change in Km and Cm respectively, A_1 refers to concentration of Km and A_2 is concentration of Cm, and c_1 and c_2 are related to cell densities of Km resistant strain and Cm resistant strains. Equations (10), (11) and (12) are growth induced models of individual strains and use the hill function for does response, M_1 for Km resistant strain and M_2 for Cm resistant strain, here $IC_{50,1}$ refers to concentration of Km at ½ inhibitory concentration likewise $IC_{50,2}$ is ½ inhibitory concentration of Cm. n1 and n2 and μ1 and μ2 are related to growth curves of strain 1 and 2.

For the simulation following parameters were considered as shown below in the code block, the values shown below were a result of simulated annealing with Cy3 strain that was previously done.

```
k = 0.636        # guess otherwise comes from annealing process

mu = 0.0014       # max growth rate of strain 1 (in 1/sec)
step_mu = 0.05/3600

Rhf = 161              # cell density for half pressure (in pixel intensity)
step_Rhf = 5

Qhf = 0.0731              # cell density for half pressure (in pixel intensity)
step_Qhf = 5

Phf = 1030              # cell density for half acceptance (in pixel intensity)
step_Phf = 5

Y = 55.2               # biomass yield (in pixel intensity per unit S0)
step_Y = 5

# Glucose
```

```
Ks = 13.3         # rate of nutrient consumption by Monod kinetics (in unit log10S0)
step_log10Ks = 0.1

# Diffusion
DC = 603            # spreading coefficient of cells (in um^2)
step_DC = 50

DS = 80.6              # diffusion coefficient of glucose (in um^2/sec)
step_DS = 50
# Initial condition
S0 = 1                # glucose concentration in feed medium
```

4.0 Results

Sample Experimental results:

Here is a time lapse of some results, including some of the sample frames shown below. The time lapse shows only Cy3 and CFP channels turned on. Phase information is not shown to avoid cluttering. Phase information is however shown in the images below, that goes over specific time points. Note that this set of experiments contain only 17 frames, since they were accidentally taken with 3-hour intervals. Here is another time lapse that was taken with 1hr intervals. It is recommended that you watch the video at slower speed or frame by frame.

Sample 1:

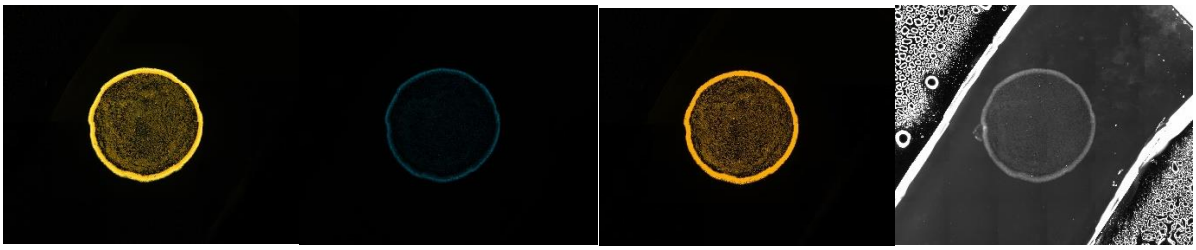

Figure 1:Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the first hour

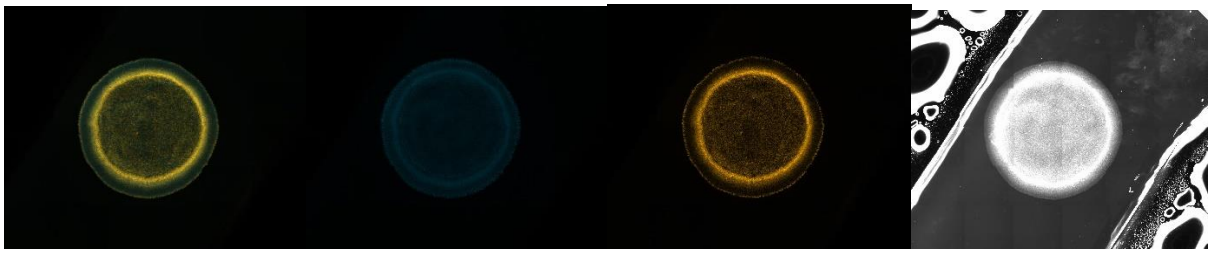

Figure 2: Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the 15th hour

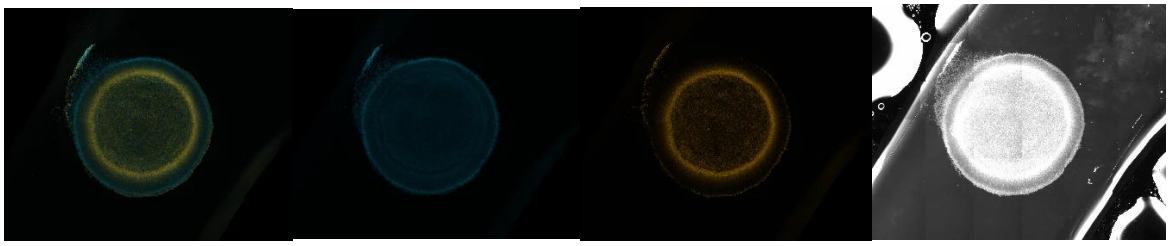

Figure 3: Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the 27th hour

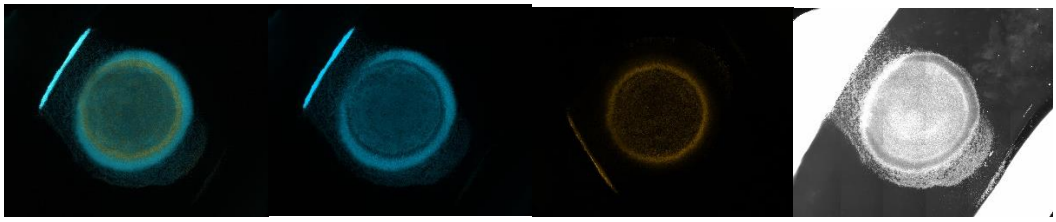

Figure 4: Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the 48th hour

Sample 2:

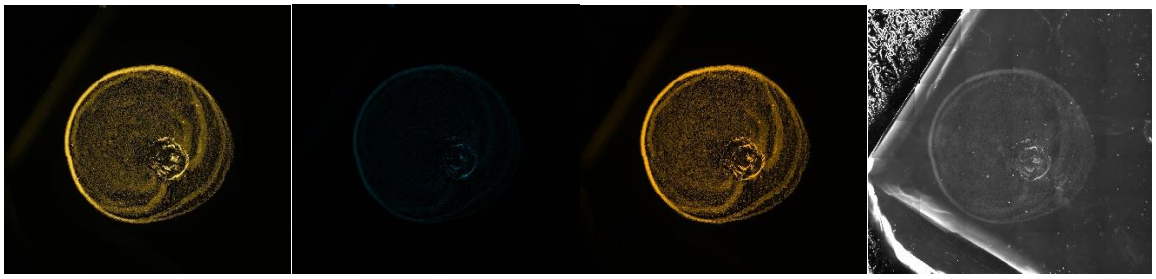

Figure 5:Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the first hour

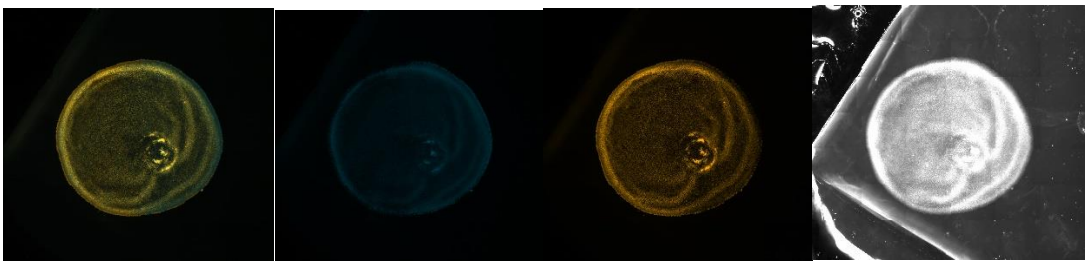

Figure 6: Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the 15th hour

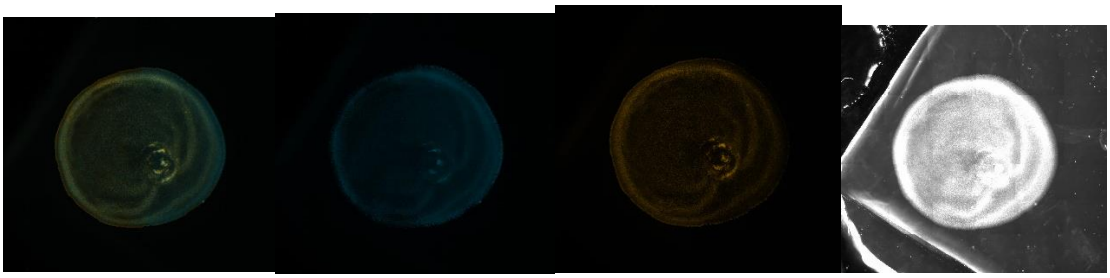

Figure 7: Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the 27th hour

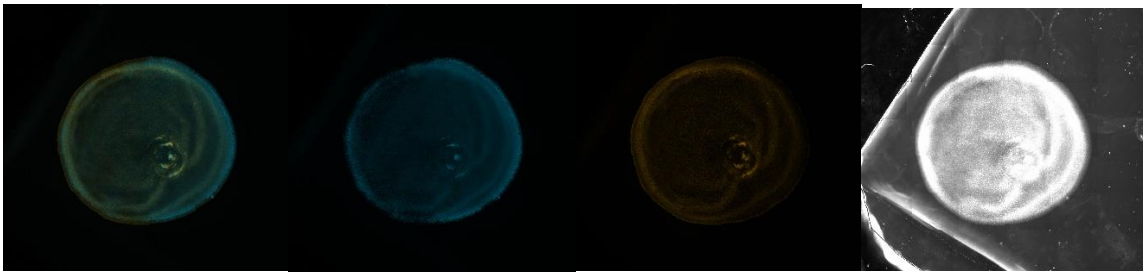

Figure 8: Time lapse images of Both channels, CFP, Cy3 and Phase channel respectively at the 48th hour

Modeling Results Based on Sample 1:

Videos are provided in the links below.

CFP simulation, Cy3 Simulation

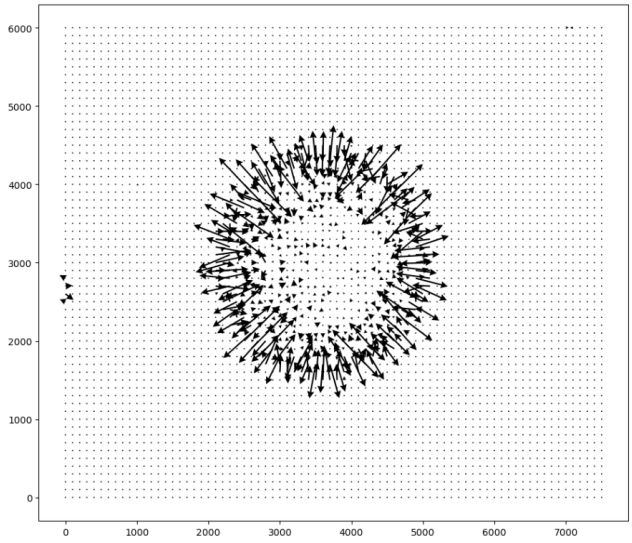

Figure 9: Arbitrary vector field depicting growth of CFP strain at t=0.

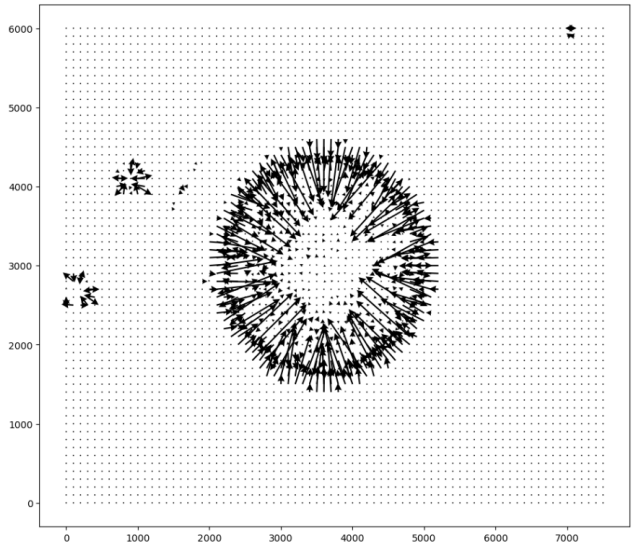

Figure 10: Arbitrary vector field depicting growth of CFP strain at t=24h

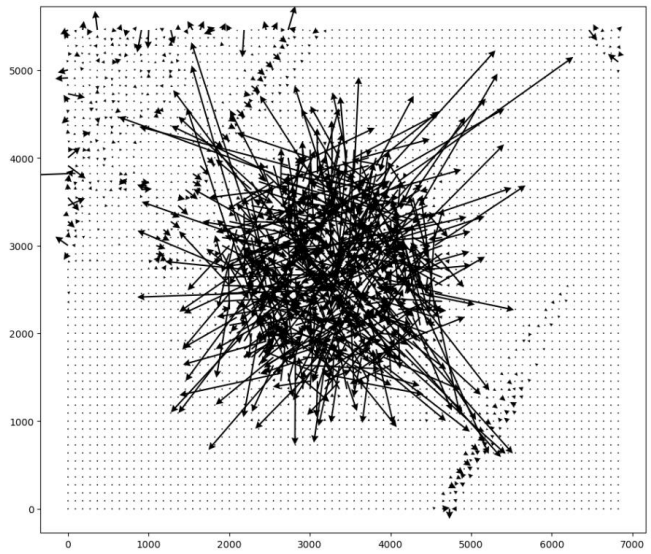

Figure 11: Arbitrary vector field depicting growth of Cy3 strain at t=0

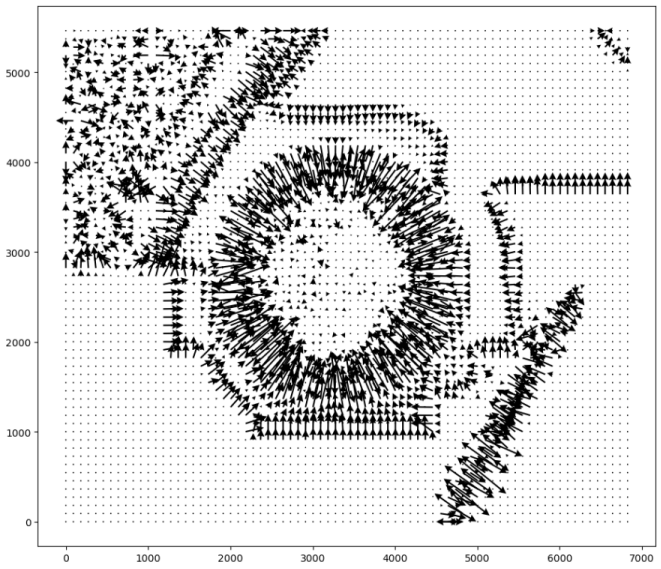

Figure 12: Arbitrary vector field depicting growth of Cy3 strain at t=24h

5.0 Discussion

The results of our study suggest that the spatiotemporal dynamics of bacterial co-cultures can be effectively modeled using partial differential equations (PDEs). Our mathematical model was able to predict the behavior of the two strains of E. coli in co-culture, considering their interactions and the effects of glucose and antibiotic diffusion. It is to be noted that the exact parameters were not calculated for each experiment. This can be done and was done by Sultan for the Km resistant strain through simulated annealing previously. The simulation or for annealing takes a long time to run (over a month) and was therefore out of the scope of this project but it gives good accuracy for the parameters. For this study, we used the same parameter estimates (as shown in the code block above) for consistency. The growth observed in the simulation was similar to what we saw in the experiments. The parameters sometimes did not work for CFP and the simulation would blow up, this is when the parameters would instead be guessed, around similar values. However, this was not always due to the parameters alone. Comparing figures 9 and 10 with 11 and 12, one can notice that there are things happening outside of the region of interest. These are debris, and edges of pads, which can often reflect the fluorescent beams (figure 4) that can interfere with the simulation. Some of the debris and artifacts can be removed through methods such as background subtraction, but it is not effective for bigger debris. Here is an example of before and after background subtraction, experiment shown is a monoculture experiment with the CFP strain. Moreover, in some of the later stages after 18-24 hours we can also see the agar pad shrinking which could also disrupt the simulation, since the overall size of the images becomes smaller. To stop the shrinking process, in an earlier experiment a slightly damp tissue outside a parafilm boundary was used to provide a humid environment but it had no significant effects, but rather caused issues with presence of water.

Another interesting observation was that the YFP fluorescence intensity tended to fade away over time even though initially the intensity increases, whereas the CFP fluorescence intensity tended to get brighter with time which is expected with steady growth. This may be due to differences in the stability of the fluorescent proteins or the differential expression of the genes encoding these proteins but there are no conclusive reasons. This observation may imply that CFP cells

outcompete Cy3 cells, however, this behavior was consistent with Cy3 monocultures, where growth was observed in the phase channel despite fading seen in Cy3 channel.

One interesting finding was the formation of a "coffee ring" pattern, where the bacteria appeared to aggregate around the edges of the growth medium. This is a well-known phenomenon in fluid dynamics and has been observed in various other systems [18]. This is due to capillary pressure forcing the cells outwards as the droplet dries on the agar pad [18]. It is interesting to note that the rate of evaporation can cause different observed patterns, i.e., even though the spread is concentrated at the edges the distribution can be more uniform based on evaporation rate and some other factors as mentioned in Anyfantakis and Baigl (2015) [18]. This was not necessarily tested since it was not the main goal of the project, but we can see difference in distribution from Results 1 and Results 2 or from Figure 1 and Figure 5.

6.0 Conclusion and Improvements

In this study, we developed a mathematical model using partial differential equations to predict the behavior of two strains of E. coli in co-culture. The model was able to effectively capture the spatiotemporal dynamics of bacterial growth, considering their interactions and the effects of glucose and antibiotic diffusion. We observed interesting phenomena such as the formation of the "coffee ring" pattern and differences in fluorescence intensity between the two strains. Overall, our results demonstrate the potential of mathematical modeling to provide insights into bacterial co-cultures and highlight the importance of considering the spatiotemporal dynamics in such systems.

One major limitation with this experiment involves the mastery in the procedure. It can be difficult to flip the pads onto the glass slide, if not done carefully this can cause bubbles to be trapped or sometimes for the circular pattern to be distorted. This causes strange artifacts as seen. This could be improved by more practice and trying to keep gel debris out as much as possible. Or in the case of post-processing, we can consider machine learning, or other image or signal processing techniques to de-noise the images. This would also help in improving the simulations.

Calculating the exact parameters for each experiment using simulated annealing could improve the accuracy of the model predictions. Although this approach takes a long time to run, it could

lead to more reliable and consistent results, rather than trying to guess around the literature value or an arbitrary value.

Furthermore, investigating the kinetics of antibiotic inhibition for both strains could provide valuable insights into the interactions between the two strains. Flow cytometry could be an effective technique to investigate these kinetics and verify the co-culture models. Overall, our study demonstrates the potential of mathematical modeling in providing a deeper understanding of bacterial co-cultures and lays the groundwork for future research in this field.

Acknowledgement

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