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Danny Contreras
Northeastern University

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Investigating Bacterial Growth Rates by Image Analysis

Honors Thesis Project

Danny Contreras

Professor Epstein

May 2, 2006

The growth of bacterial populations is an area of great scientific interest. As a scientist, the documentation of cell growth leads to more developed knowledge regarding the life cycle of bacteria species. Growth models are created to quantitatively represent data measurements. These models are particularly interested in the lag time of growth. During this period, growth proceeds at a relatively slow rate, but at some point during growth, the rate rapidly increases towards a period of exponential growth. By using such models, scientists can apply the data to estimate the time when bacteria will begin to multiply on foodstuffs, for example. The shelf-life of packaged food relies on a bacteria-free environment in order to stay fresh.

It may seem a simple matter to take a large loop of a bacterial sample and observe the bacteria using a microscope. However, this is a fact that is rarely the case. Bacteria are typically only a few micrometers in length and live in almost any environment imaginable. To this date, research has led to the discovery of only one percent of all microbial species! Only one percent of the living microbes on Earth have been viewed and documented by scientists. The main obstacle in the path of documentation is the overwhelming diversity of each bacterial species towards its living requirements. Bacteria rely on a delicate balance of chemicals and nutrients found in their environment, that once removed from this environment, they can no longer survive due to lack of nutrients. The mode of choice to artificially grow bacteria in a lab is via Petri dishes. Petri dishes can be composed of a wide variety of nutrients that are specific to the growth of a particular organism. However, our knowledge of this “cookbook recipe” of the “ingredients of life” can only be applied to the handful on microorganisms currently known. The microbes that we can grow use the nutrients in the Petri dish as a substitute

for their natural environment. Over the past century, microbiology has grown through careful experimentation of different combinations of media that lead to the growth of new microorganisms that previously could not grow on Petri dishes. Although many bacterial species have been accounted for using the Petri dishes, this method is not the solution for growth of microorganisms from a fresh water sample, for example, where the necessary nutrients are just not present in this media.

The paradox of bacteria uncultivability stems from the inadequate media preparations of Petri dishes. Therefore, a novel approach has been created that uses a diffusion chamber. This chamber acts as a simulation of the natural environment for bacteria. The bacteria are able to survive in this chamber as well as continue their growth cycles. The dimensions of the chamber are 1mm x 20mm, which allows for a small amount of space for liquid media to be pored into the well.

Materials and Methods

The measurements of growth are obtained by creating microscopic slides that contain media that closely resemble the natural environment of the organism. By doing so, the growth rate should be similar to the one found in nature. The slides are prepared using an incubation chamber, such as the one shown, that allows for liquid media to be observed on a microscope slide. The imaging chamber simply is placed on a glass slide where approximately 250 µL of liquid LB agar is pored into the well. After twenty minutes have passed, the liquid agar has solidified neatly in the well. During the passing time, a loop of bacteria is placed into approximately 1ml of distilled water, so that the bacterial sample will be diluted when viewed under the microscope. From that water

suspension, 2 μL are pipetted onto the now solidified LB agar. This is no more than a tiny drop, which is important so that the bacteria will have little area to move around once they are placed onto the agar. Ideally, the bacteria will not be able to move, but still grow and divide. If they move too much, then it becomes nearly impossible to track them for an extended amount of time. Once the slides have been prepared, they are looked at through a microscope. Often, a high magnification (1000X) is needed to adequately depict growing cells. It is the job of the investigator to capture digital pictures of growing cells. The digital images are then analyzed to understand the growth rates.

This bacterial experiment used Escherichia coli as the test microorganism. This microorganism is fairly easy to cultivate, which is why it was used to ensure that the methodology was sufficient to capture future images of uncultivable bacteria. The images of the bacteria were taken at 1000x magnification using time-lapse photography taken every two minutes for a total of 116 minutes. Each measurement was done at exactly the same place. There was no movement of the camera whatsoever except for an occasional refocusing of the microscope.

Results

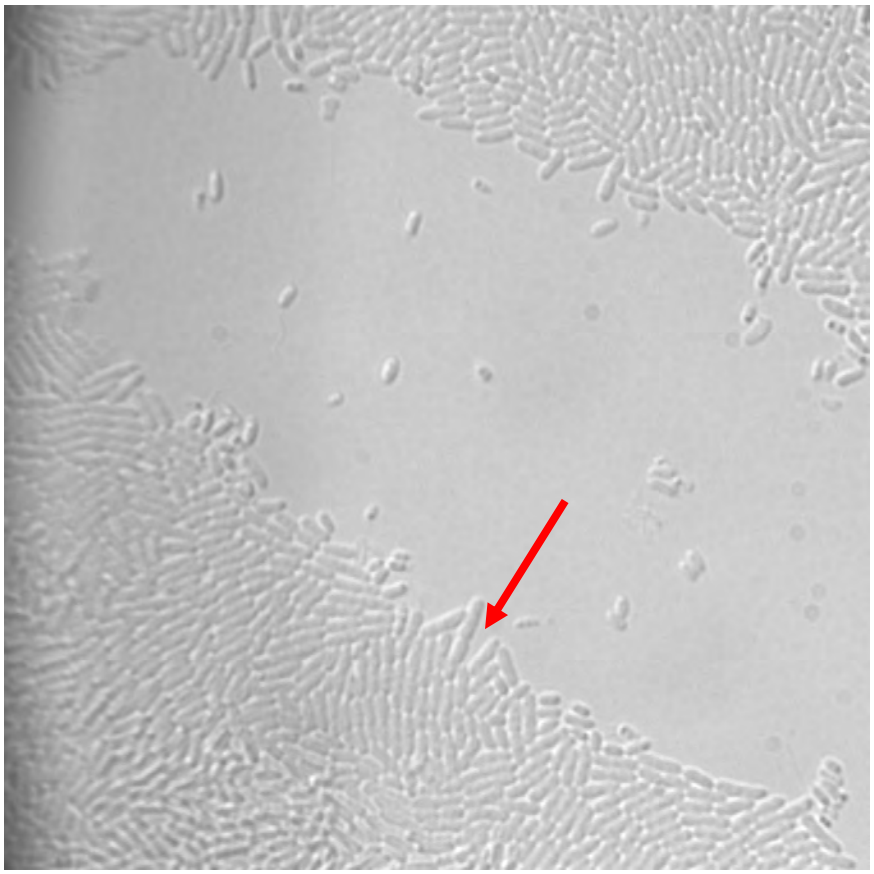

Figure 1: Initial picture at time 0. Red arrow indicates discussed bacteria

This is the first picture taken of a well defined mass of bacterial growth. The lower-left and upper right-hand quadrants contain a vast amount of cell growth, whereas the middle of the picture only contains a few isolated bacteria. Notice the arrows indicating the desired bacterium to be observed.

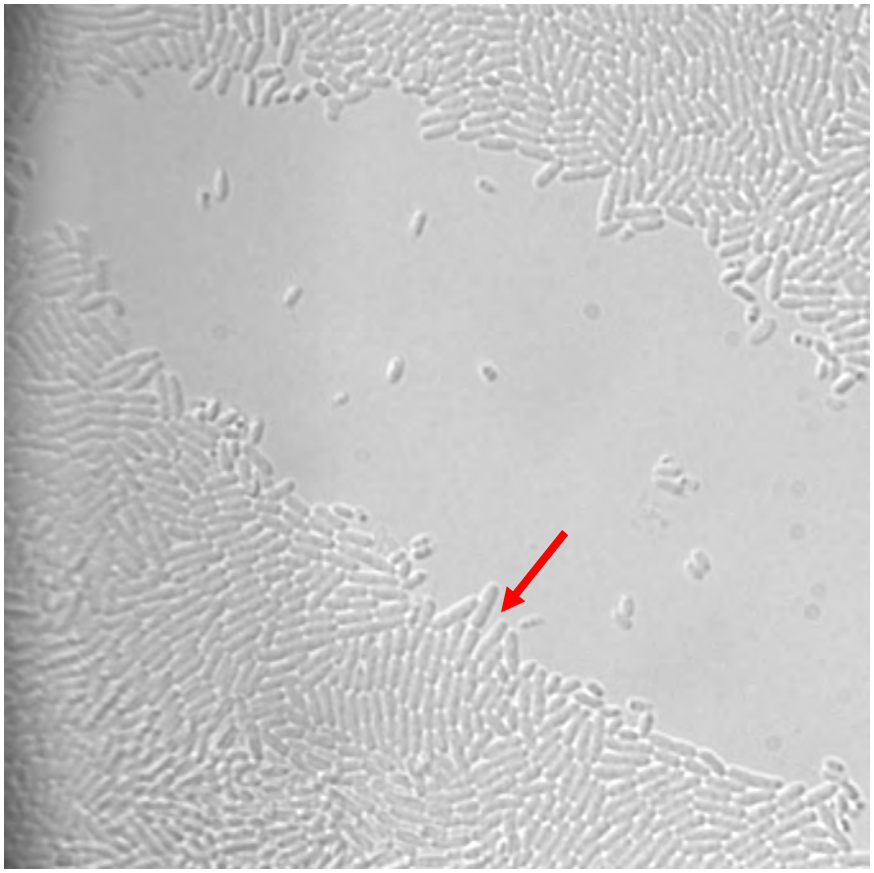

Figure 2: Time 10 minutes. Elongation of cell is noticeable.

This cell has shown visible elongation and has progressed further towards the middle of the picture. Growth of the surrounding bacteria has pushed the entire colony towards the middle.

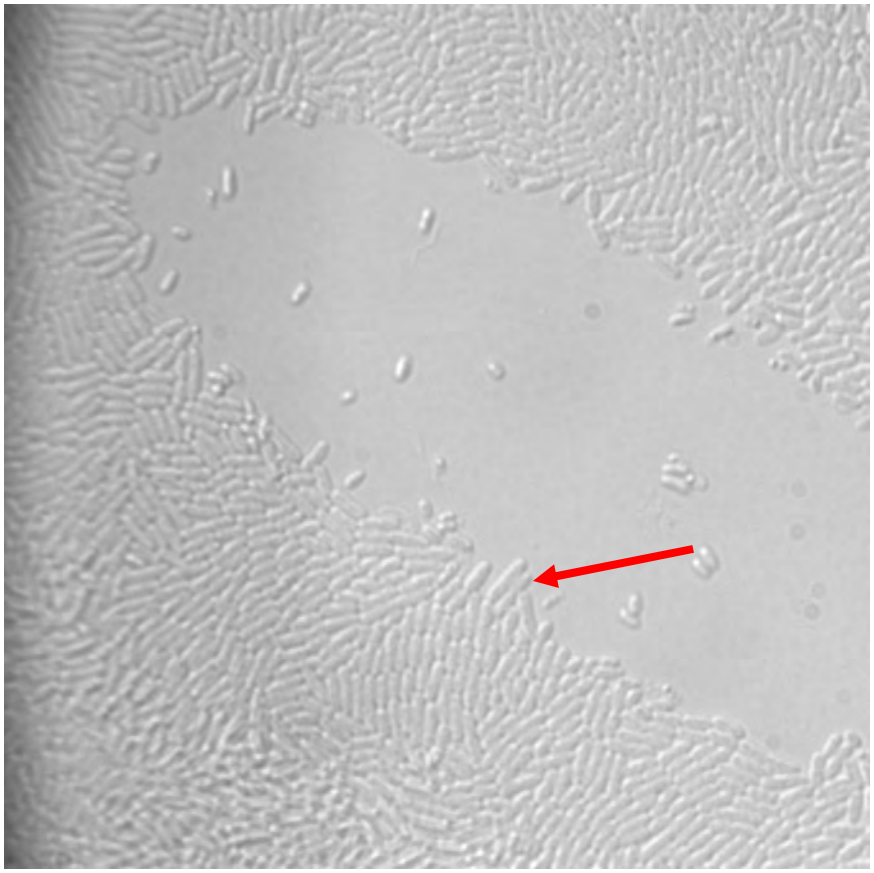

Figure 3: Time 26 minutes

The cell has continued to elongate with continued expansion of the colony. Notice how the solitary cells in the middle of the agar have not shown any growth compared to the rest of the colony.

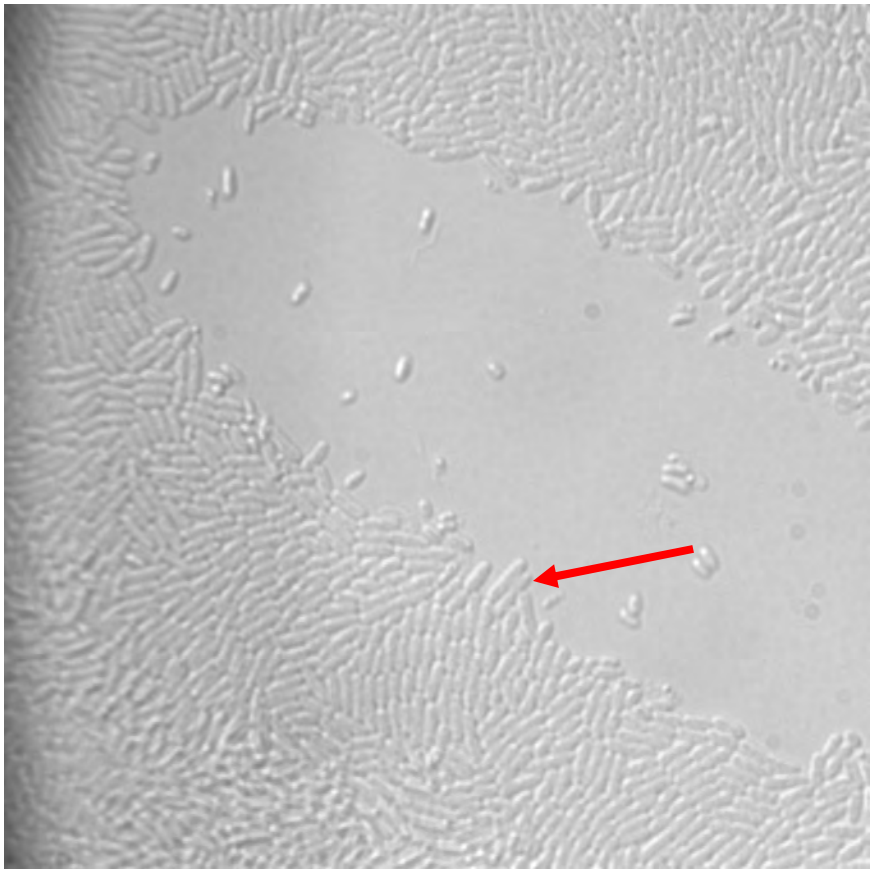

Figure 4: Time 34 minutes

Continue to notice the thinning occurring of the bacterium as it grows. It continues to feed off the agar nutrients and elongate. Also, the total growth of the colony continues to push towards the center of the picture frame. The mass of cells on both sides are growing exponentially fast in order for such a display of cell density.

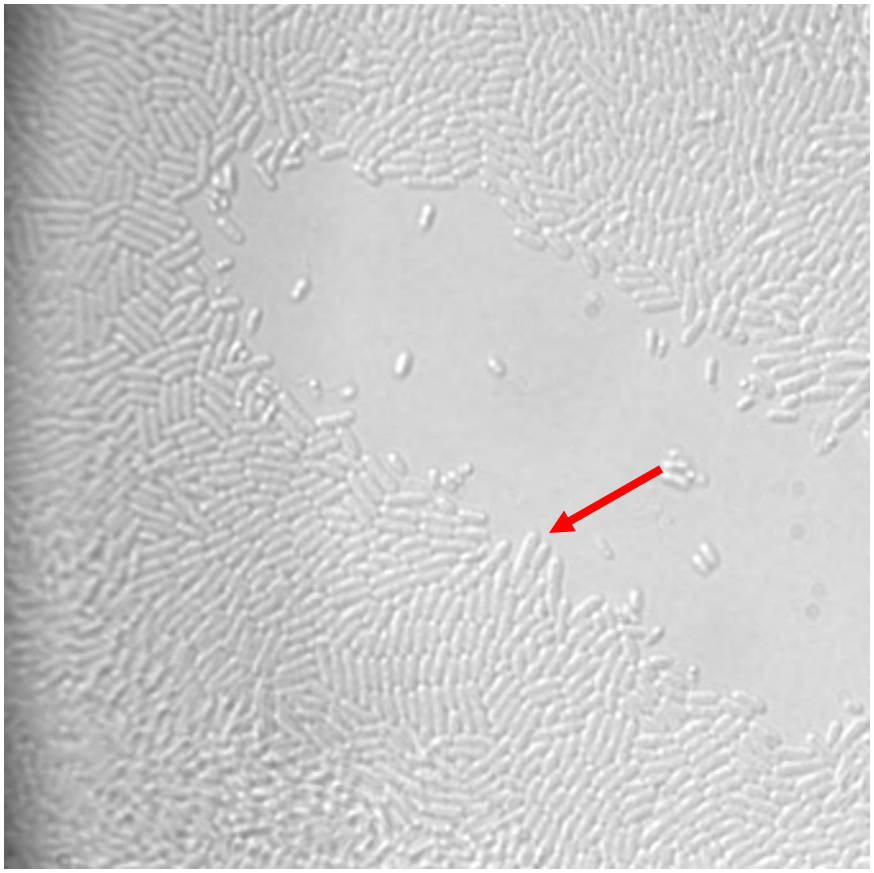

Figure 5: Time 42 minutes

Growth of the observed cell shows definite cleavage in the middle of the elongated cell. This simple delineating line represents the near culmination of the bacterium's growth cycle. The cleavage represents that two new cells have been formed from the original cell. These two cells are identical to the parent cell.

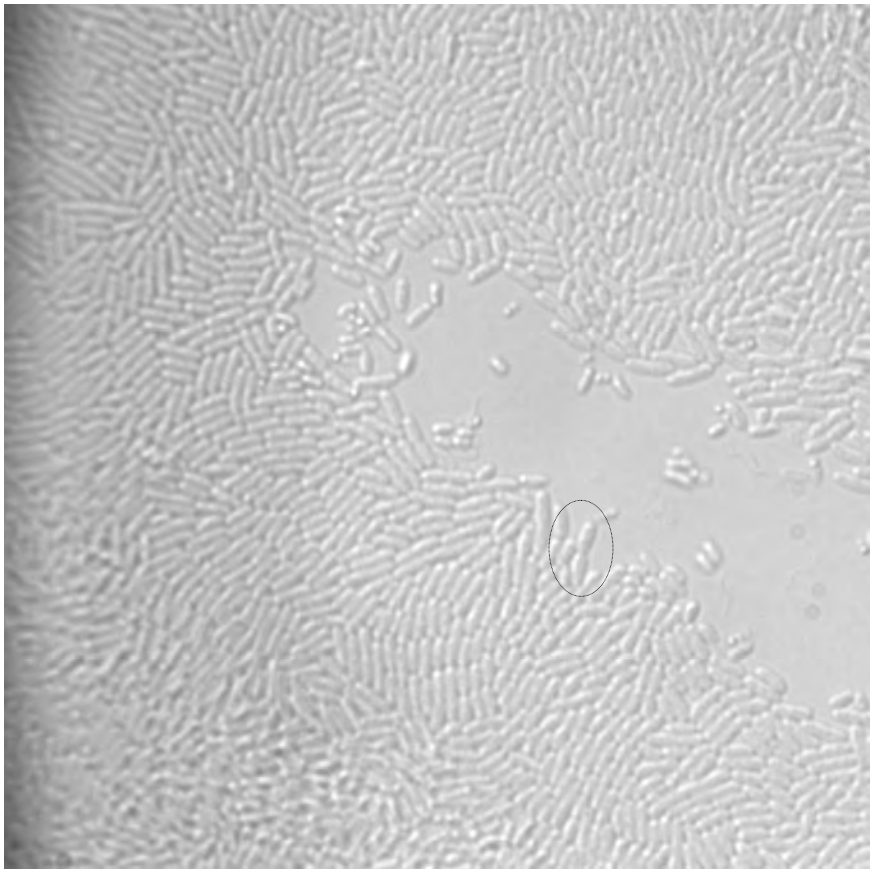

Figure 6: Time 62 minutes. Dashed circle encloses the two new cells.

This is the final picture of the series. Notice how perfectly divided the observed cell has progressed. The two cells now can undergo cellular division of their own.

Discussion

The argument can be made that bacteria will grow and divide in the proper media, however the rate at which division occurs is strongly mediated by the influence of the colony. The lag phase in growth may be attributable to bacteria that are not part of a larger colony and reside with only a few cells. The cells that showed little to no growth in this experiment were in the lag phase of their growth cycle. The exponential phase of growth, however, can occur only when a sufficiently sized colony is present to induce

this growth. The colony provides advantages to the individual bacterium attempting to grow on its own. Through the colony, bacterial communication must occur in some way that indicates the proper time to grow exponentially. Together, the bacteria grow much faster than by themselves as individuals. It seems that the bacteria need the close contact of one another in order to grow at a much faster pace, compared to their counterparts who are left isolated or with one or two other cells. When they are in close proximity, they are able to provide one another additional signaling/cues that tell them that it is safe and appropriate to divide and grow at this exact time point. A valid conclusion for this research is that sufficient growth of *E. coli* is possible only when they exist together as a colony.

Acknowledgements

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