



## **Abstract:**

The goal of our experiment was to see how a temperature above or below the optimum temperature for the growth of *Escherichia coli* (*E. coli*) affected the growth rate. We decided to address this problem by investigating the growth rate at 37 °C, which is body temperature, at 22 °C, which is room temperature, and at a raised temperature, 45 °C. Three identical samples were made by adding 13.5mL of Luria-Bertani (LB) broth and 1.5mL of the overnight culture of bacteria. Each sample was put in three different shakers set at 250 revolutions per minute (rpm) and incubated at the temperatures indicated above. We recorded absorbance readings (in Optical Density) using a spectrophotometer set at 600nm at time zero and every 20 minutes after that. Since the samples became cloudier with the emergence of more bacteria, the growth rate was determined by the readings. The bacteria at room temperature had the lowest reading, experiencing the least growth. The bacteria at 37 °C had OD readings somewhere in between the other two temperatures, and the OD readings for the bacteria at 45 °C were the highest. From our data we were able to conclude that the *E. coli* grows much faster at elevated temperatures and at a lower temperature, the growth rate is much slower.

## **Introduction:**

*E. coli* is a bacterium that lives in the human digestive tract. It has become a model organism in the study of biology because of its simple structure and it is easily grown in the laboratory (Karp, 2005). The purpose of this experiment was to determine the effects of temperature on the growth of *E. coli*. This is related to human health because whenever there is a bacterial infection, a way the body fights the invading organism is to raise the temperature of the body, hence, the person gets a fever. The

effect of this raise will have some effect on the growth of the bacteria, which is the topic of interest. The optimal temperature for the growth of bacteria ranges between 20-40 degrees Celsius. Normal body temperature falls within this range(Todar, online). It was decided to measure the growth pattern at extremely high temperature (45 degrees Celsius), and at a relatively low temperature (room temperature, ~ 22 degrees Celsius). A culture was grown at optimal temperature (37 °C) as a control. The growth rate was determined using a Spectrophotometer, which is an instrument that detects the amount of light that passes through the culture. As bacteria grow, the solution becomes cloudier and less light is able to pass through the liquid, which gives a reading in Optical Density (OD) (Piatelli and Holthaus, 2005). The bacteria are grown in an LB broth which contains all the essential nutrients for growth, amongst which are carbohydrates, amino acids, nucleotide phosphates, salts and vitamins (Lecture 1 slides).

### **Materials and Methods:**

We turned on the spectrophotometer and set the wavelength to 600nm. We inoculated 1.5mL of *E. coli* overnight grown culture and 13.5mL of LB medium in three different vials to give three different samples. Each sample was labeled 45 ° C, 37°C, or 20°C. A cuvette was filled with 5 mL of clear LB broth in order to zero out the spectrophotometer between readings. One mL from each test tube was put in a cuvette and diluted with 4 extra mL of LB broth. The cuvettes were then placed in the spectrophotometer to take an initial reading of the optical density (OD) at the starting time (T0). Each test tube was placed in a shaking incubator corresponding to the temperature on the test tube and the cap was screwed on loosely so the bacteria could

have oxygen to grow. Readings were taken every 20 minutes in the same manner and the test tubes were placed in the incubators in between readings. The entire procedure was carried out using a sterile technique, so any time a sample, beaker, or vial was opened or closed to remove or add content, the top of the container was passed through the flame of a Bunsen burner to heat it and prevent the flow of air into the sample which could lead to contamination and erroneous results. After taking a total of nine readings (including T0), the bacterial cultures were discarded and the work area was cleaned up.

**Results:**

Table I: Optical Density of Bacterial Growth at Varied Temperature at Different Times

<b>Time (in minutes)</b>	<b>Room Temperature: 22°C (OD at 600nm)</b>	<b>Ideal T: 37°C(OD at 600nm)</b>	<b>Elevated T: 45°C (OD at 600nm)</b>
0	0.01	0.011	0.011
20	0.018	0.016	0.025
40	0.025	0.021	0.035
60	0.024	0.03	0.069
80	0.021	0.043	0.091
100	0.029	0.061	0.11
120	0.025	0.075	0.12
140	0.032	0.1	0.145
160	0.035	0.138	0.16

Graph 1: Effects of Temperature Variations on the Growth Rate of *E. Coli*

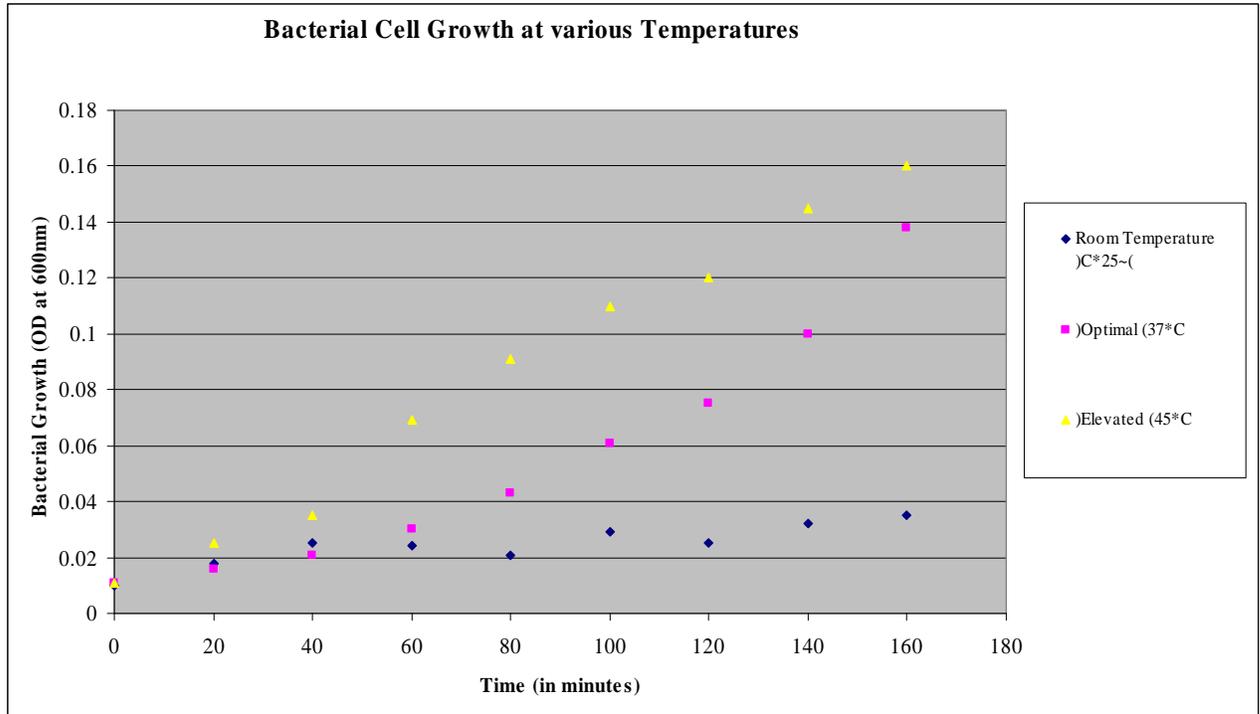


Table 1 shows the numerical results of the experiment. In column one the time readings are shown. The following three columns represent the Optical Density (OD) readings, indicating the amount of light able to pass through samples of each of the three cultures from the three different temperatures, and thus the amount of bacteria present in each division. At room temperature, the initial OD reading was very low (0.01). At each 20 minute interval the culture showed slight growth because each reading increased moderately. The different readings were 0.018, 0.025, 0.024, 0.021, 0.029, 0.025, 0.032, and 0.035. At times the points showed a decrease in absorbance, probably due to experimental error at the time of taking the readings (perhaps the cuvettes were not wiped properly). At optimal temperature, the initial OD reading was also 0.01, but as time

progressed, the additional readings increased at a faster rate than the room temperature readings did. The different absorbance readings at successive times were 0.016, 0.021, 0.030, 0.043, 0.061, 0.075, 0.100, and 0.138. The initial OD reading for the elevated temperature culture was 0.011. The other readings were 0.025, 0.035, 0.069, 0.110, 0.120, 0.145, and 0.160. This culture displayed the most growth out of the three cultures. The comparison of growth rates can clearly be seen in Graph 1.

### **Discussion:**

We predicted that at an elevated temperature the bacteria would grow at a faster rate, reach capacity and start dying off sooner; and if bacteria were incubated at a lower temperature than optimal, then the bacteria will grow at a slower rate, reach capacity and start dying off later than the control.

It can be seen in graph 1 that the growth rate at room temperature was indeed much lower than the rate at optimal temperature. In fact, at some intervals there was no growth at all. At the optimal temperature the growth displayed was higher than that of the lower temperature but was not as great as the growth displayed by the culture incubated at the elevated temperature, thus proving our hypothesis. This shows that *E. coli* has the capacity to grow in a wide range of temperatures, but it prefers warmer environments. This was further proven by the growth displayed by the culture incubated at 45°C. The data shows this culture reached exponential growth faster than the 37°C culture. It is possible that the bacteria were able to divide faster at higher temperatures because the rate of chemical reactions is directly proportional to temperature, thus, the higher the temperature, the faster the chemical reactions in the cell can be carried out. The same

holds true for lower temperatures: the lower the temperature, the slower chemical reactions are carried out, thus retarding the division rate of bacteria.

We were not able to run the experiment long enough in order to see the death phase of the cultures. Therefore, we were not able to determine if the culture incubated at an elevated temperature reached the death phase before the culture at optimal temperature did.

### **Conclusions:**

The purpose of this experiment was to determine how different temperatures of incubation would affect the growth rate of *E. coli*. There were three different cultures incubated at three different temperatures which displayed different growth rates. The bacteria incubated at room temperature (~22°C) which was lower than body temperature, where *E. coli* is known to grow moderately, displayed a much lower growth rate than the bacteria incubated at optimal body temperature (37°C). On the other hand, the bacteria incubated at an elevated temperature (45°C) displayed a much higher growth rate. In further research it would be interesting to investigate at what temperature *E. coli* will still display growth by growing cultures at even higher temperatures. It would also be interesting to see how smaller temperature variations would affect the growth. It would also be good to run the experiment long enough until the lag phase and death rate are exhibited in order to compare if a faster growth rate leads to a premature death phase.

**References:**

Karp, Gerald. 2005. Introduction to the Study of Cell Biology. In: Fitzgerald, Patrick, Osnato, Geraldine, Russiello B, editors. *Cell and Molecular Biology*. 4<sup>th</sup> Edition. Hoboken, New Jersey John Wiley and Sons, Inc; 2005: 16-17.

Piatelli, M, Holthaus, K. *Molecular Dell Biology Laboratory: investigating the Growth Phases of Bacteria*. 2005: pp. 19-39, 75-76.

<http://textbookofbacteriology.net/nutgro.html>

<http://webct.bc.edu:8900/BI310012006F/Bacteriagrowth-primer.ppt>