The effect of temperature on the growth of the bacteria *Escherichia coli DH5α*

Minh Thanh Nguyen, Saint Martin's University, 5300 Pacific Avenue SE, Lacey, WA 98503. This work was supported by Saint Martin's University.

Abstract

DH5α is specific strain of *E. coli* that is a nonpathogenic bacterium belonging to the genus *Escherichia*. I used this type of bacteria to study comparative growth rates at different temperatures (28°C, 37°C, and 45°C) and for different times (24h, 48h, and 72h). I predicted that bacteria would grow best at 37°C compared to the other temperatures, because the mean temperature of many mammals is 37°C. Mammals often serve as a host to *E. coli.* To compare growth rates, I grew the bacteria in liquid nutrient broth cultures and on nutrient agar in Petri dishes. I used the spectrophotometer to measure the optical density of bacteria growth in the liquid media and counted colonies in the Petri dishes. The number of colonies on Petri dishes did not show a statistically significant difference among the three temperatures. However, in liquid media, the bacteria grew significantly better at 37°C for the three time periods ($F = 0.06$, d.f. = 2, $P = 0.565$), compared to the other temperatures, supporting my hypothesis.

Introduction

Bacteria are one of the major domains biologists have studied for many years. They are microorganisms that we are not able to see with our naked eyes. In order to seem them, we have to use microscopes. Biologists have studied and learned about the behavior of bacteria in their populations and environmental conditions and how they are able to adapt. Even today, we are still not able to completely understand all kinds of bacteria and the ways they interact in different conditions.

We can find bacteria everywhere, in any environment that surrounds us. Bacteria are also found inside humans and many big mammals, forming symbiotic relationships. Besides the environments surrounding the human body, bacteria are found in extreme conditions such as in hot streams of water of volcanoes or in deep caves with no oxygen or sunlight. We know that bacteria can be found in two opposite environments: aerobic and anaerobic. There is a theory that bacteria from outer space were carried by asteroids that hit the earth a few million years ago. Today, in exploring comets and outer space to determine if there is life outside earth, scientists look for bacteria and other microorganisms as evidence of living organisms.

Bacteria can live in any environment as long as they have food as an energy source. Normally, they grow on organic materials and break down these materials. Besides the photosynthetic type, some bacteria use chemosynthesis. Chemosynthetic bacteria need only inorganic materials for living and do not need to have a sunlight source. Sugars are the main fuel for living organisms, but water is also needed, because all living things on earth are water-based.

Bacteria live by breaking down organic materials, so they naturally recycle the earth. The recycling of natural materials on earth is an example of the ecological role played by bacteria that break down organic materials. Bacteria also invade all

environments, including the human body, which can lead to infection and even death. Increasingly, infections are caused by bacteria that have evolved antibiotic resistance.

The complex environment of bacteria can range from relatively simple surroundings, like humans, to extreme conditions such as no photosynthesis and hot volcanic water. Besides their environments that allow them to survive well, living and growing in symbiotic relationships, they are also able to survive well and grow alone. However, there is a range of temperatures in which *E. coli* are able to survive and grow well. I examined the growth rate of *E. coli* strain *DH5α* in several temperatures. I predicted that they would grow well in 37°C compared to other temperatures, because the mean temperature of many mammals is 37°C. Mammals often serve as a host to *E. coli*.

To help identify differences among bacteria, there are three different shapes: rod (bacilius), cocci (spherical), and helical. Besides the shape that can be identified, there are many characteristics that describe bacteria type. Gram stain is one major characteristic. Gram negative bacteria contain a cell wall under a lipopolysaccharide layer. A cell wall exists in bacteria and other living species, including fungal and plant cells. Gram-positive bacteria do not have cells walls under lipopolysaccharide layers compared to gram-negative bacteria. To be able to test whether a bacterium is gram-positive or gram-negative, Gram staining is used with crystal violet dye and ethyl alcohol (Frederick *et al.,* 2003). Gram-positive bacteria appear violet under staining, indicating a single layer with resistance to the dye. Drug manufacturer share developed antibiotics to break cell walls. Examples are penicillin and cycloserine, which interfere with bacterial metabolism, break the cell wall, and kill the bacteria (Frederick *et al.,* 2003).

Some gram-negative bacteria are beneficial for the environment. Many gram-negative bacteria live on plants or in the soil, protecting plants from other invasive bacteria and fixing nitrogen for plants. Gram-negative bacteria are often harmful to humans, causing infections. They also cause wilting or even crown gall tumors in many plants (Strauss, 1999). This information suggested that I should not choose gram-negative bacteria because of health concerns for myself while conducting the study and for people around the research area.

Researchers have established and learned to use bacteria to clean up the environment for ecology. In the United States, many companies use bioengineering to control bacteria for bioprocesses to clean up the waste from factories. The beneficial clean up of the wastes can be through their break down and consumption by *E. coli* (Lindow *et al.,* 2005).

E. coli bacteria grow on broken down material like all bacteria do, but their environments can be different, with conditions not as extreme as hot steam waters of volcanoes. They would be found on the surfaces of many things that have been contaminated. Unfortunately, many types of *E. coli* have been found in improperly cooked meat like in hamburgers, which lead to harmful *E. coli* infections that hospitalize many people. *E. coli* are not only present in uncooked meat; they are also in the environment. They can be found in contaminated water and in people who consume shellfish from the contaminated water. When people eat shellfish or drink water that has *E. coli* present in it, this can lead to illness or even to death (Mallin *et al.,* 2000).

E. coli not only lives in symbiotic relationships with plants and many mammals, but also lives in symbiotic

relationships with other microorganisms. In the complex environment related to nitrates that support of food sources, they have to have live with other species that produce nitrates. The normally found *E. coli* can exist with algae in water due to high concentrations that result from a problem on a beach, lake, or shoreline. Closer to the beach, lake, or shoreline, there are not only high concentrations of *E. coli* and algae, but high levels of *E. coli* contaminated sand that would harm humans who have direct contacted with it (Byappanahalli *et al.,* 2003).

Another study showed that *E. coli* growth was directly proportional to the algal growth in lake water and sand. They also show that the best temperature was for *E. coli* growth was between 25° C and 35° C, and those temperatures correspond to the temperatures near the shore of a lake during the warm summer months (Byappanahalli *et al.,* 2003). We want to understood how *E. coli* grow with other living species and how they can be beneficial to each other when they were share an environment. We also want to isolate them to see how well they grow alone.

Humans have the potential to be infected by *E. coli* alone, but are more likely to be infected in combination with other microorganisms. Some human diseases are the result of a mixture of *E. coli* and other microbial flora. Besides the *E. coli* existence with non-pathogenic bacteria, they also exist with pathogenic organisms like yeast and other microbes. In many clinical studies, animal results show that infections by *E. coli* alone or with a virus were not severe. However, an infection can result in really severe lesions or even death when the *E. coli* are enhanced by other microorganisms. To cause disease in human, *E. coli* first has to be able to infect the mucous surface. Then, they have to penetrate into tissue and divide to increase their population. Then, in a host with high populations that weaken defense mechanisms, damage can be caused to the human (Smith, 1982).

E. coli located in the tropics can cause bladder infections, bronchopneumonia, common cold in children, and lower respiratory infections. In many clinical studies, it had been showed that the mortality was forty percent when some strains of *E. coli* were present in the bloodstream instead of infecting the surface of human skin. In the other example, influenza alone was rarely lethal, but secondary bacterial infections with simple *E. coli* made situations worse and could cause death (Smith, 1982).

E. coli, regardless of the strain, is a popular bacteria to use for research in many laboratories. Many experiments have been conducted and, the population of *E. coli* has always become better adapted due to the temperatures to which they are exposed. The temperature range is the most important physical factor for a scientist to determine for a species, as their fundamental niche, their growth, and their life expectancy are specific for the species. However, temperatures near the upper limit can lead to increased mutations for adaptation and an increasing population (Cooper *et al.,* 2001).

Exposure to the upper thermal limit can cause rapid death in the population because of changes of niches. The survivors of the upper limit gradually adapt to the temperature by increasing their thermal optimum, so that they can live within the upper thermal limit. This process is called a sliding niche and allows better adaptation to the temperatures in their environments. *E. coli* are able to change their genetic information in order to grow at their maximum rate while experiencing a constant temperature, as long as nutrition is available to allow them to grow. The population shows a consistent increase in maximum

growth, which indicates the occurred of mutations. I predicted that *DHα5* would have the highest growth at 37°C compared to the other temperatures, because the mean internal temperature of many mammals is 37°C (Copper *et al., 1999).*

Methods

Cultivation in Petri dishes

Method I I used gloves to protect myself from touching the bacteria, and then used an inoculation loop to transfer the bacteria from the stock tube to grow cultures in additional tubes. The loop was heated until it was red-hot by flaming, and then I let the loop cool down completely. I didn't touch the loop, because it would have been contaminated. I shook the tube of bacteria, removed the lid, and then flamed the mouth of the tube. A loopful of organisms were removed from the tube. After the loop had been removed from the culture, the mouth of the tube was re-flamed. After re-flaming the tube, I let it cool and closed the tube of bacteria to make sure they didn't tip over or spill. I spread the bacteria on the surface of the Petri dish gently and was careful not to puncture the gel. I spread bacteria starting at the edge of the Petri dish with the loopful of bacteria and then moved to the center of the dish. Then, I rotated the dish 180 degrees and spread again in another direction to cover the entire distance from one edge to another for one sample.

I repeated these streaking steps for more samples. I put the samples into the temperature I decided upon, and then I let them grown. I measured the growth after twenty-four, forty-eight, and seventy-two hours by putting the Petri dishes on the Quebec counter and using the mechanical hand counter to count the number of colonies. (Brown, 1998).

Method II Each tube contained twenty ml of agar that had been sterilized in the autoclave then and then cooled to below fifty degrees Celsius. I injected one loopful of bacteria from the stock into each tube. I shook each tube a few times and poured it into a Petri dish. I put the samples into the temperature I decided upon, and I let them grow. I measured the growth after twenty-four, forty-eight, and seventy-two hours by putting the dish on the Quebec counter and using the mechanical hand counter to count the number of colonies. I used this method to prevent bacteria from growing into a lawn, because the first method resulted in lawns whose colonies I was unable to count (Brown, 1998).

Cultivation in test tubes

I used thirty test-tubes for this experiment: ten test tubes per temperature, and a total of three temperatures. All the test tubes had been sterilized using an autoclave (Barnstead 14-48823). Each test tube contained four ml of nutrient broth (NB) (3g/L Beef Extract; 5g/L Bio-Gel Peptone; pH 6.8), then each received one loopful of bacteria from the stock bacteria, *DH5α.* I set the rack of tubes in the appropriate temperature (28°C, 73°C, and 45°C), let the samples grow, and then I marked the numbers on the specific tubes. I measured the optical density of the samples after twenty-four, forty-eight, and seventy-two hours by using the spectrophotometer (SPECTRONIC 20s,$^{+}$ Bausch and Lomb). I used nutrient broth to blank the spectrophotometer and recorded the absorbance of each sample at 686nm (Benson, 2005).

I analyzed my results using the Statistical Software Minitab (CRC Computer Lab Mini Tab program). I compared results using ANOVA and Tukey tests with α value of 0.05.

Results

A one–way Analysis of Variance (ANOVA) of *DH5α* bacteria grown in NB liquid medium for a periods of 24hrs, 48hrs, and 72hrs at a temperature of 28°C (Table 1) resulted in a P-value <0.0001, indicating a statistically significant difference in growth after the three time periods. A Tukey test of these 3 time periods found a statically significant difference between growth for 24hrs compared to 48hrs and 72hrs, but no difference between growth for 48hrs and 72hrs (individual confidence interval = 98.06%).

A one-way ANOVA of *DH5α* grown in NB liquid medium for 48hrs at 28°C compared to 37°C, and 48°C is described in Table 2. The result of P< 0.001 shows a statistically significant difference in growth at the three temperatures. A Tukey test of these three temperatures found statistically significant difference between 48hrs of growth at 28°C and 37°C and between 37°C and 45°C, but no difference between 48hrs of growth at 28°C and 45°C (individual confidence interval = 98.07%).

The results from a one-way ANOVA of bacteria growth in LB liquid medium at 72hrs at 28°C, 37°C, and 45°C is presented in Table 3. The result of P < 0.0001 shows a statistically significant difference among growth at the 3 temperatures. A Tukey test of growth at these three temperatures found a statistically significant between 72hrs of growth at 28°C and 37°C and between 72hrs of growth at 37°C and 45°C (individual confidence interval = 98.07%). No difference was found between 72hrs of growth at 28°C and 45°C.

Results from a one-way ANOVA comparing growth after 24hrs at 28°C, 37°C, and 45°C are show in Table 4. The result of P < 0.0001 shows a statistically significant difference among growth at the three temperatures after 24hrs. A Tukey test found these three temperatures found statistically significant difference between growth at 28°C and 37°C and between 37°C and 45°C (individual confidence level = 98.06%). No difference was found between 24hrs of growth at 28°C and 45°C.

The results of a one-way ANOVA of bacteria *DH5α* grown in nutrient agar at all periods of times were compared in table 5. Results P < 0.565 in Pool Standard Deviation were equal to 7.031, with Tukey 95% simultaneous confidence interval and an individual confidence level of 97.94%.

On the agar Petri dishes the bacteria growth was not significant according to the One-way ANOVA. The P-value was not accepted therefore was not effective result of the data to be graph or further analyze. Figure 1 depicts *E. coli* grown in NB liquid medium at different times and temperatures. Growth after 24hrs at 37°C bacteria showed the highest absorbance and 45°C had a higher absorbance than 28°C. At 48 hrs, bacteria grown at 37°C showed more growth than 28°C and 45°C. Among the 24hrs, 48hrs, and 72hrs, the 72hrs bacteria had the highest absorbance in all temperatures. The 45°C was the second highest absorbance in all temperatures. The least absorbance was bacteria growth at 28°C (Figure 1). However, the graph also shows that at thirty-seven degrees temperatures, the absorbance of the forty-eight and seventy-two hours are the same. The graph also shows that at seventy-two hours the twenty-eight and forty-five degrees were the same.

Table 1. The results comparing *E. coli* growth after 24 hours, 48 hours, and 72 hours in 28°C in NB liquid medium, descriptive statistics, and one-way ANOVA test in Minitab.

Variable	**N**	**Mean**	**StDev**		
24h-28 ºC	30	0.0713	0.0548		
48h-28 ºC	30	0.2673	0.1573		
72h-28 ºC	30	0.2687	0.0913		
Source	**DF**	**SS**	**MS**	**F**	**P**
Factor	2	0.7736	0.3868	32.16	0.0000
Error	87	1.0463	0.0120		
Total	89	1.8199			

Table 2. The results of *E. coli* growth in NB liquid medium at 28°C compared with 37°C and 45°C for 48 hours Descriptive statistics and One-way ANOVA test in Minitab.

Variable	**N**	**Mean**	**StDev**		
48h-28º C	30	0.2673	0.1573		
48h-37º C	30	0.4897	0.2350		
48h-45º C	20	0.1563	0.1057		
Source	**DF**	**SS**	**MS**	**F**	**P**
Factor	2	1.0264	0.5132	18.94	0.0000
Error	77	2.0868	0.0271		
Total	79	3.1132			

Table 3. The results of *E. coli DH5α* growth in NB liquid medium at three different temperatures over 72 hours Descriptive statistic and One-way ANOVA in Minitab.

Variable	**N**	**Mean**	**StDev**		
72h-28º C	30	0.2687	0.0913		
72h-37º C	30	0.4897	0.2350		
72h-45º C	20	0.2715	0.1857		
Source	**DF**	**SS**	**MS**	**F**	**P**
Factor	2	0.9065	0.4532	13.97	0.0000
Error	77	2.4985	0.0324		
Total	79	3.4050			

Table 4. One-way ANOVA results of *E. coli DH5α* growth after 24 hours at 28 ºC, 37 ºC, and 45 ºC.

Source	**DF**	**SS**	**MS**	**F**	**P**
Factor	2	1.2130	0.6065	50.12	0.0000
Error	87	1.0527	0.0121		
Total	89	2.2658			

Table 5. One-way ANOVA results of *E. coli DH5α* growth in the Petri dishes compared at 37°C for all times: 24 hours, 48 hours, and 72 hours.

Source	**DF**	**SS**	**MS**	**F**	**P**
Factor	2	95.2	29.6	0.06	0.565
Error	12	593.2	49.4		
Total	14	652.4			

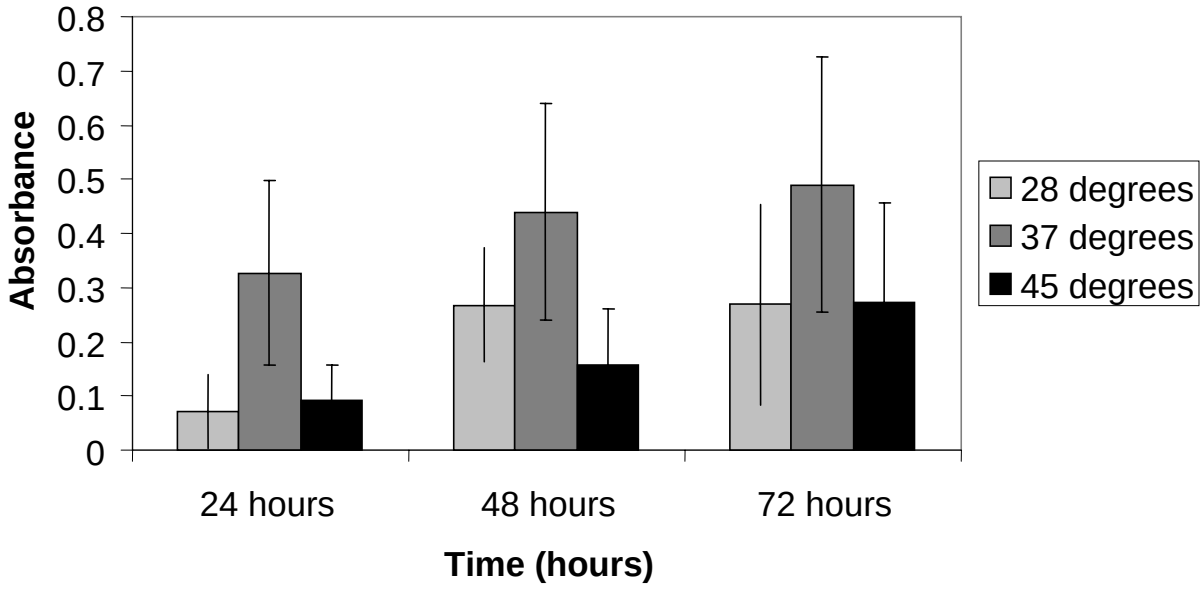

Figure 3. A comparison of E.coli growth at different times and temperatures. Absorbance was recorded after 24, 48, and 72 hours of growth at 28º C, 37º C and 45º C. Shown are the averages for 30 samples each with standard deviation error bars.

Discussion

I expected to find that the bacteria *E. coli DH5α* would grow fast and well under certain conditions. This occurred in the experiment as predicted by my hypothesis. The reason I predicted the bacteria would grow fastest at 37° C was because it was the best condition available. The best conditions available would be in an environment with a great amount of nutrients, moisture, and a warm temperature. In comparing between liquid media and solid media, bacteria grew better in the liquid media. The results showed that in Petri dishes at 45° C and at 37° C, lawn growth occurred. Therefore, I cannot tell which one had better growth by merely counting the colonies. On the 28° C Petri dish, there were no colonies, indicating that the bacteria did not grow well on solid media at this temperature.

In this experiment, I used *E. coli* strain *DH5α*. I am curious whether the entire genus of *Escherichia* would behave similarly to *DH5α*. The (28° C) temperatures I chose for this specific experiment that was not applied to the human beings average. If individual human body temperature under this temperature condition whether or not infected with the bacteria, the myocardium of the human heart has already ceased function at this specific temperature, and is therefore pathogenically irrelevant. At 37° C, I think the immune system would interfere with the growth rate of bacteria. The rate of the growth would be different between bacteria in the lab experiment and the growth rate in the circulatory system because the immune system would be affecting the growth rate of bacteria. At 45° C, cells have long since stopped functioning, with major organ failure occurring at much lower temperatures, making this temperature pathogenically irrelevant.

All the temperatures under which I have studied the growth of bacteria could happen in the lab environment or in your house. The average room temperature is about 20° C. However, an incubator for the 20° C was not available in this experiment so I used the 28° C incubator. Close to your stove, it could warm up 45° C, and 37° C could be in the hot humid temperature of a room in the hot summer time. Yes there were the results that well support my hypothesis that I was predicted. It was important that the bacteria would well fast grow inside human body at 37° C if there was no immune system interferes when they present inside of human body, they could grow at this rate.

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