Abstract

This experiment examined how different concentrations of ampicillin affected the growth rate of *E. coli* in culture. The growth rate was measured by optical density readings taken using a spectrophotometer. The culture with the highest concentration of ampicillin had the lowest growth rate while the culture with the smallest concentration of ampicillin had the greatest growth rate. The growth rates increased as the ampicillin concentrations decreased from 10x to 1x to 0.5x to 0.1x to a control of 0x. These results show that the more ampicillin that is present in a culture, the less the bacteria will reproduce because the cell walls are less likely to form due to disruptions in peptidoglycan production.

Introduction

Ampicillin kills bacteria by disrupting the formation of the cell wall by preventing the formation of peptidoglycan. If a cell wall cannot form, then the cell will be vulnerable and it ruptures (1). Bacterial growth is divided into four phases: lag phase, exponential phase, stationary phase, and death phase. The lag phase is when the bacteria acclimate themselves to their new environment; in this investigation this is the LB solution. The exponential phase is when the bacteria undergo rapid reproduction and the culture is in its healthiest state. The stationary phase is when the death rate equals the growth rate, nutrients are low, and waste product levels are high. Finally, the death phase is when the death rate is greater than the growth rate and all nutrients are exhausted (2).

This investigation sought to discover how different concentrations of ampicillin would affect the growth rate of *E. coli*. Hypothetically, the higher the concentration of ampicillin the lower the growth rate will be. Therefore, the culture with an ampicillin concentration of 10x

should have a much smaller growth rate than will the culture which has an ampicillin concentration of 0.1x, or the culture with no ampicillin, the control.

This experiment intends to find if, after a certain concentration, ampicillin has no further inhibitory effect on the growth rate of bacteria. Therefore, it would not be beneficial to administer medicinal doses of higher concentrations. The lower dosage would be more cost-effective and could eliminate unnecessary interactions ampicillin may have with other bodily functions which could lead to allergic reactions.

Materials and Methods

Five conical tubes with 12.5 mL of LB solution were inoculated with 1.5 mL of *E. coli*. Five mL of each were transferred to cuvettes and a T_0 reading was taken using a spectrophotometer set at 600 nm. The 5 mL in the cuvettes were then transferred back to the conical tubes, which were then placed in a 37°C incubator on a shaker table set at 250 rpm for 45 minutes. After an initial reading was taken, ampicillin was added to make the following concentrations: 0x (control), 0.1x, 0.5x, 1x, and 10x (where 1000x = 50 mg/mL). The cultures were returned to the incubator and every 20 minutes a spectrophotometer reading was taken until 145 minutes had passed, then a reading was taken after a 15 minute interval (3, Pg. 54-55).

Results

Test Tube	Ampicillin added (in microns)	Concentration
A	none	0
B	1.5	0.1x
C	7.5	0.5x
D	15	1x
E	150	10x

Table 1: Shows the concentration of ampicillin in each test tube.

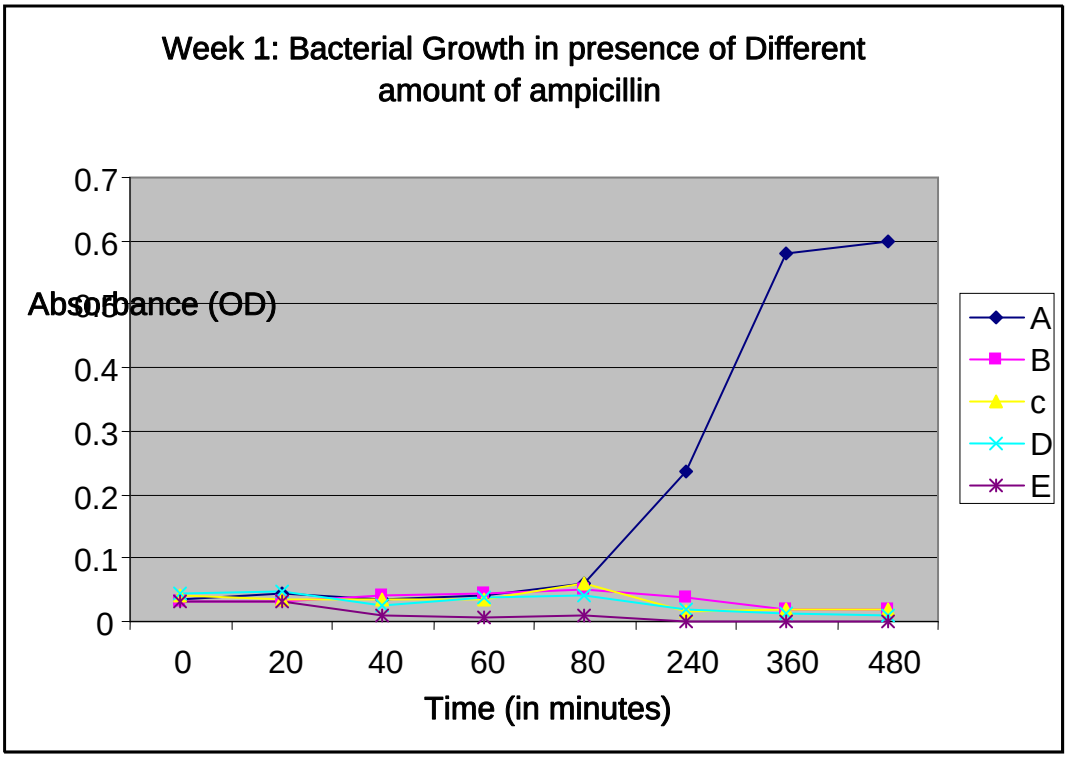
It was determined that by added a certain amount of ampicillin to the constant volume of the agar/e. coli mixture, certain concentrations would arise that were reasonably different in order to test and see significant fluctuations in the results.

Week 1 Results:

Due to certain experimental errors that occurred during the procedure since the pupils were not too sure of what to expect, the results were not very constant and showed certain undesired fluctuation. The readings were taken around every 20 minutes and then 2 hours, 4 hours and 6 hours after the lab period was over.

Time reading(in mins) / test tube		A(control)	B(0.1x)	C (0.5x)	D (1x)	E (10x)
T0	0	0.035	0.03	0.04	0.045	0.033
T1	20	0.045	0.032	0.035	0.048	0.03
T2	40	0.035	0.04	0.035	0.025	0.01
T3	60	0.042	0.045	0.035	0.038	0.005
T4	80	0.06	0.052	0.059	0.04	0.01
T5	240	0.235	0.039	0.015	0.02	0
T6	360	0.58	0.02	0.02	0.012	0
T7	480	0.6	0.02	0.02	0.01	0

Table 2 shows the absorbance of the different concentration of ampicillin at different times.



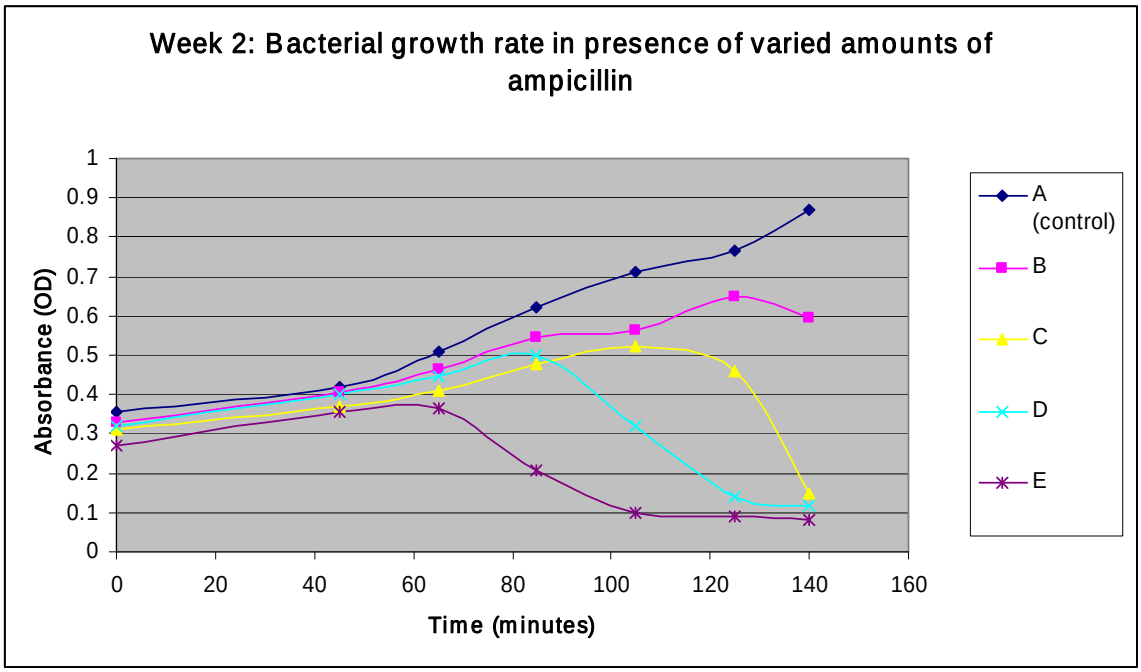
In the first week A exhibited normal growth while all the other cultures showed little to no growth.

Week 2 Results:

Time reading	Time (in mins) /test tube	A(control)	B(0.1x)	C (0.5x)	D (1x)	E (10x)
T0	0	0.357	0.33	0.312	0.32	0.271
T1	45	0.42	0.406	0.368	0.402	0.355
T2	65	0.508	0.466	0.412	0.444	0.365
T3	85	0.622	0.546	0.476	0.5	0.209
T4	105	0.71	0.562	0.522	0.322	0.1
T5	125	0.764	0.65	0.46	0.141	0.088
T6	140	0.87	0.596	0.147	0.119	0.079

Week 2 results (note: since the bacteria exhibited cell death before the lab was over, the students did not see the need to return after the lab period was over to take additional readings).

Table 2 above shows the optical density of five bacterial cultures containing varying amounts of ampicillin.



The control, the culture with no ampicillin, showed an increase of its optical density from 0.357 to 0.940, a total increase of 0.583. The 0.1x culture increased from 0.330 to 0.650, a 0.320 increase, and then it declined by 0.405 to 0.245. The 0.5x culture increased by 0.210 from 0.312 to 0.522 before dropping by 0.417 to 0.105. The 1x culture began at 0.320, went up to 0.500 with an increase of 0.180, and then it fell by 0.411 to 0.099. The 10x culture showed the

smallest increase, rising from 0.271 to just 0.365 before falling back down to 0.072, a total increase of just 0.094 and a decrease from its peak of 0.293.

The control showed the greatest increase in OD while the culture with 10x ampicillin showed the least. All cultures showed an initial upward trend in optical density, although every culture except for the control eventually began a downward trend. Figure 1 shows the growth rates in terms of the optical density of each of the five cultures. The lag phase is seen between 0 minutes and 45 minutes, and then the exponential phase begins for nearly all the cultures. The 10x culture appears to immediately be in the stationary phase after the lag phase and it reaches the death phase at only 65 minutes. Each culture successively enters the death phase, with 1x at 85 minutes, 0.5x at 105 minutes, and 0.1x at 125 minutes. The control never enters the death phase as the experiment did not last long enough for the nutrients to become exhausted and there is no ampicillin to affect its growth rate.

Discussion

This investigation sought to discover how different concentrations of ampicillin would affect the growth rate of *E. coli* bacteria. Theoretically, the higher the concentration of ampicillin, the lower the growth rate should be. Consequently, the culture with no ampicillin should have a higher growth rate than the any other culture with ampicillin. According to the data, all concentrations of ampicillin showed some inhibition of *E. coli* growth. The higher concentrations of ampicillin did indeed greatly lower the growth rates more quickly than lower concentrations did.

The control culture where ampicillin was absent showed the greatest difference in optical density from time zero to the end of the experiment. A larger difference in OD indicates greater growth, while a smaller difference indicates inhibited growth. Consequently, the addition of

ampicillin resulted in a decrease in optical density, indicating inhibited growth. The higher the ampicillin concentration present in a culture was, the lower the OD difference.

These results confirm the hypothesis that the higher concentration of ampicillin will reduce the growth rate more than the lower concentrations will. Overall, the control showed the greatest increase in optical density with 0.583 and the 10x culture showed the smallest increase of only 0.094. From the greatest increase to the lowest, it was the control with 0.583, the 0.1x culture with 0.320, the 0.5x culture with 0.210, the 1x culture with 0.180, and lastly the 10x culture with 0.094. This supports the hypothesis that the higher concentrations of ampicillin, such as the 1x and 10x, will more greatly inhibit the growth rate of the *E. coli*.

Since ampicillin inhibits the formation of peptidoglycan in the bacterial cell wall, it is logical that higher concentrations of the antibiotic would disrupt more cell walls as they formed, therefore limiting the number of cells that successfully reproduce. This explains why the higher concentrations yield a lower growth rate. If there is only a limited amount of ampicillin, such as when the concentration is low (like 0.1x or 0.5x) it can only inhibit the formation of a limited amount of cell walls, and many cells could still successfully reproduce even in its presence.

Conclusion

The goal of this research was to examine how varying concentrations of ampicillin affected bacterial cell growth. It was found that higher concentrations of antibiotic reduced the growth rate of the *E. coli* more quickly than the lower concentrations did, displaying that since there is more ampicillin present it is able to disrupt the formation of a greater amount of cell walls and thereby reduce the reproduction rate. This hypothesis could be explored further by repeating the experiment with more ampicillin concentration variations and perhaps taking more data over a longer period of time.

References

1. Class notes from lecture on September 14, 2004
2. Class notes from lecture on September 28, 2004
3. Piatelli, Michael. Lab manual for Molecular Cell Biology Lab. 2004.