BIOLOGY INTERNAL ASSESMENT

HIGHER LEVEL

Investigating the effect of varying wavelengths of light on the growth of bacteria.

Candidate Code: fyq035

Session: May 2017

INDEX

RESEARCH QUESTION

To what extent do the varying wavelengths of light affect the growth of bacteria?

INTRODUCTION

Light is a form of electromagnetic radiation, which travels in waves. It consists of photons, the number of which can be influenced by the light source, to make a certain wavelength. The electromagnetic spectrum, pictured to the right, depicts the types of waves at different wavelengths. At shorter wavelengths and higher frequencies, the energy is greater. The visible spectrum depicts the range of visible light, where the wavelength is between 400 and 700 nm. Light is absorbed as a source of energy by most organisms, to help with metabolic processes.

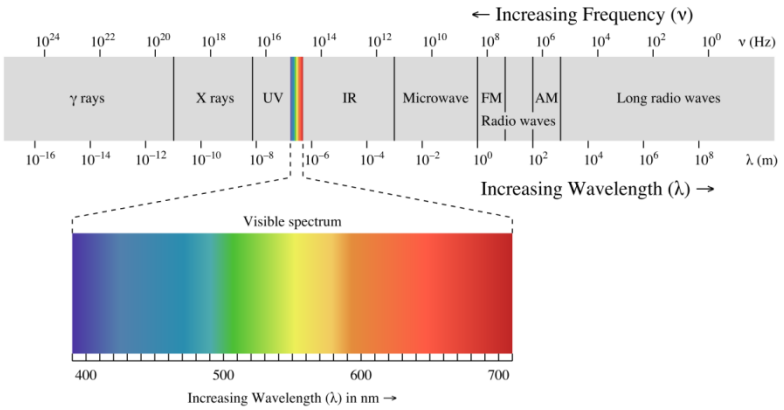


Image 1: *The electromagnetic spectrum*

Bacteria, single-celled microbes, are a type of organism which reproduces by binary fission. This process involves replication of the genetic material and reduction division, producing daughter cells, which are clones of the parent cell. Bacteria require nutrition, water, specific pH, temperature, pressure and concentration of gases (like oxygen and carbon dioxide). The bacteria that will be used in this experiment is Escherichia coli, also known as E. coli. This particular type of prokaryote has only one circular chromosome, sometimes with a circular plasmid. Despite it's simple cell structure, it can perform complicated metabolic reactions to maintain cell growth and division. Most strains of E. coli are not harmful to their hosts; through mutation and evolution, some can become harmful, causing severe disease.

An important application of the information which can be obtained from this experiment is the destruction of bacteria. The traditional approach to destroy bacteria is mainly antibiotic drugs. Sometimes, these can become inefficient due to development of resistant species. Therefore, an innovative approach to destroy bacteria is required. UV irradiation is one of these methods. However, exposure to UV radiation is dangerous for healthy tissue. Hence, through this

experiment, the wavelengths of light at which the tissue won't be at risk (the wavelength range of visible light) and at which the bacteria growth is inhibited or stopped can be found. Feursteinet et al found that blue light at 400 – 500 nm exerts a phototoxic effect on some strains of bacteria.

From this study and information, the hypothesis has been developed: The number of E. coli colonies counted will be greater in the Petri dishes which were supplied with red and white (control) light than in the Petri dishes which were supplied with green and blue light. The null hypothesis is as follows: There will be no differences in the number of E. coli colonies counted in Petri dishes supplied with red, green, blue and white light. Any difference in the number of E. coli colonies counted will be due to chance.

VARIABLES

Independent Variable		
Variable	**Why change it?**	**How?**
Color, therefore, wavelength of Light supplied to E. coli	To understand how it affects the growth of E. coli	Use differently colored (red, blue, green) sheets of glass paper to cover the Petri dishes

Table 1: *Independent Variable*

Dependent Variable	
Variable	**How?**
Growth of E. coli	It will vary according to the changes in the independent variables.

Table 2: *Dependent Variable*

Control Variables		
Variable	**Why keep it constant?**	**How?**
Temperature	Any changes in this variable would result in change in the rate of growth of bacteria.	The experiment will be conducted in an incubator, in which the temperature can be set and kept constant

Light Intensity	Any changes in this variable would result in change in the amount of growth of bacteria.	All of the bulbs inside the incubator glow with the same intensity.
Nutrients	Any changes in this variable would result in change in the amount of growth of bacteria. Also, to ensure the correct nutrient broth to agar ratio.	Mixing exact amounts of nutrient broth, Agar powder and water.
Strain of E. coli	Any changes in this variable would result in inability to compare the results.	Using the same strain of E. coli for all trials.
Type of Petri Dish	Any changes in this variable would result in inability to compare the results. Also to prevent contamination and maintain the amount of agar used.	The same type of petri dishes will be used for all trials. They will be autoclaved together and equal amounts of agar will be poured into all of the petri dishes.

Table 3: *Control Variables*

APPARATUS

- 20 petri dishes
- 1 Autoclave
- 30 grams of Nutrient Broth
- 40 grams of Agar
- E. coli Bacteria
- 1 Laminar Air Flow
- 1 Incubator
- Cotton
- 1.8 Liters of Water
- 2 Sheets of blue glass paper
- 2 sheets of red glass paper

- 2 sheets of green glass paper
- Micro pipette
- Micro pipette tips
- Spreader
- Spirit Lamp

METHOD

1. Take 30 grams of nutrient broth in 1.8L water in a conical flask.
2. Add 40 grams of agar (Agar Agar Powder) to the same mixture in the conical flask.
3. Swirl the flask until the broth and agar dissolve properly in the water.
4. For 5 Petri dishes, cover the lid with the red glass paper, securing it with a rubber band.
5. Do the same things for 5 Petri dishes each, with the green and blue glass papers.
6. Plug the mouth of the conical flask with cotton and then wrap it with aluminum foil, to ensure that no air escapes.
7. Place the flask in the autoclave for two hours at 15-20 PSI.
8. Place the flask outside to cool.
9. Pour the Agar into the sterilized Petri dishes in the laminar airflow.
10. Let it cool to let the agar solidify.
11. Label all of the Petri dishes with the color of light and their corresponding wavelengths.
12. Place the Petri dishes in an inverted position in the incubator at 37 degrees Celsius, after the Petri dishes solidify.
13. Take 10 micro liters of the cultured. Coli bacteria using a micro pipette.
14. Pour the bacteria onto the solidified agar plate.
15. Sterilize the spreader by holding it over the spirit lamp and then cooling it in the laminar airflow.
16. Use it to spread the bacteria across the surface of the Petri dish while rotating it.
17. When done, place the spreader over the flame again to sterilize it.
18. Repeat this spreading processes for the 19 other Petri dishes.
19. Keep the Petri dishes in the incubator at 33 degrees Celsius.
20. After 24 hours, count the colonies of bacteria grown.

PRECAUTIONS

- Wear gloves and eye protection when handling all materials.
- Tie hair properly to avoid contamination.
- The Petri dish must be rotated 90 degrees before streaking the bacteria.
- Streak the Agar gently, do not gash it.
- Any contaminated material must be autoclaved and discarded.
- Clean workstation after every experiment, to ensure cleanliness and discourage contamination.
- Keep the bacteria and Petri dishes in the laminar flow at all times while spreading the bacteria.
- Ensure that the cotton plug is secure before placing the flask into the autoclave.
- Label all the Petri dishes properly.

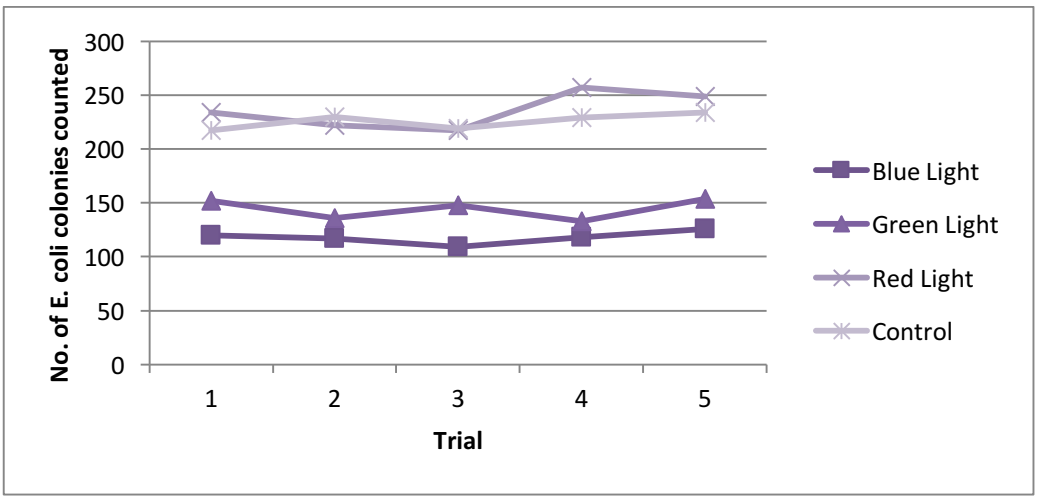
RESULTS

The raw data collected is included in Appendix 1.

This data was plotted on four separate graphs, which are included in Appendix 2.

A graph comparing all of the different growths and lights was plotted:

Comparing the growth of bacteria in all the colors of light:



Graph 1: *Trials vs. No. of E. Coli Colonies Counted*

Processed Data:

The average number of E. coli colonies counted was calculated:

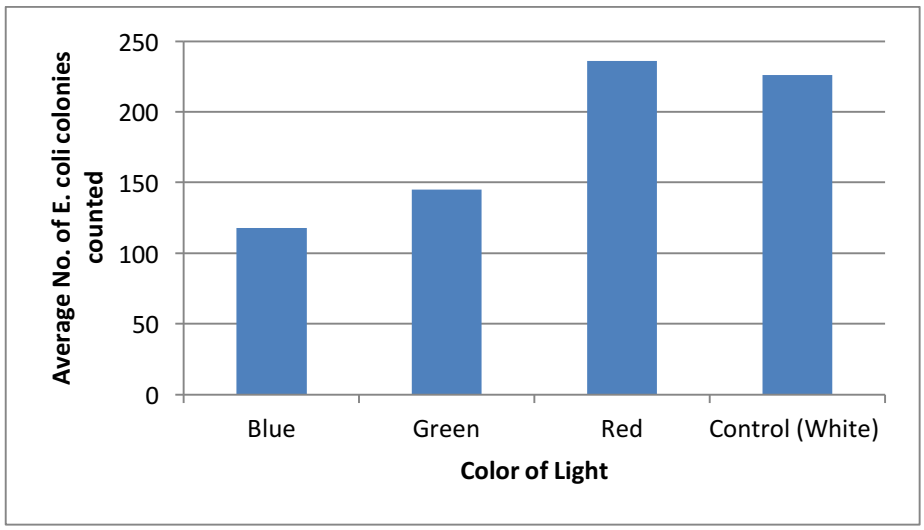
Calculating average number of E. coli colonies counted for each type of light:

Light type	**Total bacteria (of all trials)**	**Average**
Blue Light	= 120 + 117 + 109 + 118 + 126 = 590	= 590/5 = 118
Green Light	= 152 + 136 + 148 + 133 + 154 = 723	= 723/5 = 144.6 ~ 145
Red Light	= 234 + 222 + 217 + 257 + 249 = 1179	= 1179/5 = 235.8 ~ 236
Control	= 217 + 230 + 219 + 229 + 234 = 1129	= 1129/5 = 225.8 ~ 226

Table 4: *Calculating Averages*

This information was graphed:

Graph comparing the average numbers of E. coli colonies counted for each type of light:



Graph 2: *Color of light vs. Average No. of colonies*

Statistical Analysis:

A single factor ANOVA test was used to test the

A screenshot of this is included in Appendix 3.

The p value found was $8.509 * 10^{-12}$, which is much smaller than 0.05. This shows that the null hypothesis can be disregarded – There are differences in the numbers of E. coli colonies counted in the Petri dishes supplied with red, green, blue and white light. These differences are not due to chance.

CONCLUSION

The data found and analyzed clearly shows that there is a correlation between the color of light supplied and the growth of E. coli. When white light, the control variable, was supplied, the average number of colonies counted was 226. This count is closely mirrored by the average number of colonies grown in red light, 236. This shows that the bacteria count is higher in red light. However, to fully form this conclusion, further research is required. It can be stated, however, that red light did not inhibit the growth of bacteria. The same cannot be said for the bacteria grown under green and blue light. The average counts were 145 and 118 respectively. This clearly shows that the E. coli were affected by the color of light they were grown in.

Red light is of the wavelength range 620 – 720 nm, while green light is of 500 – 570 nm, and blue light is of 460 – 500 nm. The results indicate that at the higher wavelengths of 620 – 720 nm, bacteria seems to grow regularly, is perhaps even promoted. However, within the wavelength range of 460 – 570 nm, the growth of bacteria is inhibited. These results match the expectations set at the beginning of the experiment, and support the hypothesis - number of E. coli colonies counted will be greater in the Petri dishes which were supplied with red and white (control) light than in the Petri dishes which were supplied with green and blue light. The findings also support those of Feursteinet et al. The blue light has a phototoxic effect on the E. coli bacteria.

EVALUATION

Some limitations that should be considered are mentioned in the below table:

Limitations and Uncertainites
There is a possibility of contamination of the Petri dishes. This could lead to increased growth and/or growth of different types of bacteria than would have occurred without contamination.
Contamination also could have occurred due to improper autoclaving of Petri dishes or conical flasks.
While measuring the Agar Agar powder or Nutrient Broth, parallax error could have occurred.
The bacterium might not have been spread equally or evenly across the Agar.
There is ±0.05 mL uncertainity in the beakers and flasks used.
The small number of repeats (5) for each variable limits the reliability of the results.
The small number of independent variables (4) may not provide a holistic impression of the characteristics investigated in the experiment.

However, the results obtained from this experiment can be considered accurate, as they mostly match what the research predicted. The results were also reliable, as the results obtained from the many trials all had small standard deviation from the mean and were generally consistent. The uncertainity is relatively low. Additionally, the design of the experiment was succesful, as it gave precise and generally accurate results. Hence, the results are mostly reliable.

The investigation has provided evidence that radiation of the wavelength range of 460 – 570 nm greatly decreases the growth of E. coli. However, further research can be conducted to gain a deeper understanding of the effect of different wavelengths of light on bacterial growth. There are other ways of inhibiting bacterial growth, such as removing it's source of oxygen or water, as it cannot survive without these two things. Salt also inhibits the growth of bacteria – a 20% concentration or higher (in a solvent) can effectively reduce bacterial growth.

REFERENCES

Chang, Michelle, and Apiradee Sanglimsuwan. "Inhibition of Bacterial Growth by Light Wavelengths and Antibiotic Exposure." (n.d.): n. pag. Web. Jan. 2017.

Lubart, R., A. Lipovski, Y. Nitzan, and H. Friedmann. "A Possible Mechanism for the Bactericidal Effect of Visible Light." *Laser Therapy*. Japan Medical Laser Laboratory, 2011. Web. 21 Jan. 2017.

"Wavelengths of Vibgyor Colors." *GKToday*. N.p., 21 May 2013. Web. 21 Jan. 2017

APPENDIX

Appendix 1: Raw Data:

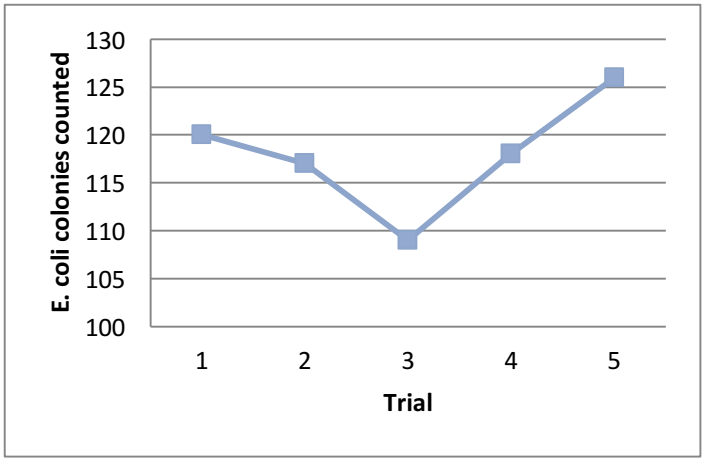
Sample	Trial No.	E. coli Colonies Counted
Blue Light	1	120
	2	117
	3	109
	4	118
	5	126
Green Light	1	152
	2	136

	3	148
	4	133
	5	154
Red Light	1	234
	2	222
	3	217
	4	257
	5	249
Control	1	217
	2	230
	3	219
	4	229
	5	234

Table 5: *Raw Data*

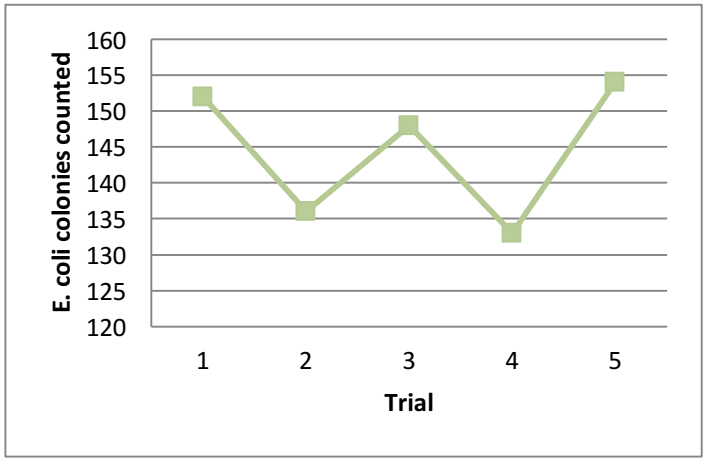
Appendix 2: Graphs of E. coli growth in all trials:

Graph of growth of E. coli in blue light:



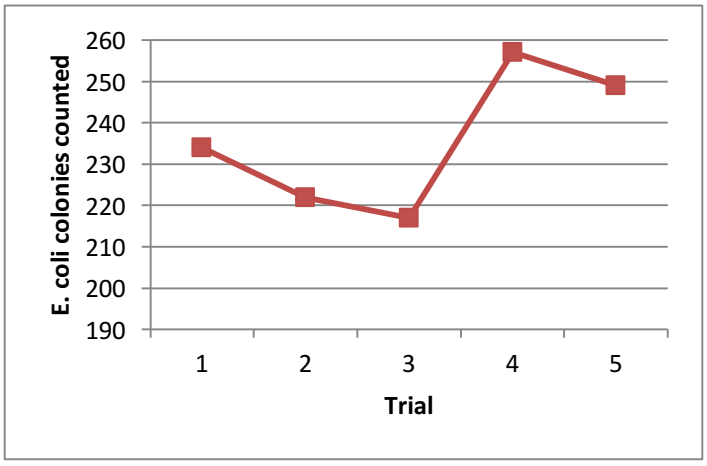
Graph 3: *Blue Light Trials*

Graph of growth of E. coli in green light:



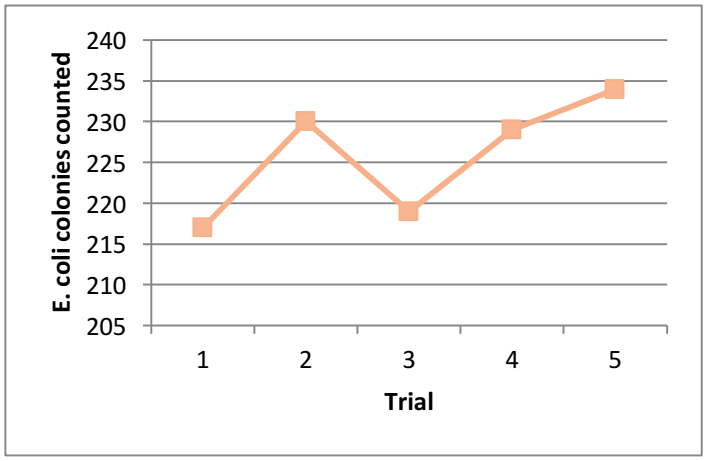
Graph 4: *Green Light Trials*

Graph of growth of E. coli in red light:



Graph 5: *Red Light Trials*

Graph of growth of E. coli in control:



Graph 6: *Control Trials*

Appendix 3: ANOVA Test:

	A	B	C	D	E	F	G	H
1	**Blue Light**	**Green Light**	**Red Light**	**White Light (Control)**				
2	120	152	234	217				
3	117	136	222	230				
4	109	148	217	219				
5	118	133	257	229				
6	126	154	249	234				
7								
8	Anova: Single Factor							
9								
10	SUMMARY							
11	*Groups*	*Count*	*Sum*	*Average*	*Variance*			
12	Blue Light	5	590	118	37.5			
13	Green Light	5	723	144.6	90.8			
14	Red Light	5	1179	235.8	292.7			
15	White Light (Control)	5	1129	225.8	54.7			
16								
17								
18	ANOVA							
19	*Source of Variation*	*SS*	*df*	*MS*	*F*	***P-value***	*F crit*	
20	Between Groups	51520.15	3	17173.38333	144.4051573	**8.50909E-12**	3.238871522	
21	Within Groups	1902.8	16	118.925				
22								
23	Total	53422.95	19					
24								

Image 2: *ANOVA Test*