Simran Johal - Science Fair Lab Report 2025

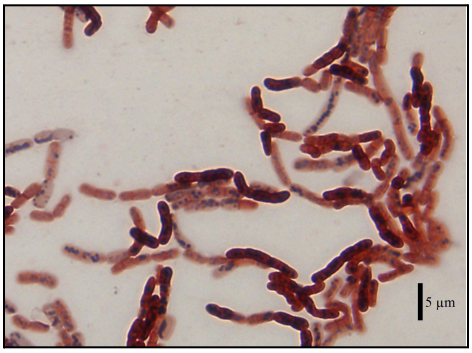
Testable Question

If Bacillus megaterium (B. megaterium) bacterial cultures are grown on petri dishes under various conditions (UV light, no light, warm (30˚C), cold (5˚C), moist, dry, and fed glucose), how do the various samples differ in abundance (which condition bred the most bacteria), reproduction (population increase in a fixed time) and resistance to a diluted bleach solution?

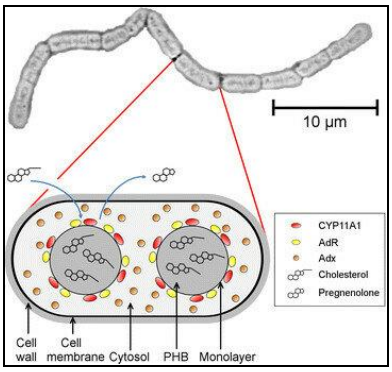
Background Research

The focus of this experiment is to investigate how environmental factors affect B. megaterium's abundance, reproduction rate and resistance to a 10% diluted bleach solution. Abundance refers to how many colonies grow. Reproduction rate alludes to the population increase over 24 hours, calculated by subtracting the number of colonies grown at 48 hours (day 2) by the number of colonies grown at 72 hours (day 3). Resistance to a diluted bleach solution indicates the bacteria's strength against a 10% water-diluted bleach solution. It will be calculated by dropping 5mL of diluted bleach into each plate, letting it sit for 24 hours and then subtracting the number of colonies remaining from the initial abundance count. A 10% diluted bleach solution was chosen as it is the typical concentration used to disinfect in student laboratories. The various conditions the bacteria will be subject to include: light, dark (no light), favorable temperature (30°C), unfavorable temperature (5°C), moist, dry, and fed glucose (⅛ tbs honey).

Bacillus megaterium is the center of this experiment. B. megaterium is a non-pathogenic, gram positive, rod shaped, motile bacteria which reproduces by spore production. Pathogenicity refers to the ability to cause disease, which B. megaterium doesn't have.

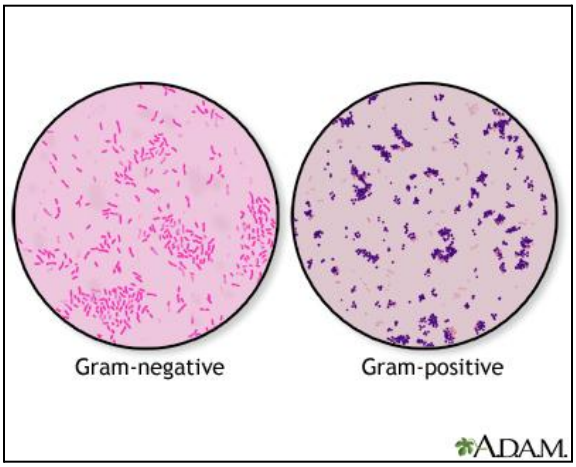


Bacillus megaterium bacteria under microscope.

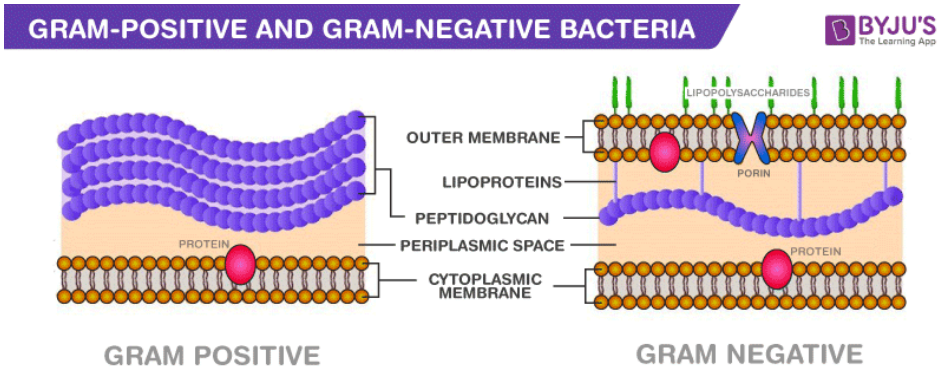


Bacillus megaterium cell diagram.

Gram positive/negative classifies bacteria based on the color they become after a gram stain. A gram stain is when crystal violet dye is added to bacterial cultures. The dye either holds onto a bacterium's cell membrane, giving it a purple color, or doesn't hold on, and gives it a red-pink tint when observed under microscope. Gram positive bacteria have thicker peptidoglycan (substance consisting of proteins and sugars which form a mesh layer) membranes than gram negative. This allows them to hold on to the dye better, whereas the thin peptidoglycan membranes of gram negative bacteria cannot. However, gram negative bacteria have a special outer membrane known as lipopolysaccharide (LPS). This membrane creates a barrier which protects the bacteria from toxic substances (including antibiotics) and other environmental factors. LPS significantly strengthens gram negative bacteria and acts as a formidle shield from antibiotics, making it much more difficult to kill (when compared to gram positive bacteria). Although gram positive bacteria lack the LPS outer wall, their layered peptidoglycan membrane still holds much importance. The thickness of the membrane prevents the bacteria from facing osmotic lysis. This is when a bacterium bursts due to excess water in the cell from failed osmosis. The osmosis regulator of the bacterium is peptidoglycan. Since gram positive bacteria have a rigid, tough peptidoglycan layer, they are better able to protect against imbalanced osmotic pressure and ensure osmosis is regulated.

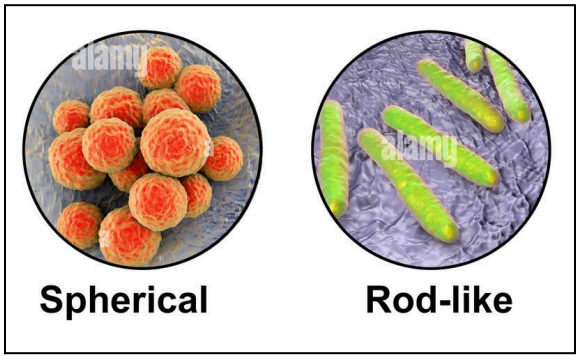


Gram negative bacteria vs. gram positive.



Gram negative vs. gram positive cell diagram.

The bacteria's shape refers to its structure when viewed under the microscope. There are two main basic shapes: cocci and rod. Cocci are spherical shaped bacteria while rods are cylindrical. Each shape has its own advantages. Rod shaped bacteria have the ability to motility (move by themselves) and absorb nutrients better. This is because they have more surface area, allowing for better nutrient intake and growth. Additionally, rod shaped bacteria have an aerodynamic shape, allowing for improved swimming skills. The cocci shape, on the other hand, is beneficial when larger surface area is unwarranted. Situations as such could be desiccation (dryness) or osmotic lysis could occur. The more surface area a bacterium has, the harder it is to resist such processes. Likewise, the less surface area, the better resistance. Rod shaped bacteria are more prevalent in aquatic, moist conditions while cocci are the opposite, enjoying dry areas better.



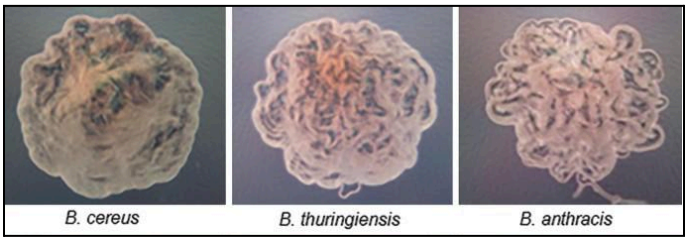
Visual representation of cocci vs. rod bacteria.

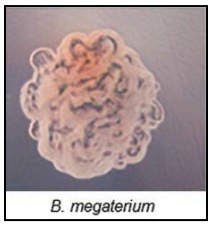
B. megaterium bacteria reproduce using spore production. Spore production is a branch of asexual reproduction in which the parent plant produces tiny, seed-like spores which eventually reproduce into new individuals. During spore production, the sporangium (structure which produces spores) initially produces a single spore which divides by mitosis. Once the sporangium is full of spores, the spores are released. Now, this is how spore production normally works, but bacteria are unicellular organisms. So, they lack the sporangium structure needed for normal spore production. B. megaterium reproduces using endospores. In endospore reproduction, the bacteria uses its endospore structure to execute mitosis within the cell wall. The endospore then forms as a swollen structure attached to the cell wall, consisting of DNA, cytoplasm and a strong outer shell.

This outer shell greatly protects the spore from environmental conditions. Unlike regular spore production, where many offspring can be produced at once, only one offspring can be produced at a time with endospores. Endospores are extremely resistant to environmental factors.

Bacillus megaterium endospore reproduction. 17,000x magnification.

Although B. megaterium itself is relatively safe, it is related to a bacterial group known as the Bacillus cereus group. This group contains 8 both pathogenic and nonpathogenic bacteria, including Bacillus cereus, anthracis, cytotoxicus, mycoides, pseudomycoides, thuringiensis, toyonensis, and weihenstephanensis. Of these 8, only 3 are nonpathogenic; B. mycoide, pseudomycoides, and toyonensis. The rest are pathogenic and can cause disease in humans. Bacillus cereus is responsible for causing gastrointestinal illnesses when ingested, such as vomiting, abdominal pain, and bowel issues (food poisoning). B. anthracis can cause an infectious disease known as anthrax, which, in humans, can cause skin, lung and digestive problems. The disease usually forms on the skin by creating black lesions. Bacillus cytotoxicus is accountable for diarrheal disease when ingested. Diseases contracted from B. cytotoxicus are rare, but deadly. Bacillus thuringiensis creates an endotoxin known as Cry toxin. This toxin is not harmful for mammals, but is for insects and is used in the Bt pesticide. B. weihenstephanensis is responsible for spoiled milk and rotten eggs, which, when consumed, can cause food poisoning.





Some Bacillus bacterial growth.

So, how does each condition impact bacterial growth? As an endospore-forming bacteria, Bacillus megaterium is resistant to environmental factors when grown in favorable conditions. Favorable growth conditions for B. megaterium include: temperature of around 30°C, moist environment, provided energy source (glucose in this experiment) and lighted environment. Presence of nutrients and a proper environment allow the bacteria to reproduce faster and so thrive better. Although B. megaterium can survive in dark conditions, UV light fosters the growth of substances called carotenoids. Carotenoids are antioxidants that protect the bacterium from oxidative damage by eliminating harmful agents in the light. Bacteria grown in the dark lack the presence of carotenoids and so put the cells at risk for oxidative damage. Presence of oxygen promotes this type of reaction, where the bacterium loses electrons during growth. This results in the collapse of its cell membrane and other structures. Temperature is a major factor in bacterial growth. In warmer conditions, organic reactions (life processes) of bacteria speed up, allowing the bacteria to grow in greater abundance. By growing bacteria in an unfavorable temperature, the growth rate slows and may even become dormant (processes stop). A moist environment provides an adequate amount of water and hydration the bacteria needs to grow and reproduce. Dry environments lack the water content needed for bacterial survival and so in dry conditions, bacteria cannot grow or reproduce at the same rate as a moist condition. Water acts as a solvent for nutrients, and so bacterial plates with greater water content will support greater bacterial growth. Glucose acts as an energy source for bacteria. Glucose creates a compound called adenosine triphosphate, which supports bacterial metabolism, generating energy that fuels bacterial life processes. Bacillus megaterium's structure also impacts the way it responds to environmental stresses. Due to its gram positive nature, the bacteria's thick peptidoglycan layer helps regulate osmosis, which is crucial for the moist bacteria. Additionally, the bacteria's rod shape helps absorb nutrients better which may enhance growth for all conditions. The typical duplication time of B. megaterium is 25 minutes when grown under desirable conditions. Bacterial resistance is heavily impacted by growth environments. Bacteria grown in favorable conditions will have increased metabolic activity, resulting in a stronger peptidoglycan layer which is more resistant to toxins such as bleach.

This project will be tested using Bacillus megaterium cultures grown on nutrient agar plates. Nutrient agar is a medium for bacterial growth composed of peptone, beef extract, agar and salt. Peptone is a protein which provides nitrogen to the bacteria. Beef extract contains most of the important nutrients, such as carbohydrates, salts, nitrogen compounds and vitamins. Agar acts as the base for the solution; the solid part. Salt helps maintain sodium levels in the agar to create an environment which resembles the cytoplasm of many organisms, encouraging growth. The bacteria will be allowed to grow for 48 hours before the first colony count. This count will be raw data needed to determine the reproduction. After 72 hours, the colonies will be counted. This number will be the abundance; whichever condition supports the most colonies will have the highest abundance. To find the reproduction, I will subtract the number of colonies after 72 hours

from the number of colonies after 48 hours. This gives the population increase. To determine resistance to bleach, I will later drop 5mL of a 10% diluted bleach solution into each plate and leave it for 24 hours. After 24 hours, I will subtract the initial number of colonies (abundance) from the colonies leftover. This will determine how many died from the bleach. Whichever condition leaves the least dead colonies will have grown the most resistant bacteria.

Hypothesis

If B. megaterium bacterial colonies are grown under various conditions, including: light, dark (no light), favorable temperature (30°C), unfavorable temperature (5°C), moist, dry, and fed glucose, then following hypotheses can be made:

1. **The plates grown at 30°C (favorable temperature) will have the highest abundance while the plates subject to the cold (5°C) will have the lowest.** A higher temperature will boost bacterial growth as it will speed up its organic processes and thus have the highest abundance. On the flip side, a cooler temperature will slow these same procedures and hinder bacterial abundance.
2. **The moist plates will have the fastest reproduction time while the dry ones will have the slowest.** Moisture levels directly impact bacterial reproduction as they influence nutrient intake and hydration. If there is a provided water source, bacteria can absorb nutrients at a much higher rate. These factors influence bacterial reproduction, higher moisture boosting it and lower moisture slowing it.
3. **Bacteria grown while fed glucose will be most resistant to bleach while bacteria subject to dry conditions will be least resistant.** Bacterial strength and resistance will depend on its nutrient intake to strengthen the peptidoglycan membrane, blocking toxins like bleach from entering. As the colonies will be grown on nutrient agar, the glucose will boost the nutrient intake and so strengthen the bacterium's peptidoglycan layer. The dry plates, on the other hand, will have trouble intaking the nutrients in the agar as they will lack the moisture needed to do so.

Variables

Controlled:

- The type of bacteria (B. megaterium)
- The amount of time the bacteria is allowed to grow for
- The amount of agar poured on each petri dish
- The amount of bacteria added to each plate
- The amount of honey added to the glucose plates
- The amount of light received by the light plates
- The amount of heat exposed to the warm plates
- The lack of heat exposed to the cool plates
- The moisture of the moist plates
- The dryness of the dry plates
- The lack of light given to the dark plates

Responding:

- Abundance
- Reproduction time
- Resistance to a diluted bleach solution

Manipulated:

- The various conditions the bacteria are grown in (light, dark, favorable temperature, unfavorable temperature, moist, dry and fed glucose)

Uncontrolled:

- The rising and setting of the Sun
- Indoor temperature
- Humidity

Materials

- 24 petri dishes
- B. megaterium bacterial cultures - mine are Merlan Scientific's Bacillus megaterium Microkwik Cultures
- 1 incubator - mine is IVYX Scientific's 5L Lab Incubator
- Aluminum foil
- 1 Spray bottle
- 1L Filtered water
- 1 Bottle of manuka honey
- ⅛ tsp measuring spoon
- 1 tsp measuring spoon
- 100g of Seaweed Solution Laboratories' Nutrient Agar
- 1L bottle of Clorox bleach
- 1 wooden mixing spoon
- 1 stove
- 3 pairs of gloves
- 1 pair of safety eye goggles
- 1 box of plastic wrap
- 3 respirator masks
- 1 box of cotton swabs
- 1 pair of scissors
- 1 bottle of Blue Lizard Sensitive Mineral Sunscreen
- 3 plastic Ziploc bags - medium sized
- 1 fridge - mine is SAMSUNG model RF28HFEDBSR
- 1 medium sized pot
- 1 large sized Ziploc airtight container
- 1 cell phone or tablet

Procedure

Making the agar:

1. Gather all materials.
2. Place your pot on the stove.
3. Turn the heat on low.
4. Pour 1L of water into the pot.
5. Open your nutrient agar packet.
6. Pour the powder into the pot.
7. Stir the mixture until partially dissolved.
8. Simmer for 10 minutes or until completely dissolved.
9. During these 10 minutes, lay out 24 petri dishes, opened.
10. After the agar has dissolved in the water, allow it to cool for 5 minutes.
11. Pour the agar mixture into each plate so that it just barely covers the bottom of the plate.
12. Allow the agar to set overnight.

Setting the dishes in their conditions:

13. Wear your PPE- gloves, eye goggles and mask.
14. After the agar sets, divide the 24 plates into 8 different groups- control, UV light, dark, glucose, moist, dry, hot and cold.

➔ Each condition should have had 3 plates. In each group, label each plate as its own trial from 1-3 and record observations accordingly.

15. Open the B. megaterium culture tube
16. Using a cotton swab, gently brush the swab around the culture.
17. In a zig-zag motion, rub this swab against the agar plate.
18. Repeat steps 16 and 17 for all 24 petri dishes. Use a new cotton swab each time.
19. For the plates you chose as UV light: Replace the lid of the plate. Place them in direct sunlight.
20. For the plates you chose and Dark: Replace the lids of the plates. Wrap each plate in aluminum foil and place the plates outside of direct sunlight.
21. For the plates you chose as Glucose: Add ⅛ tsp of manuka honey to each plate.
22. Using a clean cotton swab, gently spread the honey across each plate.
23. Replace the lids of the plates.
24. Place the Glucose plates out of direct sunlight.
25. For the plates you chose as Moist: Fill your spray bottle with filtered water.
26. Spray 3 pumps (0.6mL) of water across each plate.
27. Replace the lids of the plates.
28. Place the moist plates in direct sunlight.
29. For the plates you chose as dry: place the plates inside of your airtight container.
30. Ensure the container is completely shut - no air should be able to enter or leave.
31. Place the container in a dimly lit area.
32. For the plates you chose as hot: Plug in your incubator
33. Set the temperature to 30°C.
34. Place the plates inside and shut the door.

35. Ensure the air vents of the incubator are not obstructed and that the device is out of direct sunlight.
36. For the plates you chose as cold: Set your fridge's temperature to 5°C.
37. Place each plate inside a Ziploc bag.
38. Ensure the bags are completely sealed.
39. Change your gloves.
40. Put the plates inside a larger Ziploc bag.
41. Ensure the bag is completely sealed.
42. Place the bag inside your fridge, away from the opening (in the back).
43. For the plates you chose as control: Place the plates in a room temperature environment away from direct sunlight.
44. Allow the plates to grow for 48 hours.

Taking the Day 2 Colony Count (48 hours) - raw data needed to calculate reproduction:

45. During the 48 hours, download the @BactLAB colony counter app.
46. After the 48 hours are over, conduct your first colony count.
47. Using the app, briefly take a clear, well-lit picture of each plate and return them to their original growth conditions.
48. You will have to turn the light on for the dark plates, so be quick.
49. Run each image through the app and record the number of colonies on each plate.
50. Allow the plates to grow for another 24 hours.

Calculating Bacterial Abundance - after 72 hours:

51. After 24 hours are over, take each plate out of its condition and record observations in colony size, amount (using the app) and difference from yesterday.
52. The amount of colonies you have recorded today is the plate's colonial abundance.

Calculating Reproduction Rate - from 48 hours to 72 hours:

53. Subtract the plate's "Abundance" record from the 48 hour record.

➔ Repeat step 53 for each plate and record in observations.

➔ If colonies merge, count the larger colony as a single colony and record in observations. Track merging patterns.

Calculate Bleach Resistance - from 72 to 96 hours:

54. Dilute 100 mL of your bleach with 10 mL of water.
55. Add 5mL of this solution to every plate.
56. Shake it around a bit and leave each plate at room temperature away from direct sunlight (do not return them to original spaces).
57. Allow the bleach-filled plates to rest for 24 hours.
58. After 24 hours, using your colony counter app, record the number of colonies.
59. Subtract this number from the "abundance" number.
60. This will be the number of colonies that died from the bleach.
61. Record each plate's "bleach" number.
62. Whichever condition yielded the least dead colonies from the bleach will have the highest resistance.

Observations - After 72 Hours - Colony shape and size, and difference from Day 2.

Trial 1

Light: Colonies are medium in size, about the size of a dime. Colonies look a bit greater in abundance since day 2.
Dark: Colonies are relatively small in size, scattered around the edges rather than the centre. Colonies visually do not look like a massive increase from day 2.
Glucose: Colonies are large in size, some the size of quarters while most the size of nickels. Colonies are relatively a medium visual increase from day 2. Colonies are whiter in color than other plates.
Moist: Colonies are large in size, but not as large as glucose colonies. Colonies are clearly a massive increase in abundance since the second day.
Dry: Colonies are medium-small in size, not as big as the light plates but not as small as the dark ones. Colonies do not look like a massive increase since day 2.
Hot: Colonies are medium-large in size, slightly smaller than moist colonies. Colonies slightly look greater than day 2.
Cold: Colonies are extremely small in size and only scattered across the edges of the plates. Colonies look like a very small increase since day 2.
Control: Colonies are medium in size and look relatively as an increase since day 2.

Trial 2

Light: Colonies are of medium size with a visible increase in abundance from day 2.
Dark: Colonies are scattered towards the edges and relatively small in size. They look basically the same as day 2.
Glucose: Colonies are very large in size, and show a medium-ish increase from day 2. Colonies are again white in color.
Moist: Colonies are large in size, scattered around the entire petri dish thoroughly. Colonies are a large increase in abundance.
Dry: Colonies are small in size, and do not show an obvious increase since day 2.
Hot: Colonies are medium in size. They are scattered relatively centred in the dish. Colonies look clearly greater than day 2.
Cold: Colonies are very small in size and are accumulating towards the edges. Colonies do not show an obvious increase from day 2.
Control: Colonies are medium sized and look slightly more than day 2.

Trial 3

Light: Colonies are again medium sized, about the same as trials 1 and 2. Colony abundance increase can easily be seen since day 2.
Dark: Colonies are small in size, but slightly larger from trial 2. Colonies are in slight visible abundance from day 2.
Glucose: Colonies are very large in size, and just like trials 1 and 2, they can easily be discerned from day 2. Again, colonies are white.
Moist: Colonies are large in size, almost as big as glucose plates. Colonies have had a massive abundance increase since day 2.

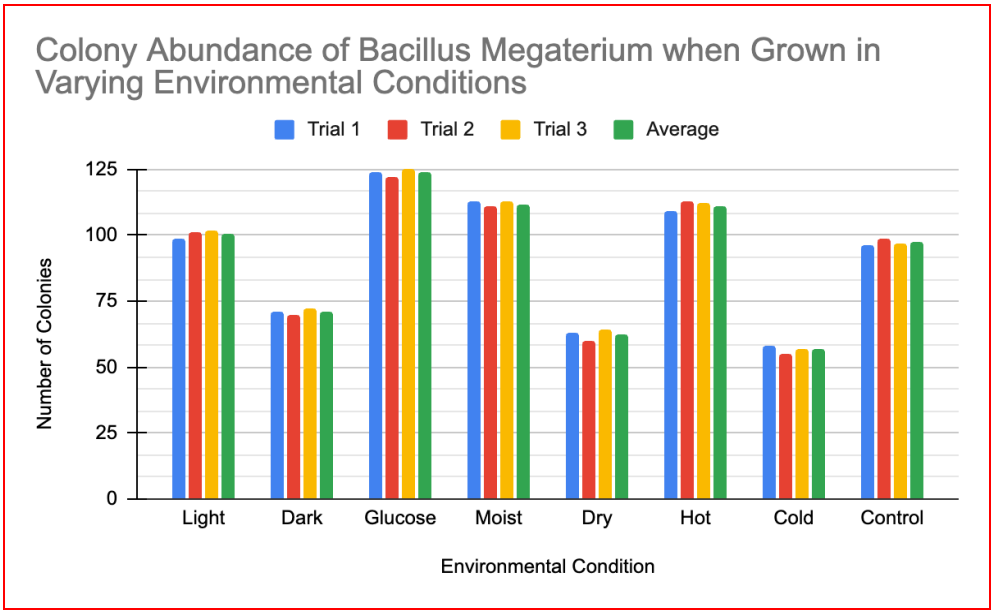
Dry: Colonies are medium in size, only slightly smaller than the light plates. Colonies are slightly in more abundance than day 2.
Hot: Colonies are large in size, however smaller than the moist plates. The colonies are in clear abundance from day 2.
Cold: Colonies are very small in size, smaller than trials 1 and 2. Abundance looks almost identical to trials 1 and 2.
Control: Colonies look in abundance from trial 2 and are medium sized.

Various bacterial plates before taking observations.

Data

Abundance Table - Colony Count after 72 Hours of Colonial Growth - Day 3

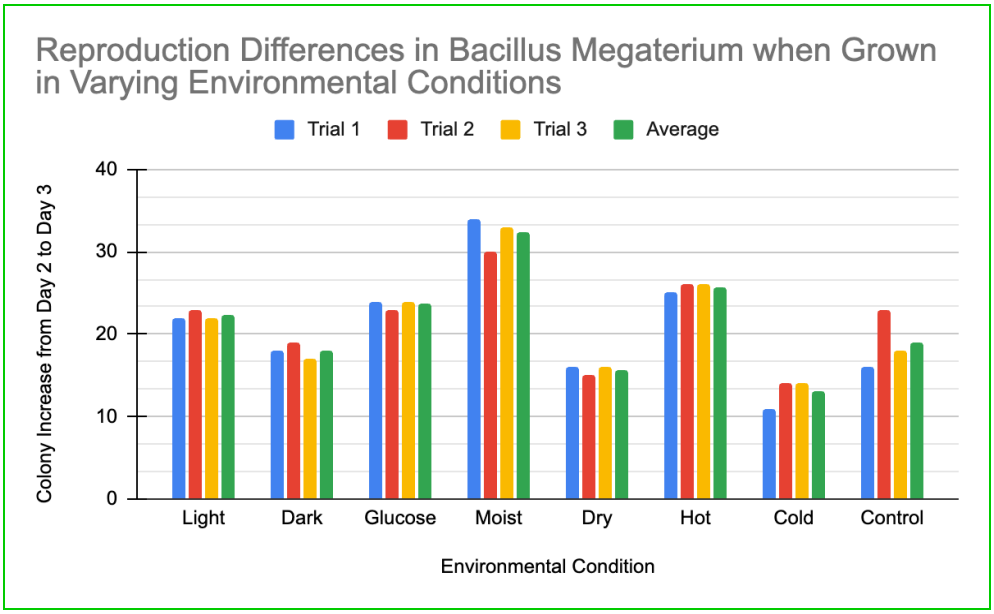
Condition ↓	Trial 1	Trial 2	Trial 3	Average
Light	99 Colonies	101 Colonies	102 Colonies	100.67 Colonies
Dark	71 Colonies	70 Colonies	72 Colonies	71 Colonies
Glucose	124 Colonies	122 Colonies	125 Colonies	123.67 Colonies
Moist	113 Colonies	111 Colonies	113 Colonies	111.67 Colonies
Dry	63 Colonies	60 Colonies	64 Colonies	62.3 Colonies
Hot	109 Colonies	113 Colonies	112 Colonies	111.3 Colonies
Cold	58 Colonies	55 Colonies	57 Colonies	56.67 Colonies
Control	96 Colonies	99 Colonies	97 Colonies	97.3 Colonies



As can be inferred, glucose plates showed the highest colonial abundance. Moist and hot plates showed similar abundance, moist only slightly ahead. Next in abundance was light plates, and after that dark. Dry plates came afterwards and lastly, cold plates had the least abundance.

Reproduction Table - Colonial Increase from Days 2-3

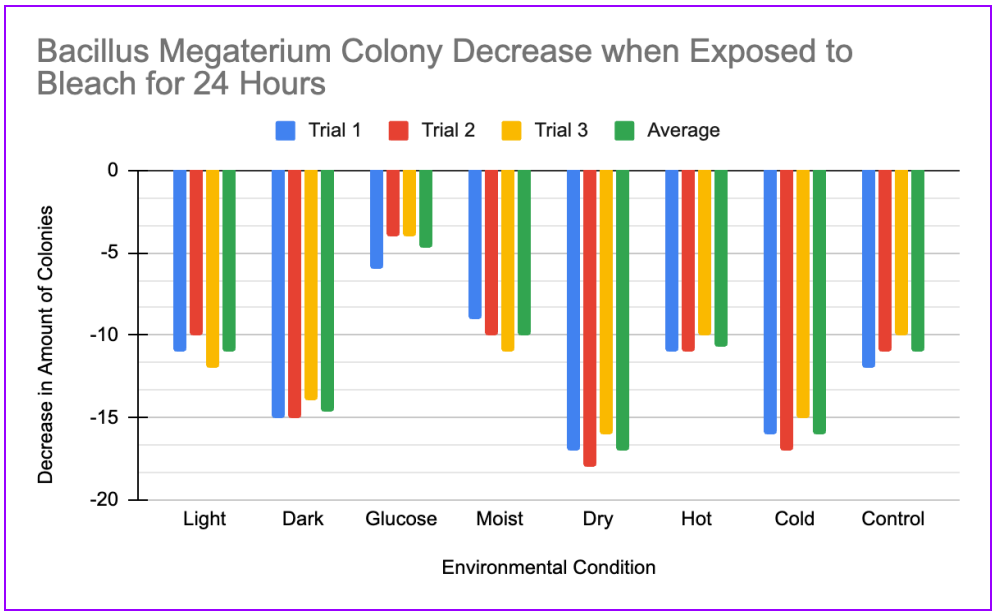
Condition ↓	Trial 1	Trial 2	Trial 3	Average
Light	+22 Colonies	+23 Colonies	+22 Colonies	+22.3 Colonies
Dark	+18 Colonies	+19 Colonies	+17 Colonies	+18 Colonies
Glucose	+24 Colonies	+23 Colonies	+24 Colonies	+23.67 Colonies
Moist	+34 Colonies	+30 Colonies	+33 Colonies	+32.3 Colonies
Dry	+16 Colonies	+15 Colonies	+16 Colonies	+15.67 Colonies
Hot	+25 Colonies	+26 Colonies	+26 Colonies	+25.67 Colonies
Cold	+11 Colonies	+14 Colonies	+14 Colonies	+13 Colonies
Control	+16 Colonies	+23 Colonies	+18 Colonies	+19 Colonies



As can be inferred, the moist plates showed the most increase in colonies from days 2-3. Behind moist plates were the hot plates, and following that were the glucose plates. Afterward came the light and dark plates. Dry plates came up behind the two and lastly, cold plates had the least colonial increase from days 2-3.

Bleach Table - Colonial Deaths after 24hr-long Bleach Exposure - Days 3-4

Condition ↓	Trial 1	Trial 2	Trial 3	Average
Light	-11 Colonies	-10 Colonies	-12 Colonies	-11 Colonies
Dark	-15 Colonies	-15 Colonies	-14 Colonies	-14.67 Colonies
Glucose	-6 Colonies	-4 Colonies	-4 Colonies	-4.67 Colonies
Moist	-9 Colonies	-10 Colonies	-11 Colonies	-10 Colonies
Dry	-17 Colonies	-18 Colonies	-16 Colonies	-17 Colonies
Hot	-11 Colonies	-11 Colonies	-10 Colonies	-10.67 Colonies
Cold	-16 Colonies	-17 Colonies	-15 Colonies	-16 Colonies
Control	-12 Colonies	-11 Colonies	-10 Colonies	-11 Colonies



As can be inferred, dry plates were most impacted by the bleach while glucose plates were impacting the least. After dry came the cold and dark plates, following up with the light plates. Behind the light plates were hot colonies, then moist colonies and lastly glucose plates, which were the most resistant to bleach damage.

Analysis

<u>UV Light:</u>

UV Light influences the production of carotenoids in bacterial colonies when exposed to it. Carotenoids act as a shield against UV damage, protect the bacterium from oxidative damage and increase flexibility of the cell membrane. This strengthens the endospores from such stresses. However, when exposed for a long period (such as 3 days, as in my experiment), bacterial colonies can become too stressed for the carotenoids and endospores to handle, resulting in slight colonial death to the UV light. Colony reaction to bleach was relatively similar to control plates due to the fact that UV light did not inherently weaken the endospore's strength, resulting in normal colony growth (normal would be considered the control). Reproduction was again normal as the colonies began experiencing slight UV damage, and so wouldn't be able to grow to the rate of moist plates.

<u>Dark:</u>

Darkness does not inherently impact the bacterial growth of Bacillus megaterium colonies. However, when colonies are grown in the dark, they become used to their surroundings and grow light sensitive. As a result, when they were briefly exposed to light to execute the day 2 check, the sensitive colonies struggled to continue growing. As a result, colonial abundance was relatively low. This also weakened the colonies, resulting in greater colonial deaths to bleach exposure.

<u>Glucose:</u>

Glucose acts as an energy source for bacterial colonies. When glucose is present in bacterial colonies, they thrive because they don't have to fuel their life processes by themselves; the glucose does it for them. As a result, colonies grow in greatest abundance. Bacterial colonies break down glucose into chemicals called adenosine triphosphate. This chemical is used by the cell's mitochondria to produce energy it can use to fuel life processes. The bacterial colonies initially grew extremely fast. However, their reproduction rate is less than moist because, after consuming all the honey, the glucose plates ran out of their energy source. As a result, their growth rate is slightly slowed. However, in all, the initial growth boost resulted in glucose colonies having the highest abundance. With more nutrients, the colonies could grow peptidoglycan membranes and other cellular structures at a stronger level, resulting in the glucose-fed colonies to have the best resistance to bleach.

Moist:

Water is necessary for bacterial growth; it acts as a solvent, allowing for nutrients to be absorbed properly. Since the growth medium was nutrient agar, the moist plates were able to sow all nutrients available. Growth was initially normal, however, as the water content within the plates evaporated and formed a moist, humid environment, colonial growth naturally exceeded as well. As a result, the moist plates had the best reproduction. Since the plates also had the ability to gain the full nutrients from the nutrient agar, they grew relatively strong and in much abundance.

Dry:

Since the dry plates lack the water content needed to properly absorb nutrients, even though the growth medium was nutrient agar, the dry plates could not harvest its nutrients. As a result, the colonies grew in less abundance. Additionally, since the colonies grew devoid of proper nutrients they were unable to weave strong peptidoglycan fibers when growing and ended up with compromised membranes. This resulted in the dry colonies having the worst resistance to bleach; the chemicals seeped through the membrane as the thin, weak layers were not a good defense. Reproduction was also impacted, as without sufficient nutrients, the colonies could not survive long enough to adequately reproduce.

Hot:

Temperature is one of the main factors influencing the rate of chemical reactions. In bacterial cells, chemical reactions occur. For example, the process of turning glucose into adenosine triphosphate is a chemical reaction. When the temperature is higher, these reactions occur faster. The particles bump into each other more, resulting in a quicker reaction. This is why the hot colonies had high abundance and reproduction rate. Colony strength was also relatively strong, as the greater temperature allowed for consistent growth which ensured the colonies grew without obstacle.

Cold:

As a greater temperature speeds chemical reactions, a lesser temperature slows it. The cold colonies grew at a much slower rate, due to the fact that the organic reactions took too much time to occur. This resulted in colonies that had trouble growing properly. Due

to such extreme stress, the bacteria in the cold plates had the least abundance. Moreover, since the bacteria did not grow properly, they couldn't support reproduction well and ended up with the worst reproduction rate. Additionally, many bacteria even went into dormancy due to the extremely unfavorable temperature. This dormancy left the bacteria vulnerable to the bleach, resulting in the cold colonies being 2nd worst resistant to bleach.

Conclusion:

The purpose of this experiment was to investigate how environmental factors impact Bacillus megaterium's colony abundance, reproduction rate and resistance to a 10% bleach solution.

It was hypothesized that the colonies grown under a favorable temperature would have the highest abundance while the bacteria grown in unfavorable temperature would have the lowest. This hypothesis was partially correct. The results of this experiment showed that the glucose fed bacteria had the highest abundance (avg. 123.67 colonies) while the bacteria subject to unfavorable temperature conditions had the lowest (avg. 56.67 colonies). Glucose, in particular, acts as a major energy source for bacterial growth. By supporting growth, the colonies grown while fed glucose were in most abundance. On the flip side, colonies grown under unfavorable temperature conditions grew extremely slowly. Their organic processes occurred at a slower rate, resulting in lesser growth and least abundance. Moreover, some bacterium may have gone into dormancy, further depleting bacterial abundance.

It was believed that the moist colonies would have the best reproduction rate while the dry bacteria would have the worst. This hypothesis was partly correct. The experiment showed that The moist colonies had the best reproduction rate (+32.3 colonies) while the unfavorable temperature bacteria had the worst (+13 colonies). Since moisture helps dissolve nutrients, the moist colonies were able to absorb all nutrients present in the agar. As a result, they flourished and had the best reproduction rate. In contrast, the cold bacteria had the worst reproduction rate due to the unfavorable temperature forcing colonies into dormancy, stopping growth completely. Since there was less growth, there was less reproduction as well. As a result, the unfavorable temperature bacteria had the worst reproduction rate.

It was theorized the bacteria grown while fed glucose would be most resistant to bleach while the colonies grown while subject to dry conditions would be the least. This hypothesis proved to be correct. The experimental outcome showed that the glucose-fed colonies had the highest resistance to bleach (avg. -4.67 colonies) while the bacteria subject to dry conditions had the lowest resistance (avg. -17 colonies). Since the glucose-fed colonies were given a provided energy source, they were able to grow stronger by consuming necessary nutrients. As a result, the glucose-fed colonies also had the best resistance to bleach. The dry colonies, on the other hand, were surrounded by nutrients, but struggled absorbing it due to lack of a water solvent. Consequently, the colonies grew malnourished and weak. Thus, they were the least resistant to bleach.

Sources of Error:

Many improvements could be made to this project. For the favorable temperature plates, I had set my incubator on the ground. Since it wasn't very tall, I once accidentally tripped over it. Luckily, nothing broke, but the incubator did fall. As a result, the bacteria inside was disrupted, and this error may have caused the favorable temperature plates to grow less bacteria than what would be grown if this mistake did not occur. In the future, improvements could be made by ensuring the incubator is set on a table where it could easily be seen. Additionally, regarding the size of petri dishes, I had 18 plates at home. However, since I needed 24, I decided to order plates from Amazon. However, I didn't check the size of these petri dishes, and ended up ordering large size petri dishes. As a result, the UV light colonies and the control bacteria were grown on petri dishes larger than the other conditions. Consequently, the colonies may have grown in larger abundance than would be grown in medium sized petri dishes. They also could've taken on different growing patterns due to the larger space. To improve, I could ensure that next time I'm ordering petri dishes, I make sure to check the size of the plates I'm buying. Variables such as the amount of agar on each plate could've been better controlled, as I did not measure the agar and instead simply poured enough so that the bottom of the dish was barely covered. This could have altered the amount of nutrients on each plate, giving advantages to some bacteria while disadvantage to others. To improve, I could use devices like syringes to ensure each plate gets the same amount of agar.

Applications:

This experiment is relevant in hospitals, daycares and elderly homes. People in these places are most vulnerable to bacterial infections and disease. By growing B. megaterium in various environmental conditions, my project gives insight on how similar bacteria would perform as well– specifically bacteria in the Bacillus cereus group. B. cereus, cytotoxicus and weihenstephanensis all cause food poisoning-type illnesses when ingested. These illnesses cause gastrointestinal issues, notably vomiting, abdominal pain, and diarrhea. Children, in particular, are at the highest risk for food poisoning. In general, around 4 million Canadians are food poisoned yearly. Of these 4 million, 11,600 people fall into critical condition and 238 die. Of the Bacillus group, B. anthracis causes a disease known as anthrax. When anthrax enters the body through the skin, large dark lesions form in the surrounding area. The lesions are itchy and develop into sores. When ingested (usually through uncooked meat), anthrax enters the bloodstream and can cause nausea, gastrointestinal illness, internal bleeding, meningitis and other membrane/mucus issues. Although anthrax is relatively rare, with only about 2000 cases yearly, it is dangerous. If left untreated, it is fatal, and even with treatment 45% die. By understanding what conditions improve bacterial growth, reproduction and strength, we can prevent bacterial growth by disrupting growth cycles and determine cleaning

methods to ensure daycares and elderly homes steer clear of food poisoning-related diseases and bacterial infections.

Next experiment:
To continue this line of study, a next experiment could be to test how different Bacillus bacteria grown at all optimal conditions respond to various antibacterial measures. In this experiment, the bacteria would be B. megaterium, cereus, anthracis, weihenstephanensis and cytotoxicus. Optimal conditions would include fed glucose, grown at favorable temperatures, and grown in moist environments. Antibacterial measures could be exposure to bleach, chlorine, boiling water, and hydrogen peroxide. This experiment would add onto safely killing Bacillus bacteria to improve sanitation measures in places where people are at most risk for bacterial-born illness, such as hospitals, daycares and elderly homes.

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