جامعة بيرزيت

BIRZEIT UNIVERSITY

Faculty Of Pharmacy, Nursing and Health Professions

Nutrition and Diet

General Microbiology laboratory

Report #2: Effect of physical agent on bacterial growth

Section: 2

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Group participants name and ID numbers:

Objective

- To study and test the effects of various physical factors on bacterial growth (filtration, temperature, osmotic pressure, radiation) and know the results, so we can regulate bacterial growth and thereby pathogenicity.
- To study the difference between the two types of heat (moist, dry heat) are used for sterilization or killing of microbes.
- To distinguish between agents that stop bacteria from growing (static effect) and those that kill them (cidal effect).
- When studying all these cases affecting bacteria, we can control the effect of bacteria on food and mitigate diseases caused by bacteria (somewhat).

Introduction

bacteria can be found in any environment we are exposed to, they are present in the bodies of humans and other large mammals, in harsh environments such as the scorching water streams of volcanoes, or in deep caves devoid of oxygen and sunlight. İt also occurs in two different types of environments: aerobic and anaerobic. Bacteria can survive in any environment if they have food as an energy source. Controlling bacterial growth is critical to preventing disease and the transmission of pathogens from one organism to another. Bacterial growth can be slowed or stopped by using physical and chemical substances that have a static or killing effect. Temperature, osmotic pressure, and radiation are examples of physical agents used in this experiment to affect bacterialgrowth.

Temperature is an important factor in controlling microbial growth. Therefore, it is important to model growth as a function of temperature and time to predict the number of organisms growing in a given area. Three cardinal temperatures have been studied and tested that can limit or promote bacterial growth, they have been described as the minimum temperature such that less than the minimum temperature inhibits microbial growth due to loss of membrane function and causes a static effect. [1]

Materials

1- Nutrient agar plates
2- Water bath at 37 ,55,75,95 c
3- Sterile swabs
4- Stock cultures of E. coli, B. subtilis, S. aureus and M. luteus.
5- Sterile glass tubes
6- Nutrient agar(control), Nutrient agar with 5% NaCl, Nutrient agar with 10%Nacl, Nutrient agar with 15% NaCl

Methods (procedure)

- Effect of temperature on spore-forming and non-spore-forming bacteria:

took some E. coli and B.subtilis were cultured in test tubes, and 8 plates were taken, each plate was divided into 8 sections according to time (0) min (control), (5) min, (10) min, (20) min, (30) min, (40) min, (50) min and (60) min. The bacteria were cultured in the first section and this was considered as the zero-time section, then the test tube was exposed to temperature for 5 min and then the bacteria were cultured on the plate, after that, the test tube was exposed to the same temperature for another 5 min and then cultured on the third section on the agar plate. Again, the test tubes were exposed to the temperature for another 10 minutes and then cultured in section 4. These steps were repeated one more time until the last section was cultured with the test tubes exposed to temperature for 60 minutes. The steps were performed twice by each group, once for E. coli and once for B. subtilis, and each group was cultured at different temperatures (4, 37, 75, 95, 55) °C, then the plates were incubated at 37 C for 24 hours.

- Effect of osmotic pressure on bacterial growth:

There were 4 plates, each plate was divided into two halves, E. coli was sprinkled in one half and S. aureus was sprinkled in the other half. The first plate was filled with nutrient agar, the second plate was filled with nutrient agar with 5% NaCl, the third plate was filled with nutrient agar with 10% NaCl, the fourth was filled with nutrient agar with 15% NaCl. Finally, all plates were stored in an incubator at 37 °C for 24 hours.

- Effect of ultraviolet light on bacteria, takes place in 4 steps:

  First, five nutrient agar plates were sprayed with M. luteus using sterile swabs, then the swab was dipped into the culture and all excess liquid was removed by pressing the swab against the side of the tube, then the plate was sprayed so that the entire surface of the agar was covered with organisms. The three plates were then irradiated with UV light as follows: the lid of the three plates was removed and a piece of cardboard with a "V" throw was placed over the agar; the first plate was irradiated with UV light for 5 seconds, the second for 10

seconds, and the third for 30 seconds. Finally, the lids were replaced and the plates were incubated at 37 °C for 24 hours. Third, the lid was put on and the box with the "V" cut out above the fourth plate was irradiated with UV light for 60 seconds and then incubated with the other plates at 37 °C for 24 hours. The fifth plate served as a non-irradiated control and was incubated for 24 hours at 37 °C with the other plates. Then the results were recorded.

Results

- Temperature effect on bacterial growth:

E. coli:

Time/ temperature	0 (control)	5	10	20	30	40	50	60
4	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth
37	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth (colonies appear)
55	Less growth	No growth	No growth	No growth	Normal growth	Normal growth	Normal growth	Normal growth
75	Normal growth	Less growth	Less growth	Less growth	Less growth	Less growth	Less growth	Less growth
95	Normal growth	Less growth	Less growth	No growth	No growth	Less growth	No growth	No growth

Bacillus subtilis:

Time / temperature	0 (control)	5	10	20	30	40	50	60
4	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth
37	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth
55	Less growth	Normal growth	Normal growth	Less growth	Normal growth	Normal growth	Normal growth	Normal growth
75	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth	Normal growth
95	Normal growth	Normal growth (colonies appear)	Normal growth	Less growth	Less growth	Less growth	Normal growth	Less growth

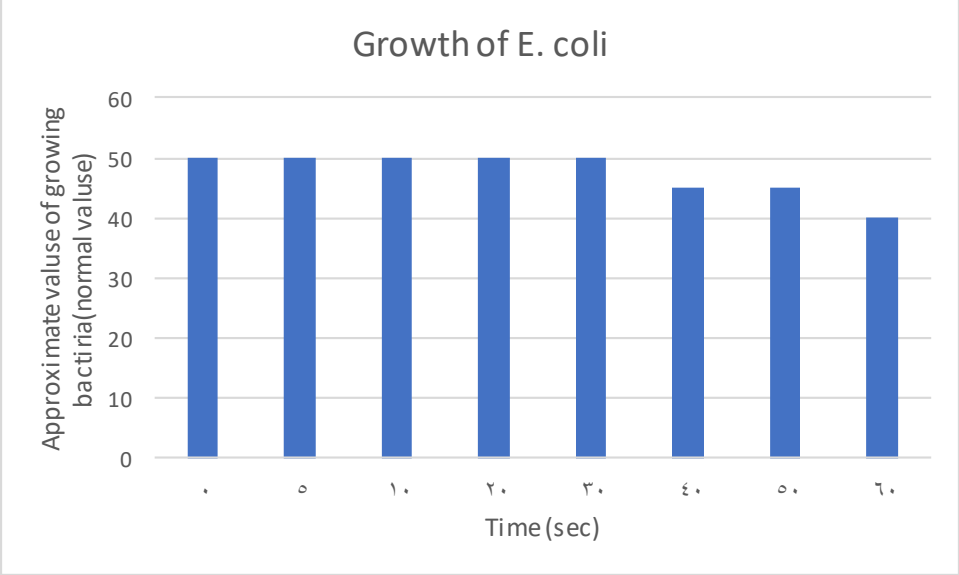

Chart 1: the relationship about growth of E. coli between time and the size or values of growth in 4° C.

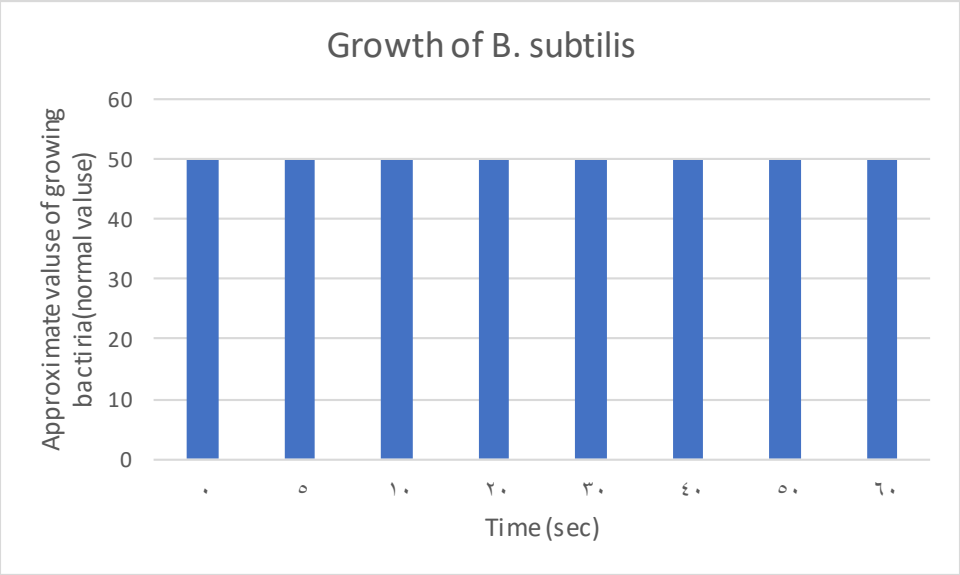

Chart 2: the relationship about growth of B.subtilis between time and the size or values of growth in 4° C.

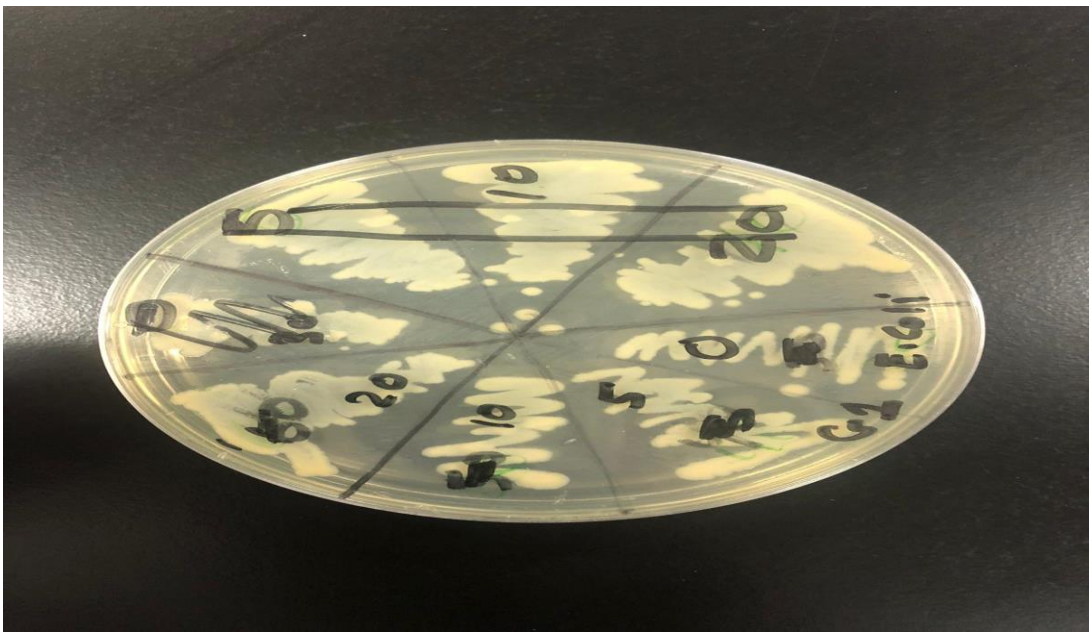

Figure 1: E. coli in 4 C temperature.

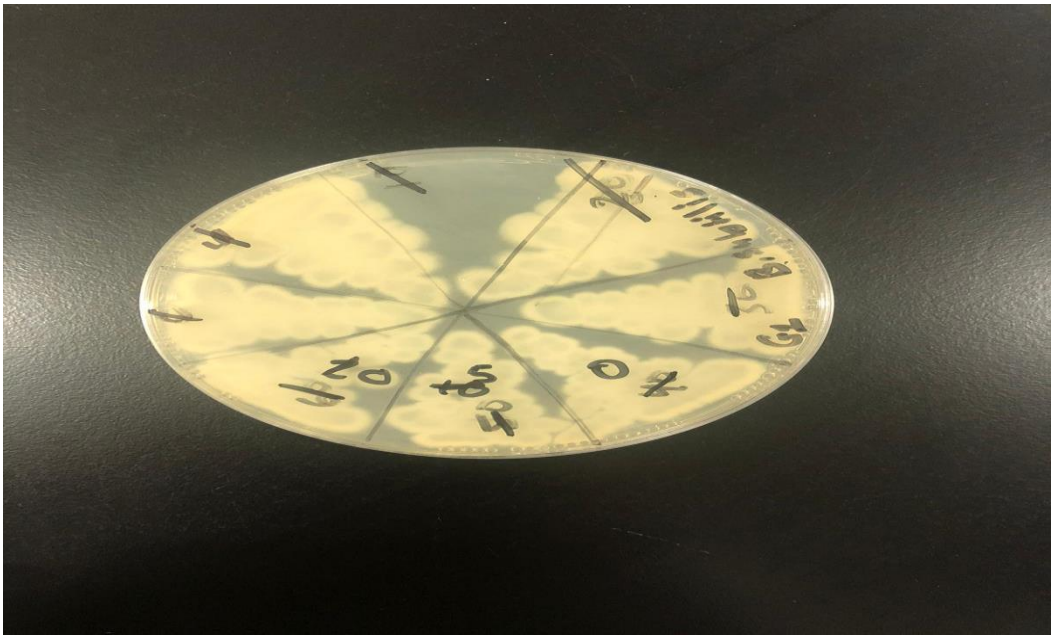

Figure 2: Bacillus subtilis in 4 C temperature.

Figure 3: E. coli in 37 C temperature.

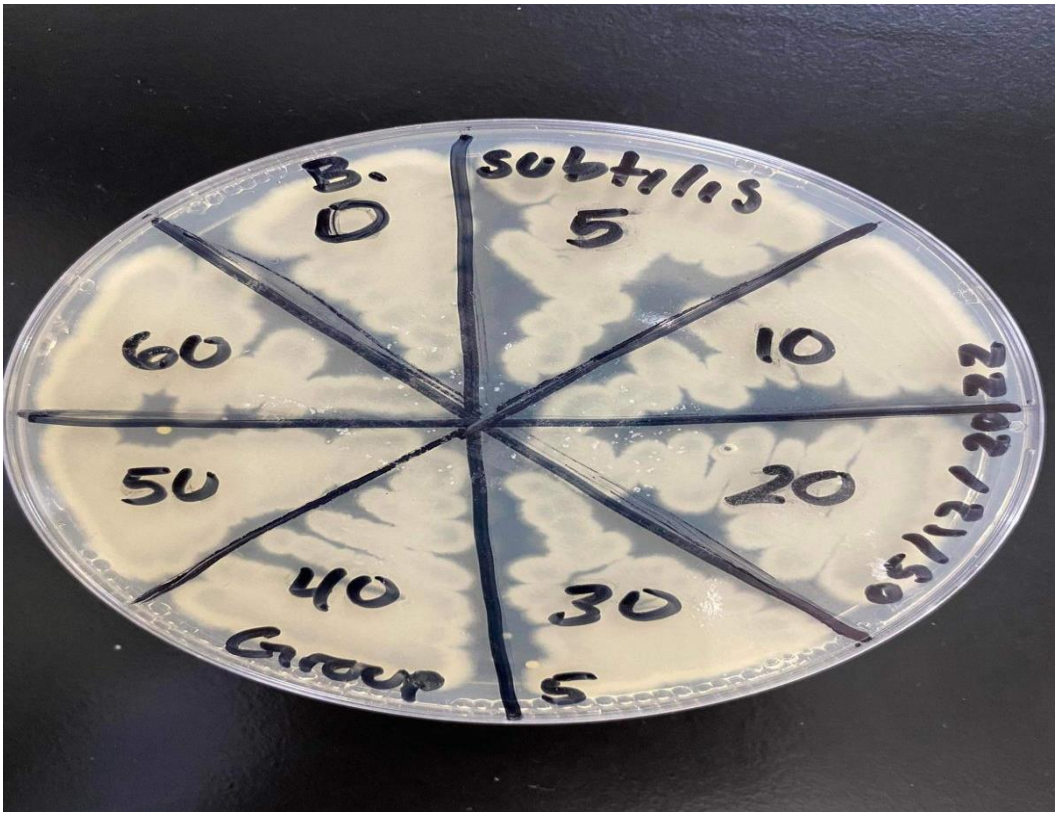

Figure 4: Bacillus subtilis in 37 C temperature.

Figure 5: Bacillus subtilis and E. coli in 55 C temperature.

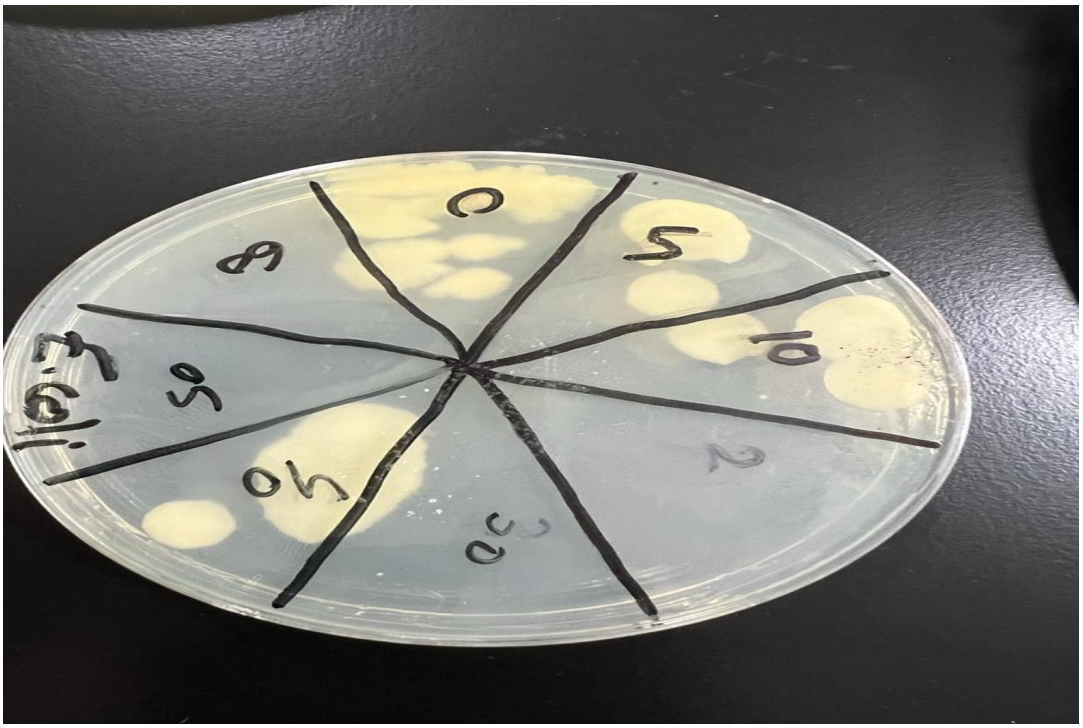

Figure 6: E. coli in 95C temperature.

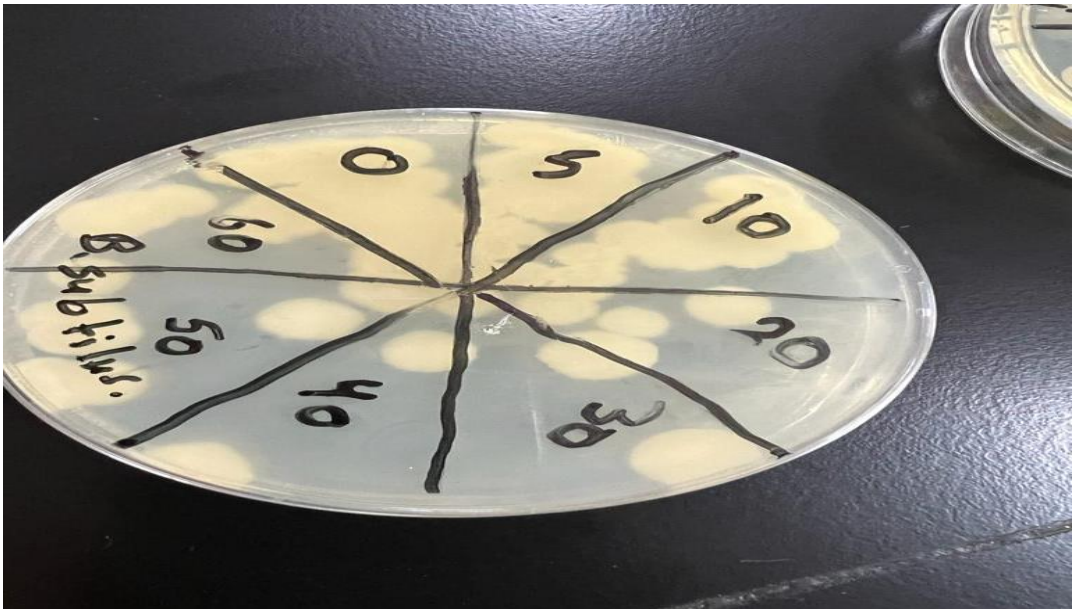

Figure 7: Bacillus subtilis in 95 C temperature.

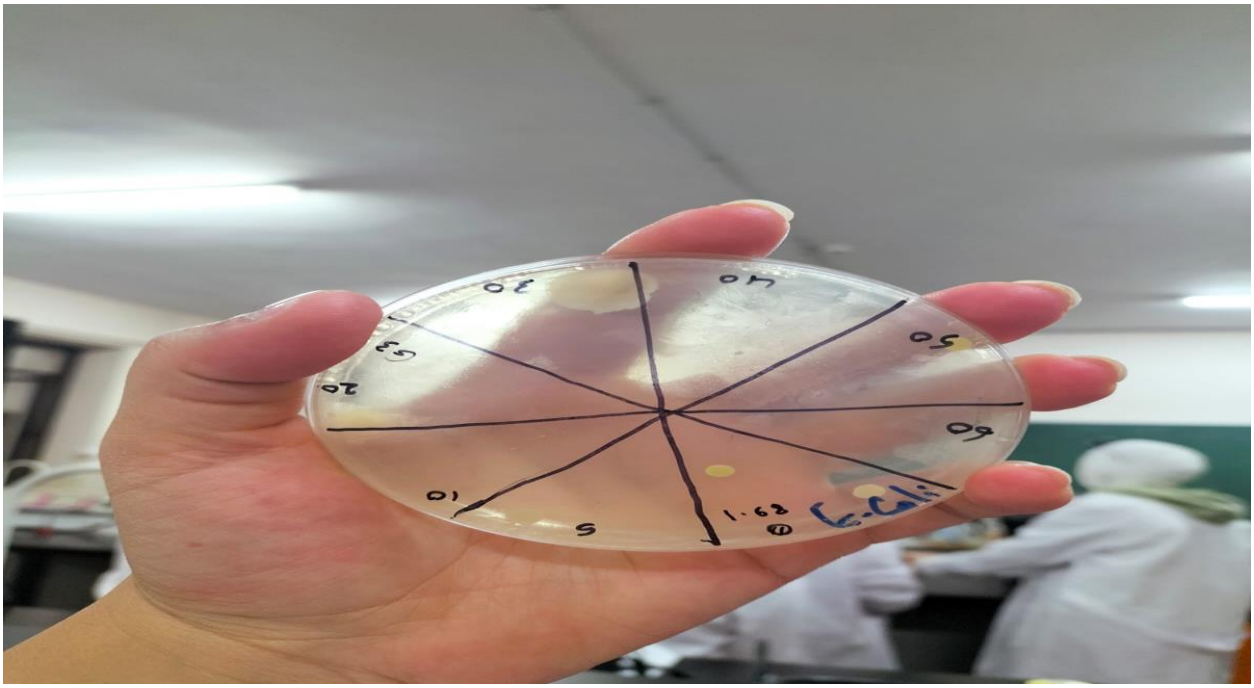

Figure 8: E. coli in 75C temperature.

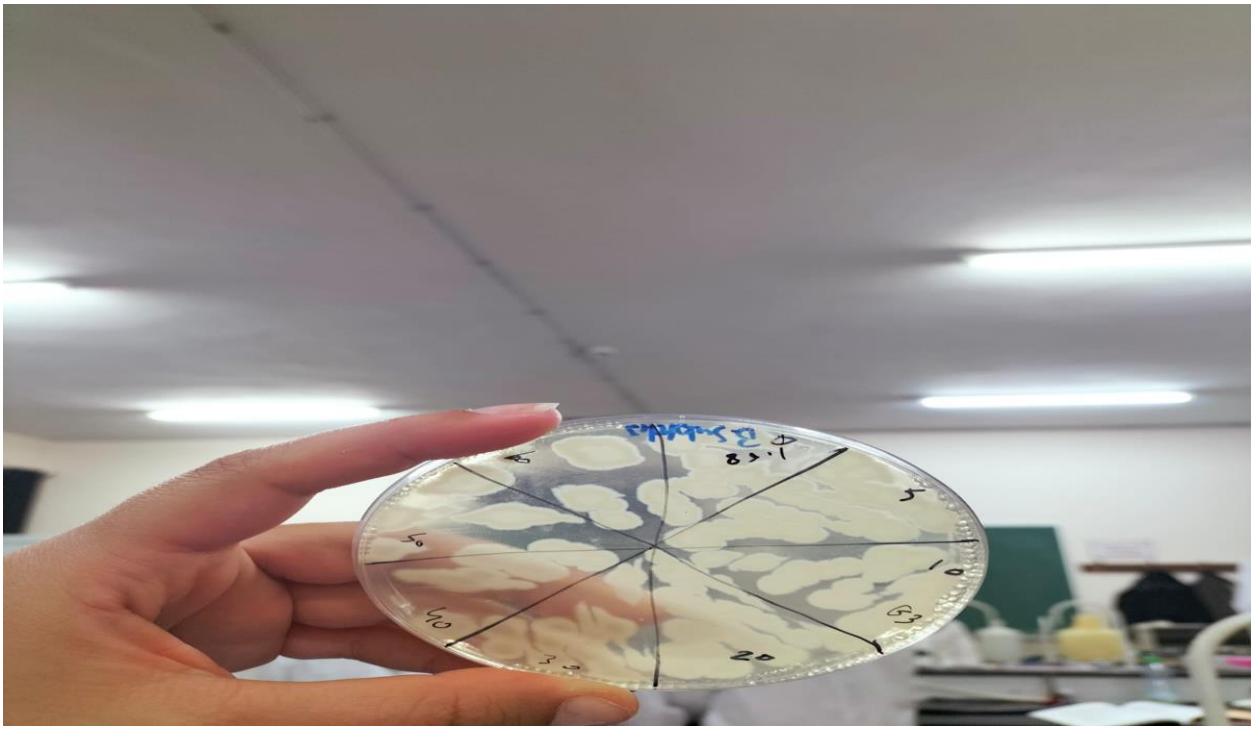

Figure 9: Bacillus subtilis in 75 C temperature

- Effect of osmotic pressure on bacterial growth:

E. coli

concentration	Control	5%	10%	15%
Effect on growth	Normal growth	Normal growth	No growth	No growth

S. aureas

concentration	control	5%	10%	15%
Effect on growth	Normal growth	Less growth	Very less growth	No growth

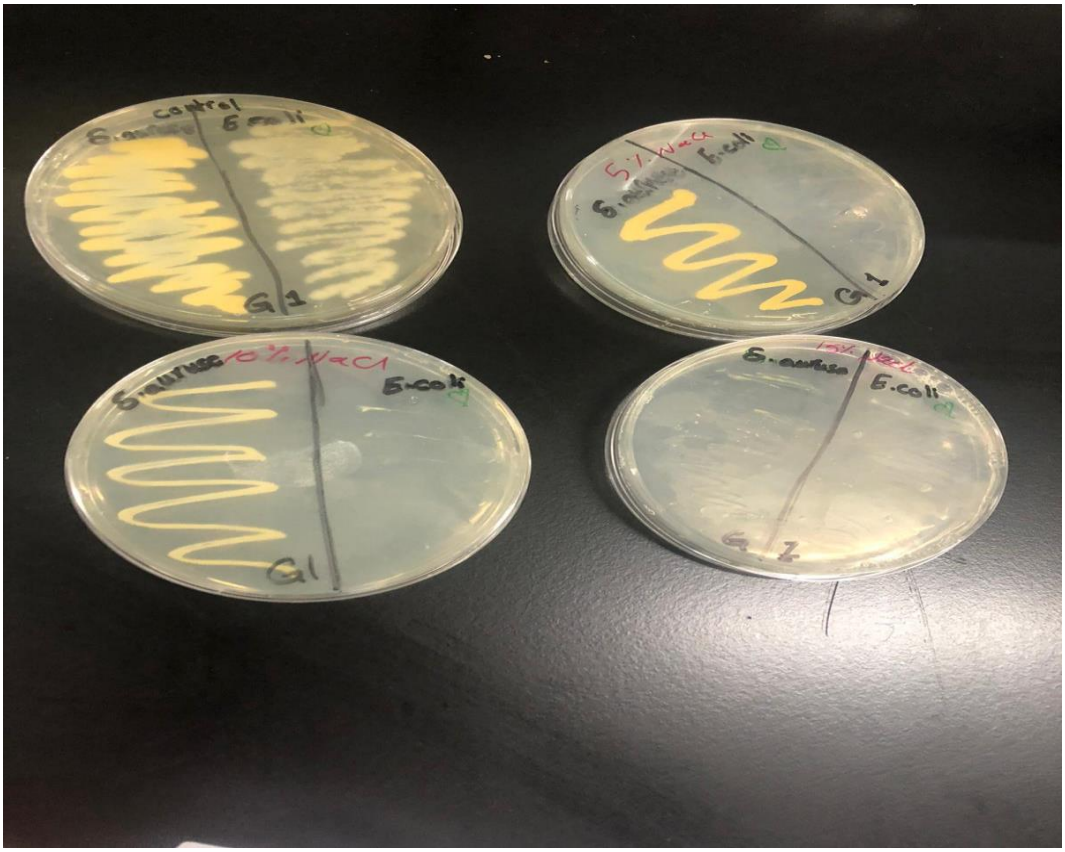

Figure 10: Bacillus subtilis and E. coli in effect of osmotic pressure on bacterial growth

- Effect of ultraviolet light on bacterial growth:

B. subtilis:

Time in seconds	1	5	10	35	45	60
Effect on growth	Growth	Growth	Growth	Growth	No growth	No growth

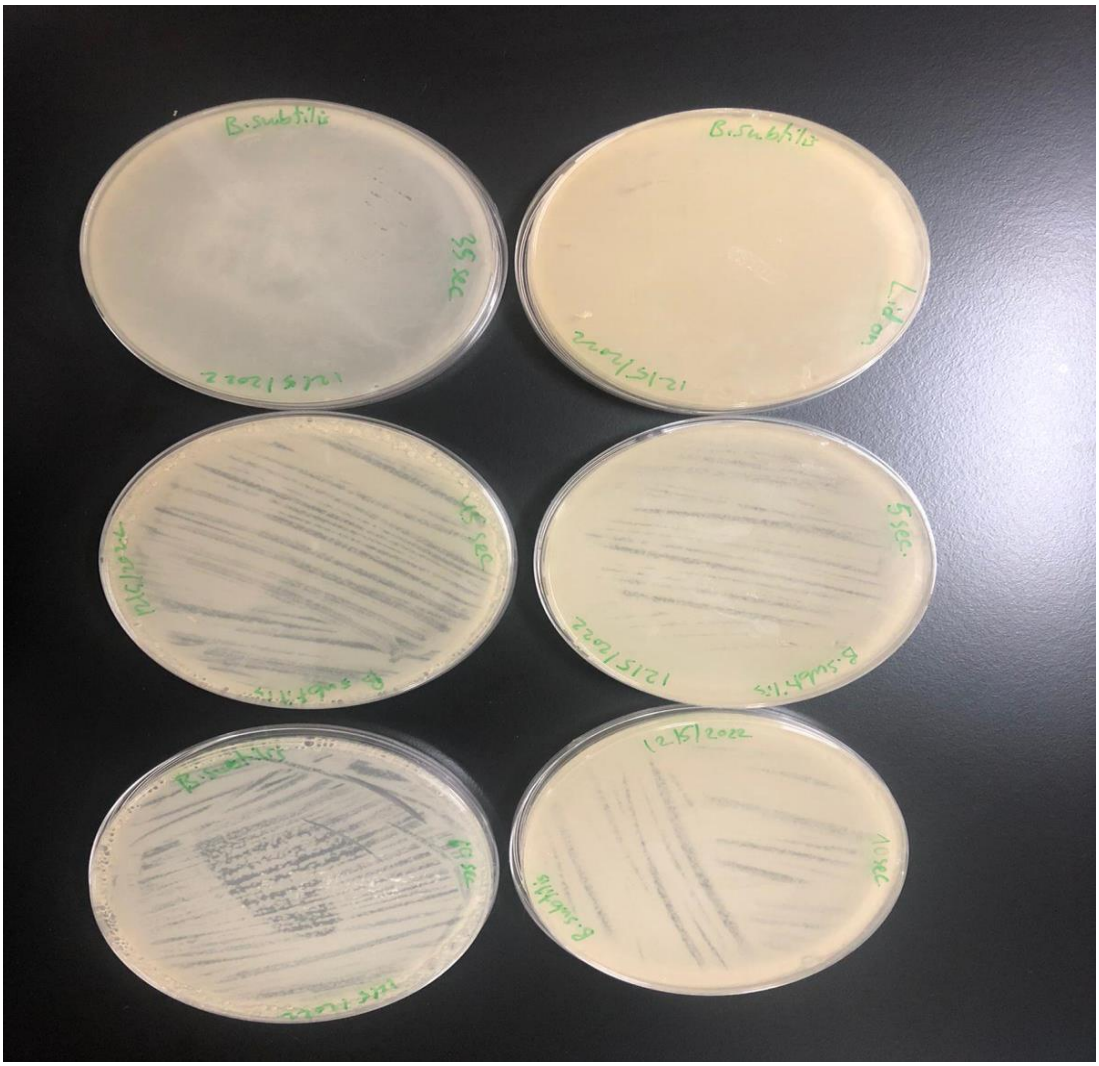

Figure 11: Effect of ultraviolet light on bacterial growth results for B. subtilis.

Discussion

- Effect of osmotic pressure on bacterial growth:

Salt nutrient agar media in the agar plate affect the isotonicity of the bacterial cell, resulting in shrinkage of the cytoplasmic membrane or plasmolysis (water flow from inside the cell to the media), which is known as a hypertonic environment. As a result, the bacterial cell becomes dehydrated and its growth is inhibited, although some can tolerate a hypertonic media, as shown in the experiment. Staphylococcus aureus is a salt-tolerant eubacterium that grows well in high-NaCl conditions (as much as 15% NaCl extremophile) [2]. E. coli can grow in a salt concentration more than (3_4) percent (non-halophile).

E. coli stopped growing after 5%, S. aureus is meant to grow till 10 %NaCl concentration. (no error happened).

In osmosis, water flows from an area of low solute concentration to an area of high solute concentration until the ratio of solute to water in the two areas is equal. Normally, solute also diffuses toward equilibrium; however, all cells are surrounded by a lipid bilayer cell membrane that allows the flow of water into and out of the cell, but restricts the flow of solute in many circumstances. When a cell is placed in a hypotonic solution, water flows into the membrane and increases its volume. Eventually, the cell membrane is enlarged so that it pushes against the rigid wall of the cell. In an isotonic solution, water flows into the cell at the same rate as it flows out. When a cell is placed in a hypertonic solution, water flows out of the cell into the surrounding solution, causing the cell to shrink and lose its plumpness. Two of the most common substances used to create a hypertonic environment for microorganisms and prevent them from growing are salt and sugar. They are commonly used in food preservation. Here, E. coli and S.aureus were used: Nutrient agar with 5% NaCl, nutrient agar with 10% NaCl, agar with 15% NaCl and the control plate were divided into two halves, an inoculating loop was used to sprinkle one half of each plate with E.coli and the other with S.aureus. They were stored in an incubator at 37 °C for 24 hours. E. coli showed no growth in 5%, 10% and 15% NaCl. But S.aureus showed growth in 5% and 10% NaCl. And there is no growth in 15% NaCl range. This means that Escherichia coli is a non-halotolerant bacterium. Because it showed no growth in the NaCl sections. Bacteria that grow in the absence of salt and in the presence of high salt concentrations are called halotolerant. S. aureus is an extremely halotolerant pathogenic bacterium with high osmotic stress tolerance that is commonly found in aquatic production and preservation (3). Therefore, it has been cultured in a high salt concentration. Staphylococcus aureus can grow in the presence of NaCl concentrations up to 3.5 M.

- The influence of temperature on bacterial growth:

The ideal temperature for E. coli 37°C, with a temperature range of 4_45°C. It can withstand refrigeration and freezing. Water heated to 60°C for 5 minutes and to 70°C or 100°C for any amount of time destroys all E. coli bacteria (4) The endospore of B. subtilis is killed at (95-100) ° C, while the bacteria is killed at (85- 90) ° C. " C (95- 100).

The results are normal, as the tow chart shows and the figures, E. coli and B. subtilis bacteria at a temperature of 4 degrees Celsius grew normal and similar growth and very close in all minutes and this is normal because the refrigerator works on the static system and does not kill bacteria.

The results of our group revealed a number of inaccuracies, including developing fungus (contamination ungraded growth), issues throughout the testing method, and other factors that were not clearly communicated to our group.

- The effect of UV light's effect on bacterial growth:

UV light's most cidal wavelength is between 260 and 270 nm, where it is absorbed by nucleic acid. UV radiation is absorbed by microbial DNA and induces adjacent DNA thymine bases on the same DNA strand to establish covalent bonds, generating thymine - thymine dimers. UV light produces mutations, which can result in incorrect protein synthesis. Bacterial metabolism is halted by enough mutations, and the organism dies. UV rays, for example. [2]

Small portable UV lamps are often used by campers to purify water from natural environments before drinking. Germicidal lamps are also used in operating rooms, biological safety cabinets, and fume hoods, which typically emit UV light with a wavelength of 260 nm. Because UV light does not penetrate surfaces and does not penetrate plastic or glass, cells must be exposed directly to the light source. For the effect of ultraviolet light on bacteria: 6 culture media were coated with M. luteus using sterile swabs and immersed in the culture. Excess liquid was removed by pressing the swab against the side of the tube. The entire surface of the plate was covered with organisms. The lid was removed from each plate and a cardboard box with the letter "M" was cut out over the agar. The first plate was exposed to UV radiation for 5 seconds, the second plate for 10 seconds, the third plate for 30 seconds, the fourth plate for 45 seconds and the fourth plate for 60 seconds. And they were incubated at 37°C for 24 hours. On the fifth plate, the lid was removed. The fifth plate was covered with the lid, marked with the letter "M", irradiated with UV light for 60 seconds and incubated at 37°C for 24 hours. The sixth plate was considered as non-irradiated control and incubated at 37°C for 24 hours. In figure 11 B. subtilis growing in 45 sec and 60 sec and that is right.

Conclusion

Among the numerous physical agents that exert a deleterious effect on microorganisms, only a few have been used for sterilization or disinfection for medical purposes. Temperature is the most important agent that, on the one hand, allows the support of the metabolic processes of psycho-, mezzo- and thermophilic microorganisms in a very wide range, but beyond these limits causes their death. A high temperature leads first to damage of the cytoplasmic membrane and then to denaturation of RNA, resulting in death. On the other hand, low temperature, which slowly drops below 0 degrees Celsius, leads to crystallization of water in cells and destruction of cytoplasmic structures. Ultraviolet radiation causes mutations that lead to disruption of DNA replication in all forms of microorganisms. The same type of lethal effect is exerted by ionizing radiation. Its kinetic energy induces mutations that affect not only individual bases but also entire operons, making gene expression impossible. Gaseous plasma is a new physical agent that has recently been used for sterilization. Using radiofrequency energy, the plasma is generated from hydrogen peroxide vapors in a high vacuum and produces reactive particles from the vapors that collide and kill microorganisms. The use of ultrasound to kill microorganisms, on the other hand, needs further study because there are major differences depending on the frequency and energy used. For example, it is not known whether the destruction of microorganisms by ultrasound is related to the phenomenon of cavitation or thermal energy. Nevertheless, even a frequency and energy range used in commercial microwave ovens kills vegetative cells of coliform rods in about 15 minutes.[3]

References

1) https://academic.oup.com/femsec/article/30/2/101/608571?login=true
2) https://www.google.com/search?q=uv+light+and+thymine+dimers&rlz=1C1GCEA_enPS950PS950&oq=uv+and+thy&aqs=chrome.2.69i57j0i13i30j0i22i30l6j0i8i13i30l2.6518j0j7&sourceid=chrome&ie=UTF-8
3) https://pubmed.ncbi.nlm.nih.gov/9432703/