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LAB EXPERIMENT # 2
STAINING: GRAM STAIN

Introduction:

Unstained bacteria are hard to observe under the microscope because they're practically as clear as the slide itself. The gram stain is one of scientific processes that can be used to identify unknown bacteria under the microscope. The results of the gram staining were successful so we can see the color, the shape, the structure, and arrangement. The gram stain method divides all bacteria into two groups: gram-positive cells that stain purple and gram-negative bacteria that do not. In terms of structure, the chemical composition of the cell wall is what causes a cell to be able to become stained during the gram stain method. The gram stain method divides all bacteria into two groups: gram-positive cells that stain purple and gram-negative bacteria that do not. In terms of structure, the chemical composition of the cell wall is what causes a cell to be able to become stained during the gram stain method. The gram staining method involves attaching a bacterial colony to a slide and then saturating the colony with various chemicals. First, crystal violet dye is applied to the bacterial cells, which causes gram-positive cells to turn purple. Iodine is used to hold the violet dye in place before washing it off the unstained cells with ethanol. Finally, gram-negative cells are stained with a red dye called safranin to make them visible. Gram-positive bacteria appear purple under a microscope when the gram stain method is completed, while gram-negative cells are looking pink.

The purpose of the experiment is to classify the bacteria into gram-positive bacteria and gram-negative bacteria after seeing them under the microscope. So when the bacteria have a pinkish color under the microscope that means it is gram-negative. When the bacteria have purplish color under the microscope, that means it’s gram-positive.

Material:

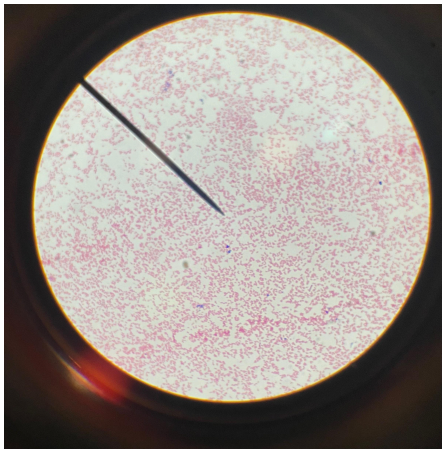
- Microscope
- Microscope slides
- Flame
- Marker
- Agar cultures of *Staphylococcus aureus, Escherichia coli*, and *Bacillus subtilis*
- Timer
- Water
- Loop
- Gram-staining reagent
    a. Hucker's Crystal violet
    b. Gram's iodine solution
    c. Decolorizer, 95% ethyl alcohol
    d. Safranin

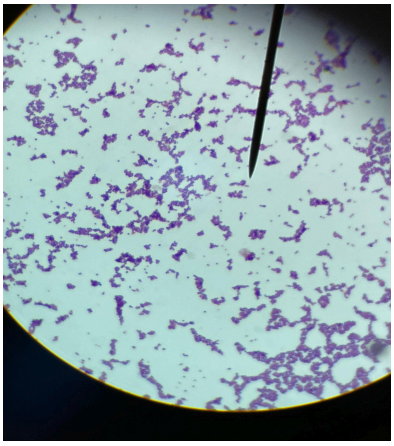
Procedure:

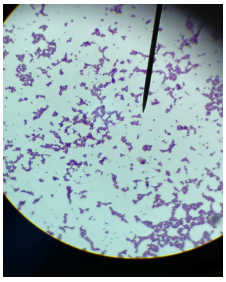
- First, we cleaned the table with disinfectant water, then we washed our hands with water and soup to avoid any contamination.
- After, we did this steps, we start our gram staining method
- The first step we did in the gram staining was label the slide with our initial and bacteria name
- We drew a circle in the middle of the slide then we flip the slide
- Then, we put 1- 2 drops of the water in the middle of the circle, after that we sterilize the loop with flame then we took some of the bacteria from the nutrient agar.

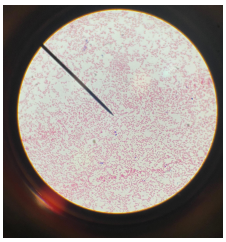
- We spread the bacteria with the water in circle that we draw it in the slide
- Then we dry the slide by hold the slide near to the flame
- After the slide dried, we fix the slide by crossing the slide in the flame 3 times
- After fixing the slide, we start follow the gram-staining reagent
- We covered the smear with Hucker's crystal violet for 1 minute, and then washed with water.
- Then, we covered the smear with Gran's iodine (mordant) solution for 1 minute, and then washed with water.
- After that we decolorized the smear by using acid- alcohol. Then we hold the slide at 45^0 angle and add decolorizer drop wise from above the stained area for 25 seconds. After decolorizing the slide, they were clear or just slightly tinged with purple, and then we washed with water.
- The last step was applying the safranin that know as the counterstain for 30 second the we washed the slide with water and dried the slide by putting the slide close to the flame
- We did the same steps dor of the bacteria

Results:





Bacteria	Shape	Color	Gram-negative/ Gram-positive	Arrangement
Bacillus subtilis 	Bacillus like rod -shaped	Purple color	Gram-positive bacteria	Single Bacillus or Streptobacilli (short chain or single chain, and clumps)
Staphylococcus aureus 	Cocci (spherical)	Purple color	Gram-positive bacteria	Staphylococci (grape of clusters)
Escherichia coli	Bacillus like rod -shaped	Pink color	Gram-negative bacteria	Single bacillus Or Diplobacilli (single cell)

Discussion:

It's so hard to see the bacteria under the microscope without using a gram staining method. The gram staining method is a great method to check different bacteria under the microscope. In this experiment, we used this method to differentiate three different types of the bacteria by gram positive bacteria or gram negative bacteria. These three bacteria were *Staphylococcus aureus, Escherichia coli* and Bacillus *subtilis.* After following the steps that I mentioned in the procedure, we put the slide under the microscope. Under the microscope, *Staphylococcus aureus* bacteria was having a purple color and had cocci shapes that formed as grape-like clusters. So after we saw these results we can say that the *Staphylococcus aureus* is a gram positive bacteria because of the purplish color. For *Bacillus subtilis* appears under the microscope as purple color and has a rod shape that forms clusters and chains. So after we saw these results we can say that the *Bacillus subtilis* is a gram positive bacteria because of the purplish color. For *Escherichia coli* appears under the microscope as pink color and has a rod shape that forms clusters and chains. So after we saw these results we can say that the *Escherichia coli* is a gram negative bacteria because of the pinkish color. The cell wall of *Escherichia coli* has the structure of an outer membrane, peptidoglycan, and plasma membrane. As a result, when alcohol dissolves the outer membrane and leaves holes in the peptidoglycan,

the violet-iodine crystals wash out and turn the bacteria pink when stained with safranin. The outer membrane is absent from the cell walls of *Staphylococcus aureus* and *Bacillus subtilis*, thus when the alcohol dehydrated the peptidoglycan, the crystal violet-Iodine crystals did not escape and the stain remained purple. The *Escherichia coli* on the nutrient agar appear as small, white, round colonies with smooth surfaces. *Staphylococcus aureus* on nutrient agar appears as large, cream or yellow color, golden yellow colonies with smooth surface. *Bacillus subtilis* on nutrient agar appears as small, white, round colonies with slightly wrinkled surfaces. In the end, I can say that the gram staining method was distinguishing between different bacteria.

Conclusion:

Based on the chemical and physical characteristics of their cell walls, the Gram stain is a differential staining technique used to classify bacteria as Gram-positive or Gram-negative. Gram stains are used to assess the presence of bacteria on samples that are placed on glass microscope slides and examined under a microscope. The result of this experiment was that the *Escherichia coli* bacteria is a gram negative bacteria by showing as a pinkish color under the microscope. We know that *Staphylococcus aureus* and *Bacillus subtilis* bacteria are gram positive bacteria by showing purplish color under the microscope. At the end of this experiment, I learnt the difference between these three different bacteria on nutrient agar and under the microscope. Now, I can say that I know the shape, color, and arrangement of the three bacteria after seeing them under the microscope.