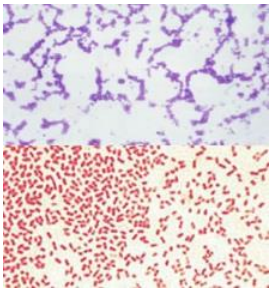


NAME_____ **DATE**_____

Gen Bio 2 Lab #1: Kingdoms Archaebacteria & Eubacteria

Archaebacteria and Eubacteria
Pre-Lab reading: Page 518-520 in 9th edition
Pre-Lab vocabulary

1. Domain

2. Kingdom

3. Culture (bacteria)

4. Oil immersion lens

5. Gram positive bacteria

6. Gram negative bacteria

7. Nitrogen fixation

8. Antibiotic resistance

Pre-Lab Questions:

1. Once thought to be a single group of organisms, the prokaryotes comprise 2 domains and 2 kingdoms: Domain Bacteria/Kingdom Bacteria and Domain Archaea/Kingdom Archaebacteria. **List 3 differences between these 2 groups.**
   a.
   b.
   c.

2. The remaining groups of organisms are combined (for now) into Domain Eukarya. **What are the Kingdoms included in this Domain?**

3. **Draw the phylogeny for the 3 Domains.**

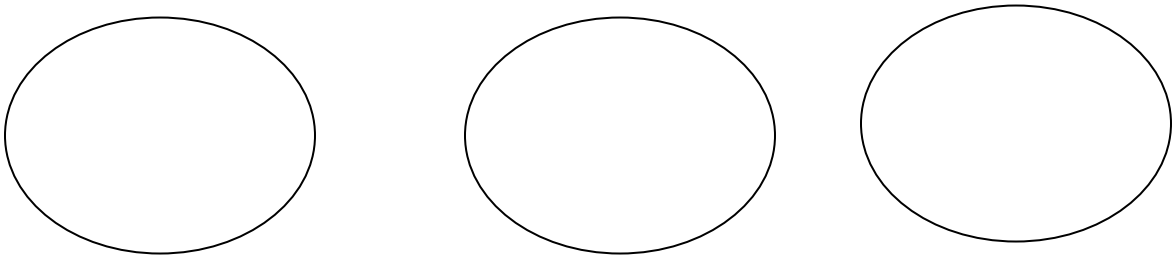
Procedure 1: Identifying shapes of bacteria

Materials
Microscope
Composite Bacteria slide
Oil

Procedure 1: Practice using the oil immersion lens to identify stained bacteria on prepared slides.

a. Using the 10X objective, focus the microscope on an appropriate field containing bacteria. Center the bacteria in the field of view, by manipulating the lateral movement knobs.
b. Rotate the nosepiece to the 40X objective and refocus with the fine focus. Again, center the object which you wish to examine.
c. Rotate the nosepiece so that an intermediate position between the 40X and 100X objectives is obtained.
d. Place a small drop of immersion oil on the center of the viewing area of the slide.
e. Continue to rotate the nosepiece so that the 100X objective is rotated into the oil.
f. **Do not under any circumstances place the 40x objective in the oil**
g. Use only the fine focus and refocus your specimen.
h. Determine the shapes of the bacteria and draw them in the place provided.
i. Immediately after using the oil, remove any residual oil from the slide and from the front of the 100X objective by gently rubbing with lens paper.

The slides include the 3 different shapes. **Observe the 3 different areas and draw the bacteria you see**.



_____ _____ _____

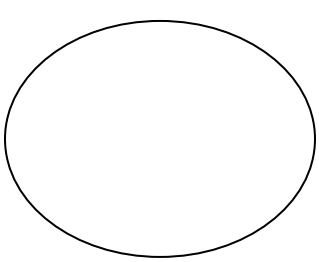
Procedure 2: Gram Staining
Materials
Microscope
Microscope slide and cover slip
Wire loop
Bunsen burner
Distilled water
Crystal Violet
Decolorizer
Safranin

Procedure 2: Gram Staining: We will not culture bacteria, but observing the size and shape of colonies is one way to classify bacteria. One standard procedure used to classify bacteria is the Gram Stain.
Question: What is the basis for the Gram Stain, i.e. what makes bacteria positive or negative?

Now perform the Gram Stain procedure on the 2 different types of bacteria available today, _____ & _____. **Read the procedure carefully before proceeding. Your instructor will demonstrate the technique of transferring the bacteria to a slide.

Gram Staining

1. Work in groups of 2 each pair doing on bacteria and sharing their results with others at their table that do the other bacteria.
2. On a clean slide place a drop of distilled water.
3. Flame your wire loop ----run it though the open flame
4. Using the wire loops provided obtain a small sample of bacteria and rub it around in the drop of distilled water.
5. Flame your wire loop
6. Heat fix your bacteria to the slide-use clothes pin provided holding one end of slide while you pass the slide over the flame until dry. DO NOT OVER HEAT!!!!!!!!!! Let cool to room temperature before continuing.
7. Once cool cover your bacteria with Crystal violet—let set for 1 minute.
8. Rinse off with water.
9. Cover bacteria with iodine—let set for 1 minute.
10. Rinse off with water.
11. Decolorize using the DECOLORIZER (pour over slide continuously) until no color is running off of slide. No more than 30 seconds
12. Rinse off with water.
13. Cover bacteria with Safranin-let set for 1 minute.
14. Rinse off with water.
15. Blot dry or let air dry (best).
16. Examine under microscope. –find dark shape—then use oil emersion.
17. Draw **your bacteria** and **find a lab mate with the other sample of bacteria and draw theirs** as well.
18. To clean your slides: dip several times in bleach solution and then wash, rinse and dry each slide and place back in the dish.



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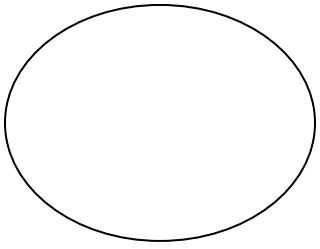

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Questions:

What is your conclusion about the 2 bacteria i.e. are they Gram + or Gram -?

What is the shape of each of the bacteria types?

Look up each bacterium online and list 3 facts about each one, citing your sources. (*Wikipedia is not a source, because it is crowd-edited***)**

Procedure 3: Root nodules

Materials:
Preserved Specimen
Prepared slide

Most people are familiar with bacteria as disease causing organisms, however, bacteria also perform many beneficial functions. Observe the root nodules in the preserved specimen and then observe the prepared slide of a cs. (cross section) of one of the root nodules. Look for the black "stuff" on the slide. See **Figure 55-9** in your text for an example image of nitrogen fixation.

Questions:

What do these bacteria do for the plant?

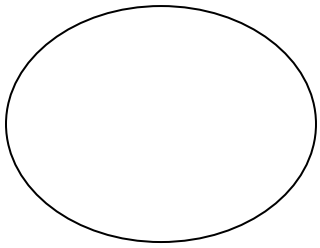
What does the plant do for the bacteria?

The relationship between the plant and the bacteria is called _____.

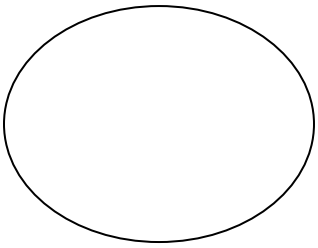
Procedure 4: Cyanobacteria

Materials:

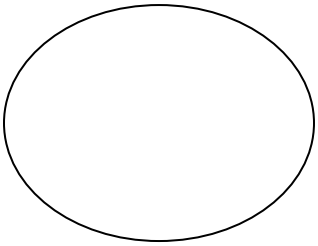
Procedure 4 Most cyanobacteria like to live together in a gelatinous "*condominium colony*" (i.e. they are ***colonial***). Observe each of the prepared slides under the microscope and draw each one.



Oscillatoria



Gleocapsa



Anabaena

Questions:

What causes their blue-green color?

What beneficial function do they perform?

CLEAN UP:

1. Make sure all of your slides have been washed, rinsed, dried and returned to the dish.

2. **Microscope clean-up:** clean the objective lenses using lens paper (70% alcohol on lens paper will remove oil), clean the stage with 70% alcohol, and any other spills on them. Do this before returning your scopes to their cabinets.

2. **Table clean-up:** First, use 70% alcohol on your table and wipe clean with paper towels. Second, wipe down with Fabuloso.

3. **Self clean-up:** Wash your hands with hot water and soap, scrubbing all surfaces for at least 30 seconds.

Questions to e x p a n d your mind.

1. List and explain 5 beneficial uses for bacteria.

2. You may have read in the newspaper or heard your parents talking about bacteria that are resistant to antibiotics. This means that streptococcus or harmful bacteria may not die when a person takes an antibiotic. Based on what you have learned about bacteria, why do you think they can become resistant?

3. What was the single most surprising fact you learned about bacteria?