AP BIOLOGY
DIVERSITY OF LIFE
ACTIVITY #2

NAME______________________

DATE___________HOUR_____

PROKARYOTES

PART I: GRAM STAINING OF BACTERIA

INTRODUCTION

Gram stain is commonly used to assist in bacterial identification. This stain, first developed in 1884, separates bacteria into groups, depending on their reaction to this stain. Bacteria react by testing **gram-positive, gram-negative**, **gram-variable**, with the first two groups being the most common. The response of cells to the stain is due to differences in their cell walls. Cell walls of gram-positive bacteria are predominantly a complex protein-carbohydrate polymer, **peptidoglycan**, located outside the plasma membrane. Cell walls of gram-negative bacteria are thinner, with less peptidoglycan present. The wall in gram-negative bacteria is a polysaccharide-protein-lipid complex. Studies of bacterial taxonomy have show that these differences define major taxonomic groups.

Gram stain relies on the use of two dyes, crystal violet (purple) and safranin (pink). Cells with the peptidoglycan wall retain the purple dye and are **gram-positive**. Those cells that retain the pink safranin are **gram-negative**.

In this lab, you will prepare and stain slides of five different bacterial species to determine how each reacts to gram stain.

MATERIALS

At the "smear" station:

- Bunsen Burner
- Burner striker
- Inoculating loop
- Beaker of distilled water
- Bacteria cultures
- Clean microscope slide

At staining station:

Staining jars of:
- Crystal Violet
- Gram's Iodine
- 95% Ethyl Alcohol
- Safranin

1000 mL beaker with tap water

PROCEDURE -- "SMEAR" PREPARATION

In this section of the lab, your group will prepare a smear of each of the bacteria cultures. Make sure you carefully follow the steps below to avoid contamination of the bacteria cultures and of the lab area.

1. Carefully light the burner. Adjust the burner so you have a blue flame.

2. Sterilize the end of the inoculating loop by placing it in the flame of the burner until the wire turns bright red.

   CAUTION: *Use care with the open flame of the burner. Do not touch the hot end of the inoculating loop. The loop may still be quite hot after the red glow has faded.*

3. With a wax pencil, label one end of the slide #1. Place a loopful of distilled water on a clean, dry microscope slide. Sterilize the loop. Allow the loop to air cool before proceeding to the next step.

4. Lift the petri dish lid of the bacteria culture. Gently touch the sterile inoculating loop to a bacteria colony. The bacteria should be barely visible on the loop. Replace the petri dish lid. Spread the bacteria sample on the microscope slide by mixing it with the drop of water.

5. Sterilize the loop. Allow the loop to air cool before proceeding to the next step.

6. Allow the smear to dry by waving the slide over the Bunsen burner flame. To prevent the smear from cracking and peeling from the slide, do not heat the slide more than the fingers can comfortably stand.

7. After the film is thoroughly dry, FIX it by passing it quickly through the flame two or three times. Be sure the specimen side of the slide is up when fixing. Allow the slide to air cool before proceeding to the staining steps. Turn the burner off.

8. Repeat steps 1 through 7 for each culture of bacteria. Be sure to label each slide with the number of the bacteria culture.

PROCEDURE -- GRAM STAIN

9. Use a clothes pin to hold the slide during the staining procedure. Place the smear in the crystal violet for 60 seconds.

10. Rinse the slide quickly (a few seconds) in tap water and immediately place it in the Gram's iodine solution for 60 seconds.

11. Rinse the smear gently in tap water and dry it by blotting with a clean paper towel. (**CAUTION:** Blot gently so that the bacteria are not rubbed from the slide.)

12. Place the smear in 95% ethyl alcohol, a decolorizing agent, for 30 seconds. Blot the slide again with a clean paper towel.

13. Immerse the slide in safranin, a couterstain, for ten seconds.

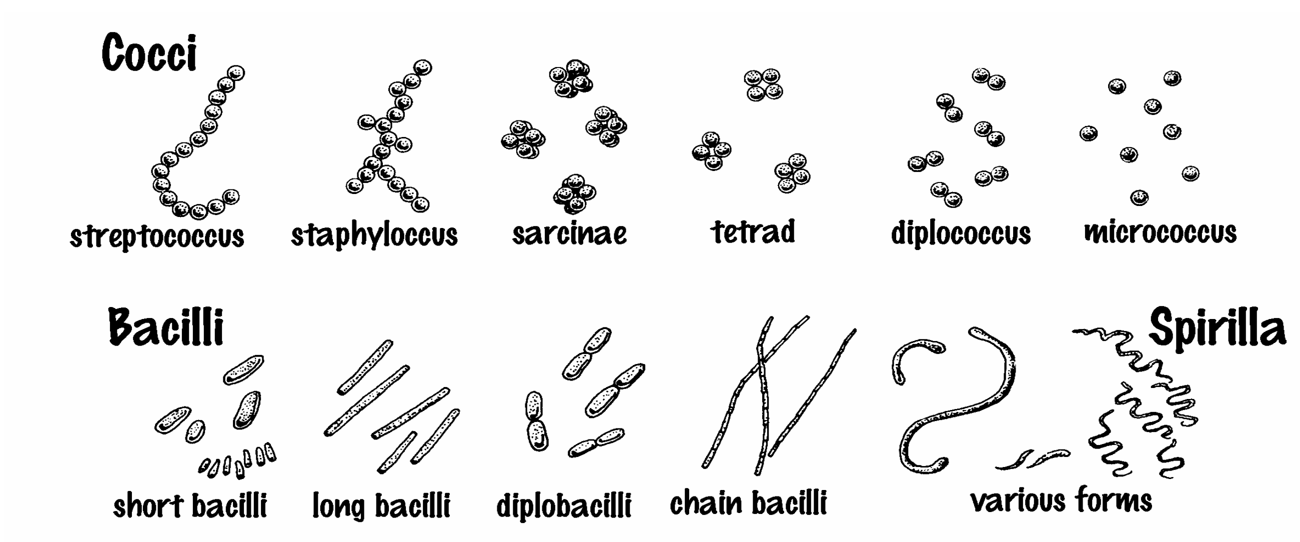
14. Wash the slide in tap water and blot it dry.

15. Examine the slide under high power of the microscope. The gram positive bacteria cells will appear violet in color and the gram negative cells will be red or pink in color.

OBSERVATIONS

16. Complete the following chart. Use the pictures at the bottom of the page to help you identify the cell shape and cell arrangement.

Culture	1	2	3	4
Drawing (in color)				
Magnification				
Cell Size				
Cell Shape				
Cell Arrangement				



17. Use the information below and the information you collected in this lab to match the bacterial culture (1, 2, 3, or 4) to the correct species.

______ *Bacillus cereus* (gram-positive, rod)

______ *Micrococcus luteus* (gram-positive, coccus)

______ *Rhodospirillum rubrum* (gram-negative, spiral)

______ *Serratia marcescens* (gram-negative, rod)

PART II: QUESTIONS

1. At present, the placement of all prokaryotes into Kingdom Monera is being challenged. Why?

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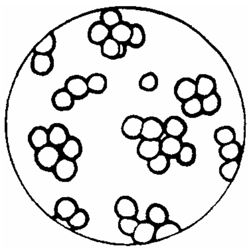
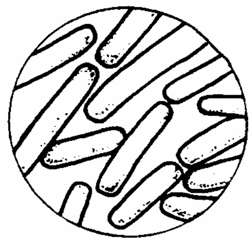
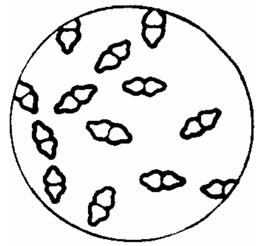
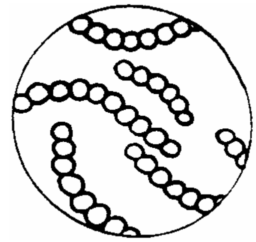
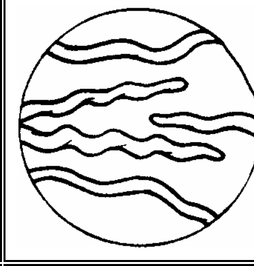
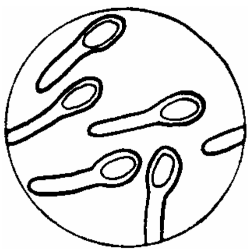
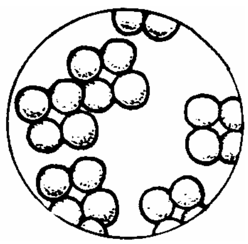
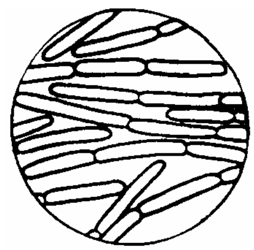
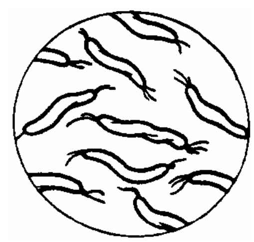
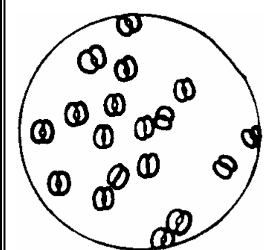
2. The shape and arrangement of bacterial cells are used to identify bacteria. On page 5 are drawings of several disease-causing bacteria. Use the key below to identify the bacteria in each picture.

1	a. Bacillus bacteria	go to 2
	b. Coccus bacteria	go to 4
	c. Spirillum bacteria	go to 7
2.	a. Bacteria arranged in chains	Rat Bite Fever Stain A
	b. Bacteria appear singly	go to 3
3.	a. Bacteria with swollen endospore	Tetanus
	b. Bacteria without swollen endospore	Anthrax
4.	a. Bacteria appear in pairs	go to 5
	b. Bacteria appear in chains	Strep Throat
	c. Bacteria appear in clumps	go to 6
5.	a. Bacteria have pointed ends	Pneumonia
	b. Bacteria without pointed ends	Gonorrhea
6.	a. Bacteria appear in groups of 4 (Sarcina)	Human skin bacteria
	b. Bacteria appear in clusters	Staph infection
7.	a. Bacteria with flagella	Rat Bite Fever Stain B
	b. Bacteria without flagella (Spirochete)	Syphilis

3. What are three functions of the cell wall in prokaryotes?

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____

4. What are peptidoglycans?

____

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5. How do antibiotics affect peptidoglycans?

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6. Identify each of the following as true of gram-positive (**+**) or gram-negative (**-**) prokaryotes.

_______ Simpler cell walls

_______ Cell walls contain large amounts of peptidoglycans

_______ Cell walls more complex

_______ Cell walls contain smaller amounts of peptidoglycans

_______ Have a membrane containing lipopolysaccharides outside the cell wall

_______ Stain purple with Grams stain

_______ Stain pink with Grams stain

7. Why are gram-negative bacteria more often disease causing than gram-positive?

____

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8. List the 3 types of motility used by motile prokaryotes.

____

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9. Match the term with the correct definition

_______ Movement toward or away from a stimulus	A. Chemotaxis
_______ Movement toward or away from light	B. Phototaxis
_______ Movement toward or away from a certain chemical	C. Taxis

10. Explain why a photosynthetic bacterium would exhibit a positive phototaxis. A positive taxis occurs when the bacterium moves toward the stimulus.

____

____

11. The prokaryotic flagellum is not homologous to the eukaryotic flagellum. Use the key below to indicate if the characteristics listed below are true of prokaryotic or eukaryotic flagella.

P = Prokaryotic **E = Eukaryotic**

_______ smaller width	_______ larger width
_______ covered with plasma membrane	_______ not covered with plasma membrane
_______ composed of flagellin	_______ composed of tubulin
_______ 9+2 arrangement of microtubules	_______ no 9+2 arrangement
_______ Rotating motion	_______ Undulating motion
_______ Filament is solid	_______ Made of microtubules

12. Match the term with the correct description of definition.

A. Binary fission **C. Genophore**
B. Endospores **D. Plasmids**

_______ Prokaryotic chromosome

_______ Usually double-stranded; forms a ring; found in nucleoid region

_______ Small rings of DNA

_______ Consist of only a small number of genes

_______ Endows cell with antibiotic resistances or the ability to metabolize unusual nutrients

_______ Method of reproduction in prokaryotes

_______ Resistant cells that contain one copy of the chromosome surrounded by a durable wall

_______ Allows some prokaryotes to withstand harsh conditions

13. Why doesn't mitosis or meiosis occur in prokaryotes?

____

____

14. You have a bacterial infection and the doctor gives you an antibiotic that blocks the protein synthesis of the bacteria in your body. What effect does this antibiotic have on the protein synthesis in your cells? Explain.

____

____

15. Match the definition with the correct term.

_______ Change in DNA base sequence	A. Conjugation
_______ Origin of a new trait	B. Mutation
_______ Major source of variation in prokaryotes	C. Transduction
_______ Direct transfer of genes between two prokaryotes	D. Transformation
_______ Cell takes in and incorporates DNA from the environment	
_______ Transfer of genes between cells via a virus	

16. Use the key below to identify the nutrition type being described.

PA = Photoautotrophs **PH = Photoheterotrophs**
CA = Chemoautotrophs **CH = Chemoheterotrophs**

_______ Energy source is light; Carbon source is organic

_______ Energy source is oxidation of inorganic substances (H_2S, NH_3, Fe^{2+}); Carbon source is CO_2

_______ Energy source is organic; Carbon source is organic

_______ Energy source is light; Carbon source is CO_2

_______ Two modes of nutrition restricted to certain prokaryotes; not found in other organisms

_______ Many prokaryotes, many protists, all fungi, and all animals

_______ Most plants

17. Identify each of the following as true of **S**aprobes or **P**arasites

_______ Decomposers

_______ Absorb nutrients from dead organic matter

_______ Absorb nutrients from the body fluids of a living host

_______ The Chlamydias live inside the cells of a host; leading cause of blindness and causes the most common form of STD in the US.

_______ Prokaryotes found in or on food

_______ Chemoheterotrophs

18. Match the description or definition with the correct term.

_______ Poisoned by oxygen

_______ Cannot grow without oxygen

_______ Cannot use oxygen

_______ Use oxygen if it is present; uses fermentation oxygen is absent

A. Facultative anaerobes

B. Obligate aerobes

C. Obligate anaerobes

19. Listed below are characteristics of each group of Domain Archaea. Use the key below to indicate which group the characteristic applies.

M	=	Methanogens
EH	=	Extreme halophiles
T	=	Thermoacidophiles

_______ Found in mineral springs and thermal volcanic vents on ocean floor

_______ Found in waters of extreme salinity (15 to 20%)

_______ Some are important symbionts in termites & other herbivores

_______ Some are important decomposers in marshes and swamps

_______ Contain the pigment bacteriorhodopsin

_______ Use H_2 to reduce CO_2 to CH_4

_______ Habitat is hot (60^{o}C to 80^{o}C) and acidic (pH 2 to 4)

20. How is photosynthesis in cyanobacteria similar to that in plants?

____

____

21. Bacteria are important in the chemical cycling that occurs on earth. Match the bacterial group with the correct role.

_______ Convert N_2 to NH_3 and NO_3^{--}	A. Autotrophic bacteria
_______ Convert NH_3 to NO_2^{-} & NO_3^{--}	B. Decomposers
_______ Convert NO_3^{-} to N_2	C. Denitrifying bacteria
_______ Fix CO_2	D. Nitrifying bacteria
_______ Support food chains	E. Nitrogen-fixing bacteria
_______ Some produce oxygen gas	
_______ Break down dead organisms and waste	
_______ Return elements (C and H) to the environment	

22. Match the definition or description with the correct term.

_______ Ecological relationship between organisms of different species that are in direct contact	A. Commensalism
_______ Organisms involved in symbiosis	B. Host
_______ Usually the larger symbiont	C. Mutualism
_______ Symbiotic relationship where both symbionts benefit from the relationship	D. Parasitism
_______ Symbiotic relationship where one symbiont benefits and the other is neither harmed nor helped	E. Symbiont
_______ Symbiotic relationship where one symbiont benefits at the expense of the other	F. Symbiosis

23. Legumes are a group of plants that contain nitrogen-fixing bacteria in root nodules.

    a. What does each organism get from this relationship:

       The plant gets:____

       The bacteria get:____

    b. What type of symbiotic relationship (mutualism, commensalism, or parasitism) does this represent?

       ____

24. Humans exploit bacteria and other prokaryotes for scientific and commercial purposes. List 5 of those uses.