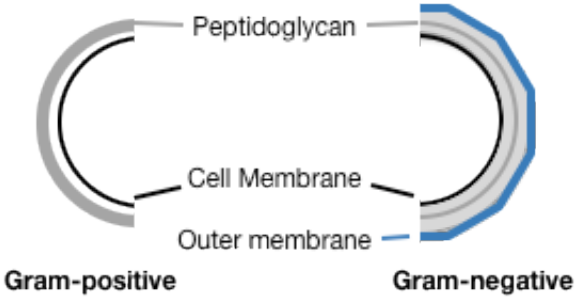
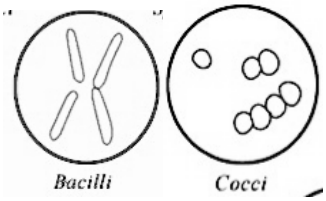
Gram Stain

When a bacteria is isolated from the environment, or from an infected individual it is important to identify the specific type of bacteria that is being dealt with. One of the first steps to identifying bacteria is to perform a Gram Stain. Most bacteria can be classified into one of two groups, each of which will react differently to Gram staining. Gram-positive cells appear purple and Gram-negative cells appear pink due to a difference in the outer layers of the bacterial cell. This differential staining also allows the shape of the bacteria to be better visualized.



Materials



Sterile sticks

LB broth (25 µl)
E. coli culture (50 µl)
Micrococcus luteus culture (50 µl)

3 microscope slides
Grease pencil
Sterile wooden sticks
Bunsen burner/alcohol burner
Gram stain kit
Staining tray

Protocol

1. Using a sterile stick, take a colony from your environmental isolate plate and mix with 25 µL of LB broth in the microcentrifuge tube provided.
2. Label 3 microscope slides E. coli, M. luteus, and Isolate.
3. Using the grease pencil, draw a circle on the slide creating a well.

4. Pipette 5 µl of each liquid bacterial culture into this well (E. coli, M. luteus, and your isolate) on the appropriately labeled clean microscope slide.
5. Spread the drop into a thin film using the pipette tip and allow to air dry. This can take several minutes and will result in a white film on the slide.
6. When the smear is dry, pass the slide through the flame of a Bunsen burner 3 times to heat fix the bacterial cells to the slide. The teaching fellow will have burners set up and assist you.
7. After the slide cools, cover the smear with a thin layer of crystal violet solution, and allow it to sit for 1 minute. (Binds to peptidoglycan)
8. Rinse the slide with water, and then shake off excess.
9. Add a few drop of iodine solution, and allow it to sit for 1 minute.
10. Rinse the slide with water, and then shake off excess water.
11. Add a few drops of 95% ethanol, tilt slide back and forth before draining off ethanol.
12. Repeat step 11 until the ethanol runs clear (additional purple color does not come off).
13. Rinse the slide with water, and then shake off excess.
14. Add a few drop of Safranin, and allow to sit for 1 minute. (Pink, binds to cell membranes)
15. Rinse the slide with water, and then shake off excess water.
16. Dry by carefully blotting between pieces of paper towel.
17. Pass the slide once through flame.
18. Examine the slide with the microscope. The teaching fellow will have several microscopes set up to assist you.