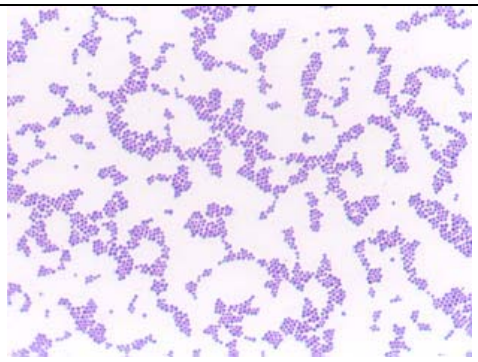
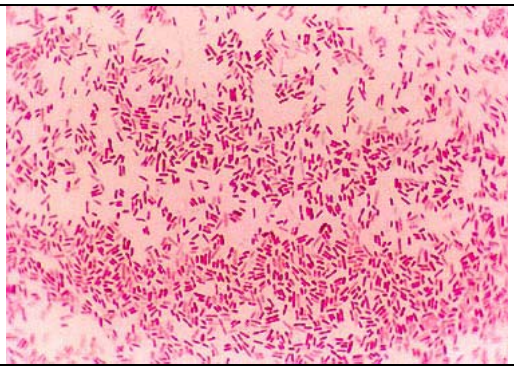
Gram Characteristics of Bacteria

Introduction

Bacteria are interesting little critters in that they are not easily visualized without some sort of stain. In classical methodology, two different kinds of stains have been used to identify bacteria: acidic and basic dyes. Acidic dyes are so called because they consist of a salt that has a cation that transfers no color, but the anion portion is colored and does give off color. Basic dyes consist of an anion that does not give off any color and a cation that does stain biological samples. Examples of acidic dyes include acid fuchsin and eosin; examples of basic dyes include crystal violet, methylene blue and safranin.

As a general rule, basic dyes are attracted to the surface of a bacterial cell (due to ionization of the carboxyl groups in the fatty and amino acids present in the cell wall/membrane). Basic dyes are attracted to the nucleic acid component of the bacterial cell, as well. As a general rule, acidic dyes are attracted to basic (alkaline) subcellular components. The most common stains used to stain bacteria are basic dyes. The most common background stains are acidic dyes since they do not cross the cell wall/membrane particularly easily. A background stain is a stain used to stain everything other than the bacteria.

The Gram stain is one of the oldest, most cost-efficient, yet most under-utilized, staining method used to identify bacteria. It consists of a basic dye (the primary stain crystal violet), a mordant (Gram's iodine), decolorizer (acetone:alcohol) and a counter stain (safranin). A mordant is a compound which helps hold the primary stain to the bacteria. In this case, the iodine forms a complex with the crystal violet to "lock it into" the cell wall/membrane. The decolorizer is used to decolorize the bacteria that "do not like" the primary stain. The counter stain is used to stain those bacteria which were decolorized by the acetone:alcohol. Bacteria which retain the crystal violet:iodine complex are purple-colored and are called Gram positive bacteria. Bacteria that are decolorized by the acetone:alcohol and stained by the safranin are pinkish-orange and are called Gram negative bacteria. While Gram reactions get bandied about on a regular basis, remember that the phrases "Gram positive" and "Gram negative" only apply to the Gram stain -- not any other stain reactions.

	
Gram Positive Cocci	Gram Negative Rods

The special property that differentiates between Gram positive and Gram negative bacteria has been demonstrated to occupy the cell wall. The experiment that demonstrated this phenomenon involved removing the cell wall from Gram positive bacteria and decolorizing the "naked" bacteria after the staining. That which makes this method even more mystical is that even though the chemical composition of the two different kinds of cell walls is well known, the mechanism which retains the crystal violet:iodine complex during decolorization has yet to be elucidated.

The composition of Gram positive cells includes a thick peptidoglycan (carbohydrate/protein molecule) layer, which contains teichoic acid; the cell envelope of Gram positive bacteria contains very little lipid-containing molecules. The composition of Gram negative cells includes a THIN peptidoglycan layer with NO teichoic acid; the cell envelope of Gram negative bacteria contains a large amount of lipid-containing compounds. It is thought that this composition in the Gram negative bacteria is the basis for the removal of the crystal violet:iodine complex by the acetone:alcohol. Lipids are soluble in acetone and alcohol. Perhaps the mechanism is that the decolorizer makes the cell wall/membrane of Gram negative bacteria "leaky" so that the dye complex is "leached" from the bacteria. In any matter, this method serves in a manner analogously for the study of cell membrane receptors.

Experimental

Materials

The table, below, summarizes the supplies you will need to complete this experiment successfully:

Crystal violet	Gram's iodine	Decolorizer
Safranin	Prepared Gram Control Slide	Bunsen burner
Burner tubing	Striker	Disinfectant
Microscope	Bibulous paper	Lens paper
Immersion oil	Staining tray	China marking pencil -- RED

CAUTION: E. coli is pathogenic. Use care when working with it. The decolorizer is flammable. Use no flames in its proximity. Immersion oil loosens glue on the microscope. When you are done with the microscope, wipe the oil off the oil immersion objective and check the others for possible accidental immersion in oil.

Method

Prepare your Gram stain slides as you did the simple stain slides. Remember to label the slides with the name of the bacteria you smeared on them. Let the slides air dry, then heat fix them.

Once the slides are heat fixed, you may begin staining. Flood the target circles with crystal violet for between 15 seconds and one minute. The time is not crucial AS LONG AS you use the same period of time with the Gram's iodine. Once the slides have stained with the crystal violet, rinse the slides with water. Immerse the target circles under Gram's iodine for however long you stained them with the crystal violet. Rinse with water.

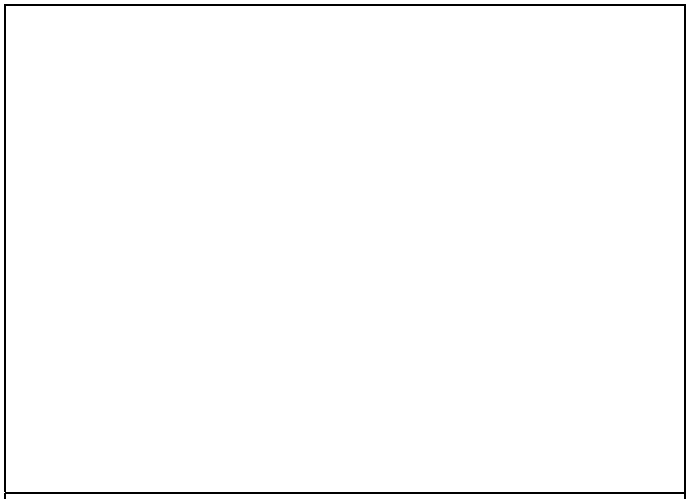
Remove the lids to the bottles of decolorizer and safranin. One slide at a time, now, rinse with decolorizer for 3-5 seconds. To stop the decolorizing, you may do one of two things:

1) Rinse with water, then flood the target circle with safranin for 30 seconds, or,

2) Immediately flood with safranin and let stain for 30 seconds.

When staining is completed, rinse with water and blot on bibulous paper. Repeat for the second slide, as well.

Once the slide has dried, examine it under the microscope as you have in the previous experiments. Record your sketches and observations in the space below:



Gram Stain Results

Since the control bacteria are spherically and rod-shaped, you ought to see rods and cocci under your microscope. The Gram negative bacterium is pinkish-orange, and the Gram positive bacterium is purple. Most rods are obviously rod-shaped and very easy to observe. E. coli, however, is subtly a rod -- in all likelihood, it is really more a coccobacillus than a rod, although it really is longer than it is wide. It, nevertheless, takes a very discerning eye to detect its shape.

When you have completed your work, wipe the oil off the objectives, discard your slides and clean up your bench.

References

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3. Tortora, Case and Funke: Microbiology: An Introduction, Fourth Edition. (Benjamin Cummings: Redwood City, CA) ©1992.

4. Zubay: Biochemistry. (Addison Wesley: Reading, MA) ©1983.