

BIO 201 Lab 3

Experiment 3 Results

Professor Diane Hilker



Overview

I. **Exp. 3: Collection of Microbes**

1. Isolation of molds
2. Isolation of bacteria

Exp. 3: Isolation of Molds

- ▶ Where you successful in isolating molds?



Pure Culture



Not a Pure Culture

Exp. 3: Isolation of Bacteria

- ▶ Where you successful in isolating individual bacterial colonies with the T-Streak method?



- ▶ **Colony:** a visible mass of microbial cells originating from one cell.

Exp. 3: Isolation of Bacteria

- ▶ Mixed Culture Broth: 3 types of microbes
 - Med., pink–red, creamy colonies: *Serratia marcescens*
 - Large, beige, dry–like colonies: *Escherichia coli*
 - Small, pin–point or dot–like, white colonies: *Staphylococcus epidermidis*

Exp. 3: Isolation of Bacteria

- ▶ Mixed Culture Broth: 3 types of microbes

Serratia marcescens



Escherichia coli



Staphylococcus epidermidis



BIO 201 Lab 3

Experiment 4

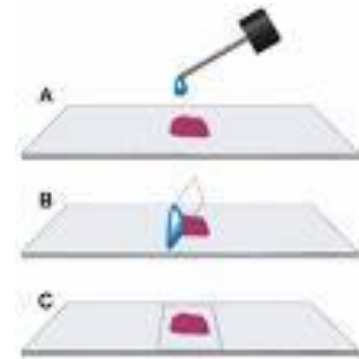
Professor Diane Hilker

I. Exp. 4: Bacterial Morphology

- ▶ **Purpose:** To become familiar with several staining procedures and to compare morphological features, such as size & shape of various microbes.
- ▶ **Today:**
 1. Wet Mount
 2. Heat Fixation: required prior to staining
 3. Simple Stain
 4. Gram Stain
 5. Review Stains: Endospore, Capsule & Acid-Fast Stains

I. Exp. 4: Bacterial Morphology

- ▶ **Wet Mount:** observing living cells
 - **Motility and size of cells**
 - Place 1 drop dH₂O on center of slide
 - Using a sterile loop, remove a small amount of growth from the colony.
 - Mix cells in the drop of H₂O; spread to ½ inch
 - Focus on edge of coverslip with scan (dim light)
 - Move toward center of slide
 - Observe under low & high powers
 - powers
 - Slides will dry out quickly



I. Exp. 4: Bacterial Morphology

▶ Wet Mount

◦ Bacteria: *E. coli*

- Must observe under 400x
- Very small & motile
- Looks like specks of sand
- Hard to discern shape
- Smaller than yeast

& protozoa

- ## ▶ Instructor to provide demonstration & instructions



I. Exp. 4: Bacterial Morphology

▶ Heat Fixation

- Done prior to staining a slide
 - Done for 2 reasons
 - Allows organism to attach to the slide
 - Kills microbe by denaturing proteins
 - Refer to Lab Manual for directions
- ▶ Instructor to provide demonstration & instructions



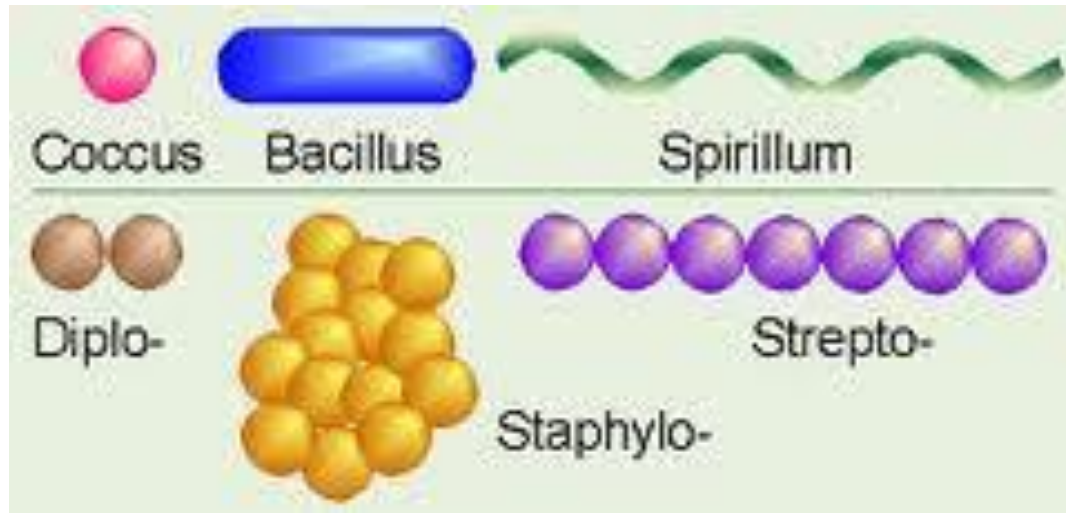
I. Exp. 4: Bacterial Morphology

▶ Simple Staining

- Stain bacteria to make them more visible
- One reagent: **crystal violet**
- All cells will stain **blue/purple**
- Must be viewed under oil-immersion
- Allows you to see: **Shape**
Size
Arrangement

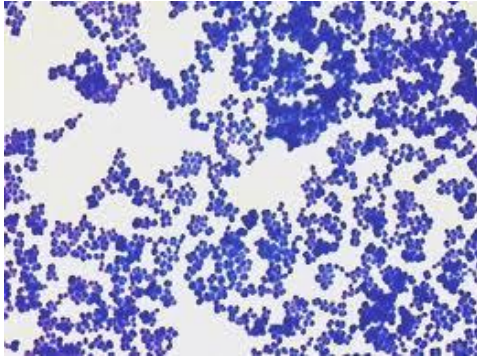
I. Exp. 4: Bacterial Morphology

▶ Shape & Arrangement

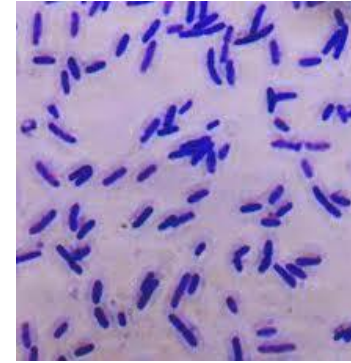


- ▶ **Size:** large or small cocci
long or short rods/bacilli

I. Exp. 4: Bacterial Morphology



Staph.: cocci in clusters



E. coli: rods, no arrangement

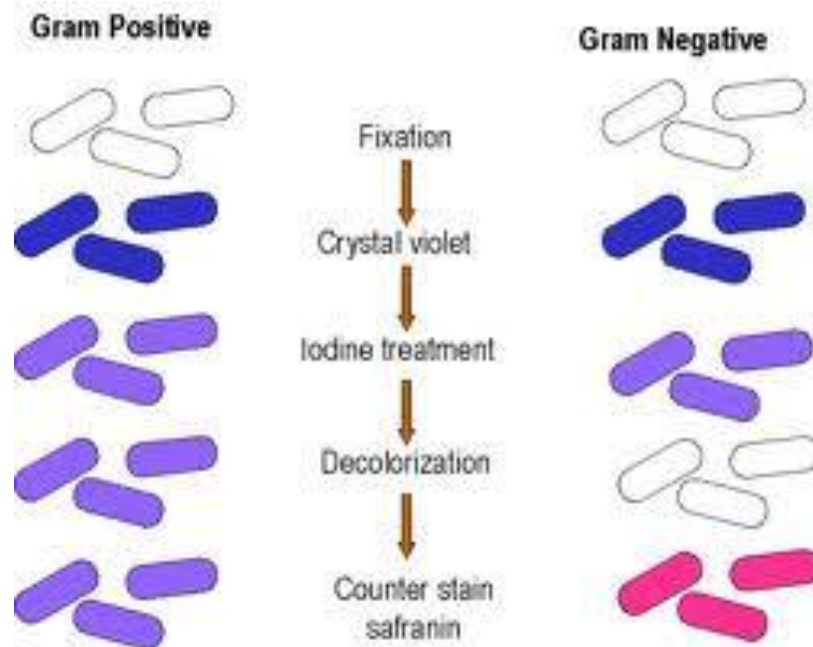
- ▶ Refer to Lab Manual for directions
- ▶ Instructor to provide demonstration & instructions

I. Exp. 4: Bacterial Morphology

- ▶ **Gram Stain:** also see size, shape & arrangement
 - **Differential stain:** stain pink/red or blue/purple
 - **4 Reagents:**
 - Primary Stain**–crystal violet
 - Mordant**–iodine
 - Decolorizer**–ethanol
 - Counterstain**–safranin (red)
 - **Results:** Gram + bacteria: blue/purple
Gram – bacteria: pink
 - **Why?** Cell wall composition (PG)

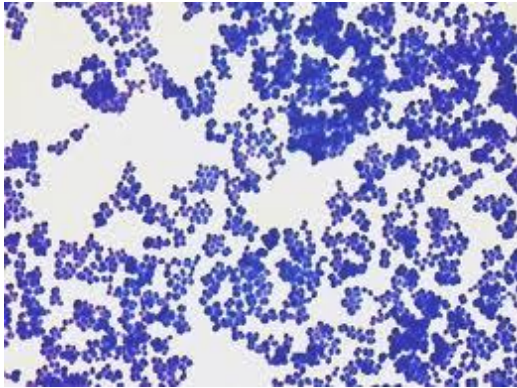
I. Exp. 4: Bacterial Morphology

- ▶ Gram Stain: must view under 1000x

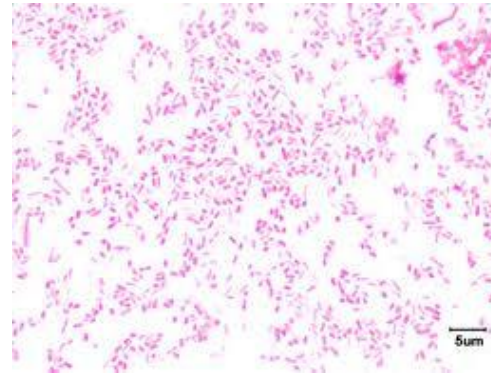


I. Exp. 4: Bacterial Morphology

▶ Gram Stain



Staph: Gram positive cocci in clusters



E. coli: Gram negative rods (no arrangement)

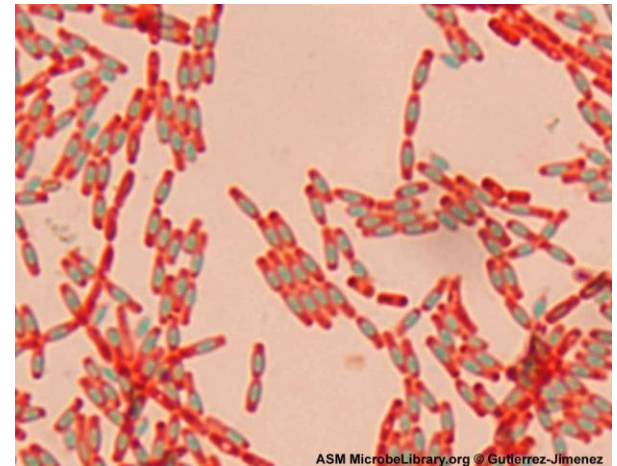
- ▶ Refer to Lab Manual for directions
- ▶ Instructor to provide demonstration & instructions

I. Exp. 4: Bacterial Morphology

▶ Review of Other Stains

◦ Endospore

- Allows the organism to resist adverse environmental conditions: heat, cold, chemicals, radiation, drought, starvation
- Malachite green & safranin as you heat slide
- *Clostridium sp.* & *Bacillus sp.*



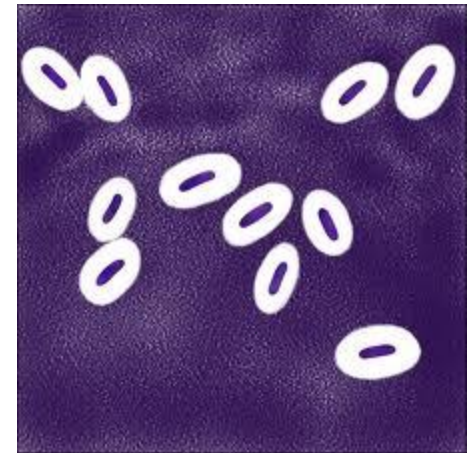
ASM MicrobeLibrary.org @ Gutierrez-Jimenez

I. Exp. 4: Bacterial Morphology

▶ Review of Other Stains

◦ Capsule: virulent

- Allows the organism to resist host defenses: lysozyme & phagocytosis
- Mucopolysaccharide outer coat
- Negative stain since you stain the background
- India Ink stain
- *Pasteurella multocida*

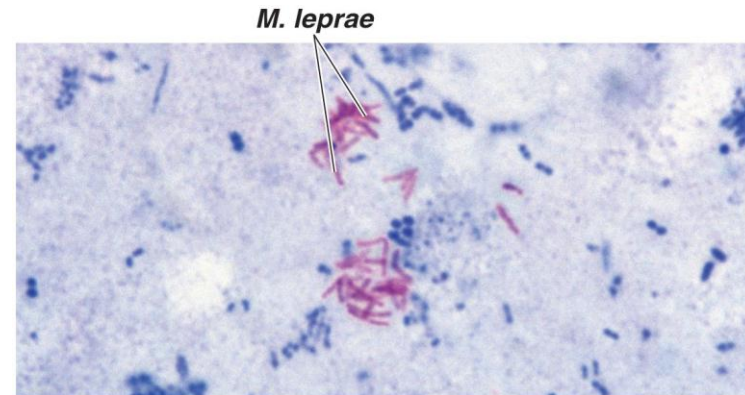


I. Exp. 4: Bacterial Morphology

▶ Review of Other Stains

◦ Acid-Fast Stain: AFB

- Cell wall contains wax
- Carbol-fuchsin stain & heat



LM 5 μ m

Copyright © 2010 Pearson Education, Inc.

- *Mycobacterium tuberculosis* & *M. leprae*
- Acid -fast positive: cells stain red
- Acid-fast negative: cells stain blue