

# BIO 201 Lab 3

# Experiment 3 Results

Professor Diane Hilker



# Overview

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

### 1. Isolation of bacteria

# 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 species of bacteria
  - 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 species of bacteria

*Serratia marcescens*



*Escherichia coli*



*Staphylococcus epidermidis*



# BIO 201 Lab 3

# Experiment 4

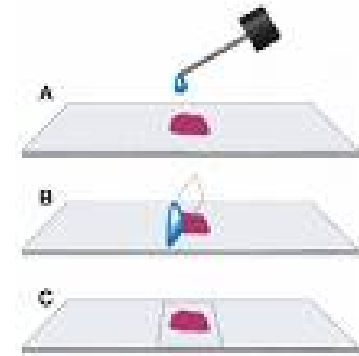
Professor Diane Hilker

# I. Exp. 4: Bacterial Morphology

- ▶ **Purpose:** To become familiar with several staining procedures and to compare morphological features, such as size & shape of various bacteria.
- ▶ **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 dH<sub>2</sub>O on center of slide
    - Using a sterile loop, remove a small amount of growth from the colony.
    - Mix cells in the drop of H<sub>2</sub>O; spread to ½ inch
    - Focus on edge of coverslip with Scan (dim light)
    - Move toward center of slide
    - Observe under Low & High 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:
    1. Allows organism to attach to the slide
    2. Kills bacteria by denaturing proteins
  - Refer to Lab Manual for directions
- ▶ Instructor to provide demonstration & instructions



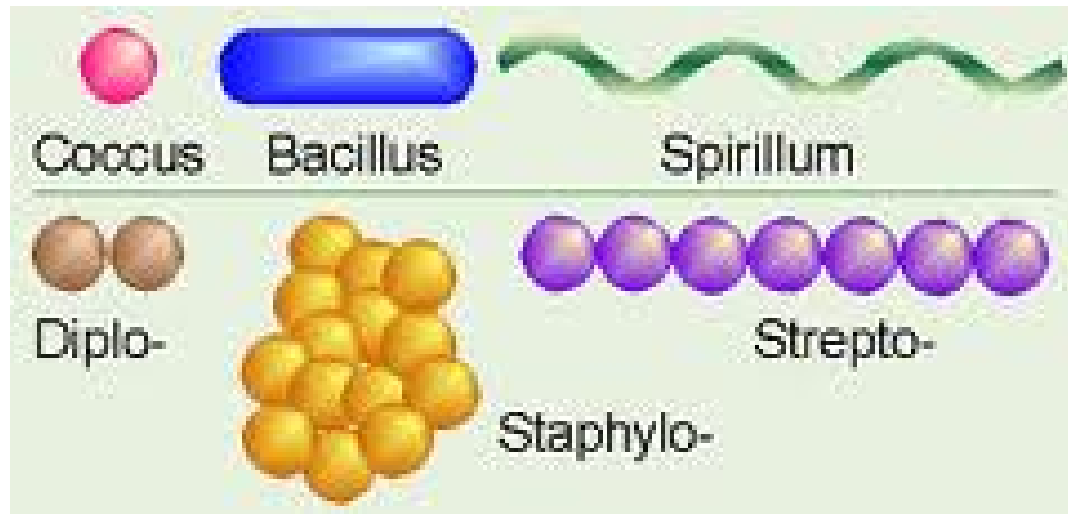
# I. Exp. 4: Bacterial Morphology

## ▶ 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 Power
- Allows you to see: **Shape**  
**Size**  
**Arrangement**

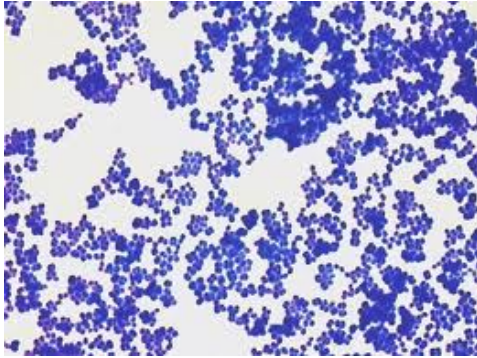
# I. Exp. 4: Bacterial Morphology

## ▶ 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

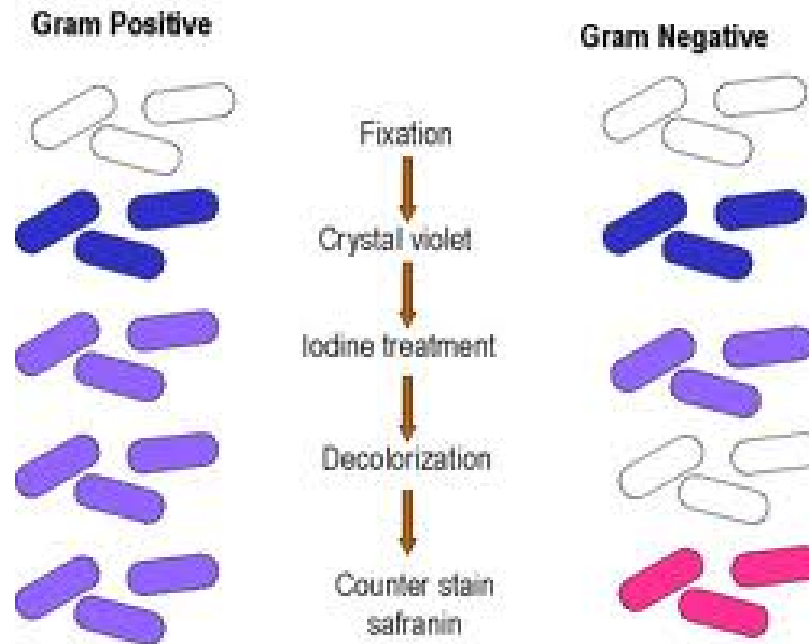
- ▶ Refer to Lab Manual for directions
- ▶ Instructor to provide demonstration & instructions

# 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)

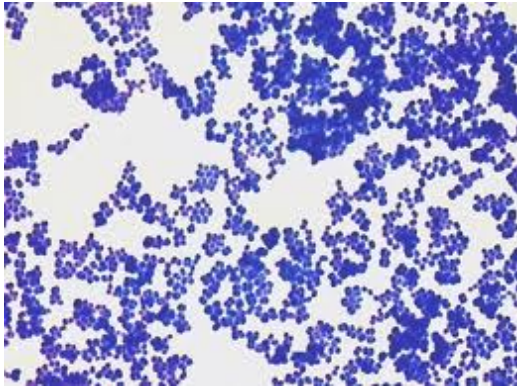
# I. Exp. 4: Bacterial Morphology

- ▶ **Gram Stain:** must view under Oil-Immersion Power (1000x)

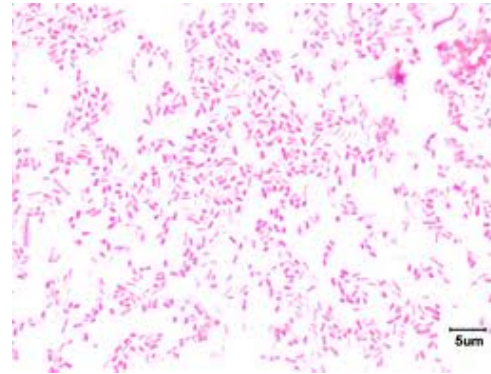


# I. Exp. 4: Bacterial Morphology

## ▶ 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

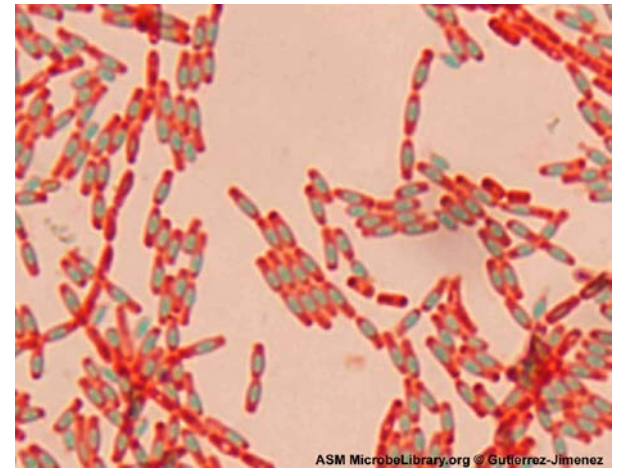


# I. Exp. 4: Bacterial Morphology

## ▶ 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.*



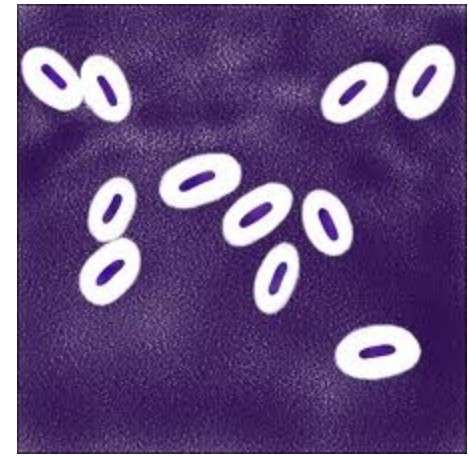
ASM MicrobeLibrary.org @ Gutierrez-Jimenez

# 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*

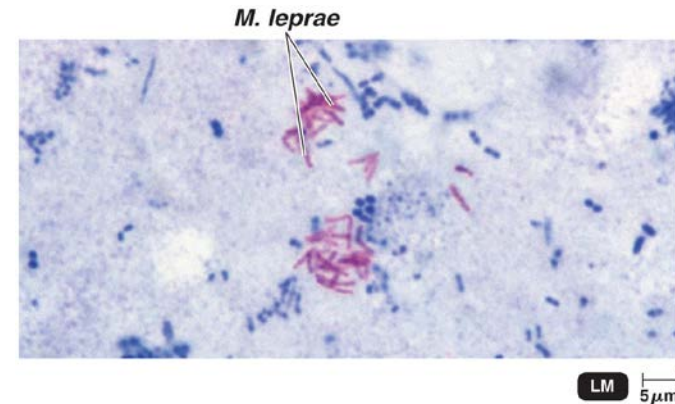


# I. Exp. 4: Bacterial Morphology

## ▶ Review of Other Stains

### ◦ Acid-Fast Stain: AFB

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



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