222 MBIO
Microbial Fine Structure

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Bacterial Structure

Learning Objectives

By the end of this lab, students will be able to:

- Identify the main structural components of bacterial cells
- Distinguish between essential and accessory bacterial structures
- Explain the role of bacterial structures in pathogenicity
- Understand the principle of Gram staining
- Compare Gram-positive and Gram-negative bacteria

Why Study Bacterial Cell Structure?

1. Understanding pathogenicity

- Structural components influence how bacteria cause disease, for example:

- **Cell wall** ⟶ protection and immune interaction

- **Capsule** ⟶ immune evasion

- **Flagella** ⟶ motility and virulence

- **Lipopolysaccharide (LPS)** in the outer membrane of Gram-negative bacteria ⟶ triggers strong immune responses

2. Identifying antibiotic targets

- Knowing the structure helps in designing drugs that are both **effective and selective.**

- Many antibiotics work by disrupting bacterial structures.

- **For example:**

  - **Penicillin** targets peptidoglycan synthesis in the cell wall.

3. Bacterial identification and classification

- Structural features help classify bacteria.
- Used in diagnostic microbiology.
- **For example:**
  - **Cell wall** ⟶ protection and immune interaction
  - **Flagella** ⟶ Number and arrangement are species-specific
  - **Capsules** ⟶ Affect colony morphology, often associated with virulence

		Essential structures vs particular (or accessory) structures	
Essential structures	Present in almost all bacteria Required for survival,	**Cell wall**	Provides shape, rigidity/strength , and protection
		Plasma membrane	Controls transport of nutrients and waste
		Cytoplasm	Contains enzymes and structural components.
		Nucleoid	Region containing the bacterial chromosome (DNA)
		Ribosomes	Sites of protein synthesis
Accessory structures	Not found in all bacteria, Provide specialized functions	**Capsule**	Protects against phagocytosis; aids in biofilm formation
		Flagella	Enables motility; may contribute to virulence
		Pili	Adhesion to surfaces or host cells; involved in conjugation

Bacterial Structure (Cell Wall)

Cell Wall Functions

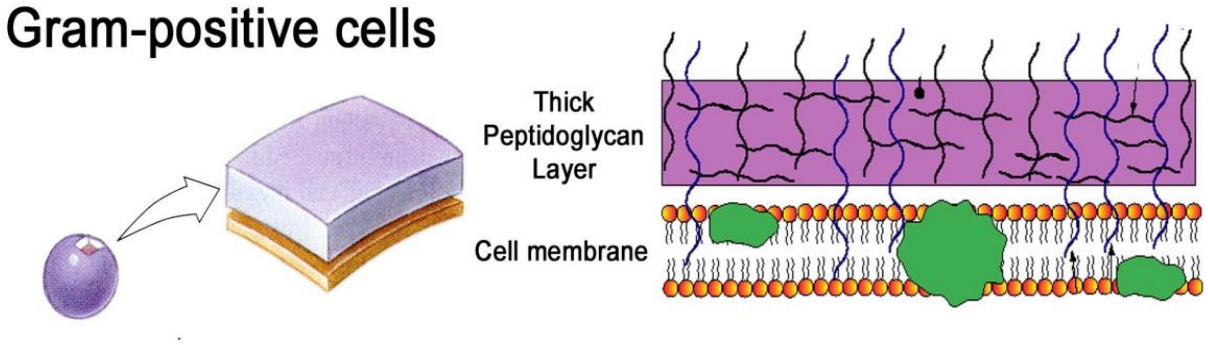
Function	Description
Shape Maintenance	Determines and preserves the characteristic shape (cocci, bacilli, spirilla).
Osmotic Protection	Prevents cell lysis by resisting turgor pressure from high internal solute concentration.
Barrier Against Threats	Shields against lysozymes, and environmental stressors.
Antigenic Properties	Surface molecules (e.g., LPS) act as antigens, aiding/helping immune recognition.
Site for Antibiotic Action	Many antibiotics (e.g., β-lactams) target peptidoglycan synthesis.
Virulence Factor	Structural components contribute to pathogenicity and immune evasion.

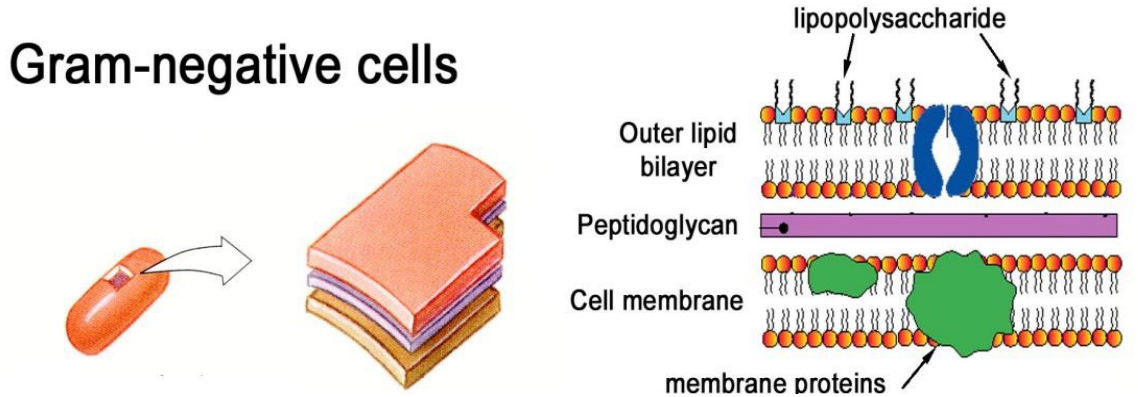
Differential Staining for Classification

- The principle behind **Gram staining** lies in the structural differences in bacterial **cell walls**, particularly:

- **The thickness** of the peptidoglycan layer.

- The **presence or absence** of an outer membrane.





Experiment 1:
Smear Preparation, Simple Stain, and Gram Stain

- Lab 1 -

1. Smear Preparation

❖ The aim

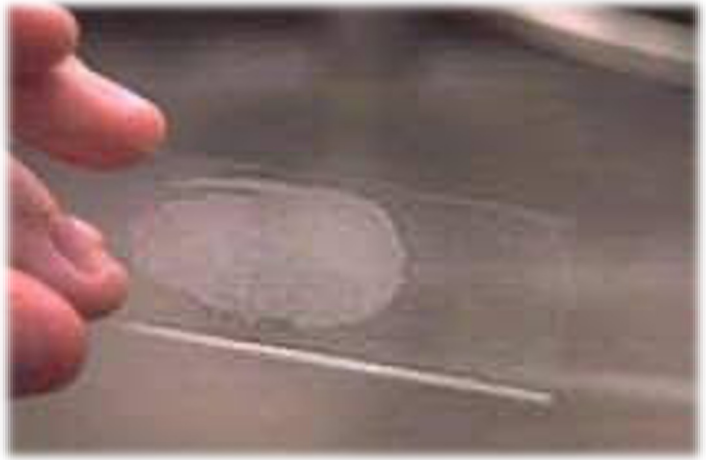
- Prepare microorganisms for microscopic observation.

- Cells from a culture are spread in a thin film over a small area of a microscope slide, dried, and then fixed to the slide by heating or another chemical fixatives.

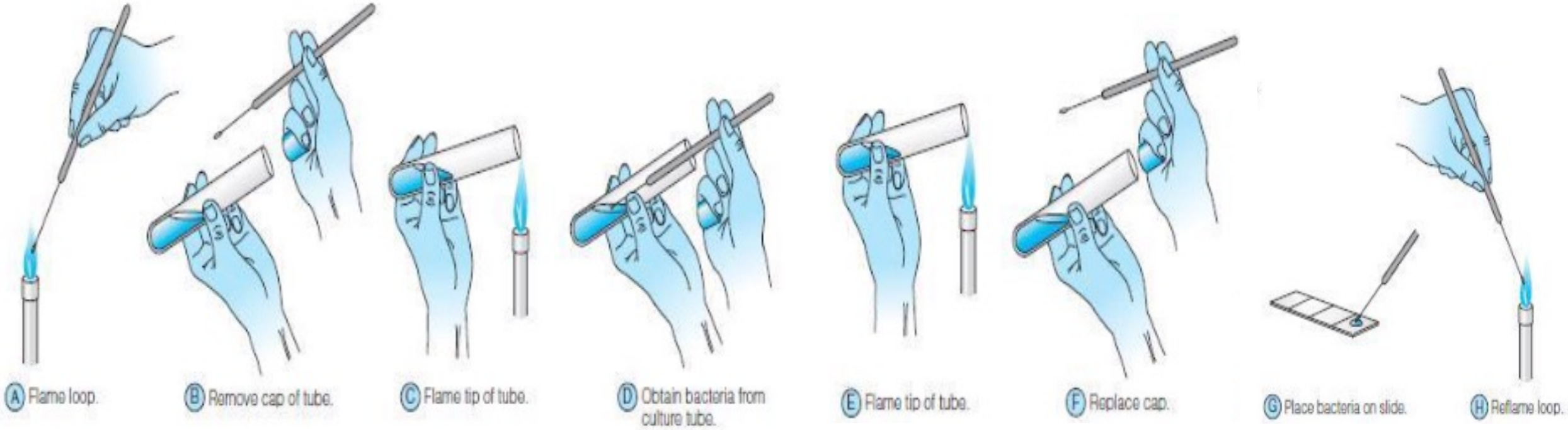
- **A good smear preparation is the key to a good stain.**

❖ Important Notes While Preparing

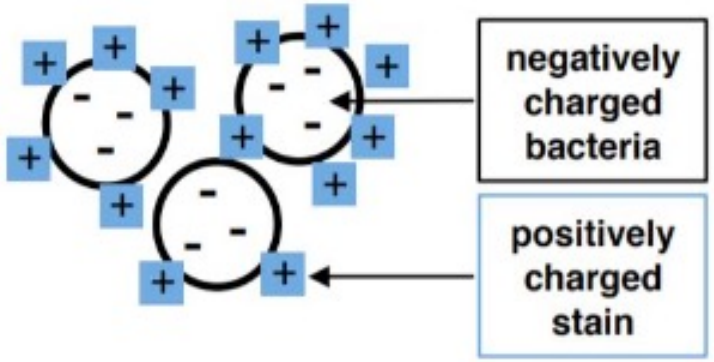
- **If too much inoculum** is taken, you will **not** obtain a good smear due to the flaking of **cell aggregates** upon drying.

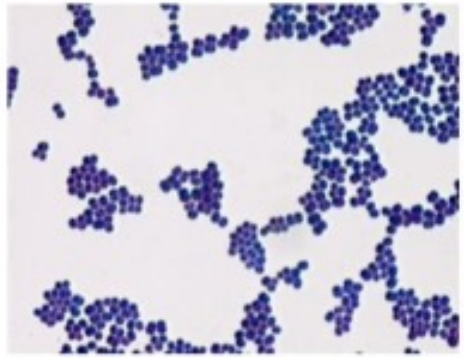


- When the slide is dry, the specimen may be hardly visible especially if the culture was taken from a broth; however, the surface of the slide will be dull and not shiny.


A Flame loop.
B Remove cap of tube.
C Flame tip of tube.
D Obtain bacteria from culture tube.
E Flame tip of tube.
F Replace cap.
G Place bacteria on slide.
H Reflame loop.

2. Simple Staining Technique





❖ The aim

- Observe stained microorganisms and identify their size, shape, and arrangement.

- All types of bacteria appear as **the colour of that stain**.
- Commonly used stain include **crystal violet**, **safranin**, and **methylene blue**.
- Simple stains can be used to **determine** a bacterial species' **morphology** (cell shape) and arrangement (single, chains, clusters, etc.)

3. Gram Staining Technique

❖ Principle

- **Crystal Violet** ⟶ the primary stain, penetrates all bacterial cells.
- **Iodine (fixing the dye)** ⟶ the mordant, forms a complex with crystal violet (CV-I complex), making it larger and less soluble.

When exposed to alcohol (the decolourizer):

Gram-positive bacteria

- Have a **thick** peptidoglycan layer surrounds the cell
- The solvent **dehydrates** the layers which causes closure of pores in the cell wall
- The stain gets **trapped** into this layer and the bacteria turned **purple.**

Gram-negative bacteria

- Have a thin peptidoglycan layer that does not retain crystal violet stain.
- Has a **thick lipid layer** which dissolved easily upon decolorization with alcohol.
- Cells counterstained with safranin and turned red.

Gram (+)

Gram (+) cell walls have single membrane enclosed by thick, cross linked peptidoglycan

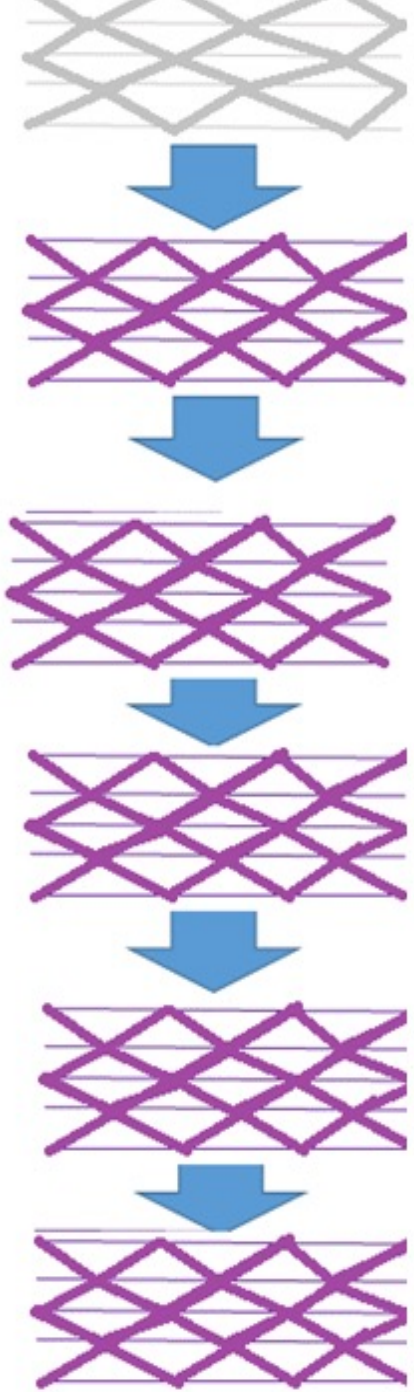
Thick peptidoglycan takes up dye. Appears purple.

Dye and mordant complex forms. Adheres firmly to thick peptidoglycan layer.

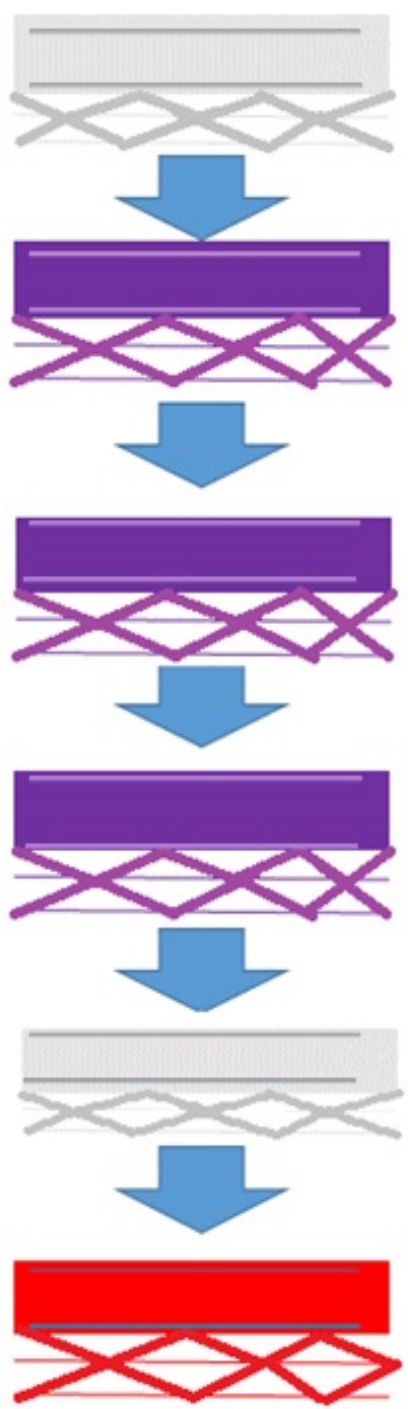
Alcohol cannot wash out the dye-mordant complex because it is firmly secured in the thick peptidoglycan layer.

Saturated with the crystal violet dye, the cell counter stain has little to no effect.

Cell wall ranges in color from mid to dark purple.



1. Heat fix cells to slide
2. Saturate with crystal violet dye for 60 seconds
3. Add iodine (mordant) for 60 seconds
4. Rinse slide with alcohol for 20 seconds
5. Stain slide with safranin (counter stain)



Gram (-)

Gram (-) cell walls have a thin layer of peptidoglycan in the periplasmic space within its inner and outer lipid membranes

Cell wall takes up dye. Appears purple.

Dye and mordant form complex, but does not adhere to the thin layer of peptidoglycan.

Dye and mordant complex is easily removed from peptidoglycan layer with alcohol.

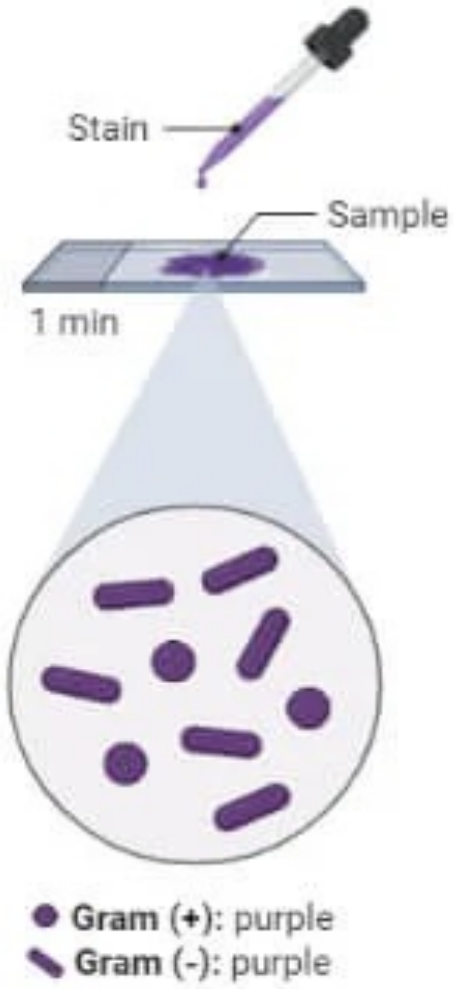
Colorless cell wall can easily take up counter stain.

Cell wall, counter stained with safranin, ranges in color from pink to red.

Step 1

Crystal violet

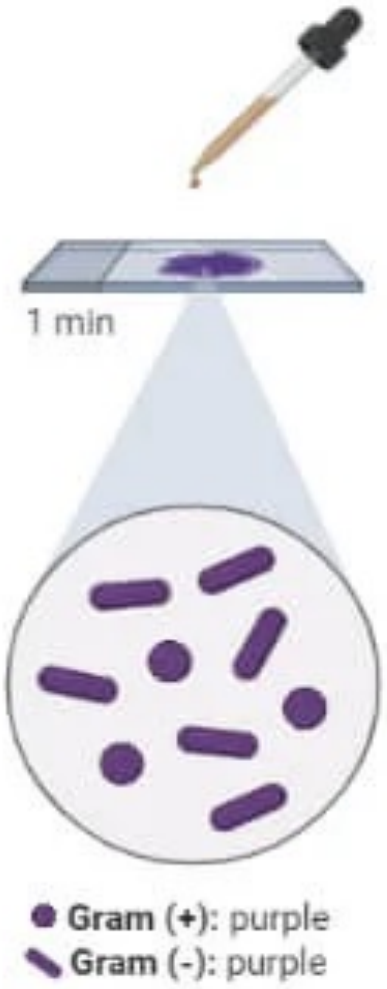
Primary stain added to specimen smear.



Step 2

Iodine

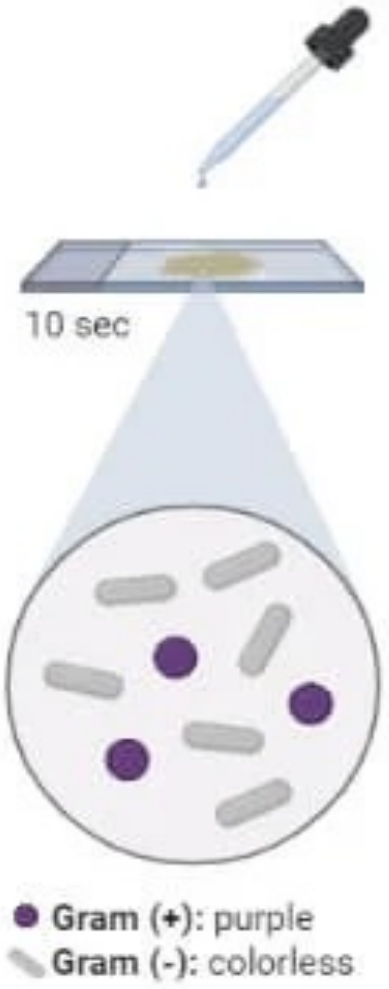
Mordant makes dye less soluble so it adheres to cell walls.



Step 3

Alcohol

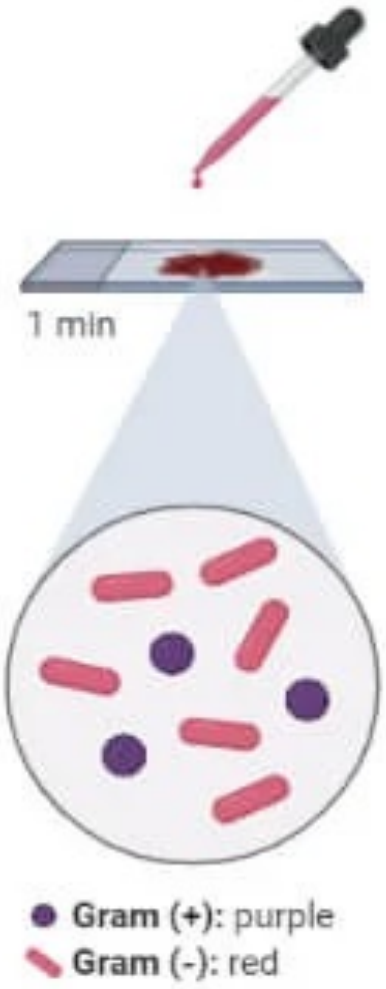
Decolorizer washes away stain from gram (-) cell walls.

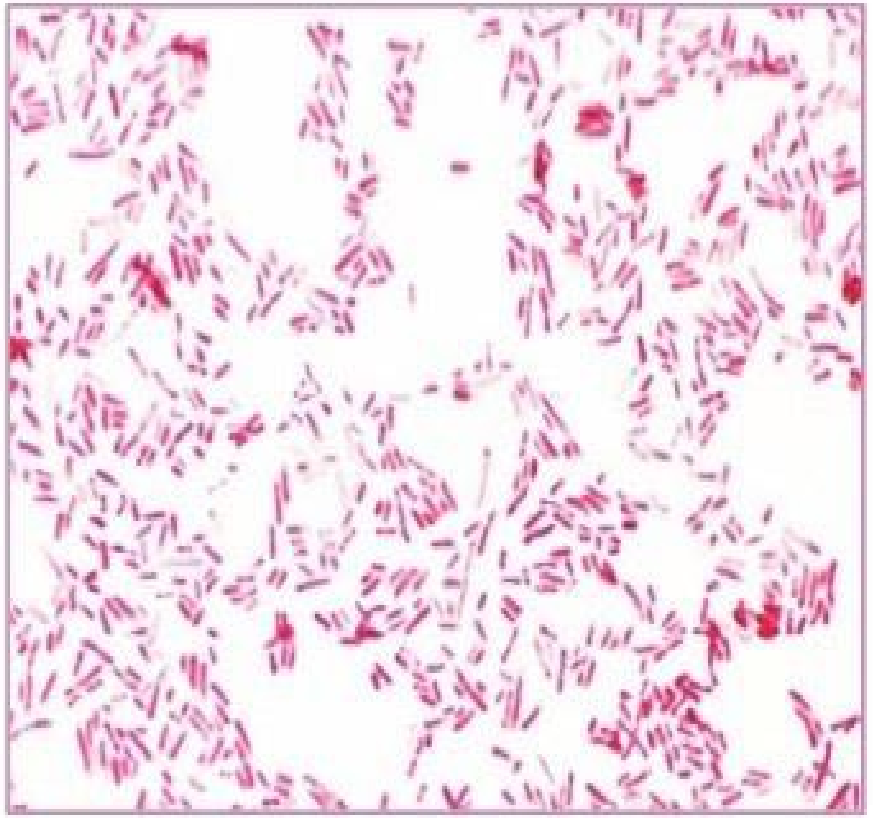


Step 4

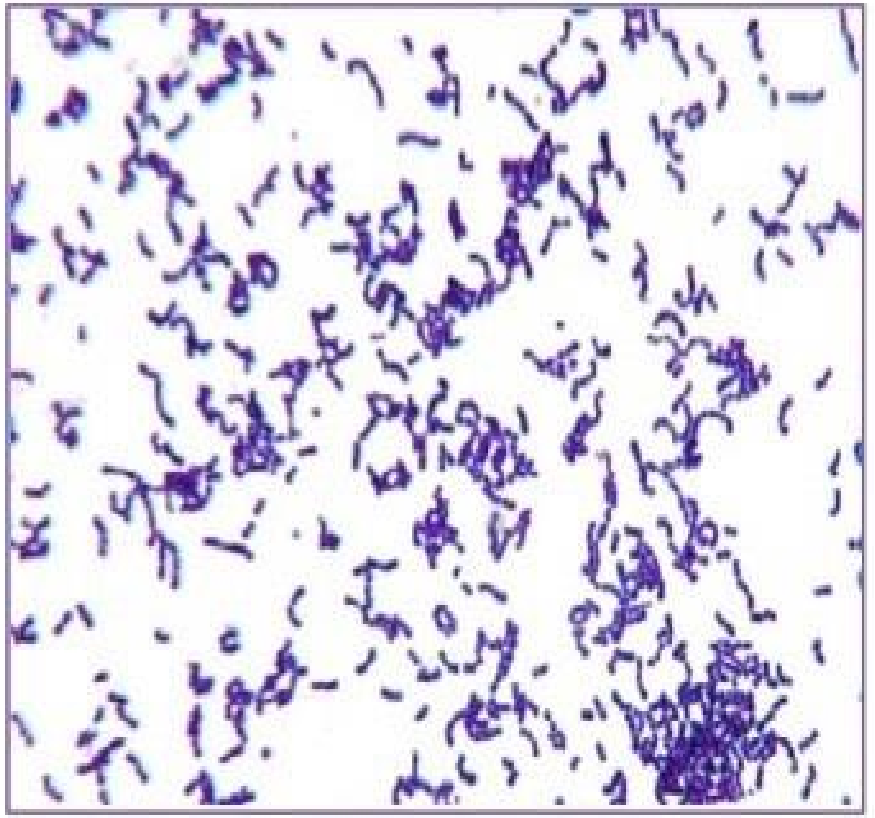
Safranin

Counterstain allows dye adherence to gram (-) cell walls.





Gram-Negative Bacteria



Gram-Positive Bacteria

"Success in this course comes from practice, attention to detail, and responsibility in the laboratory. Engage actively and make the most of every practical session.."

Enjoy the course 🤓