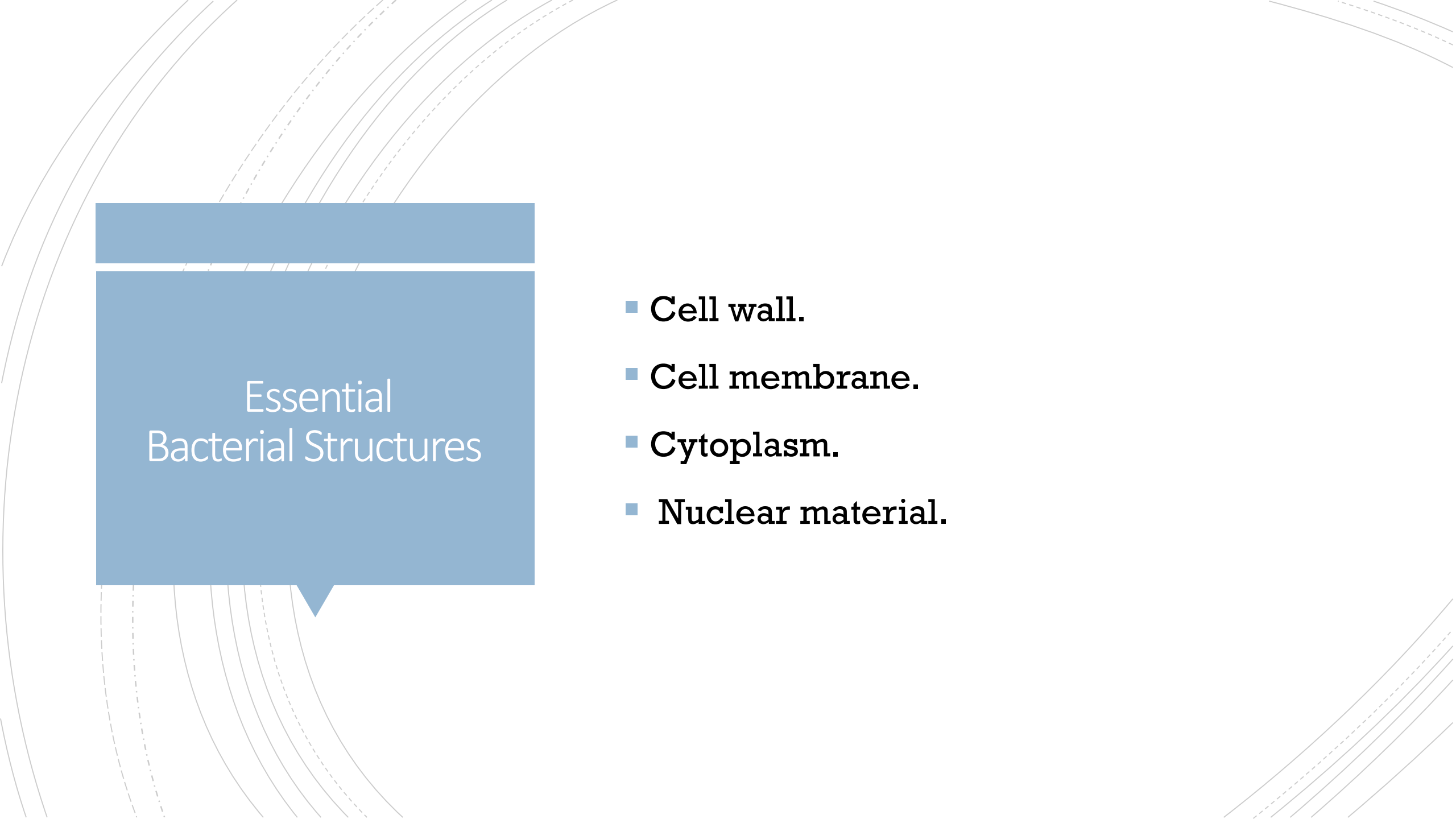
The background features a series of concentric circles in light gray, some solid and some dashed, creating a ripple effect. A large, solid blue rectangle is centered on the page, containing the title and author information. The rectangle has a small downward-pointing triangle at its bottom center.

# Bacterial Structure (Lab 5)

Rana Alqusumi

The background features several concentric, curved lines in shades of gray, some solid and some dashed, creating a sense of depth and movement. A blue rectangular box with a white border and a small white triangle at the bottom center is positioned on the left side of the slide.

## Essential Bacterial Structures

- **Cell wall.**
- **Cell membrane.**
- **Cytoplasm.**
- **Nuclear material.**

The background features several sets of concentric, curved lines in shades of gray, some solid and some dashed, creating a sense of depth and movement. A blue rectangular box with a white border and a small white triangle at the bottom center is positioned on the left side of the slide.

## Particular Bacterial Structures

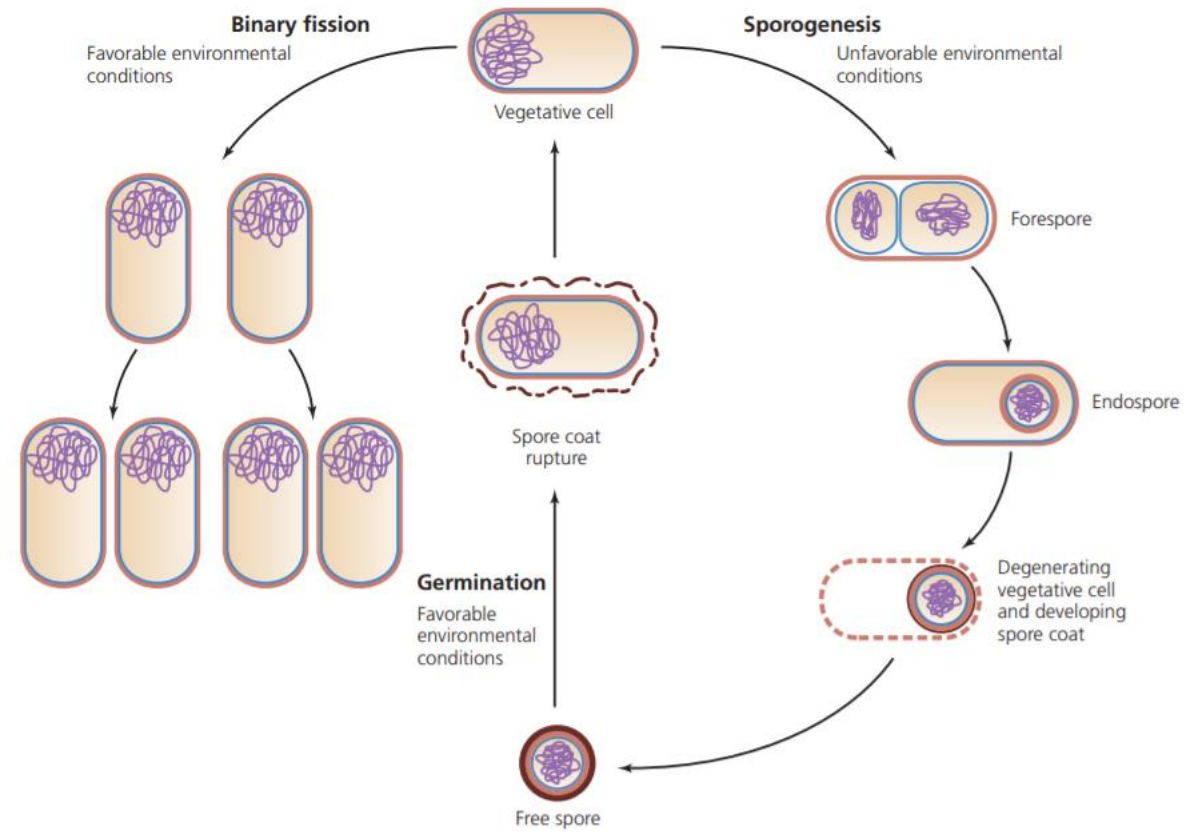
- **Capsule.**
- **Flagella.**
- **Pili.**
- **Fimbriae.**
- **Spore.**

## Sporogenesis

- Members of the anaerobic genera *Clostridium* and the aerobic genus *Bacillus* are examples of organisms that have the capacity to exist either as metabolically active vegetative cells or as highly resistant, metabolically inactive cell types called spores.
- When environmental conditions become unfavorable for continuing vegetative cellular activities, particularly with the exhaustion of a nutritional carbon source, these cells have the capacity to undergo sporogenesis and give rise to a new intracellular structure called the endospore, which is surrounded by impervious layers called spore coats.
- As conditions continue to worsen, the endospore is released from the degenerating vegetative cell and becomes an independent cell called a free spore.

## Spore Stain (Schaeffer-Fulton Method)

- Because of the chemical composition of spore layers, the spore is resistant to the damaging effects of excessive heat, freezing, radiation, desiccation, and chemical agents, as well as to the commonly employed microbiological stains. With the return of favorable environmental conditions, the free spore may revert to a metabolically active and less resistant vegetative cell through germination (see Figure 13.1).
- It should be emphasized that sporogenesis and germination are not means of reproduction but merely mechanisms that ensure cell survival under all environmental conditions.



**Figure 13.1** Life cycle of a spore-forming bacterium

## Spore Stain (Schaeffer-Fulton Method)

➤ **The spore stain uses two different reagents:**

▪ **1-Primary Stain(Malachite Green ):**

unlike most vegetative cell types that stain by common procedures, the free spore, because of its impervious coats, will not accept the primary stain easily. For further penetration, the application of heat is required. After the primary stain is applied and the smear is heated, both the vegetative cell and spore will appear green.

## Spore Stain (Schaeffer-Fulton Method)

➤ **The spore stain uses two different reagents:**

▪ **Decolorizing Agent(Water):**

once the spore accepts the malachite green, it cannot be decolorized by tap water, which removes only the excess primary stain. The spore remains green. On the other hand, the stain does not demonstrate a strong affinity for vegetative cell components; the water removes it, and these cells will be colorless.

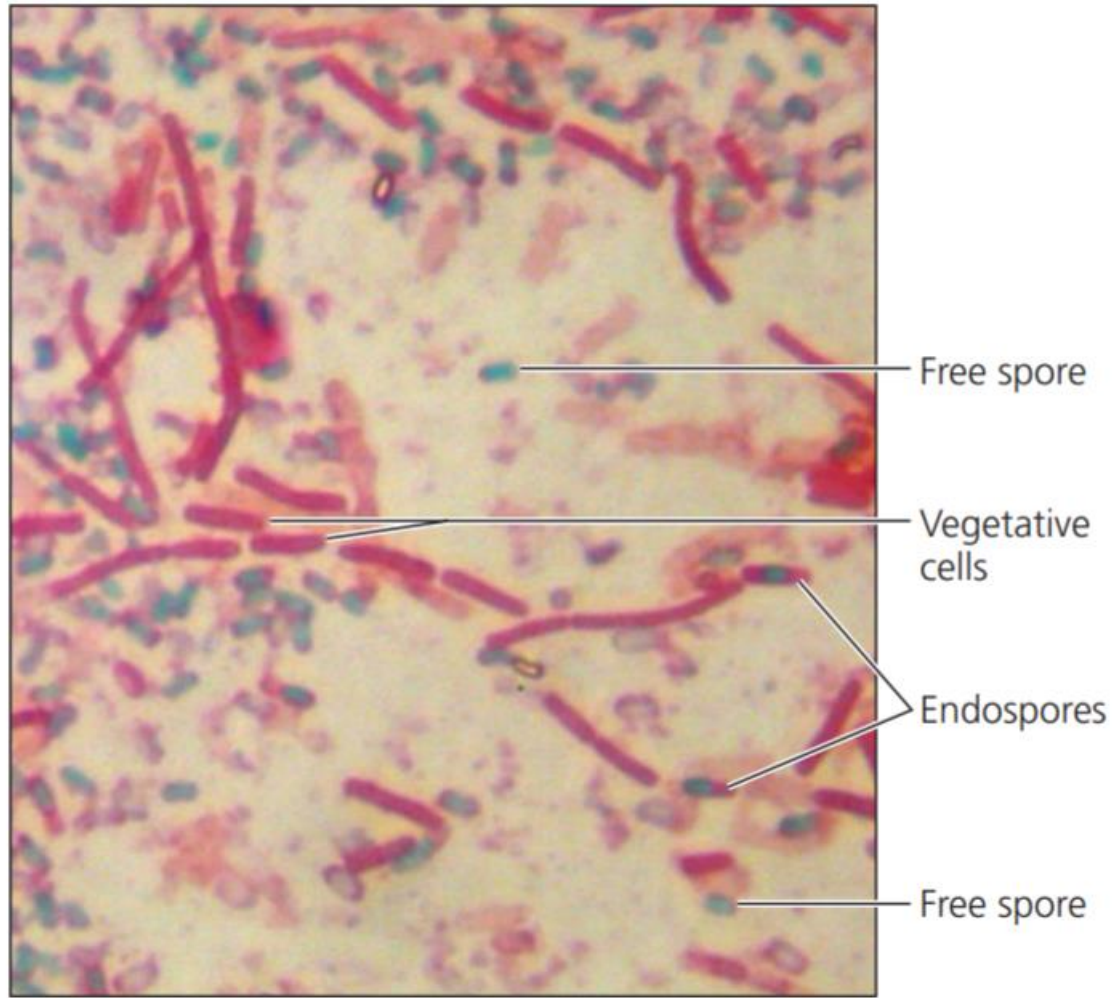


## Spore Stain (Schaeffer-Fulton Method)

➤ **The spore stain uses two different reagents:**

▪ **2-Counterstain(Safranin):**

This contrasting red stain is used as the second reagent to color the decolorized vegetative cells, which will absorb the counterstain and appear red. The spores retain the green of the primary stain. A micrograph of spore-stained cells appears in Figure 13.2.



**Figure 13.2** Spore stain showing free spores and vegetative bacilli

## At the bench (Schaeffer-Fulton Method)

### ➤ **Materials**

- **Cultures:**

48- to 72-hour culture of bacteria that have spores.

- **Reagents:**

Malachite green and safranin.

- **Equipment:**

Bunsen burner, hot plate, staining tray, inoculating loop, glass slides, bibulous paper, lens paper, and microscope

## At the bench

(Schaeffer-Fulton Method)

### ➤ **Procedure:**

#### ■ **Smear Preparation:**

1. Obtain two clean glass slides.
2. Make individual smears in the usual manner using aseptic technique.
3. Allow smear to air-dry, and heat fix in the usual manner.

## At the bench (Schaeffer-Fulton Method)

### ➤ **Procedure:**

#### ■ **Spore Staining**

Steps 1–5 are illustrated in Figure 13.3

1. Flood smears with malachite green and place on top of a beaker of water sitting on a warm hot plate, allowing the preparation to steam for 2 to 3 minutes. Note: Do not allow stain to evaporate; replenish stain as needed. Prevent the stain from boiling by adjusting the hot plate temperature.
2. Remove slides from hot plate, cool, and wash under running tap water.
3. Counterstain with safranin for 30 seconds.
4. Wash with tap water.
5. Blot dry with bibulous paper and examine under oil immersion.

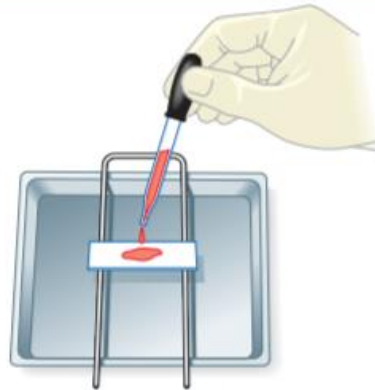
## PROCEDURE



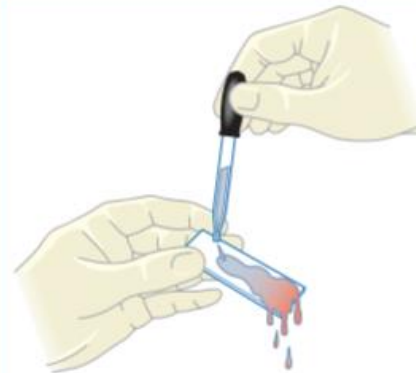
1 Flood smears with malachite green and steam over a beaker of water placed on a hot plate.



2 Cool and wash off stain with tap water. The water also serves as the decolorizing agent.



3 Counterstain with safranin for 30 seconds.



4 Wash off the safranin with tap water.



5 Blot the slide dry with bibulous paper.

Figure 13.3 Spore-staining procedure

# Reference

- **James G. Cappuccino, Natalie Sherman. 2014. Microbiology a laboratory manual. 10<sup>th</sup> ed.**
- <https://fac.ksu.edu.sa/sites/default/files/362mic-lab3.pdf>