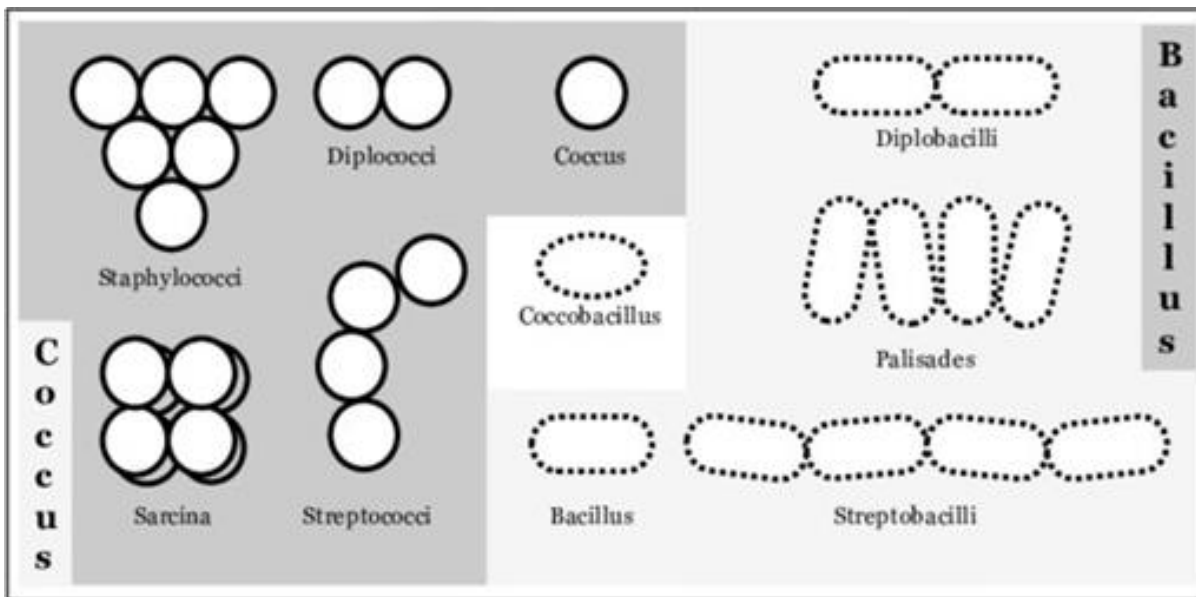


2nd Tutorial session's correction

Exercise 1: Bacterial shape and arrangement



Exercise 2: Study of the bacterial cell wall

1. Compare the thickness:

- Gram-positive bacteria have a thick cell wall: 20–80 nm.
 - Gram-negative bacteria have a thin cell wall: 2–7 nm
- ➡ □ Thickness is mainly due to the **amount of peptidoglycan.**

2. Main chemical components of the bacterial cell wall

- Peptidoglycan (murein)
- Teichoic acids (in Gram-positive)
- Lipopolysaccharides (LPS, in Gram-negative)
- Proteins (e.g., porins in Gram-negative)
- Lipoproteins (in Gram-negative)

3. Elements specific to:

- **Gram-positive:** Thick peptidoglycan, teichoic acids, lipoteichoic acids.
- **Gram-negative:** Thin peptidoglycan, outer membrane with LPS, periplasmic space, porins.

4. Why Gram staining differentiates them:

In Gram-positive bacteria, the crystal violet–iodine complex is retained within the thick peptidoglycan layer after alcohol decolorization. In contrast, in Gram-negative bacteria, alcohol removes lipids from

the outer membrane, increasing permeability; the thin peptidoglycan layer cannot retain the dye, which is washed out, leaving the cells pink/red after counterstaining.

Exercise 3: the role of the bacterial cell wall

General principle

The bacterial cell wall:

- maintains the **normal shape** of the bacterium,
- protects the cell against **osmotic pressure**,
- is essential for survival in **hypotonic environments**.

Lysozyme is used in this experiment to **damage the bacterial cell wall**.

Gram-positive bacterium (*Bacillus subtilis*)

Tube 1: Hypotonic medium, no lysozyme

Observation: Cells remain intact and maintain their **normal bacillary (rod-shaped) form**.

Explanation: Although water enters the cell by osmosis, the intact cell wall resists internal pressure and prevents membrane rupture.

Conclusion: The cell wall protects the bacterium against osmotic lysis.

Tube 2: Isotonic medium + lysozyme

Observation: Cells lose their bacillary shape and become **spherical**.

Explanation: Lysozyme damages the cell wall, which is responsible for maintaining cell shape. The isotonic medium prevents excessive water entry, so the cell does not burst.

Conclusion: The cell wall determines cell shape, but osmotic pressure is required for lysis.

➡ □ The spherical cell obtained is called a **protoplast**.

Tube 3: Hypotonic medium + lysozyme

Observation: Cells swell and burst (**lysis**).

Explanation: Lysozyme removes the protective cell wall. In a hypotonic medium, water enters the cell freely. The cytoplasmic membrane alone cannot withstand the internal pressure.

Conclusion: Lysis occurs when the cell wall is destroyed and osmotic pressure is applied.

Summary for Gram-positive bacteria:

- Intact wall → protection
- Wall removed + isotonic medium → shape change only
- Wall removed + hypotonic medium → lysis

Gram-negative bacterium (*Escherichia coli*)

Tube 4: Isotonic medium + lysozyme

Observation: No visible change in cell shape.

Explanation: In Gram-negative bacteria, the **outer membrane** protects the cell wall and prevents lysozyme from reaching it.

Conclusion: Lysozyme alone is ineffective against Gram-negative bacteria.

Tube 5: Isotonic medium + lysozyme + EDTA

Observation: Cells become **spherical** but remain intact.

Explanation: EDTA weakens the outer membrane, allowing lysozyme to act on the cell wall. The isotonic medium prevents osmotic lysis.

➔ The resulting structure is called a **spheroplast**.

Conclusion

- Gram-positive and Gram-negative bacteria respond differently due to differences in cell wall structure.
- Lysis requires both: destruction of the cell wall + a hypotonic environment.

Synthesis and interpretation

1. **Protoplast vs. spheroplast:**

- **Protoplast:** A Gram-positive bacterium completely devoid of its cell wall (only cytoplasmic membrane).
- **Spheroplast:** A Gram-negative bacterium with partially removed cell wall, retaining some outer membrane material.

2. **Survival in hypotonic environments:**

The rigid cell wall counteracts turgor pressure, preventing osmotic lysis.

3. **Complete the sentence:**

The bacterial cell wall, mainly composed of **peptidoglycan**, plays an essential role in maintaining **cell shape and integrity** and protecting the cell against **osmotic lysis**.