Tutorial session 2: Bacterial cell structure

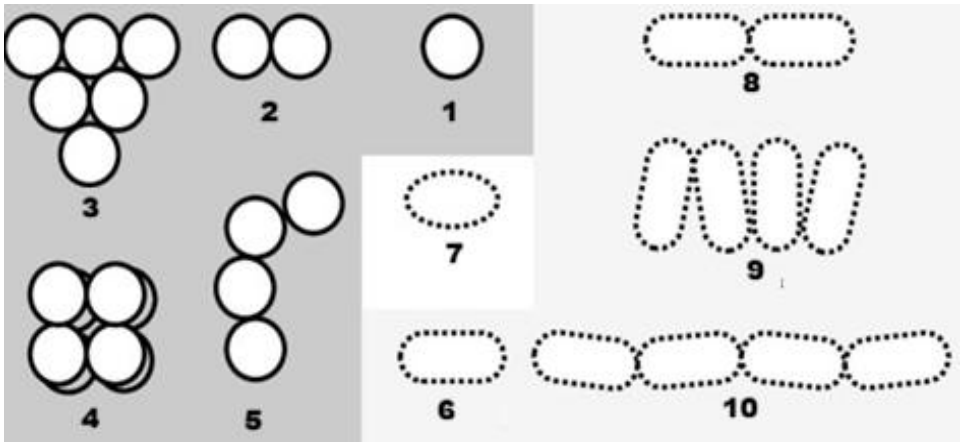
Learning objectives:

- Identify bacterial shapes and arrangements from microscopic observations.
- Compare the cell wall structure of Gram-positive and Gram-negative bacteria.
- Explain the role of the bacterial cell wall in maintaining cell integrity under osmotic stress.

Exercise 1: Bacterial shape and arrangement

Bacteria observed under the light microscope after simple staining may show different shapes and cellular arrangements. These characteristics are important for bacterial identification.

Task: Examine the image below and indicate the shape and arrangement of each bacterium.



1..... 2 .....

3..... 4.....

5..... 6.....

7..... 8.....

9..... 10.....

Exercise 2: Study of the bacterial cell wall

The bacterial cell wall is a fundamental structure that differs significantly between Gram-positive and Gram-negative bacteria.

Two bacteria are studied:

- Bacterium A: Gram-positive
- Bacterium B: Gram-negative

Questions:

1. Compare the thickness of the cell wall in Gram-positive and Gram-negative bacteria.

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2. List the main chemical components of the bacterial cell wall.

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3. Indicate the elements specific to:
   - Gram-positive bacteria

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   - Gram-negative bacteria

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4. Explain why Gram staining allows differentiation between these two groups.

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Exercise 3: The role of the bacterial cell wall

Two bacterial species are studied **separately**:

- **Bacterium 1:** *Bacillus subtilis* (Gram-positive)
- **Bacterium 2:** *Escherichia coli* (Gram-negative)

Each bacterium is suspended in different media, under controlled conditions.

Gram-positive bacterium *(Bacillus subtilis)*

The same bacterial suspension is divided into three separate test tubes.

Tube	Medium	Additional treatment
1	Distilled water (hypotonic)	None
2	Isotonic solution (physiological solution / sucrose)	Lysozyme
3	Distilled water (hypotonic)	Lysozyme

Note: Target of lysozyme : Lysozyme degrades β (1–4) glycosidic bonds between NAM and NAG in peptidoglycan

Observed results

- Tube 1: Cells keep their normal rod shape.
- Tube 2: Cells become spherical.
- Tube 3: Cells swell and burst.

Questions

1. Why do the bacteria not lyse in tube 1 despite the hypotonic medium?

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2. Why do the bacteria become spherical in tube 2?

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3. Why does lysis occur only in tube 3?

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4. What conclusion can be drawn about the role of the cell wall?

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Gram-negative bacterium *(Escherichia coli)*

The experiment is repeated using *E. coli*.

Tube	Medium	Additional treatment
4	Isotonic solution	Lysozyme
5	Isotonic solution	Lysozyme + EDTA

EDTA (ethylenediaminetetraacetic acid) is an agent that destabilizes the outer membrane of Gram-negative bacteria.

Observed results

- Tube 4: No visible change in cell shape.
- Tube 5: Cells become spherical but do not burst.

Questions

1. Why does lysozyme alone have no effect in tube 4?

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2. Why are the cells spherical but still intact in tube 5??

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Synthesis and interpretation

Answer the following questions using the previous exercises:

1. Define protoplast and spheroplast.

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2. Explain why bacteria can survive in hypotonic environments.

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3. Complete the following sentence:

"The bacterial cell wall, mainly composed of....., plays an essential role in maintaining ..... and protecting the cell against ....."