**Guided work: 3**

**Exercice1:**

In a hypotonic medium, *B. subtilis* behaves normally, but when lysozyme is added, the bacteria swell and burst due to the degradation of the peptidoglycan. In an isotonic medium with lysozyme, *B. subtilis* forms spherical structures called **protoplasts**, which lose their antigenic properties, cannot divide, fix bacteriophages, or move. Similarly, *E. coli* forms **spheroplasts** in an isotonic medium with lysozyme, but they retain their initial properties. These observations highlight several roles of the bacterial cell wall: maintaining cell shape, providing protection against intracellular osmotic pressure, possessing antigenic properties (e.g., teichoic acids in Gram+ and LPS O-antigens in Gram-), enabling bacteriophage fixation, supporting flagellar mobility, and contributing to Gram- toxicity via the LPS endotoxin. Additionally, the wall allows small molecules to pass while limiting others. The space between membranes in Gram- bacteria, called the periplasmic space, contains enzymes for nutrient processing, unlike Gram+ bacteria, which excrete these enzymes externally.

**Questions**

1- What is the primary role of the bacterial cell wall demonstrated here?

2- Why do bacteria burst in a hypotonic medium when lysozyme is added?

3- What is the difference between a protoplast and a spheroplast?

4- Why do protoplasts lose their antigenic properties?

5- What are the key antigens in Gram+ and Gram- bacteria?

6- Name at least three roles of the cell wall demonstrated by this experiment.

7- How does the cell wall contribute to bacteriophage fixation and bacterial mobility?

8- Why do Gram+ bacteria excrete enzymes externally while Gram- bacteria retain them in the periplasmic space?

**Exercice 2:**

Bacteria possess essential structures that ensure their survival and adaptation. Three experiments are proposed to analyze membrane permeability, the presence of storage vacuoles, and the role of plasmids in antibiotic resistance.

Cultivate E. coli and B. subtilis in liquid medium until the exponential phase. Take three aliquots and place them respectively in:  
a) An isotonic solution (0.9% NaCl)  
b) A hypotonic solution (distilled water)  
c) A hypertonic solution (10% glucose)

Observe turbidity variations and examine bacteria under the microscope.

2- Stain bacteria with Nile blue and Sudan III red to detect lipid granules and polyhydroxybutyrate (PHB) reserves. Compare the results between B. subtilis and E. coli.

3- Cultivate two E. coli strains: one containing a resistance plasmid and the other without a plasmid. Inoculate these bacteria on a simple LB medium and an LB medium containing ampicillin. Compare bacterial growth on both media.

### ****Questions****

1- What is the primary function of the plasma membrane?  
2- What effect is observed on bacteria placed in each medium (isotonic, hypotonic, hypertonic)? Justify your answer.

3- What is the purpose of storage vacuoles in bacteria?  
4- Why do some bacteria accumulate lipid reserves?

#### 5- What is the role of plasmids in bacterial resistance? 6- What happens to E. coli without a plasmid on LB + ampicillin medium? Why? 7- How could one experimentally prove that resistance is due to a plasmid?