University Center Mila
Module: General Microbiology *Dr. RABHI Nour El Houda*
Level: 2nd Year biotechnology

Guided work 04

Exercice:

Three experiments are carried out simultaneously on *E. coli* and *B. subtilis* cultured in LB liquid medium until the exponential growth phase.

Experiment 1 – Membrane permeability**:** Three aliquots of each culture are placed respectively in an isotonic solution (0.9% NaCl), a hypotonic solution (distilled water), and a hypertonic solution (10% glucose). Turbidity and cell morphology are observed after 30 minutes under the microscope.

Experiment 2 – Lipid reserves: Smears of *E. coli* and *B. subtilis* are stained with Nile Blue and Sudan III, then observed under the optical microscope to detect the presence of intracellular lipid granules.

Experiment 3 – Plasmid-mediated resistance: Two *E. coli* strains — one carrying an ampicillin resistance plasmid and one without — are inoculated onto standard LB medium and LB medium supplemented with ampicillin (100 µg/mL). Growth is observed after 24 hours at 37°C.

Questions

Q1. What is the primary function of the plasma membrane? Why is it described as semi-permeable?

Q2. For each of the three solutions (isotonic, hypotonic, hypertonic), describe the expected effect on bacteria and justify your answer using the concept of osmosis.

Q3. What is the role of PHB granules in bacteria? Under what conditions are they synthesized?

Q4. Why does *B. subtilis* accumulate more PHB than *E. coli*? What survival advantage does this provide?

Q5. Explain the principle of Nile Blue and Sudan III staining. What results do you expect for each bacterial species?

Q6. What is a plasmid? How does it differ from the bacterial chromosome?

Q7. What mechanism allows *E. coli* carrying the resistance plasmid to survive in the presence of ampicillin? What happens to the strain without a plasmid? Justify.