BACTERIAL GROWTH

Bacterial Growth

1. Bacterial Division

Bacteria reproduce by **binary fission**: the bacterium grows and then divides into two daughter cells separated by a division septum formed by the cell wall. During division, DNA and other cellular components duplicate. Various enzymatic systems of synthesis and degradation participate in cell division.

2. Growth Dynamics

Growth is generally defined as the **orderly development of an organism's components**, resulting in an increase in size and volume.
Bacterial growth, however, is more about **population growth**, leading to an increase in the number of individuals within a colony.
As the population increases, nutrient availability and living space decrease, while waste products accumulate, affecting **pH**, **redox potential**, **conductivity**, **osmotic pressure**, **surface tension**, and **viscosity**.

3. Methods of Study

To monitor bacterial population evolution, several parameters can be measured:

- Number of cells per unit volume,
- Microbial mass,
- Concentration of a stable cellular component,
- Bacterial activity.

A. Measuring Cell Number

a. Cell counting per unit volume

- **Breed's method**:
  A known volume (0.01 mL) of liquid culture is spread on a clean glass slide over a 1 cm² area, dried, and stained (e.g., Gram stain or methylene blue).
  Bacteria are counted under the microscope using an ocular micrometer.

Advantages: simple method.
Disadvantages: counts both live and dead bacteria.

b. Plate count method (dilution and culture in agar medium)

- **Procedure**:
  1. Perform **serial decimal dilutions** (10^{-1}, 10^{-2}, etc.) using tubes containing 9 mL of physiological saline.
  2. Mix 1 mL of each dilution with melted, cooled agar at 40–45°C in sterile Petri dishes.
  3. After solidification, incubate plates at 37°C for 18 hours.
  4. Count colonies; choose plates with **30 to 300 colonies** for reliable counts.

Result:

$$N = \text{bacteria (CFU) per mL}$$

B. Measuring Biomass (Mass of Living Matter)

a. Direct measurement: dry weight

- Culture is centrifuged, washed with buffer, and dried to a constant weight.
- Rarely used due to multiple disadvantages.

b. Indirect measurement: turbidity

- Measure suspension turbidity using a spectrophotometer at **650 nm**.
- Follows the **Beer-Lambert law** for low cell densities.

C. Measuring Activity

- Measure **substrate consumption**, **production of a cellular constituent**, **excretion of a molecule**, or **physicochemical changes** in the medium.

4. Growth Kinetics: Population Evolution Over Time

A. Growth Constants

- **Generation time (G)**:
  Time for the bacterial population to double.
- **Growth rate (μ)**:
  Number of divisions per unit time.

Formulas:

$$G = \frac{t}{n} \quad ; \quad \mu = \frac{n}{t}$$

B. Mathematical Expression of Growth

At different times t, the biomass N changes.

If N_0 is the number of bacteria at time t_0, after n generations:

- After 1 generation: $N_1 = 2N_0$
- After 2 generations: $N_2 = 2^2 N_0$
- After 3 generations: $N_3 = 2^3 N_0$
- After n generations: $N_n = 2^n N_0$

Using growth rate μ: $N_n = 2^{\mu t} N_0 \quad \text{or} \quad N_n = N_0 e^{\mu t}$

C. Growth Curve

Plotting **number of bacteria vs time** shows different phases:

1. **Lag Phase**:
   - No division (μ = 0).
   - Adaptation to the new environment.
   - No lag if transferred into identical medium.

2. **Acceleration Phase**:
    - Increase in growth speed.
3. **Exponential Phase**:
    - Maximum growth rate ($\mu = \mu max$).
    - Constant doubling time.
    - Minimal cell death.
4. **Deceleration Phase**:
    - Growth slows due to nutrient depletion and waste accumulation.
5. **Stationary Phase**:
    - Growth rate is zero ($\mu = 0$).
    - Number of new cells equals number of dying cells.
6. **Decline (Death) Phase**:
    - Growth rate negative ($\mu < 0$).
    - Cells die and lyse.
7. **Cryptic Growth Phase**:
    - Some surviving cells grow using nutrients released by dead cells.

D. Factors Influencing Growth

Main factors:

- **Temperature**
- **pH**
- **Substrate concentration**
- **Water activity (Aw)**

5. Continuous Culture Growth

Growth remains **exponential** when the culture medium is continuously renewed and waste products are removed.
The growth rate (μ) stays **constant and maximal**.

Two main devices:

- **Chemostat**:
    - Nutrient influx at constant rate.
    - Growth controlled by limiting nutrient availability.
- **Turbidostat**:
    - Nutrient influx adjusted automatically based on optical density (OD).
    - Maintains constant cell density.

6. Synchronous Growth

All bacteria divide simultaneously.
Growth curve shows **successive steps** (staircase pattern).
Useful for studying **cell division separately from growth**.

7. Diauxic Growth Phenomenon

When two carbon sources are present:

- Growth shows a **biphasic curve** (two distinct exponential phases separated by a lag).

Principle:

- On a synthetic medium with two carbon substrates, some combinations give a classic growth curve; others give a biphasic curve.

Results and Explanation:

- **List A (glucose, fructose, mannose, mannitol)**: classic curve.
- **List B (arabinose, maltose, inositol, sorbitol)**: biphasic curve.

Explanation:

- Enzymes for List A substrates are **constitutive**.
- Enzymes for List B are **inducible**.
- First substrate represses the synthesis of enzymes for the second substrate (**catabolic repression**).

Basic Concepts of Bacterial Culture

- A **culture medium**: a nutrient solution for microbial growth in the lab.
- **Simple bacteria** grow on many types of media; **fastidious bacteria** require specific media.
- Some bacteria cannot grow in any artificial medium.
- **Inoculum**: the microbial sample introduced into a culture.
- **Culture**: microbes growing and multiplying in or on a medium.

Medium Requirements:

- Nutrients (ions, growth factors, carbon/energy sources),
- Proper humidity, pH, osmotic pressure, and oxygen concentration,
- Must be initially sterile.

Solid media: made by adding **agar** (gelatinous extract from seaweed).

- Solidifies at 40°C.
- Used in tubes or Petri dishes (slanted or deep).

Types of Culture Media

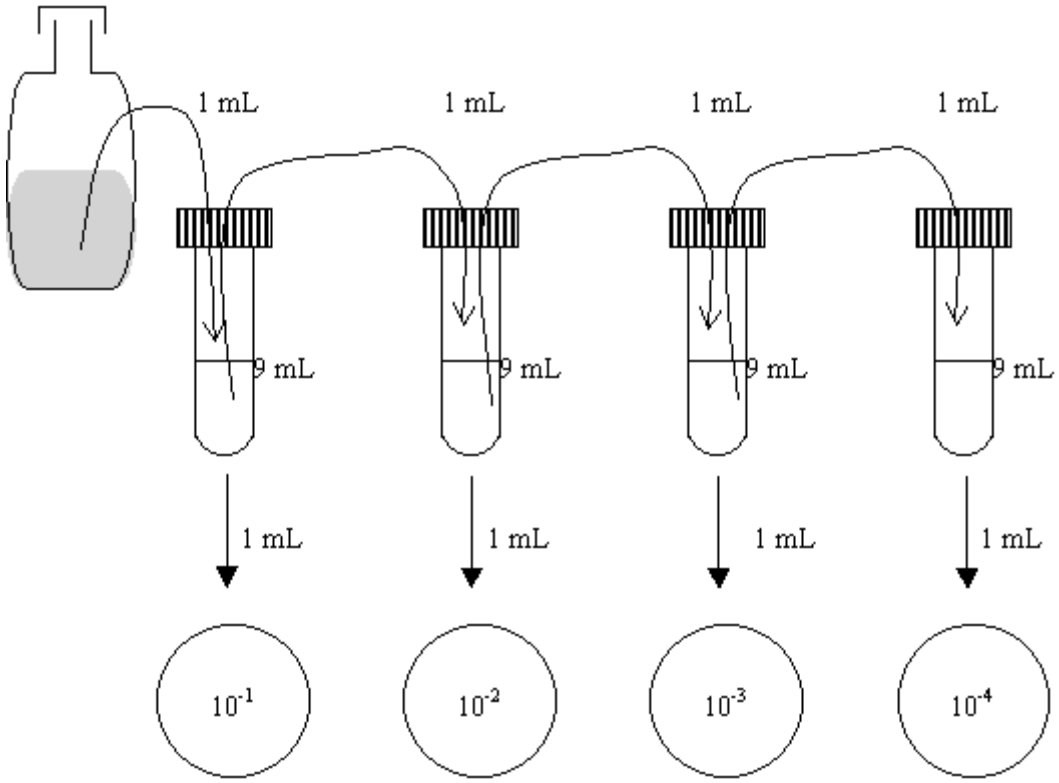
- **Synthetic media**: exact known chemical composition.
- **Complex (empirical) media**: made from natural extracts (e.g., yeast, meat extracts).
- **Selective media**: inhibit unwanted microbes, allow target microbes.
- **Differential media**: distinguish colonies based on their properties.
- **Enrichment media**: promote growth of rare microbes.

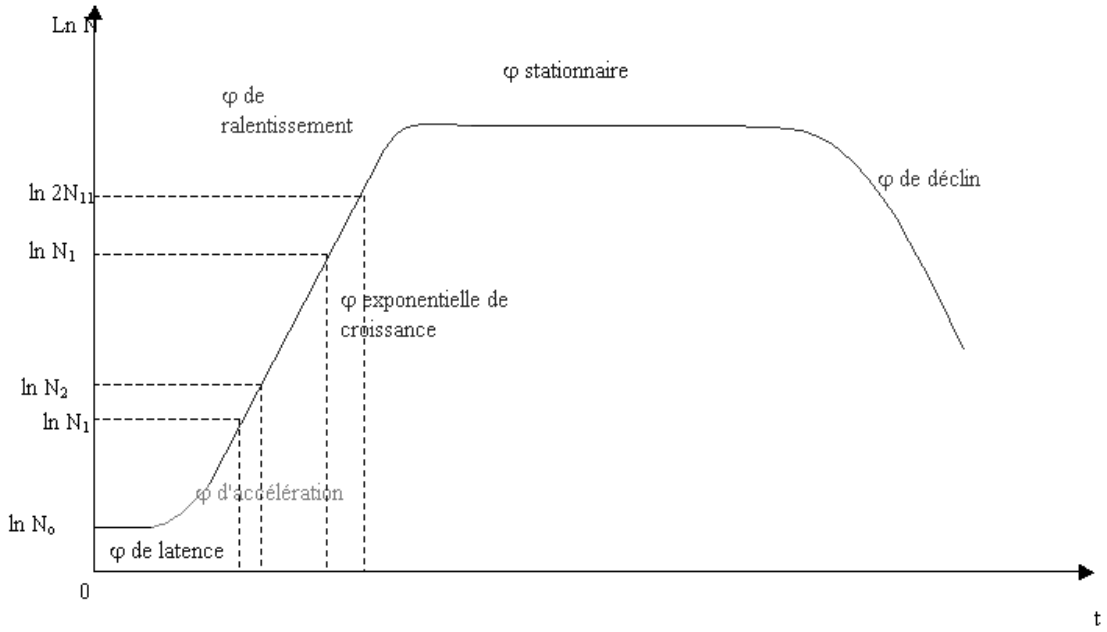
Isolation and Identification Media

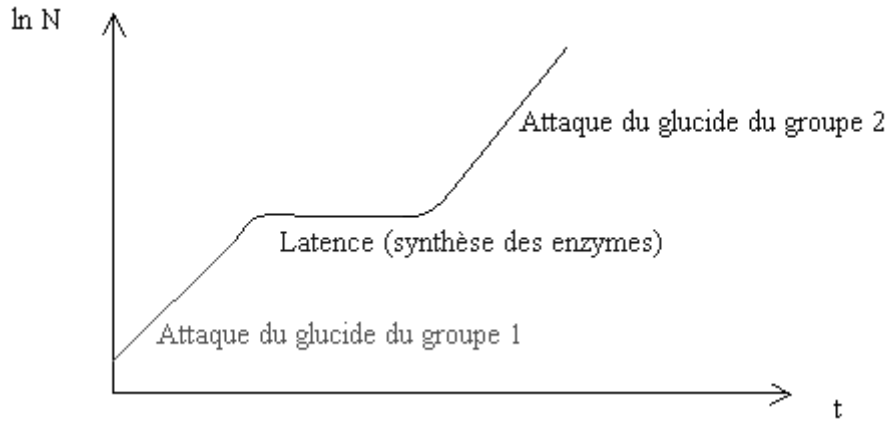
- Solid media like **nutrient agar** allow for colony formation and isolation of pure cultures.
- Identification media test for metabolic properties (e.g., sugar fermentation, gas production, enzyme activity).

Pure Cultures

- Essential for bacterial study.
- Obtained by isolating different species from a mixed sample.
- **Pure culture**: a population derived from a single cell.



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