Practical Work N° 2

Macroscopic and microscopic observation of microorganisms

Introduction

Understanding the morphology, behavior, and characteristics of microorganisms is essential for their identification and study. This practical work focuses on two key observational techniques: **macroscopic observation** of microbial **colonies** and **microscopic examination** of bacterial **motility**.

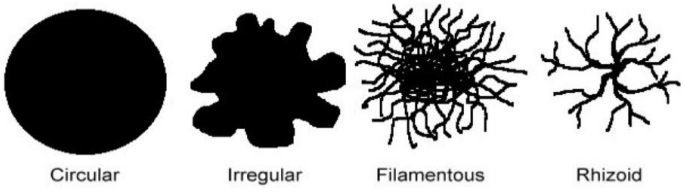
Objective

- To describe and analyse the morphological characteristics of well-isolated microbial colonies
- To use the optical microscope and perform focusing.
- To observe bacterial motility using wet mount microscopy.

1- Macroscopic observation: (Description of colonies)

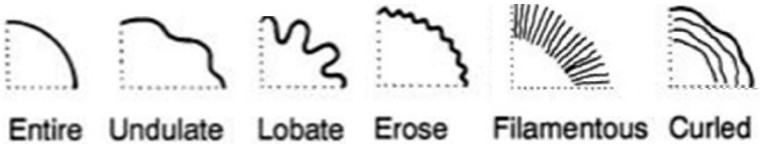
To identify a microbial strain, the first step in microbial diagnosis and biotyping is the macroscopic description of well-isolated colonies. The main characteristics to examine are :

1) **Shape**: Many bacterial species form **round** colonies, while others produce colonies with **varied** shapes.



2) **Elevation** There are several types of elevations observed in microbial colonies.

3) **Margin:** The margin of a colony refers to its edge or boundary.



4) **Size:** The size of a bacterial colony can sometimes be difficult to assess. The most commonly used terms include: **Punctiform colonies**, **small colonies**, **medium colonies** and **large colonies**

5) **Surface:** It is an important criterion as it is often linked to other characteristics, including pathogenicity. Colonies are classified as smooth (S) or rough (R).

6) **Colour:** it can be:

- **Natural**: Due to the production of one or more pigments by the bacteria.
- **Artificial**: Due to a dye or pH indicator present in the medium.

7) **Opacity**: colonies can be opaque, transparent or translucent

8) **Consistency**: assessed during sampling using a sterile, cooled platinum loop. Colonies can be dry, greasy, creamy, or mucoid.

2- Microscopic observation

Microscopic observation enables the morphological study of microorganisms, including:

- Wet mount microscopy examination
- Examination after staining

2.1. Wet Mount Microscopy

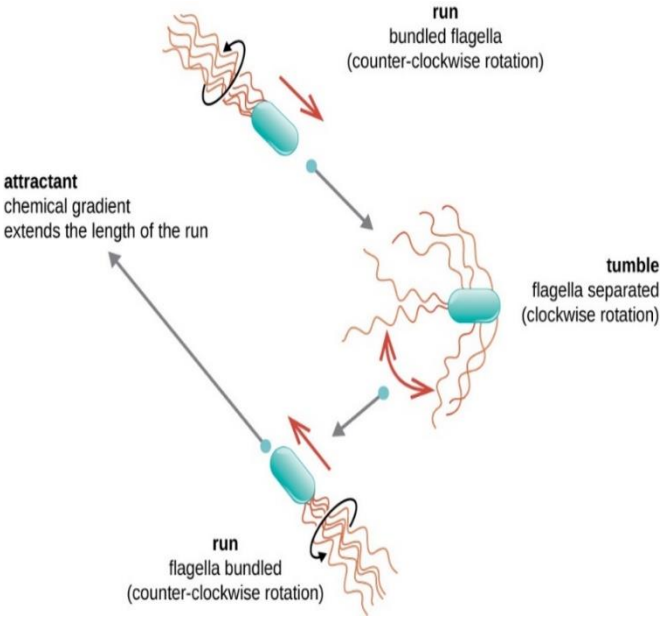
Wet mount microscopy involves the microscopic examination of living microorganisms. It allows for the assessment of locomotor ability using an optical microscope, provided the species being studied is motile.

Bacterial motility: Some bacteria are motile, using one or more **flagella** to move in a characteristic '**run and tumble**' pattern

During the "*run*" phase, bacteria swim straight by rotating their flagella **counterclockwise**, while "*tumbles*"—brief, random reorientations—occur when flagella rotate **clockwise**.

This movement is regulated by the **chemotaxis signalling network,** which adjusts tumble frequency, allowing bacteria to navigate toward favourable environments or away from harmful ones.

In dilute suspensions, cells move **independently**, while in dense populations, they exhibit **collective motion**, forming dynamic clusters, whirls, and jets.

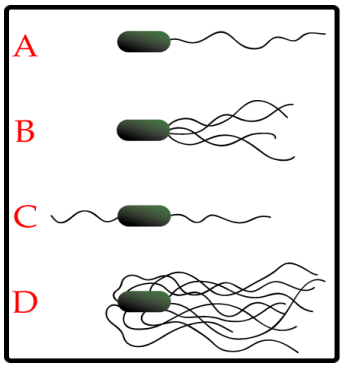


Bacterial flagella: are **long** and **flexible** protein appendages. Their number and arrangement can vary among different bacterial species. Some bacteria possess a single flagellum, while others may have up to ten, and certain species like *Candidatus Ovobacter* can have more than 400.

The positioning of flagella on the bacterial cell surface varies among species. The '**flagellar arrangement**,' also known as '**ciliation**,' describes the distribution of flagella.

Depending on the number and location of flagella, the arrangement is classified as:

a. Polar ciliation (flagellation): In this type, flagella are positioned at one or both poles of the bacterium. It can be classified as follows:



✓ **Monotrichous**: A single flagellum at one pole of the bacterium (e.g., *Vibrio*), enabling linear, directed movement.

✓ **Lophotrichous**: A cluster of flagella at one pole (e.g., *Pseudomonas*), allowing both forward propulsion and oscillatory motion.

✓ **Amphitrichous** (e.g., *Spirillum volutans*): A single flagellum at each of the two opposite poles. Only one flagellum functions at a time, enabling rapid directional reversal by alternating which flagellum is active.

b. Peritrichous ciliation: Flagella are distributed across the entire cell surface, rotating in a coordinated manner to generate a helical, propeller-like movement, ensuring efficient navigation (e.g., *Escherichia coli*).

Work to do

Materials

- Pure bacterial suspensions (*Escherichia coli* and *Bacillus* sp.).
- Microscope slides, cover slips, and platinum loops.
- Bunsen burner, microscopes.
- Immersion oil.
- Absorbent paper.

Technique

A. From a liquid culture:

- Using a platinum loop or Pasteur pipette, place a small drop from a young broth culture onto a clean slide.
- Cover the drop with a cover slip, avoiding the trapping of air bubbles.
- Do not extend the observation beyond 3 to 10 minutes.
- Ensure the liquid does not overflow.

B. From a solid culture:

- Place a small drop of distilled water or sterile physiological saline on the slide.
- Using a sterile platinum loop, collect a small portion of a colony from the agar culture medium.
- Gently emulsify (to avoid damaging the flagella) to obtain a homogeneous suspension.
- Cover with a cover slip, avoiding air bubbles.
- Do not extend the observation beyond 3 to 10 minutes.
- Ensure the liquid does not overflow.

Observation

Conduct an observation using the 40x objective or 100x (with immersion oil), ensuring proper focus under low light conditions by lowering the condenser and slightly closing the diaphragm.

- Bacteria are considered motile if they exhibit distinct trajectories (movement in various directions).
- Do not confuse this with liquid flow, which moves all bacteria uniformly in the same direction and speed (this can occur if the cover slip is moved).

Note: A motile bacterium may appear immobile if observation conditions are not optimal:

- Flagella must not be damaged during preparation or destroyed by an overheated instrument.
- The bacterium must come from a young, actively growing culture.
- Incubation temperature can also influence motility: some bacteria that are immobile at 37°C may become motile at 22°C *(e.g., Yersinia, Hafnia).*
- Motility can be confirmed by inoculating a soft agar medium.

Safety and cleanup

After observation:

1. Dispose of slides and cover slips in a disinfectant solution, such as bleach.
2. Clean the microscope objective with lens paper and cleaner to remove immersion oil.
3. Wash hands thoroughly after handling live bacteria.