CHAPTER 5 : Techniques for Studying and Identifying Microorganisms

Introduction

The identification of microorganisms is a key step in several fields such as health, industry, and the environment.

Objectives of microorganism identification :

• **Health :** It enables the diagnosis of infections and the selection of appropriate treatments. For example, identifying bacteria responsible for diseases such as tuberculosis or urinary infections allows effective patient management.

• **Industry :** It is used in quality control of food, pharmaceutical, and cosmetic products. Detecting pathogenic microorganisms, such as *Salmonella* in meat, is essential for ensuring product safety.

• **Environment :** It helps monitor microorganisms present in water and soil to protect ecosystems. For instance, detecting *Escherichia coli* in water can indicate contamination.

Thus, microorganism identification is essential for ensuring public and environmental safety and for guaranteeing the quality of industrial products.

1. Cultural Characteristics

Observing microorganisms on specific culture media allows the study of their growth properties and facilitates their identification. Cultural characteristics include visible criteria that vary depending on the type of microbe.

Main features observed :

• **Colony shape :** Colonies may be circular, irregular, or filamentous.
• **Color :** Some bacteria produce specific pigments that aid in identification. For example, *Pseudomonas aeruginosa* produces a green pigment.
• **Colony size :** Colonies may be small or large depending on the species and culture medium.
• **Surface appearance :** Colonies may be smooth, rough, mucoid, or dry.
• **Growth rate :** Some bacteria grow rapidly (e.g., *E. coli*), while others grow slowly (e.g., *Mycobacterium tuberculosis*).

Examples of culture media :

• **Solid media :** Blood agar allows observation of hemolysis (destruction of red blood cells). *Streptococcus pyogenes* causes complete (β) hemolysis.
• **Liquid media :** Nutrient broth is used for general bacterial growth. Depending on the microorganism, the broth may show uniform turbidity or sediment at the bottom.

2. Morphological and Structural Characteristics

Morphological and structural characteristics allow differentiation of bacteria by observing their cellular features using various staining methods.

2.1. Gram Staining

Gram staining is a differential staining method that divides bacteria into two groups based on the structure of their cell wall: Gram-positive and Gram-negative.

Principle of Gram staining :

• **Gram-positive :** These bacteria have a thick cell wall rich in peptidoglycan, which retains crystal violet.
• **Gram-negative:** These bacteria have a thin cell wall and an outer membrane. They do not retain crystal violet but take the pink color of safranin after decolorization.

Steps of Gram staining:

1. **Crystal violet :** Stains cells purple.
2. **Iodine (mordant) :** Strengthens dye binding in Gram-positive cells.
3. **Alcohol decolorization :** Removes crystal violet from Gram-negative cells.
4. **Safranin :** Stains Gram-negative bacteria pink.

Results:

• **Gram-positive :** Bacteria appear purple (e.g., *Staphylococcus*, *Bacillus*).
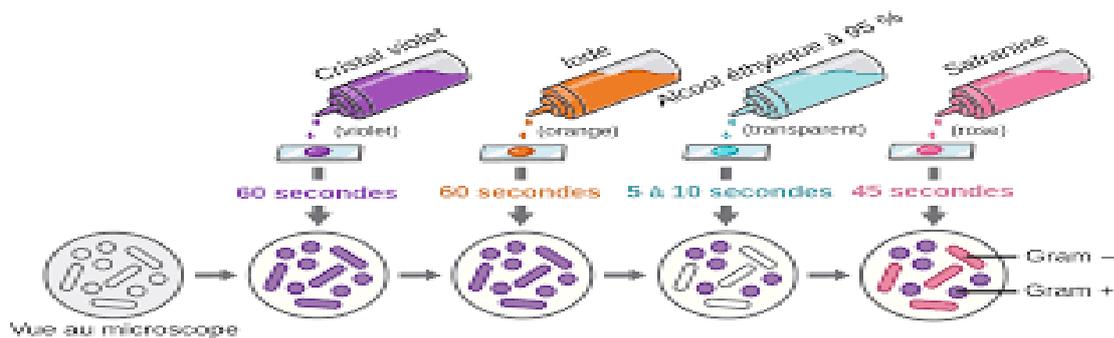• **Gram-negative :** Bacteria appear pink (e.g., *Escherichia coli*, *Salmonella*).

Figure 1: The steps of Gram staining.

2.2. Endospore Staining

Some microorganisms form endospores when exposed to harsh environmental conditions. These structures are highly resistant and allow the bacterium to survive.

Principle of endospore staining:

• Malachite green is used to stain the endospores, while vegetative cells are stained with safranin.

• This staining method allows differentiation between spore-forming and non-spore-forming bacteria.

Example: *Bacillus subtilis* is a spore-forming bacterium that produces spores when exposed to unfavorable conditions.
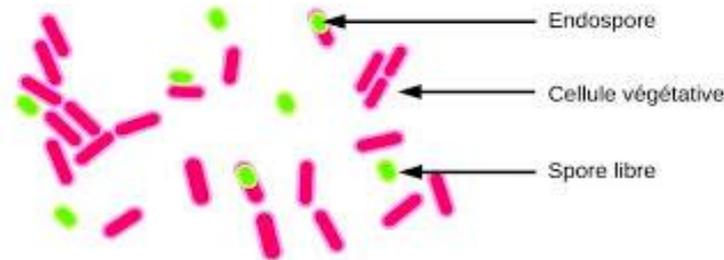

Figure 2 : Spore-forming cell (free spore) and non-spore-forming (vegetative) cell.

3. Biochemical and Physiological Characteristics

Biochemical and physiological tests are used to observe bacterial reactions to various reagents and conditions. These tests help determine the enzymes present and the metabolic capabilities of bacteria.

3.1. Key Enzymatic Tests

• **Catalase test :**

The catalase test detects the enzyme catalase, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. If the bacterium is positive, oxygen bubbles appear.
Example : *Staphylococcus* is catalase-positive, while *Streptococcus* is catalase-negative.

• **Oxidase test :**

The oxidase test detects the enzyme cytochrome c oxidase. A positive reaction produces a blue or purple color.
Example : *Pseudomonas* is oxidase-positive, while *Escherichia coli* is oxidase-negative.

Figure 3 : Typical results of catalase and oxidase tests.

3.2. Sugar Fermentation and Motility

• Sugar fermentation :

This test verifies whether a bacterium can ferment specific sugars to produce acids or gases. A positive test shows a color change (for instance, the tube turns yellow if acids are produced). **Example :** When *Escherichia coli* ferments glucose, the medium turns yellow.

• Motility test :

The motility test is performed in a semi-solid medium. Motile bacteria spread throughout the medium, while non-motile bacteria remain at the site of inoculation.

Figure 4 : Motility test and sugar fermentation test.

3.3. Urea Degradation

The urea degradation test detects the enzyme urease, which breaks down urea into ammonia. This raises the pH of the medium and produces a pink coloration.

Example : *Klebsiella* is urease-positive (medium turns pink), while *Escherichia coli* is urease-negative.

Figure 5 : Urea test results.

4. Multiple Biochemical Studies (API Gallery)

The API gallery (Analytical Profile Index) is a tool used in microbiology for rapid and standardized identification of microorganisms, especially bacteria and yeasts. Each API

gallery contains a series of miniaturized biochemical tests arranged in microtubes, allowing characterization of a microorganism based on its metabolic profile.

Use :

1. **Inoculation :**
   The tests are inoculated with a bacterial suspension.
2. **Incubation :**
   The gallery is incubated to allow enzymatic or biochemical reactions to occur.
3. **Reading the results:**
   Positive or negative reactions are observed (color change, precipitation, etc.).
4. **Identification :**
   The biochemical profile is compared with a database to identify the organism.
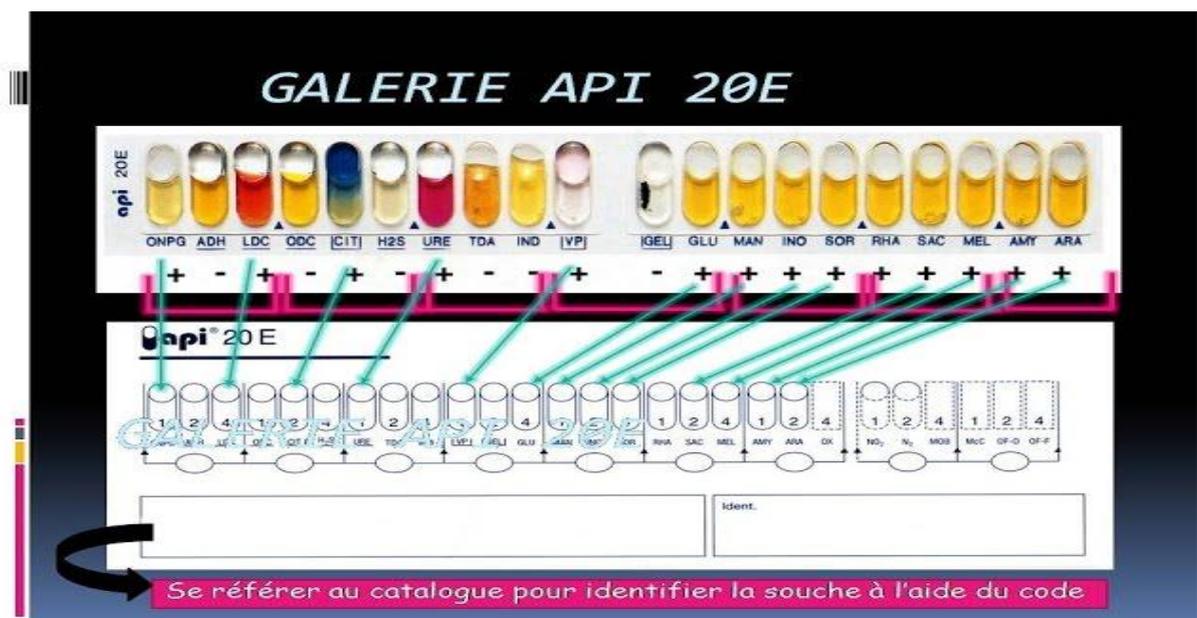

Figure 7 : Example of typical API gallery results.

Conclusion

In conclusion, the identification of microorganisms relies on the analysis of their cultural, morphological, structural, biochemical, and physiological characteristics. These techniques not only help distinguish different types of bacteria but also guide medical diagnosis, industrial quality control, and environmental monitoring.

These classical identification methods are often combined with more advanced molecular techniques such as PCR and DNA sequencing, which allow for even more precise identification of microorganisms.

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