Tutorial session 5: Biochemical identification tests for bacteria

Introduction

Bacterial identification is a critical step in laboratory diagnosis of diseases or contamination. A series of biochemical tests is used to identify bacteria based on their metabolic activities. A simple visual detection of the growth of the organism in the presence of essential nutrients by increased turbidity in the liquid medium is often used. In other tests, the results are based on the change in colour of the medium as a result of the change in its pH.

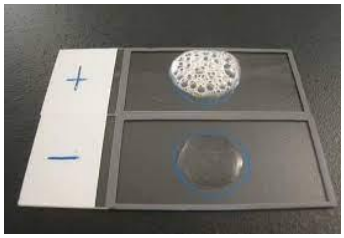
Generally, a single biochemical test is insufficient to identify an unknown bacterium, but by combining multiple tests in a classical or miniaturized biochemical strip (e.g., API systems), precise species identification can be achieved.

Conventional biochemical tests for respiratory, carbohydrate, or protein metabolism are routinely used in laboratories to differentiate or identify bacteria.

A. Classical biochemical tests

Numerous conventional biochemical tests are used in microbiology for bacterial identification. Among the most widely employed:

1. Catalase Test

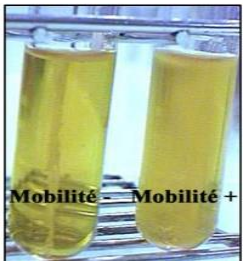


This test identifies organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen ($2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$). The formation of bubbles due to oxygen gas production clearly indicates a positive catalase result.

2. Mannitol-Motility test

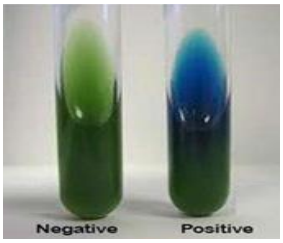
The mannitol motility medium is a combined medium used to determine three characteristics: mannitol fermentation, bacterial motility, and the presence of nitrate reductase.

- If the medium turns yellow: the bacterium is mannitol positive (+).
- If the medium remains red: the bacterium is mannitol negative (-).
- If the bacteria are motile, they will spread from the inoculation stab, creating turbidity in the medium. Otherwise, the bacterium is non-motile (growth only around the central stab).



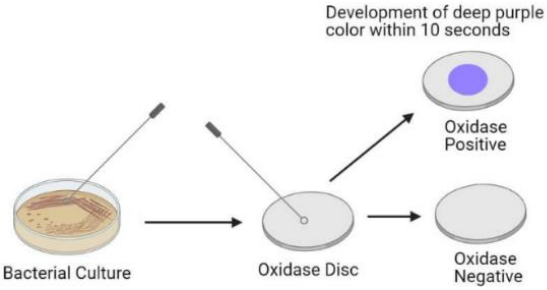
3. Simmons' Citrate test

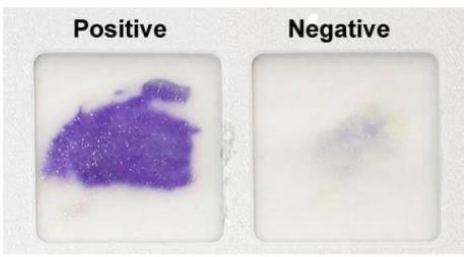
This medium is used to demonstrate the ability of bacteria to utilize citrate as the sole source of carbon and energy. Citrate is hydrolyzed into pyruvic acid and CO_2, which reacts with components of the medium to produce an alkaline compound. This causes the pH indicator (bromothymol blue) to change from green to blue.



4. Oxidase test

This test identifies microorganisms containing the enzyme cytochrome oxidase (important in the electron transport chain). In the oxidase test, artificial electron donors and acceptors are provided. When the electron donor is oxidized by cytochrome oxidase, it turns dark purple.





5. Triple Sugar Iron (TSI) agar

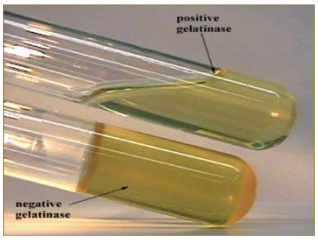
This medium contains glucose, sucrose, lactose, peptone, ferric ammonium citrate, and a sulfur source. It is widely used for identifying *Enterobacteriaceae*.

The microbial strain first utilizes glucose, then sucrose or lactose in the second stage, and finally peptones in the third stage. It may also produce hydrogen sulfide (H_2S).

Triple sugar iron agar tubes: from the left:
1, Acid slant/acid bottom with gas, no H_2S (A/A).
2, Alkaline slant/acid butt, no gas, H_2S-positive (K/A H_2S+).
3, Alkaline slant/alkaline butt, no gas, no H_2S (K/K).
4, Uninoculated tube (negative control).

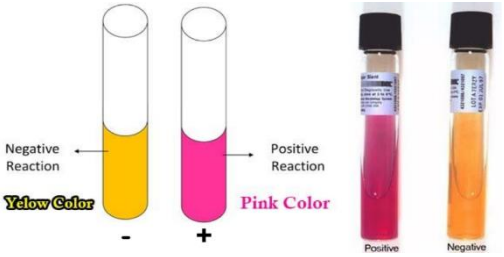
6. Gelatin hydrolysis test

This test detects the ability of an organism to produce gelatinase (a proteolytic enzyme) that liquefies gelatin. Hydrolysis of gelatin indicates the presence of gelatinases.



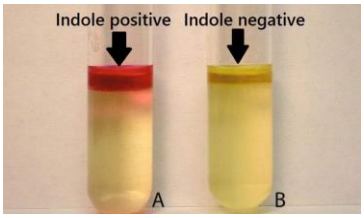
7. Urease test

Urease detection is performed using Ferguson's medium (Urea-Indole medium), which contains tryptophan, urea, and the pH indicator phenol red. This medium helps identify urease, tryptophan deaminase, and indole production (key traits for identifying *Enterobacteriaceae*).



8. Indole Test

The indole test determines the ability of bacteria to produce indole from tryptophan using various enzymes. Indole is detected in a bacterial culture by adding Kovacs' reagent to the medium (often following the urease test).

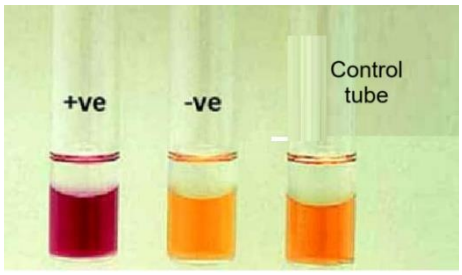


9. Decarboxylase tests

The decarboxylase test is used to identify a bacterium's ability to produce an enzyme called decarboxylase, which removes a carboxyl group (-COOH) from an amino acid (lysine, ornithine, or arginine), producing an amine and increasing the pH of the medium.

a. In the first stage, the bacteria use glucose in the medium, acidifying it and turning the indicator yellow.

b. In the second stage, decarboxylation of the amino acid alkalizes the medium, turning the indicator back to violet-blue, while the control tube remains yellow.



- Positive test (presence of decarboxylase): The color changes to purple (alkaline pH).
- Negative test (absence of decarboxylase): The color turns yellow (acidic pH, due to the fermentation of sugars present in the medium).

B. Miniaturized API-type system

1. **Definition**:

The API system is a miniaturized and standardized biochemical test strip, compatible with comprehensive identification databases. The most well-known is the API 20E (20 tests for *Enterobacteriaceae*).

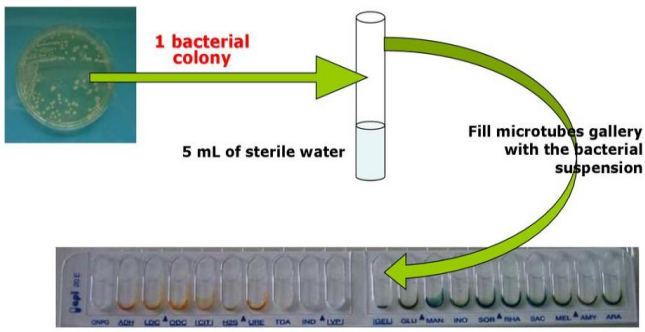
The API 20E strip consists of a plastic strip with 20 microtubes containing dehydrated media. Each tube is designed to detect enzymatic activity, primarily related to carbohydrate fermentation or protein and amino acid catabolism by the inoculated organisms.

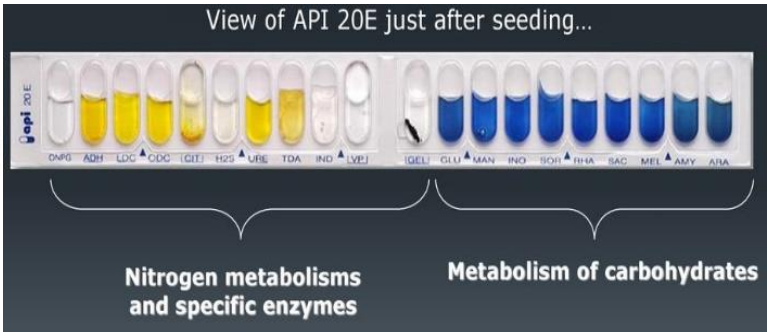
2. **Principle**:

A bacterial suspension is used to rehydrate each microtube. During incubation, metabolic activity produces spontaneous colour changes or changes revealed by adding reagents. All positive and negative test results are compiled to generate a **profile number**, which is then compared to profile numbers in a commercial codebook (or online database) to identify the bacterial species.

3. **Interpretation of results**:

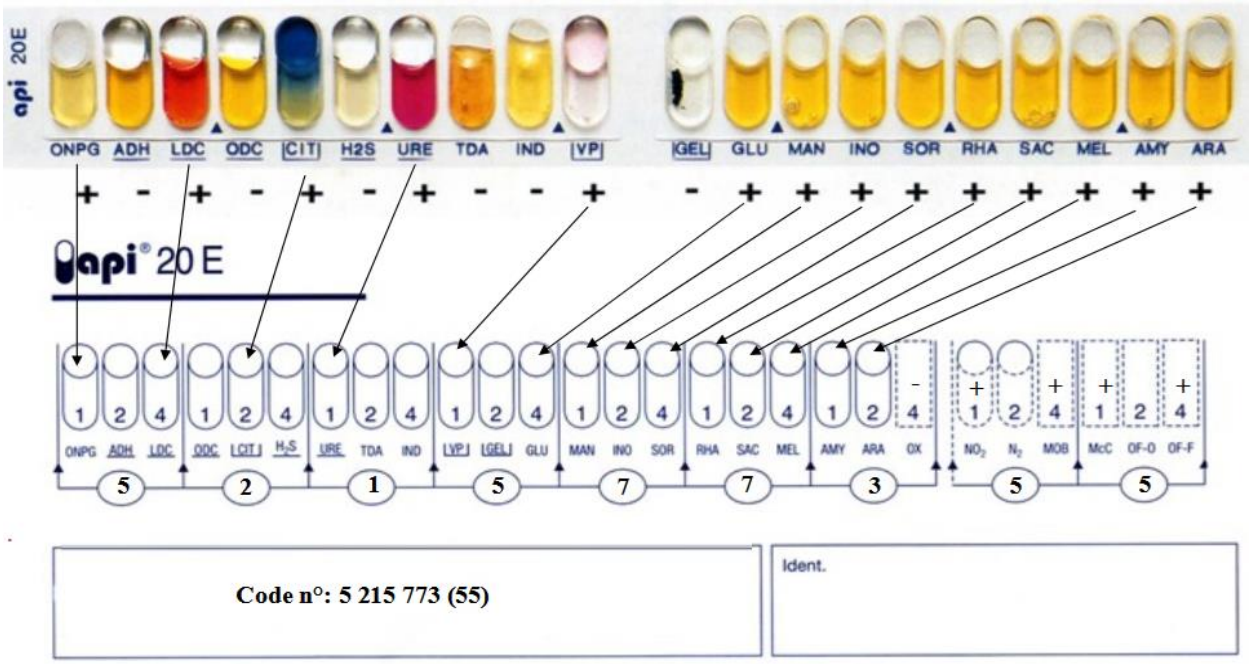
- For some compartments, colour changes can be read immediately after 24 hours, but for others, reagents must be added before interpretation.
- Fill out the API reading sheet by marking each test as positive or negative. The wells are grouped into triplets marked by black triangles, and scores are assigned accordingly.
- Add up the scores for positive wells within each triplet.
- Three test reactions are summed at a time to create a **7-digit number**, which can then be looked up in the codebook.
- Identify the organism using the API catalog or online database





API 20 E after incubation...Positive results for all tests :

API 20 E after incubation...Negative results for all tests :



The most used API system...

API Strep
→ Identification of *Streptococcus* species

API Staph
→ Identification of *Staphylococcus* species

API 20NE
→ Identification of **N**on **E**nterobacteria
(*Pseudomonas* for example)

API 20E
→ Identification of **E**nterobacteria