**Differential biochemical tests** to characterize a species of bacteria

For every biochemical process they perform, they need to have the right enzyme(s).

Enzyme expression is **species-specific**

Therefore, by looking at a pattern of biochemical activity, you can identify the organism.

**Goal:** determine biochemical profile of organism. No single test can tell you!

- Substrate use
- End product formation

### #13: Fermentation of sugars

Differential tests. Q: can UNK ferment the sugar? Choices: glucose, sucrose, lactose

Fermentation: ATP production without using O₂.

**Obligate aerobes:** can’t ferment

**Facultative anaerobes/ Indifferent/ Obligate anaerobes:** can ferment various sugars depending on species

Sucrose ➔ Glycolysis substrates (G6P, Fructose 6 P) ➔ fermentation ➔ acid production +/- gas

Lactose ➔

First, look for turbidity (cell growth). If positive, ask:
- **acidic end products** produced?
- **Gas end products (CO₂, H₂) may** also be produced.

**Durham tube:** collects gas as a bubble. In these tests, the gas is H₂. CO₂, when produced, is water soluble. H₂ is not.

Sucrose and lactose are **disaccharides**. In order to ferment these sugars, bacteria must possess enzymes for cleavage, and isomerization to glycolytic substrates.

In these tests: one sugar per tube. Ask: acid? (yellow=fermented); gas?

### #14: Triple Sugar Iron (TSI): see textbook table 6.6 & Dr. Metcalf’s photos

Differential test. Very useful for distinguishing enteric bacteria (mostly gram – bacilli)

**Key points:**
- All THREE sugars are in this media.
- There is lots of oxygen present on the slant surface; less inside; and very little at the butt

**Questions to ask of TSI results:**

1. Do the bacteria ferment? If so, which sugars?

All 3 sugars are present in TSI, but 10x more sucrose & lactose than glucose

In presence of abundant S&L, even when oxygen is available, bacteria will preferentially ferment (if they can) and turn off cellular respiration pathway.
- In the butt: little or no oxygen, so only fermentative metabolism can occur. Yellow butt = acid pH = glucose fermented. No color change = no glucose fermentation
- In the slant: fermentation of high concentration sucrose and/or lactose if possible (yellow, acid); otherwise oxidative metabolism occurs (red, alkaline)

2. Cysteine metabolism: production of H₂S end product, appears as a black ppt
3. Gas production: cracks in medium

pH indicator is phenol red. 7.3 = red  <6.8 = yellow (acid= + fermentation)

Where does fermentation occur? Natural oxygen gradient in an agar slant: lots on surface, little at bottom of tube.

Interpretation is best if you can compare colors of multiple tubes, including uninoculated control.

Results expressed as slant/butt  A=acid, K=alkaline; gas or no gas; H₂S or no gas

Interpretation:
- Butt: yellow = glucose fermented; red or unchanged = can’t ferment glucose
- Slant: yellow (A) = other sugar(s) fermented
  - red (K) = cannot ferment others, glucose used aerobically, runs out of glucose and aerobically metabolizes proteins/aminoacids producing alkaline products (NH₃)

#15: Catalase & oxidase tests
Catalase and oxidase are enzymes containing a distinctive prosthetic group, or chemical attachment, called an iron porphyrin group. Classic example is hemoglobin. Others are various proteins of the electron transport chain, called cytochromes. The electron transport system is central to aerobic respiration, carrying electrons from NAD/FAD to oxygen, while making ATP. Another iron porphyrin protein is catalase, which breaks down toxic H₂O₂ into H₂O and O₂.

If a cell can make catalase, it can make iron porphyrin groups and is expected to make cytochromes. A positive test for catalase, therefore, indicates cells which can respire aerobically. Test is especially useful for differentiating Gram + cocci.

There are MANY cytochromes, however. Different bacteria produce different ones. The oxidase test specifically detects cytochrome C, which is actually rare in bacteria. Pseudomonas genus is positive.

Catalase - = ferment only, cannot respire aerobically (THIS DOES NOT MEAN anaerobe); usually very small colonies as fermentation is less efficient than aerobic respiration
Catalase + = can make iron porphyrin groups; can make cytochromes & respire aerobically
Oxidase + = all of above plus specifically makes cytochrome C
Oxidase - = no cytochrome c; ??catalase or aerobic respiration

Inoculate:
#13: Using unk SLANT & loop (not broth as it says in lab manual), inoculate glucose, lactose & sucrose broths (make sure no gas bubble present). CHECK for bubbles in Durham tube (shouldn’t be any); bring labels with you so you don’t mix up the sugars; use aseptic technique
#14: Using unk slant with needle, inoculate TSI stab then streak. Aim for center of media.
#15: Streak for colony isolation: NAG plate with unk from BROTH