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 a 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 O_2.

Obligate aerobes: can't ferment
Facultative anaerobes/ Indifferent/ Obligate anaerobes: can ferment various sugars depending on species

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

First, look for turbidity (cell growth). If positive, ask:

- **acidic end products** produced?

pH indicator: Brom Cresol Purple (BCP glucose/sucrose/lactose)
7.0 purple <5.4 yellow (+ test) >8.0 blue (products of cellular respiration)

- **Gas end products (CO_2, H_2) may** also be produced.

Durham tube: collects gas as a bubble. In these tests, the gas is H_2. CO_2, when produced, is water soluble. H_2 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 H_2S 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; H_2S or no
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 H_2O_2 into H_2O and O_2.

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