Biochemical activity of bacteria

- **Different bacteria use different metabolic pathways and the differences between metabolic patterns can be used for their identification**

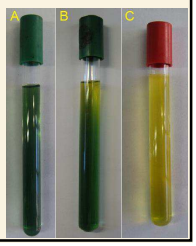
Carbohydrate metabolism

- **Aerobic: glycolysis, citrate cycle, terminal oxidation, terminally CO_2 and H_2O are produced.**
- **Anaerobic: glycolysis and fermentation, organic acids, alcohol and CO_2 are produced.**

Fermentation patterns can be used for identification of bacteria.

5. Oxidation-fermentation test

- The bacterium is inoculated into media containing glucose and bromethimol-blue indicator
- *E. coli* - If bacteria are capable of fermentative metabolism, the colour of the medium turns into yellow due to the acid production. (C)
- *Pseudomonas aeruginosa* - Oxidative microbes which are capable of respiration only, grow only on the top of the tube, turning the colour of the tube into yellow. (B)
- *Acinetobacter lwoffi* – No change in the colour of the media (A) .

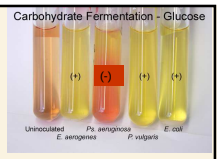


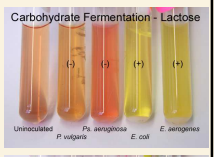
1. Carbohydrate fermentation

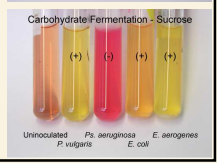
- **to determine the ability of an organism to ferment various simple carbohydrates (lactose, glucose, sucrose, maltose, mannit).**
- **pH indicator (phenol red, decolorized fuchsine) is used for determination of acid production during fermentation of carbohydrates.**
- **in some cases, gas is also produced during the fermentation, which is entrapped in Durham tube.**
- **in positive cases, change of colour and (in some cases) gas production can be seen.**

E. coli: **lactose fermentation ➔ yellow**

Proteus: **can not ➔ red**



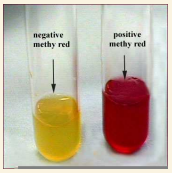


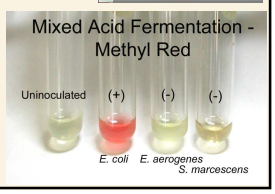


2. Methyl red test

- To test the ability of a bacterium to produce and maintain stable acid end products from glucose fermentation (pH < 4,2)
- Glucose broth is inoculated with the bacteria and incubated overnight. Then a few drops of methyl red indicator is added.

E. coli, Yersinia are positive ➔ red.
Klebsiella is negative ➔ yellow.

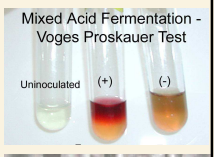


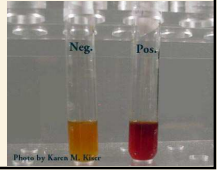


3. Voges-Proskauer reaction

- To identify organisms that are able to produce acetoin (acetylmethyl-carbinol) during butanediol fermentation.
- Glucose broth is inoculated and incubated. Add 3 ml alpha naphtol, followed by 1 ml of 40% KOH. Mix and allow to stand for 30 minutes.

Klebsiella, Enterobacter are positive ➔ pink.
E. coli is negative ➔ no change.





4. Esculine hydrolysis

- When an organism hydrolyses esculine (a special carbohydrate), esculetine is produced, which forms a black precipitate in the presence of ferric citrate.

Enterococcus faecalis is positive ➔ black precipitate.
Streptococcus pyogenes is negative➔ no precipitate.

TSI-triple sugar iron medium

- Slant agar contains 3 different sugars: glucose, lactose, sucrose (10-fold amount of lactose and sucrose than glucose) and indicator.
- No colour change: non-fermentative bacterium. (*Pseudomonas)*
- Strong oxidative fermentation: colour change on the top (*Acinetobacter baumanni)*
- If bacteria ferment only glucose, yellow colour can be seen only on the bottom of the medium because of the produced acids (fermentation). The top of the medium will not turn yellow as the low amount of acids is oxidized to CO_2 and H_2O.
  (Shigella flexneri, Morganella morganii)
- If bacteria ferment lactose and/or sucrose in addition to glucose, the whole medium will become yellow because a lot of acids are formed. *(E. coli)*
- H_2S production: indicated by ferrous sulfate (black precipitate).
  (Proteus vulgaris)

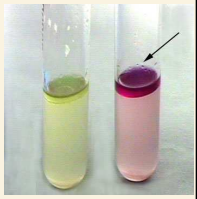
Amino acid and nitrogen metabolism

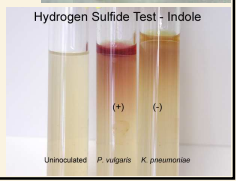
1. Indole production test

- Used to identify bacteria capable of producing indole from tryptophane.
- Can be detected by Kovacs's reagent.

E. coli is positive ➔ red ring is seen on the top of the broth.

Enterobacter, Klebsiella are negative ➔ yellow ring.





2. Urease test

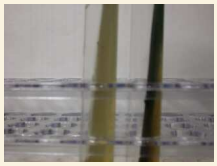
- Used to differentiate bacteria based on their ability to hydrolyse urea with the enzyme urease.
- Useful in distinguishing the genus Proteus from other enteric bacteria.
- If urea is hydrolysed, ammonia is produced and pH increases. So the colour of the medium turns into pink (indicator)

Proteus, Klebsiella are positive ➔ pink colour.
Salmonella, E. coli are negative ➔ yelllow.

3. Phenyl-alanine deaminase production

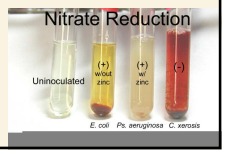
- Deaminase removes amino-group, and the resulting keto-acid will form a greenish complex with iron (from 10% ferric-chloride).

Proteus is positive.



4. Nitrate reduction test

- detects the ability of an organism to reduce nitrate (NO_3^-) to nitrite (NO_2^-) or some other nitrogenous compound, such as molecular nitrogen, using the enzyme nitrate reductase.
- bacteria are subcultured in media containing nitrate and incubated overnight
- reagents (alpha-naphthilamine and sulphanilic-acid) are added to test for the presence of nitrite
- Red colour ➔ nitrite production (E. coli)
- in case of a negative reaction, **add some zinc** (zinc can produce nitrite from nitrate)
  - After the addition of zinc powder:
- If no colour change can be seen, bacteria reduced nitrate to nitrogen gas. Gas can be seen with the aid of Durham-tube (Pseudomonas)
- If red precipitate is formed after the addition of zinc, the bacteria did not reduce nitrate at all (Acinetobacter anitratus)



5. Gelatine digestion test

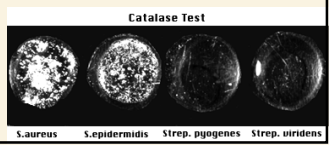
- Used to determine the ability of a microbe to produce hydrolytic exoenzymes called gelatinases that digest and liquefy gelatine.
- There are gelatine cubes with active carbon in bouillon. If bacteria produce gelatineses, gelatine become liquid and carbon sink onto the bottom of the tube.

Pseudomonas, Staphylococcus aureus are positive.
E. coli is negative.

Other tests

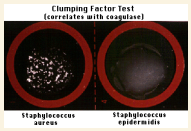
1. Catalase test

- Used to determine those organisms that produce catalase enzyme.
- When bacteria produce catalase, O_2 and H_2O are produced from hydrogene-peroxide and we can visualize the bubbles of O_2.
  Staphylococci are positive.
  Streptococci are negative.



2. Coagulase test

- Used to detect the ability of certain *Staphylococcus* species to clot citrated plasma.
- It is used to identify *Staphylococcus aureus*.
- Clumping test:
  - Put anti-coagulated serum on a slide and suspend bacteria into it.
  - If bacteria produced coagulase, plasma is clotted, fibrin is coagulated.

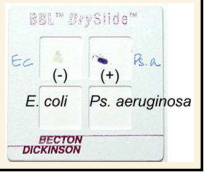


3. Oxidase test

- Used to identify bacteria containing the respiratory enzyme cytochrome oxidase.
- Is useful in distinguishing Enterobacteriaceae (-) from Pseudomonaceae (+).
- The test:
  - Put a piece of filter paper onto a glas slide and drop reagent onto.
  - Take a colony from the bacterium with another glass slide.
  - Streak bacteria on the filter paper soaked with the reagent.

Pseudomonas is positive➔
purple patch on the filter paper.

E. coli or *Proteus* is negative ➔ no colour change.



4. Citrate utilization

- Used to determine the ability of a bacterium to use citrate as a sole carbon source.
- Koser's liquid medium (containing citrate) is used.
- Positive: bacteria are able to grow (indicated by turbidity or change in the colour of an indicator).

Klebsiella: positive.
E. coli: negative.