Family: Enterobacteriaceae

Domain: **Bacteria**
Phylum: **Proteobacteria**
Class: **Gammaproteobacteria**
Order: **Enterobacteriales**
Family: **Enterobacteriaceae**

Genus: ***Escherichia, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus***

General Characteristics **:** The Enterobacteriaceae are a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. They are gram-negative rods, either motile or nonmotile; grow well on MacConkey's agar; grow aerobically and anaerobically (are facultative anaerobes); are catalase-positive, oxidase-negative, and reduce nitrate to nitrite Capsules are large and regular in *klebsiella*, less so in *enterobacter*, and uncommon in the other species.

Enterobacteriaceae can be subdivided into:

Enterobacteriaceae

Lactose fermented rapidly	**Lactose fermented slowly**	**Lactose non-fermenter**
Escherichia coli	***Edwardsiella***	***Salmonella* species**
Enterobacter aerogenes	***Serratia***	***Shigella* species**
***Klebsiella* species**	***Citrobacter***	***Proteus* species**
	Arizona	
	Providencia	
	Erwinia	

Lactose fermenter Enterobacteriaceae

- ***Escherichia coli****:* gram-negative rods, motile, flat, nonviscous colonies. ***Can cause:***
  1. Infants epidemic diarrhoea, indicate faecal contamination.
  2. Urinary tract infection (UTI).
  3. Wound infection.
  4. Bloody diarrhoea.
  5. Sepsis.
  6. Meningitis.
- ***Klebsiella:***

K. pneumoniae*:* very viscous, mucoid growth, nonmotile.
It causes**:** 1. a small proportion (about 1%) of bacterial pneumonias.
2. produce urinary tract infection
3. bacteremia.
4. hospital-acquired infections.

K. oxytoca: ***causes:*** hospital-acquired infection

Klebsiella ozaenae **and** ***Klebsiella rhinoscleromatis:*** ***<u>are associated</u>:*** with inflammatory conditions of the upper respiratory tract.

- ***Enterobacter aerogenes:*** This organism has small capsules, may be found free-living as well as in the intestinal tract. ***<u>Causes</u>:***
  1. Urinary tract infections**.**
  2. Sepsis.
- ***Serratia: S. marcescens*** is a common opportunistic pathogen in hospitalized patients. ***Serratia*** (usually nonpigmented) ***<u>causes</u>:***

  1. Pneumonia.
  2. Bacteremia.
  3. Endocarditis.

  Only about 10% of isolates form the red pigment (prodigiosin) that has long characterized *S. marcescens.*
- ***Providencia: Providencia*** **species (*Providencia rettgeri, Providencia alcalifaciens,* and *Providencia stuartii*)** are members of the normal intestinal flora. ***<u>All cause</u>*** urinary tract infections and occasionally other infections and are often resistant to antimicrobial therapy.
- ***Citrobacter: Citrobacter <u>can cause:</u>*** urinary tract infections and sepsis.

<u>Specimens</u> ***:*** Specimens included urine, blood, pus, spinal fluid, sputum, or other material, as indicated by the localization of the disease process. Specimens are plated on both blood agar and differential media.

<u>Culture and Growth Characteristics</u> ***:*** *E. coli* and most of the other enteric bacteria form circular, convex, smooth colonies with distinct edges. *Enterobacter* colonies are similar but somewhat more mucoid. *Klebsiella* colonies are large and very mucoid and tend to coalesce with prolonged incubation. Some strains of *E coli* produce haemolysis on blood agar.

<u>Classification</u> ***:***

1. Serological classification upon antigens (O-Ag, H-Ag, K-Ag).
2. Biochemical classification and sugar fermentation.
3. DNA-DNA hybridization / G:C ratio.

<u>Laboratory diagnostic tests:</u>

1. **Gram stain** (Gram-negative rods).
2. **MacConkey agar (Selective and Differential media)**

<u>Selective because contains</u>: bile salts and crystal violet that inhibited the growth of Gram-positive bacteria and some fastidious Gram-negative bacteria.
<u>Differential because contains</u>: lactose as source of carbon and pH indicator neutral red (yellow at alkaline pH, pink at acid pH) that differential between lactose fermenter and non lactose fermenter.
Lactose fermenting bacteria produce a mixed of acids that conversion of the neutral red to red color so that produce pink to red colonies.

Lactose non fermenting bacteria produce colonies appear colorless or transparent.

3. **Eosin methylene blue agar (EMB) (Selective and Differential media)**

Selective because contains: the aniline dyes (eosin and methylene blue) that inhibited the growth of Gram-positive bacteria and some fastidious Gram-negative bacteria.

Differential because contains: the aniline dye also combines to form a precipitate at acid pH (appearing as a metallic green sheen) thus serving as indicator of acid production from lactose.

4. **Triple Sugar Iron Agar (TSI):**

The triple sugar iron (TSI) agar test is generally used for the identification of enteric bacteria (Enterobacteriaceae). It is also used to distinguish the Enterobacteriaceae from other gram-negative intestinal bacteria.

TSI agar slants contain:

0.1% glucose, 1% lactose, and 1% sucrose,
pH indicator: phenol red (pink at alkaline pH, yellow at acid pH).
Substrate for H_2S production: sodium thiosulfate (Na-thiosulfate)
Indicator for H_2S production: ferrous sulphate ($FeSO_4$)

$$\text{Na-thiosulfate} + H_2 \longrightarrow H_2S$$
$$H_2S + FeSO_4 \longrightarrow FeS\downarrow \text{ (black precipitate)}$$

Once H_2S is produced, it combines with the ferrous sulfate, forming an insoluble, black ferrous sulfide precipitate.

The results:

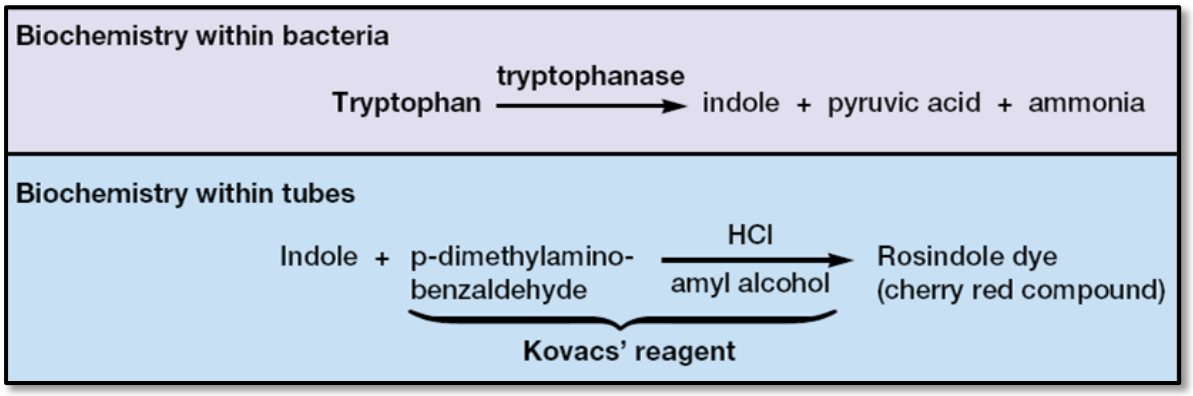
1. Yellow butt (A) and red slant (K) due to the fermentation of glucose. The slant remains red (alkaline) (K) because of the limited glucose in the medium and, therefore, limited acid formation, which does not persist.
2. Yellow butt (A) and slant (A) due to the fermentation of lactose and/or sucrose due to the high concentration of these sugars leading to excessive acid formation in the entire medium.
3. Red butt (K) and slant (K) indicates that none of the sugars were fermented and neither gas nor H_2S were produced.
4. Gas formation (CO_2) noted by bubbles or splitting of the agar.
5. Gas formation (H_2S) seen by blackening of the agar (black precipitate).

5. **IMViC test (indole, methyl red, Voges-Proskauer, and citrate) (the "i" is for ease of pronunciation)**

This test is using to the differentiation and identification of Family Enterobacteriaceae.

Indole Production: This test is using to detect the production tryptophanase enzyme that can hydrolyze tryptophan (amino acid) to its metabolic products, namely, indole, pyruvic acid, and ammonia. The pyruvic acid and ammonia are using nutrition of bacteria while indole is not used and accumulates in the medium. Kovacs' reagent reacts with the indole, producing a bright red compound on the surface of the medium.

Medium: peptone water or tryptic soy broth.
Substrate: tryptophan
Reagent: Kovacs' reagen

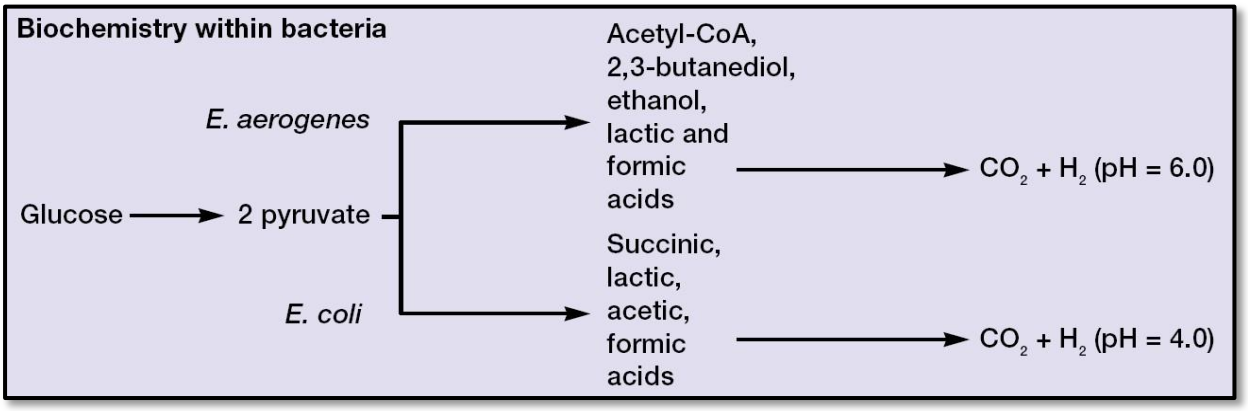


The results:

Positive (+): red layer on the surface of the medium (red ring).
Negative (-): the absence of a red color (no change).

Methyl Red test (MR): glucose full fermentation (Mixed acids fermentation and thus acidify the medium). This test distinguishs between *E. coli* (a mixed acid fermenter) and *E. aerogenes* (a butanediol fermenter).

Medium: MR-VP broth medium
Substrate: glucose
Indicator: methyl red (yellow at alkaline pH, red at acid pH)



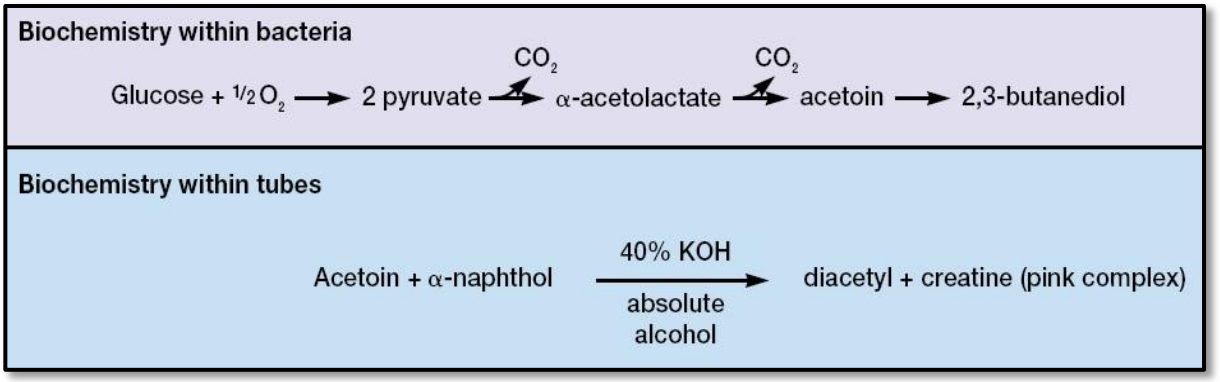
The results:

Positive (+): red color
Negative (-): yellow color

Voges-Proskauer test (VP): glucose partial fermentation (ferment glucose, leading to 2,3-butanediol accumulation, the pH of the medium does not fall as low as during mixed acid fermentation). This test detect the presence of acetoin - a precursor in the synthesis of 2,3-butanediol.

Medium: MR-VP broth medium
Substrate: glucose

Indicator: 40% KOH and 5% solution of alpha-naphthol in absolute ethanol (Barritt's reagent)



The results:

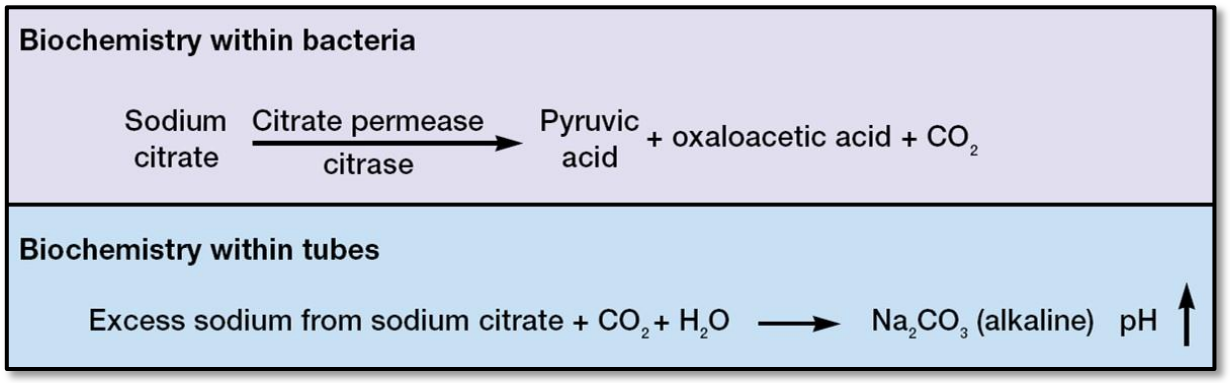
Positive (+): Development of a red color in 15 minutes
Negative (-): absence of a red color

Citrate Utilization Test: This test determines the ability of bacteria to use citrate as a sole carbon source for their energy needs. This ability depends on the presence of a citrate permease that facilitates transport of citrate into the bacterium.

Medium: Simmons citrate agar slants

Substrate: citrate

pH Indicator: bromothymol blue (blue at alkaline pH, yellow at acid pH) When bacteria oxidize citrate, they remove it from the medium and liberate CO_2. CO_2 combines with sodium (supplied by sodium citrate) and water to form sodium carbonate an alkaline



product. This raises the pH, turns the pH indicator to a blue color.

The results:

Positive (+): blue color
Negative (-): no color change and no growth in the medium

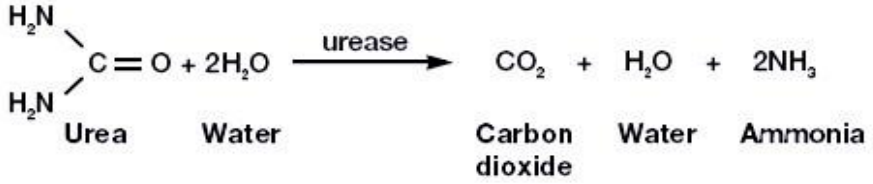
6. Urease test :

Some bacteria are able to produce an enzyme called urease that attacks the nitrogen and carbon bond in amide compounds such as urea, forming the end products ammonia, CO_2, and water. When urea is hydrolyzed, ammonia accumulates in the medium and makes it alkaline (increase in pH).

Medium: urea broth

Substrate: urea
pH Indicator: phenol red

Biochemistry within bacteria:



Biochemistry within tubes:

Ammonia + phenol red ⟶ deep pink or purplish red

The results:

Positive (+):deep pink or purplish red
Negative (-): no deep pink color or (yellow color)

7. **Motility test:** stab method in semisolid media

8. **Sensitivity test:** on Mueller-Hinton Agar

The sulfonamides, ampicillin, cephalosporins, fluoroquinolones, and aminoglycosides have marked antibacterial effects against the enterics, but variation in susceptibility is great.

Test	***E.coli***	***K. pneumonia***	***Enterobacter***
MacConkey agar	**Pink colonies, smooth**	**Pink colonies, mucoid, larger than *E.coli***	**Pink colonies**
EMB	metallic green sheen	No metallic green sheen	No metallic green sheen
TSI	**A/A + -**	**A/A + -**	**A/A + -**
Indole	**+**	**-**	**-**
MR	**+**	**-**	**-**
VP	**-**	**+**	**+**
Citrate	**-**	**+**	**+**
Uraese	**-**	**+**	**-**
Motility	**+**	**-**	**+**