# Bactria Classification

***Escherichia coli***

**Lab 8**

# Scientific Classification of *Escherichia spp.*

**Domain: Bacteria**

**Phylum: Proteobacteria**

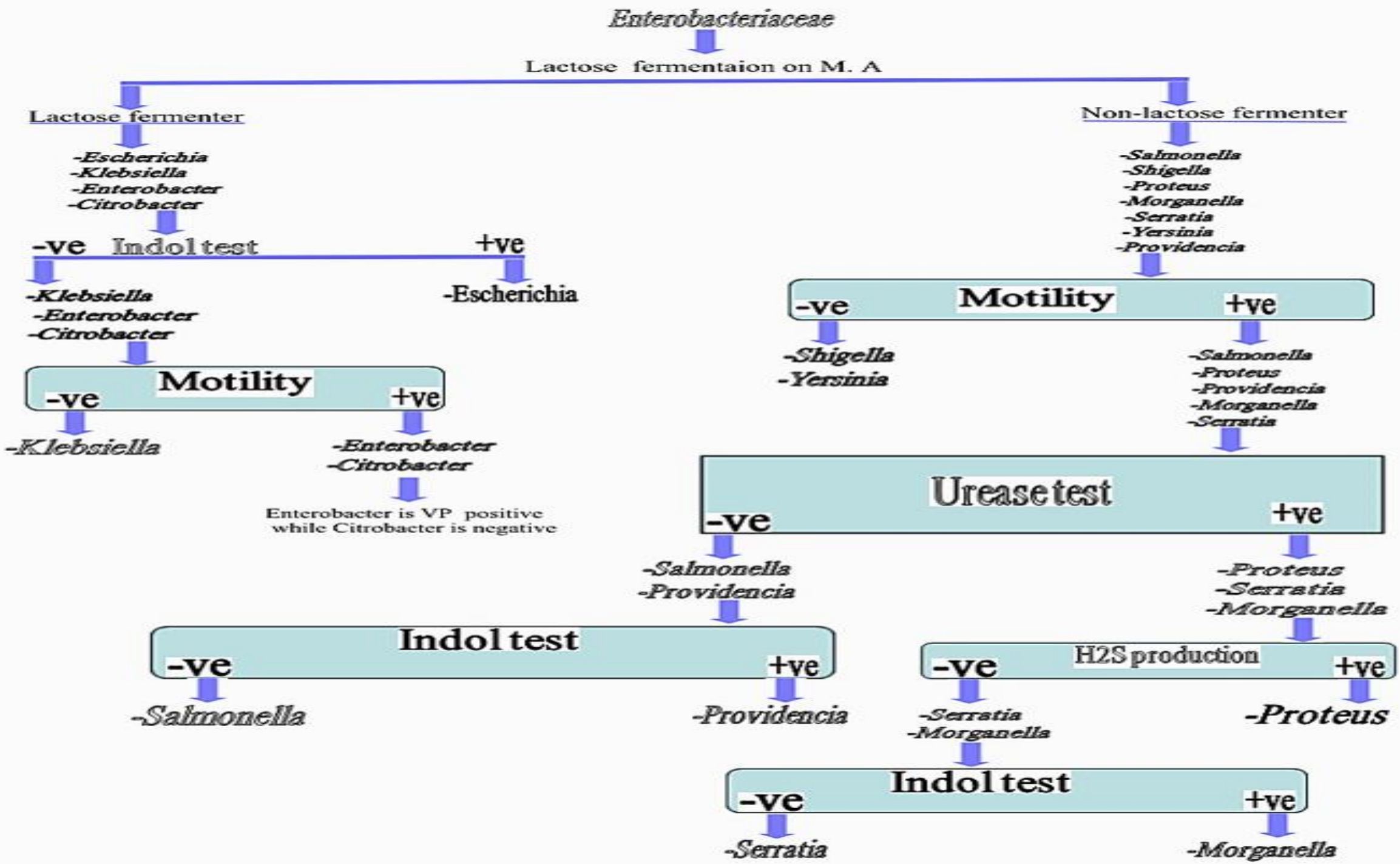
**Class: Gammaproteobacteria**

**Order: Enterobacteriales**

**Family: Enterobacteriaceae**

**Genus: Escherichia**

**Species: *E. coli***


Enterobacteriaceae
Lactose fermentaion on M. A
Lactose fermenter
-Escherichia
-Klebsiella
-Enterobacter
-Citrobacter
-ve Indol test +ve
-Klebsiella
-Enterobacter
-Citrobacter
-Escherichia
Motility
-ve +ve
-Klebsiella
-Enterobacter
-Citrobacter
Enterobacter is VP positive while Citrobacter is negative
Non-lactose fermenter
-Salmonella
-Shigella
-Proteus
-Morganella
-Serratia
-Yersinia
-Providencia
Motility
-ve +ve
-Shigella
-Yersinia
-Salmonella
-Proteus
-Providencia
-Morganella
-Serratia
Urease test
-ve +ve
-Salmonella
-Providencia
-Proteus
-Serratia
-Morganella
Indol test
-ve +ve
-Salmonella
-Providencia
H2S production
-ve +ve
-Serratia
-Morganella
-Proteus
Indol test
-ve +ve
-Serratia
-Morganella

# The IMViC Tests

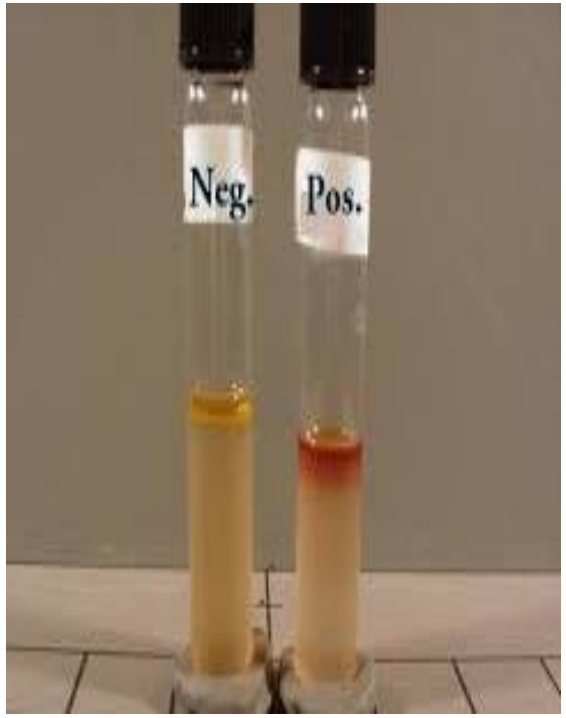
**Indole, Methyl red, Voges-Proskauer, and Citrate**; the "**i**" is for ease of pronunciation.

The amino acid **tryptophan** is found in nearly all proteins. Bacteria that contain the enzyme **tryptophanase** can hydrolyze tryptophan to its metabolic products, namely, indole, pyruvic acid, and ammonia. The bacteria use the pyruvic acid and ammonia to satisfy nutritional needs; indole is not used and accumulates in the medium. The presence of indole can be detected by the addition of **Kovacs' reagent**. Kovacs' reagent reacts with the indole, producing a bright red compound on the surface of the medium. Bacteria producing a red layer following addition of Kovacs' reagent are **indole positive;** the absence of a red color indicates tryptophan was not hydrolyzed, and the bacteria are **indole negative.**


**Citrate test**


**Methyl Red Test**


**Voges-Proskaeur test**




**Indole Production**

**Procedure**

a. Label each of the Tryptophan Broth tubes with the name of the bacterium to be inoculated ( *E. coli* ), (If tryptophan broth medium is unavailable, tryptic soy broth is a good substitute for testing indole production.)
b. Using aseptic technique, inoculate each tube by a loopful of culture.
c. Incubate the tubes for about 24 hours at 35°C.
d. Remove the tubes from the incubator and add **0.5** ml (about **10** drops) of ***Kovacs' reagent*** to each tube, and shake the tube gently. A deep red develops in the presence of indole. Negative reactions remain colorless or light yellow.

## 2- Methyl Red Test

**All enteric bacteria catabolize glucose for their energy needs; however, the end products vary depending on the enzyme pathways present in the bacteria. The pH indicator methyl red detects a pH change to the acid range as a result of acidic end products such as lactic, acetic, and formic acids. This test is of value in distinguishing between *E. coli* (a mixed acid fermenter) and *Enterobacter aerogenes* (a butanediol fermenter).**

**Mixed acid fermenters such as *E. coli* produce a mixture of fermentation acids and thus acidify the medium.**

**Butanediol fermenters such as *E. aerogenes* form butanediol, acetoin, and fewer organic acids. The pH of the medium does not fall as low as during mixed acid fermentation. At a pH of 4, the methyl red indicator turns red, a positive methyl red test. At a pH of 6, the indicator turns yellow, a negative methyl red test.**

## Procedure

a. **Label each of the Methyl red - Voges- Proskauer ( MR-VP) broth- media tubes with the name of the bacterium.**

| Typical Formula | Gram /litter |
|---|---|
| Peptone | 7.0 |
| Glucose | 5.0 |
| Phosphate buffer | 5.0 |
| pH 6.9 ± 0.2 | |

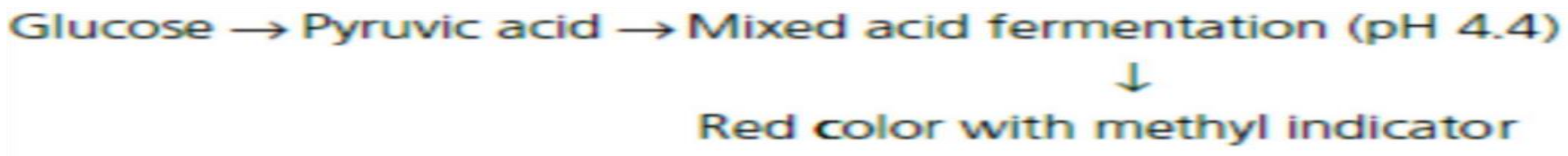

**Methyl Red Test**

b. **Inoculate each tube with the appropriate bacterium by means of a loop inoculation.**

c. **Incubate all tubes at 35°C for 24 to 48 hours. For slow fermenters, it may take four to five days.**

d. **Transfer 1/3 of each culture into an empty test tube and set these aside for the Voges-Proskauer test.**

e. **To the 2/3 of the culture remaining in each tube, add 0.2 ml (about 4 to 5 drops) of methyl-red solution indicator, and read the colour on the surface of the medium immediately, any color change (a red color is MR positive).**



# 3- Voges-Proskauer Test

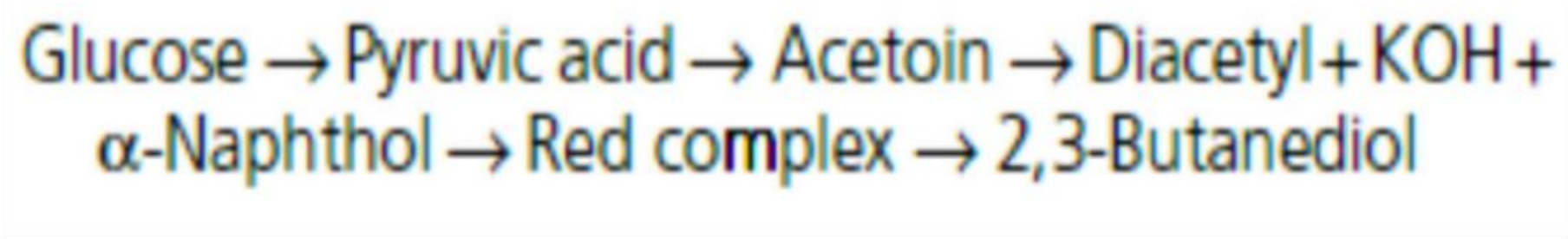
The Voges-Proskauer test identifies bacteria that ferment glucose, leading to 2,3-butanediol accumulation in the medium. The addition of (40% KOH and a 5% solution of alpha-naphthol) will detect the presence of acetoin- a precursor in the synthesis of 2,3-butanediol.

In the presence of the reagents and acetoin, a cherry-red color develops. Development of a red color in the culture medium 15 minutes following the addition of reagent represents a positive VP test; absence of a red color is a negative VP test.

## Procedure

- Use the $\frac{1}{3}$ aliquot from the methyl red test. While wearing disposable gloves, add 0.6 ml of 5% alpha naphthol (15 drops) followed by 0.2 mL of 40% KOH (5 drops) to each culture.

- Shake vigorously to aerate. Positive reactions occur at once or within 20 minutes and are indicated by the presence of a red color.



## Procedure

The **citrate utilization 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. Once inside the bacterium, citrate is converted to pyruvic acid and CO2. **Simmons citrate agar** slants contain sodium citrate as the carbon source, ammonium ($NH4^+$) as a nitrogen source, and the pH indicator bromothymol blue. This test is done on slants since O2 is necessary for citrate utilization. When bacteria oxidize citrate, they remove it from the medium and liberate CO2. CO2 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, and represents a **positive citrate test;** absence of a color change is a **negative citrate test**. Citrate-negative cultures will also show no growth in the medium.

# Procedure

a. **Inoculate each bacterium into Simmons citrate agar slants tube by means of a *stab-and-streak* inoculation.**

b. **Incubate these cultures for 24 to 48 hours at 35°C.**

c. **Examine the slant cultures for the presence or absence of growth and for any change in color from green to blue. The development of a deep blue color is a positive test.**


**Citrate test**