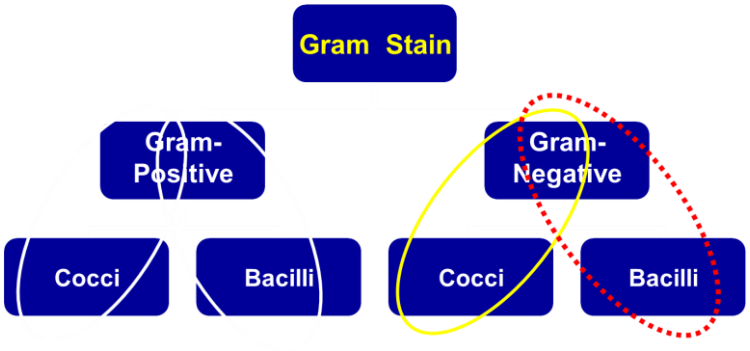
Non-Fermentative Gram-Negative Rods

Pseudomonas spp

Classification of Bacteria



Characters of *Pseudomonas*

- Gram-negative bacilli belonging to ***Pseudomonadaceae***
- Motile by means of a single polar flagellum.
- Non spore forming
- non Capsulated "Polysaccharide capsule"
- Aerobic
- Breakdown glucose by oxidation i.e. Oxidative
- Oxidase and catalase positive
- It has very simple nutritional requirements i.e. non fastidious
- The most important pathogenic organism is *Ps. aeruginosa*
- Optimum temperature is 37 C, and it is able to grow at 42 C
- It is resistant to high concentrations of salts, dyes, weak antiseptics, and many antibiotics
- Common inhabitants of soil, water, GITe`ewe
- ***Ps. aeruginosa*** is opportunistic pathogen and associated with a variety of infections including:
  - Urinary tract infections

- Wound and burn with blue green pus
- Respiratory system infections (Pneumonia)
- Eye infection and may lead to blindness
- Ear infection (external ear or otitis media)
- Meningitis
- A variety of systemic infections

- *Ps. aeruginosa* produce two types of soluble pigments:
  - **Pyoverdin or fluorscein:** It is yellow-green pigment and fluorescent
  - **Pyocyanin**: It is a blue-green pigment and non-fluorescent

Identification of *Ps. Aeruginosa*

Laboratory diagnosis

- **Specimen:**
  - Urine, pus, sputum, CSF, blood, skin swap according to the type of infection
- **Microscopical Examination**
  - **Gram Stain:** Gram-negative rods
  - Motility Test:
    - **Hanging Drop Techniques**
    - **Semisolid agar medium**

Cultural Characteristics

- On Nutrient agar:
  - Colonies are surrounded by bluish green coloration
- On selective media "Cetermide"
  - Pigments are more obvious
- On Blood agar

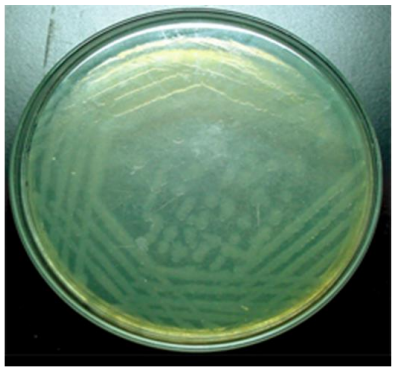
β-hemolytic colonies

- On MacConkey agar

Pale yellow colonies i.e. non lactose fermenters

- Ps. aeruginosa able to grow at 42 C for 3 days

Cultural Characteristics



Ps. aeruginosa **on cetrimide agar**

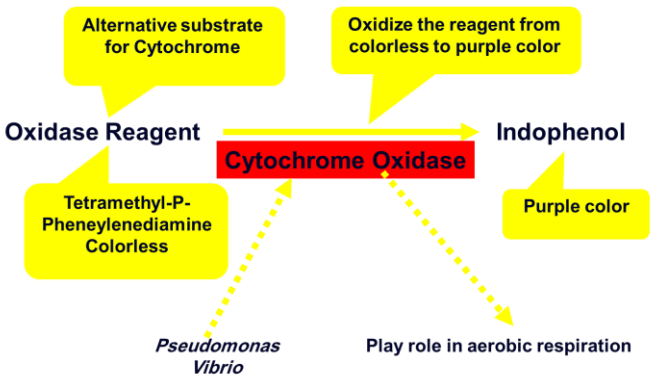
Gram Stain of *Pseudomonas*

Ps. aeruginosa **on Nutrient agar**

Biochemical Reactions

- **Oxidase positive**
- **Breakdown glucose oxdatively**
- **Nitrate Reductase negative**
- **Gelatinase positive**
- **Utilize Citrate**

Oxidase Test: Principal

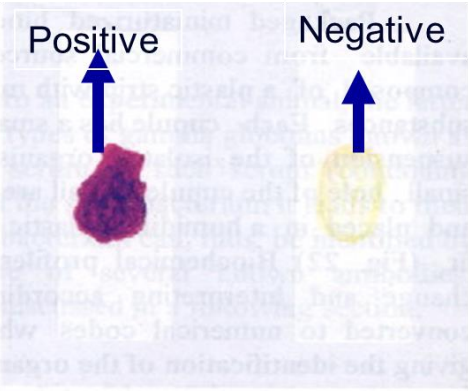


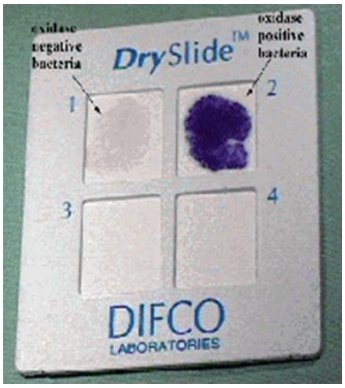
Method:

- hold a piece of the oxidase test paper with forceps and touch onto an area of heavy growth
- Use platinum loop (not used nichrome) or wood stick

Results

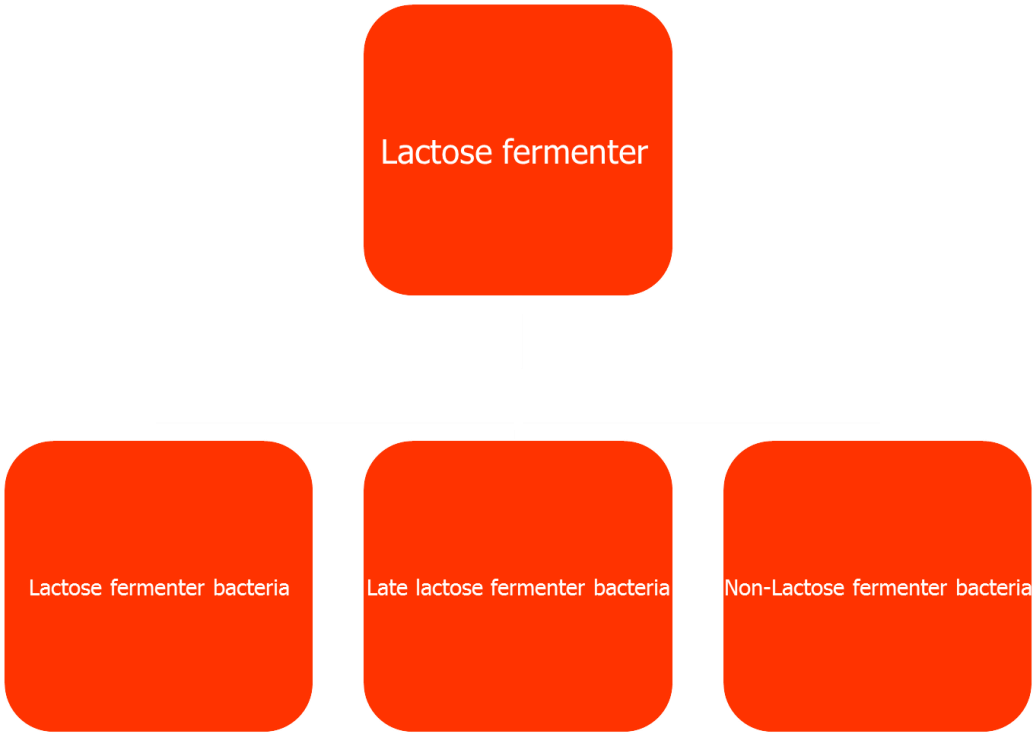
- Color change to purple within:
  - 10 seconds = positive
  - 10 - 60 seconds = delayed positive
  - >60 seconds = negative

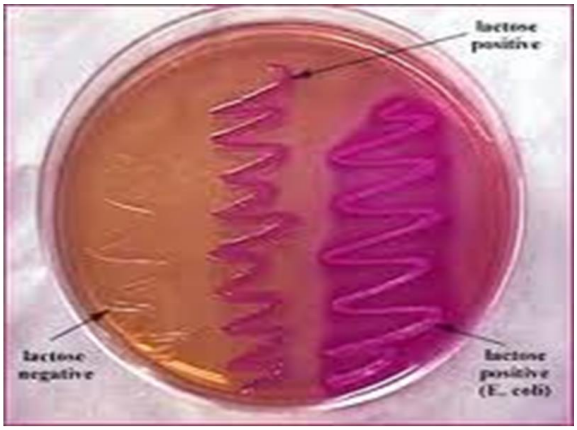


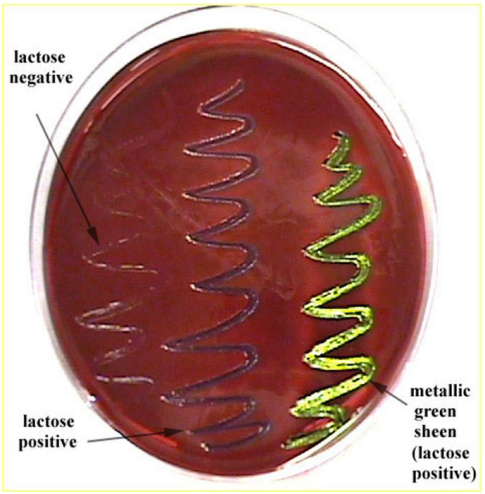


ENTEROBACTERIACEAE

- Gram negative non-spore forming bacilli.
- Aerobic and facultative anaerobic.
- Grow easily on ordinary media.
- Most are motile with peritrichous flagella .
- All species ferment glucose with production of acid or acid and gas.
- Catalase +ve
- Oxidase –ve
- Inhabit the intestinal tract of man and animals naturally.
- Many species are pathogenic.
- Genera have been defined on biochemical basis.







Escherichia

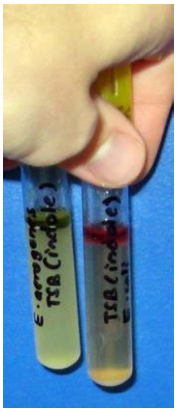
- *Escherichia coli*: cause UTI, diarrhea, bacteremia or meningitis.
- Microscopical appearance: Gram-negative short rods. Most strains are motile.
- Cultural characteristics: Colonies on MacConkey agar are pink (lactose fermented with bile precipitation.
- Biochemical reactions: IMViC + + - -

IMViC tests
Indole

• *Enterobacteraerogenes* –

•*Escherichia coli*(pink/red) +

Kovac's reagent detects if tryptophan has been hydrolyzed to indol/tryptophanase

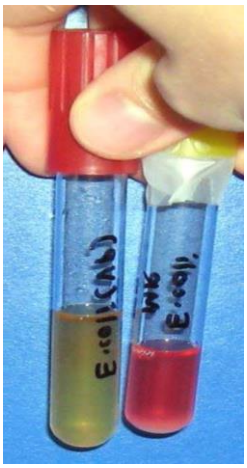


Methyl Red (MR)

•*Enterobacter aerogenes*(left) –

•*E. coli*(bright red) +

•Reagent: Methyl red indicator identifies pH change due to mixed acid fermentation

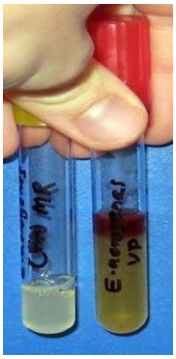


Voges –Proskauer (VP)

• *Enterobacter aerogenes* +

•*E. coli* –(left)

•Barritt's reagent Tests for acetoin, precursor to 2,3 butanediol fermentation•Addition of alpha-naptholand KOH



Citrate

•*E. coli* (left green) –

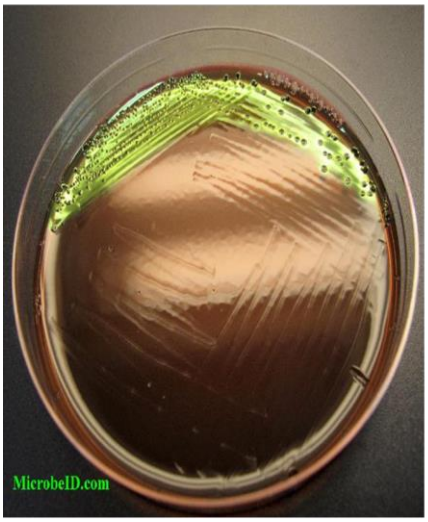
•*Enterobacter aerogenes* (right royal blue) +

•Reagent: Bromothymol blue indicator tests for ability to use citrate as sole carbon source/citrate permease

Urease

- *E. coli*–(left)
- *Proteus vulgaris*+
- Phenol Red a pH indicator turns tube bright pink because NH3 decreases the pH
- CO(NH3)2+ 2 H2O –urease → CO2+ H2O + 2 NH3

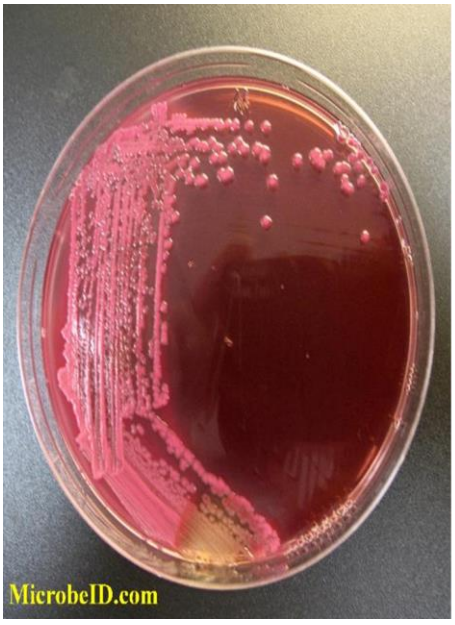
E.Coli on EMB



E.Coli on maccoky agar

Klebsiella

- The most pathogenic species is *K. pneumoniae* that causes pneumonia.
- Microscopical appearance: G _ve short rods. Non-motile. Capsulated both in tissues and on *in vitro* cultivation.
- Cultural characteristics: Colonies on MacConkey agar, are large, mucoid and show pink color (lactose fermenter)
- Biochemical reactions: IMViC - - + +,



Enterobacter

- Is considered as an opportunist. They are frequently isolated from urinary tract infections, septicemia. They are found in soil, water, dairy products & animals & human intestine. The most common species: *E. cloaca, E.aerogenes*.
- Microscopical appearance: G _ve short rods, and motile.
- Cultural characteristics: Colonies on MacConkey agar, are large, pink in color (lactose fermented).

Serratia marcescens

- Free living & common opportunistic pathogen in hospitalized patients. Cause pneumonia, bactermia, endocarditic, UTI, meningitis or wound infection.
- Microscopical appearance: G -ve short rods and motile
- Cultural characteristics: Produce a red pigment (prodigiosin) at room temp. Colonies on MacConkey agar are late lactose fermenters, i.e give pink colonies after 48 hr.
- Biochemical reactions: IMViC - - + +, TSI Alkaline slant/Acid butt (K/A).

Citrobacter

- Considered as an opportunist. Can cause UTI & bacteremia. G-ve short rods, motile & late lactose fermenters on MacConky agar.
- Biochemical reactions: IMViC V + - +, Urease V, TSI Alkaline slant/Acid butt(K/A).

IMViC Reactions

	I	M	Vi	C
Escherichia coli	+	+	–	–
Edwardsiella tarda	+	+	–	–
Proteus vulgaris	+	+	–	–
Klebsiella pneumoniae	–	–	+	+
Klebsiella oxytoca	+	–	+	+
Enterobacter spp.	–	–	+	+
Serratia marcescens	–	–	+	+
Citrobacter freundii	–	+	–	+
Citrobacter koseri	+	+	–	+

Dr.T.V.Rao MD 23