Isolation and identification of Enterobacteriaceae

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Enterobacteriaceae

- Enterobacteriaceae is a large diverse family of bacteria commonly referred to as the fermentative, gram negative, enteric bacilli, indicating that they are gram-negative rods which can ferment sugars.

- Many are normal flora of the intestinal tract of humans and animal, some infect the intestinal tract.

infection	
E. coli spp	*Salmonella spp*
Klebsiella spp	*Shigella spp*
Proteus spp	*Serratia spp*
Enterobacter spp	

Microscopic Morphology

- Gram-negative nonspore forming rods.
- Most Enterobacteriaceae are motile, with the exception of the common isolates *Klebsiella*, *Shigella*,, the motile strains possess peritrichous flagella.
- Many Enterobacteriaceae also possess fimbriae and sex pili .
- Fimbriae are important for the ability of bacteria to adhere to specific host cell receptors.
- Sex pili facilitate genetic transfer between bacteria.

- The heat-stable lipopolysaccharide (LPS) is the major cell wall antigen and consists of three components:

1) The O polysaccharide (important for the epidemiologic classification of strains within a species).
2) The core polysaccharide (important for classifying an organism as a member of the Enterobacteriaceae).
3) lipid A (is responsible for endotoxin activity, an important virulence factor).

Biochemical Characteristics Of Enterobacteriaceae

- All members can grow rapidly, aerobically and anaerobically (facultative anaerobes)
- The Enterobacteriaceae

A-have simple nutritional requirements,

B- ferment glucose,

C- reduce nitrate,

D-catalase positive and

E- oxidase negative.

Colonial Morphology

- Characteristics of the organisms' colonies on different media have been used to identify common members of the family Enterobacteriaceae. For example, the ability to ferment lactose (detected by color changes in lactose-containing media such as MacConkey agar).

- lactose-fermenting strains *pink-purple colonies*
- (e.g., *Escherichia, Klebsiella, Enterobacter*)
- Non lactose-fermenting strains * colorless colonies*

(e.g., *Proteus, Salmonella, Shigella*.).

- Resistance to bile salts in some selective media has been used to separate enteric pathogens (e.g., Shigella, Salmonella) from commensal organisms that are inhibited by bile salts (e.g., gram-positive and some gram negative).

- Most have similar colonial morphology in blood agar plate.
  - moist, smooth, gray colonies.
  - some strains are beta hemolytic.

MacConkey Agar

Purpose: Selective and differential medium; identification of Enterobacteriaceae
Media: Contains 1- bile salts to inhibit most Gram (+) bacteria except *Enterococcus* and some species of *Staphylococcus*, 2- peptone, and 3- lactose.
Reagents/Indicators: Contains crystal violet and bile salts, which inhibit Gram (+) bacteria, and neutral red dye, which stains microbes fermenting lactose (and thereby decreasing the pH) a pink color.

Lactose fermenter(left) and non-lactose fermenter on MacConkey Agar

Mechanism/reactions: By utilizing the lactose available in the medium, Lac+ bacteria such as Escherichia coli, *Enterobacter* and *Klebsiella* will produce acid, which lowers the pH of the agar below 6.8 and results in the appearance of red/pink colonies. Non-Lactose fermenting bacteria such as *Salmonella*, Proteus species and Shigella cannot utilize lactose, and will use peptone instead. This forms ammonia, which raises the pH of the agar, and leads to the formation of white/colorless colonies.

Directions: Streak agar in a straight line and incubate for 24 – 48 hours.

Interpretation:
(+) = Lactose fermentation, red /pink colonies
(Slow) = Some organisms ferment lactose slowly or weakly, and are sometimes put in their own category – these include *Serratia* and *Citrobacter*
(-) = non-lactose fermenters, white/colorless growth

Eosin methylene blue agar (EMB) Agar

Purpose: Selective and differential medium; identification of Enterobacteriaceae. Used primarily to distinguish **coliform** (Escherichia, Klebsiella, Enterobacter and Citrobacter) from **non-coliform** bacteria in water testing.

Media: Eosin, Methylene Blue, lactose, sucrose

Reagents/Indicators: Eosin Y and Methylene Blue

Mechanism/reactions: Selects for Gram Negative bacteria, and differentiates those enteric which ferment lactose (coliforms) from those which do not ferment lactose (non-coliforms). Indicators form a dark purple precipitate at low pH (due to fermentation products) and also inhibit gram positive bacteria. ***E. coli*** will often produce a green metallic sheen due to strong fermentation and precipitation of acid and indicator complex.

Directions: Streak agar in a straight line and incubate for 24 – 48 hours.

Interpretation:

(+) = Lactose fermentation, dark purple colonies with dark center. Weak fermenters will have pink mucoid growth.

Green sheen = vigorous fermentation of lactose

(-) = non-lactose fermenters, colorless (or very faint pink) growth.

Two lactose fermenters growing on EMB.


Thank you