Culture Media of Enterobacteriacea I

Clinical Bacteriology II
CLS 413

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General characteristics of the enterobactericeae

- Rod shaped
- Gram negative
- Catalase positive
- Oxidase negative
- Facultative anaerobes
- Most ferment glucose, some are LF
- Most reduce nitrate to nitrite

Members of the Enterobacteriaceae Family

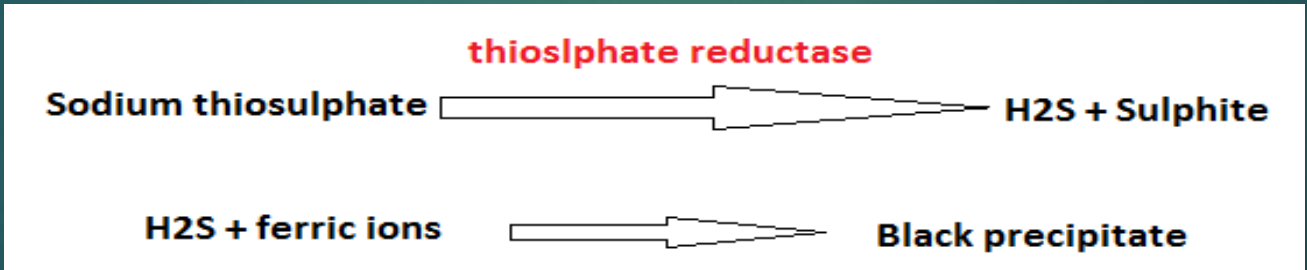
- E.coli:
  - lactose fermenter- releases acids as a product of fermentation. Color (Ph) indicators change the color of the medium in response to the acidic environment.
  - Usually produces dry LF colonies
- Klebsiellah Pneumoniae:
  - Lactose fermenter
  - Mucoid LF colonies

Members of the Enterobacteriaceae Family

- Proteus:
  - Non lactose fermenter (NLF)
  - Tends to swarm on most culture media
  - CLED agar prevents swarming
  - Can produce H2S in sodium thiosulfate-containing media
- Salmonella:
  - NLF
  - Produces H2S in sodium thiosulfate-containing media
- Shigella:
  - NLF
  - Non H2S producer

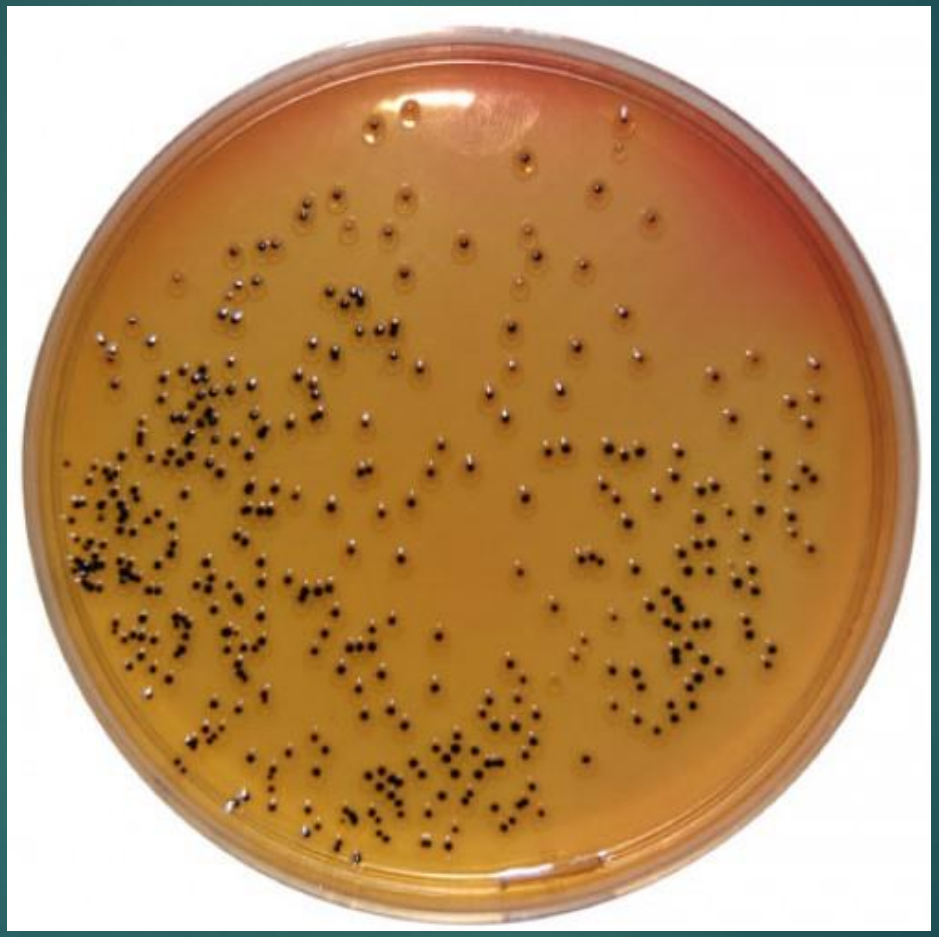
Desoxycholate Citrate Agar (DCA):

- Differential and selective.
- Selective for gram –ve
- Inhibitory substances: sodium desoxycholate and sodium citrate
- Differentiates between LF and NLF and between H2S producers and non producers
- Neutral red is pH indicator
- H2S producers give black-centered colorless colonies on DCA (salmonella). Shigella sp. produce colorless colonies without a black center.



- LFs give pink colonies, while lactose NLFs produce colorless or pale colonies

H2S-producers on DCA



Salmonella on DCA

Shigella (non H2S producer) on DCA

Hektoen Enteric Agar (HE)

- Differential and Selective
- Selective for gram –ve bacteria (Contains bile salt inhibitors)
- Differentiation based on lactose fermentation and H2S production
- Carbohydrate sources are lactose sucrose and salicin
- Bromothymol blue is color indicator
- Determines H2S production through sodium thiosulphate(as an H2S source)
- H2S producers give clear colonies with black centers (fish eye colonies)
- Lactose fermenters give orange colored colonies (sometimes called salmon colonies). Non fermenters give green-blue colonies

Lac –ve, H2S -ve colonies on HE

Lac +ve, H2S -ve bacteria on HE

Salmonella on HE, H2S +, Lac -

Characteristic Fish-eye Colonies

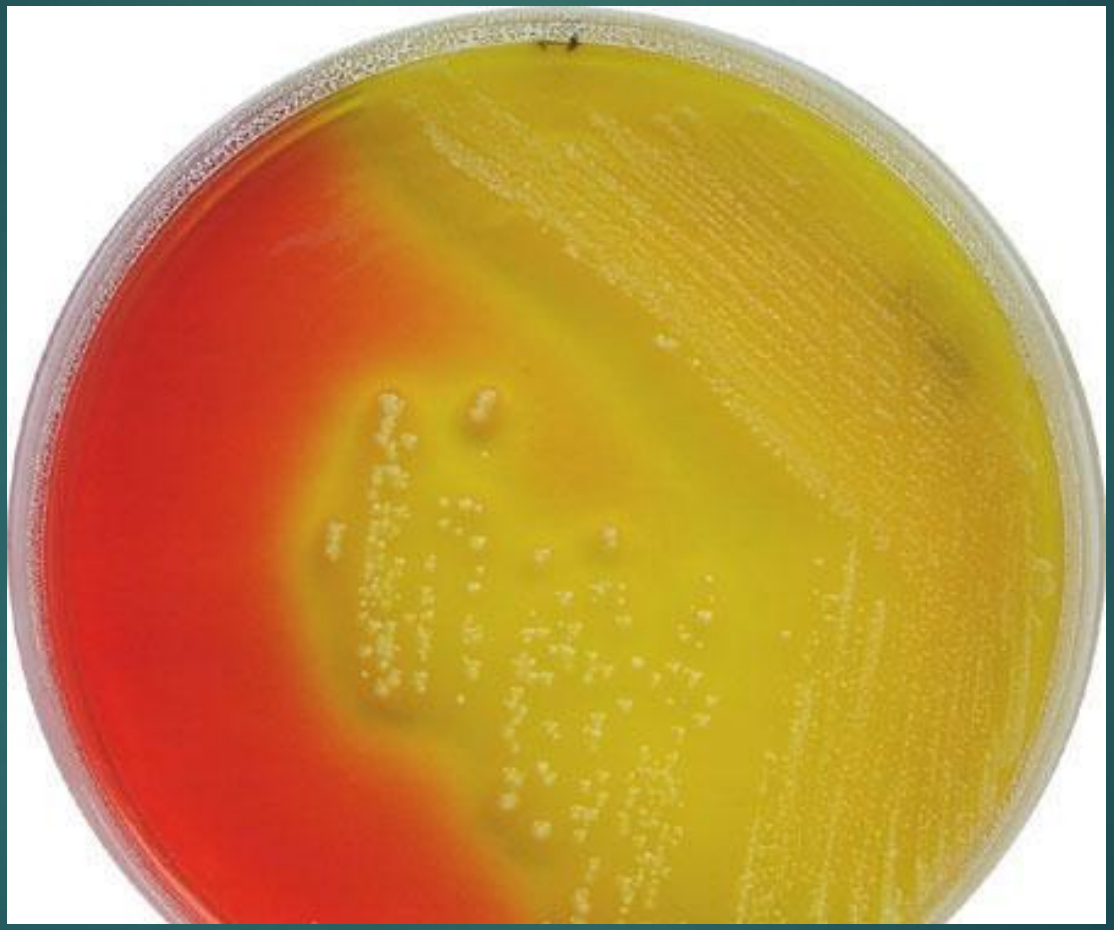
Xylose Lysine Desoxycholate Agar (XLD):

- A Selective and differential medium
- Sodium desoxycholate is inhibitor
- Phenol red: pH indicator
- Differentiation: LF, H2S production
- Contains xylose ,lactose (sugars) and lysine (amino acid)
- LFs produce yellow colonies
- H2s producers (salmonella): red colonies with black centers.
- Non H2S produces red colonies without black centers
- **All enterics, except shigella, ferment xylose→ this explains why proteus –even though it's NLF- gives yellow colonies on XLD
  - Salmonella ferments xylose but, does not give yellow colonies
  - Why? because salmonella decarboxylates lysine in the medium leading to alkaline products (if the environment is alkaline, the plate remains red)

Salmonella on XLD: Lac –, H2S + red colonies with black centers

Shigella on XLD: lac -ve, H2S –ve: red colonies w/out black centers

Lac +ve, H2S - colonies of on XLD



E.coli

Diseases Caused by Enteric Bacteria

- **E.coli**: Opportunistic infections; some strains Cause diarrheas, Enteritis 0157:H7 toxin (food poisoning), and UTIs
- **Shegilla**: bacillary dysentry
- **Salmonella:** Salmonella Typhi: typhoid or enteric fever, Salmonella paratyphi: mild enteric fever, Other species cause food poisoning .
- **Klebsiella pneumoniae:** pneumonia, wound infections and UTIs
- **Proteus species:** UTIs

Assignment

Salmonella and Proteus are both motile organisms. And some species of Proteus produce H2S like Salmonella. How can we tell the difference between H2S producing Proteus species and salmonella? (I want an answer other than swarming)

Tasks to be done today

1. Prepare a smear from the provided organism and stain it by the gram stain method. View the slide under the microscope.
2. Culture the given organism on your agar plates. Then incubate at 3 degrees Celsius. Return to the lab within 24 hrs to record your results
3. After recording your results, prepare a table of growth characteristics (which you will include in your report).
4. Prepare your lab report as described previously

Organism / Media	E.coli	Kleb.	Salmonella	Shigella	Proteus
MAC					
CLED					
EMB					
XLD					
DCA					
HE					

Describe:

- Growth
- Color
- Lac fermentation
- H2S +/-
- Specfic characteristics (mucoid colonies, swarming..etc)

Before you leave:

- Turn off your microscopes
- Turn off the incinerators
- Clean your bench with disinfectant spray and tissue
- Wash your hands