

Practical Pathogenic Bacteria

LAB:5

Bacterial Identification

(Cultural Media of Gram Negative Bacteria)

Culture Media Used for Gram Negative

1. MacConkey Agar

- It is both selective and differential agar.
- It detects the ability of bacteria to ferment lactose and tolerate crystal violet and bile salts (inhibitors of G+ve non-enteric bacteria).
- Sugar: lactose, pH-indicator: neutral red.

Why this medium is selective?

- It allows the growth of G-ve enteric bacteria and inhibits G+ve non-enteric bacterial growth.
- G+ve inhibitor: crystal violet.
- Non-enteric bacteria inhibitor: bile salts.

Why this medium is differential?

It differentiates lactose-fermenting bacterial species from non-lactose fermenting ones.

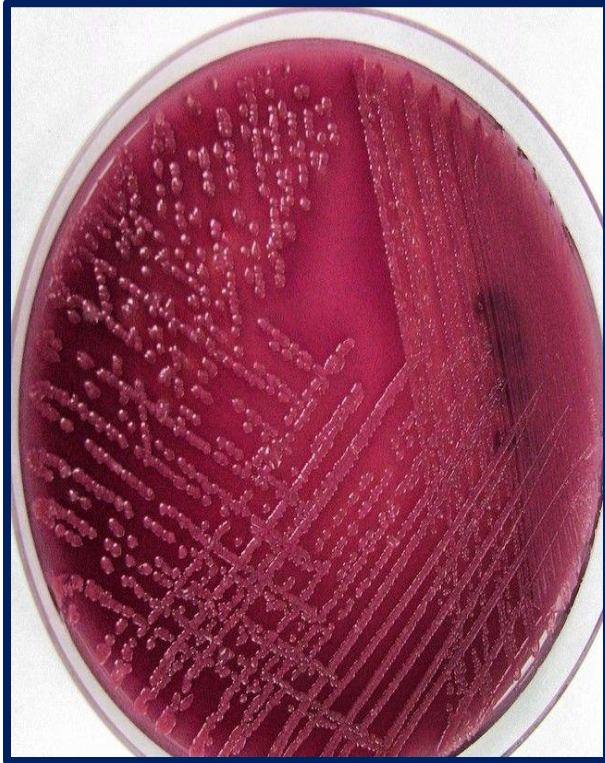
Lactose Fermenters and Non-lactose Fermenters on MacConkey Agar



Pink colonies

**Colourless or pale
colonies**

Bacterial Species on MacConkey Agar



E. coli
Lactose fermenter



Klebsiella
Lactose fermenter



***Pseudomonas
aeruginosa***
Non-lactose fermenter

1. Eosin Methylene Blue Agar (EMB)

- It is both selective and differential agar.
- It detects the ability of bacteria to ferment lactose and tolerate eosin and methylene blue (inhibitors of G+ve bacteria).
- Sugar: lactose and sucrose, pH-indicators: eosin and methylene blue.

Why this medium is selective?

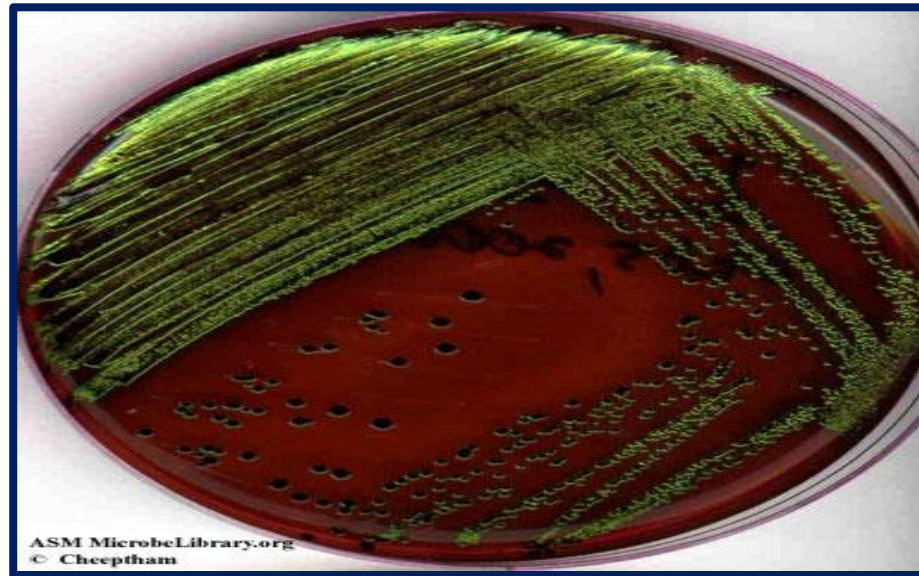
- It allows the growth of G-ve fecal coliforms and inhibits G+ve bacterial growth.
- G+ve inhibitor: eosin and methylene blue.

Why this medium is differential?

- It differentiates lactose-fermenting coliforms from non-lactose fermenting enteric bacteria.

Lactose Fermenters on EMB

- Strong lactose fermenter like *Escherichia coli*, typically produce colonies with an intense green metallic sheen.



***E. coli* (lactose fermenter)**
Blue black colonies with green metallic sheen

Lactose Fermenters on EMB

- A lesser degree of lactose fermentation is indicated by the pink colour colonies compared to strong fermenters.



***Klebsiella* (lactose fermenter)**
Pink to purple mucoid colonies without green metallic sheen

Non-lactose Fermenters on EMB

- Colourless colonies typically indicate non-lactose fermenting bacteria.



***Salmonella, Shigella, Proteus and Pseudomonas* (non-lactose fermenters)
Colourless colonies**

Levine Eosin-Methylene Blue (EMB) Agar

Lactose fermenter
(high acid producer:
black colonies)

Non-lactose fermenter



Gram positive
(growth inhibited)

Lactose fermenter
(low acid producer:
pink colonies)

Results on EMB Agar



No
growth

Gram +Ve Bacteria



Colorless
colonies

Lactose non fermenting G-ve



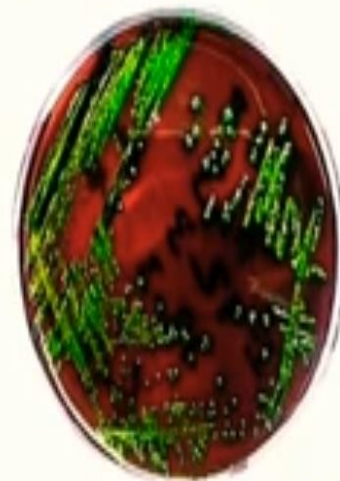
Pale pink
To purple

Weak lactose fermentor



Purple to
Black

Strong Lactose fermentor



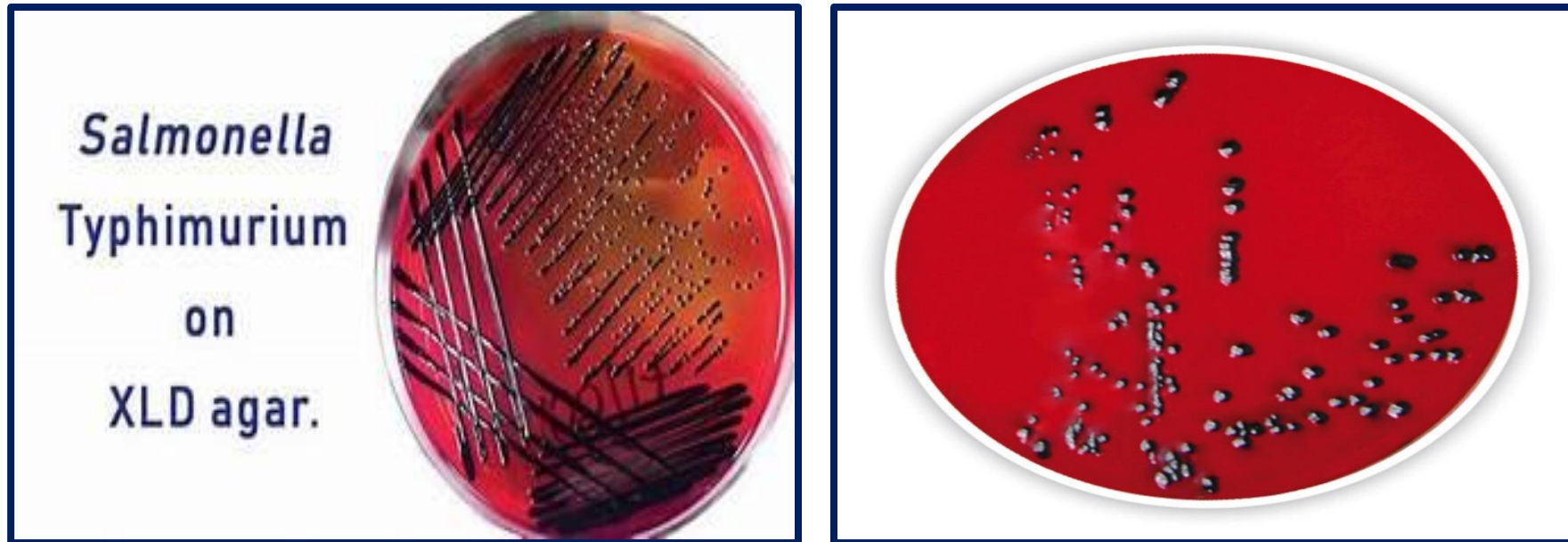
Green
Mettalic
Sheen

E.coli (Typical)

Xylose Lysine Deoxycholate Agar (XLDA)

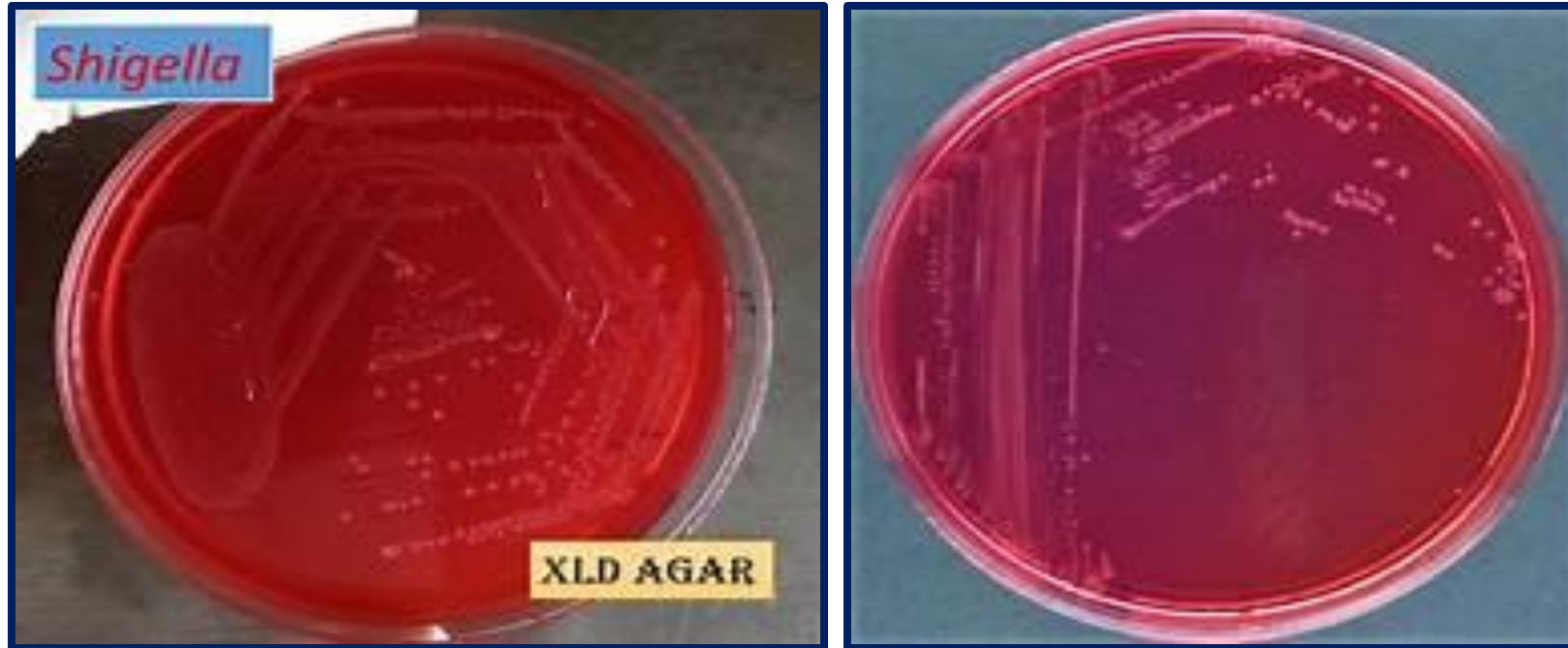
- It is both selective and differential medium for *Salmonella* and *Shigella*.
 - Sugar: xylose.
 - pH-indicator: phenol red.
 - H₂S indicator: Sodium thiosulphate.
 - G+ve and G-ve inhibitor (selective agent): Sodium deoxycholate.
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- It has a pH of approximately 7.4 leaving it with a bright pink to red appearance due to the pH-indicator (phenol red).
 - Xylose is fermented by most enteric bacteria and *Salmonella*, except for *Shigella* which are differentiated from other species.
 - Sugar fermentation results in acid production which lowers the pH causing the change of phenol red to yellow.
 - After exhausting xylose, *Salmonella* decarboxylate lysine (via lysin decarboxylase) causing the pH to rise (neutral or slightly alkaline).

***Salmonella* Colonies on XLD Medium**



Xylose fermenter: red colonies with black centres (*Salmonella*)

***Shigella* Colonies on XLD Medium**



Non-xylose fermenter: red colonies without black centres
(*Shigella*)

***Salmonella* and *Shigella* Colonies on XLD Medium**



Salmonella on XLD agar



Shigella on XLD agar

Salmonella-Shigella Agar (SSA)

- Sugar: lactose.
- pH-indicator: neutral red.
- H₂S indicator: sodium thiosulphate.
- G+ve and G-ve inhibitor: Brilliant green.
- A non-lactose fermenter: pale colonies with black centres (*Salmonella*).
- A non-lactose fermenter: pale colonies without black centres (*Shigella*).

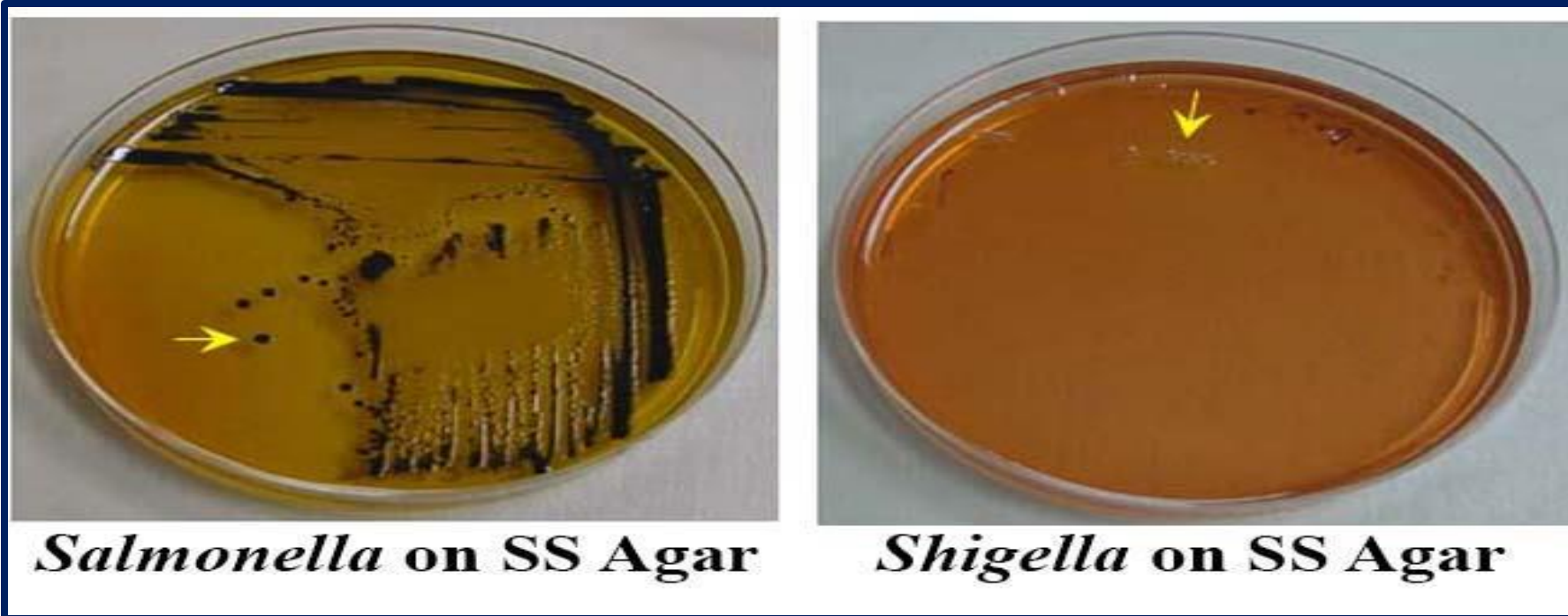
On Salmonella Shigella Agar

- is used for the isolation of *Salmonella spp.* and some strains of *Shigella spp.*
- inhibit gram-positive bacteria, most coliform bacteria, and inhibit swarming *Proteus spp.*, while allowing *Salmonella spp.* to grow.



Salmonella will not ferment lactose, but do produce hydrogen sulfide gas. The resulting bacterial colonies will appear colorless with black centers.

***Salmonella* and *Shigella* Colonies on S-S Medium**



- Salmonella* colourless colonies with black centres (do not ferment lactose, produce H₂S gas).
- Shigella* colourless colonies (do not ferment lactose and do not produce H₂S gas)

Genus: *Salmonella* spp.

- It belongs to the Enterobacteriaceae family.
- There are two species of *Salmonella*: *S. enterica* and *S. bongori*.
- Strains of *Salmonella* cause illnesses such as typhoid fever, paratyphoid fever, and food poisoning (salmonellosis) .
- It is a rod-shaped (bacillus) G-ve bacterium.
- It is a non-spore forming, predominantly a motile bacterium.
- It is a facultative anaerobic bacterium.
- It is a non-lactose fermenting bacterium.
- Most subspecies of *Salmonella* are H₂S(+ve), which can be detected by growing the bacteria on media containing ferrous sulfate, such as the triple sugar iron medium (TSI).

Genus: *Shigella* spp.

- It is a rod-shaped (bacillus) G-ve bacterium.
- It is a facultative anaerobic bacterium.
- It is a non-spore forming, non-motile bacterium.
- It is a non-lactose fermenter bacterium except *S.sonnei*.
- It is H₂S (-ve).
- It genetically closely relates to *E.coli*.
- It is the causative agent of shigellosis.
- It is classified to four serogroups:
 - Serogroup A (*S. dysenteriae*)
 - Serogroup B (*S. flexneri*)
 - Serogroup C (*S. boydii*).
 - Serogroup D (*S.sonnei*).