Lecture-6: Cultivation and Isolation of Viable Pathogen Laboratory media

Constituents of culture media: The basic constituents of culture media are:

1- Water: Distilled water or potable water with low mineral content is used for media preparation.

2- Electrolytes: sodium chloride or other electrolytes.

3- Peptone: it is a complex mixture of partially digested proteins.

Agar: it is used for solidifying the culture media. It is commercially available in powder form; melts in water after boiling and jellifies after cooling also called 'agar-agar

Preparation of agar media: The appropriate amount of agar powder is added to water and the mixture is dissolved and then sterilized by placing it in an autoclave.

When the temperature of the molten agar comes down to 45°C, it is poured to the Petri dishes and then allowed to set for 20 minutes.

Types of culture media:

A. Based on consistency:

1. Liquid media (or broth).
2. Semisolid media.
3. Solid media.

B- Based on the growth requirements

1. Routine laboratory media: They can further be classified into

Simple media; Enriched media; Enrichment broth; Selective media; Differential media; Transport media; Anaerobic media.

2. Synthetic media:

Simple media

Many bacteria will grow in or on simple media such as nutrient broth/nutrient agar that contains peptone

Enriched media

These contain additional nutrients for the isolation of more fastidious bacteria that require special conditions for growth like agar containing whole blood (blood agar) or agar containing lysed blood (chocolate agar).

Selective media

These are designed to facilitate growth of some bacteria, while suppressing the growth of others, and include:

Mannitol salt agar which include increases NaCl (salt) concentration for the recovery of staphylococci.

MacConkey agar which contains bile salt and allows the growth of bile-tolerant bacteria only (such as gram negative bacteria).

Antibiotics, which are frequently added to the media to allow only some bacteria to grow while suppressing or killing others type of bacteria.

Indicator media

These are designed to aid the detection and recognition of particular pathogens. They are often based on sugar fermentation reactions that result in production of acid and the subsequent color change of a pH indicator, such as

MacConkey agar contains lactose and a pH indicator (neutral red); lactose-fermenting bacteria (Escherichia coli) produces acid and form pink colonies, whereas non-lactose fermenting bacteria (Salmonella spp.) do not produce acid and form pale yellow colonies.

This property facilitates the recognition of possible Salmonella colonies among normal bowel flora. Note that indicator media may also contain selective agents including antibiotics or substances such as bile salts and crystal violet to suppress growth of most Gram- positive microorganisms.

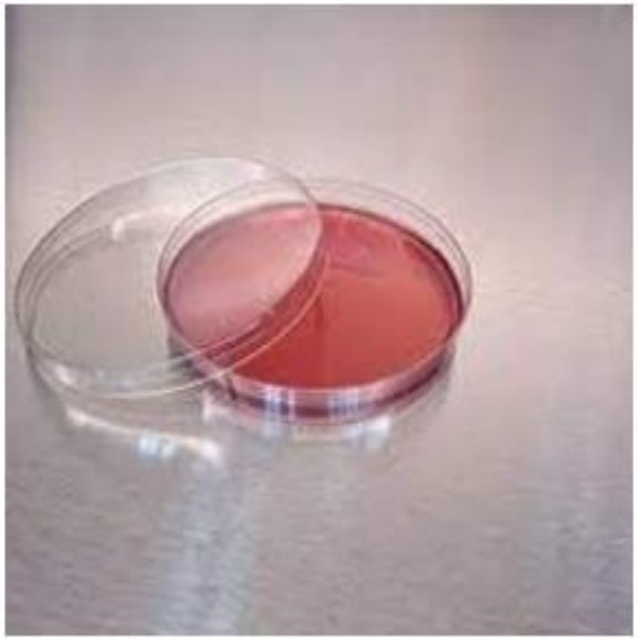
MacConkey agar is therefore both a selective medium and an indicator medium.

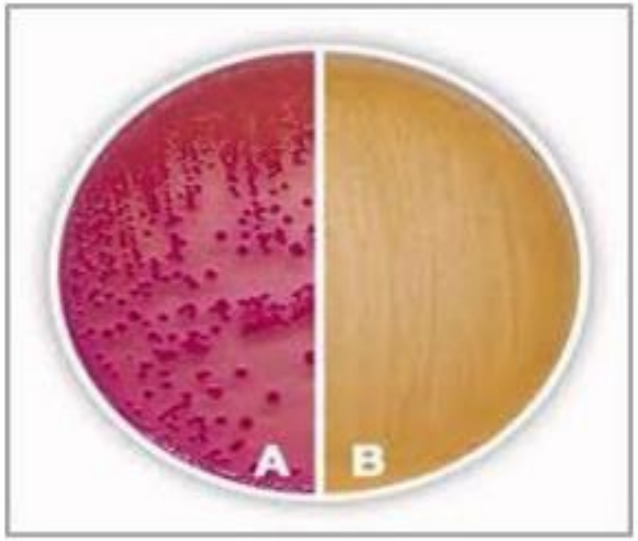
MacConkey agar:

Selective: it contains crystal violet (inhibits G+ve) and bile salts (inhibits G-ve other than enteric bacteria)

Differential: differentiates between Lactose fermenter (pink colonies) e.g. (E.coli) and lactose non-fermenter (pale colonies) e.g. (Salmonella)

indicator: Neutral red(yellow in alkaline pH and pink in acid pH)



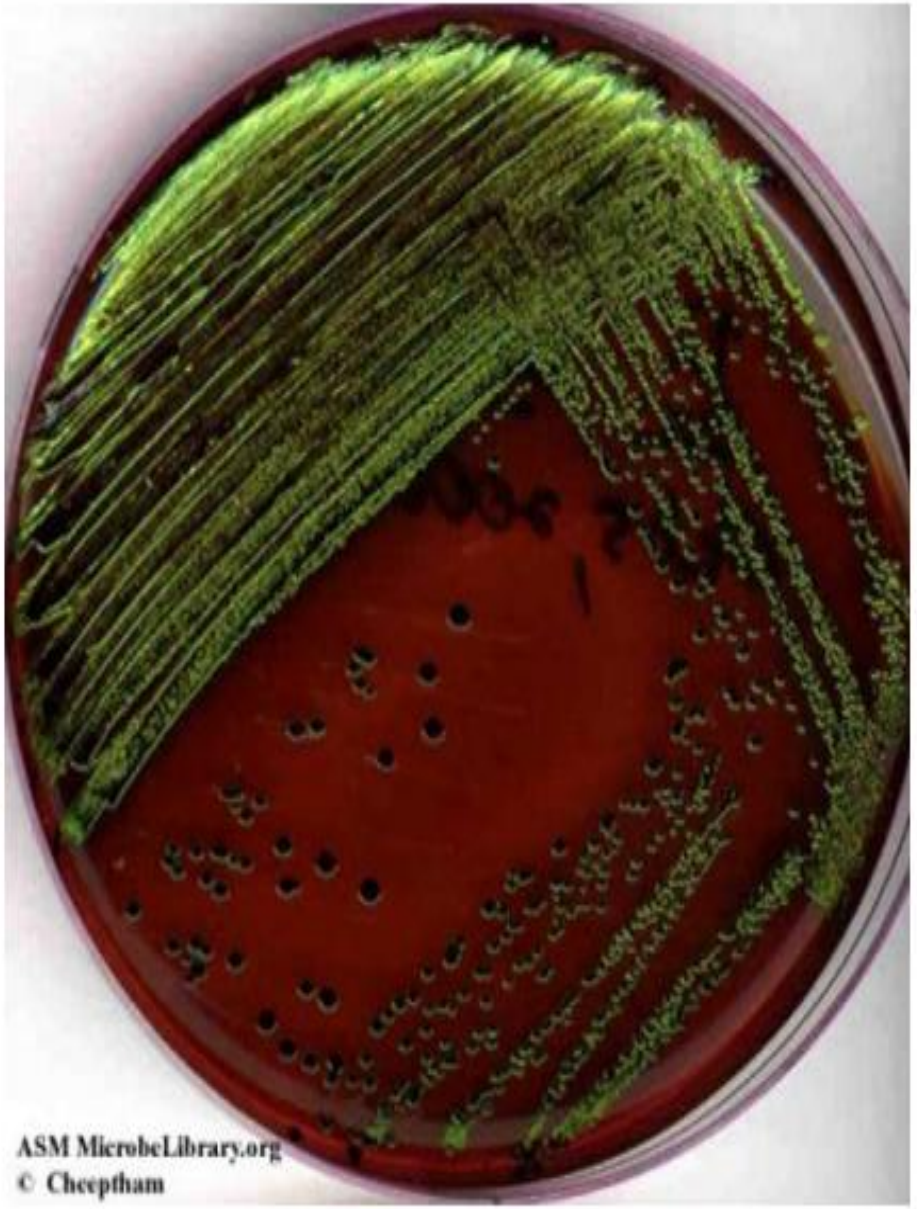


Lactose fermenter& lactose non-fermenter

Eosin methyline blue agar (EMB):

Selective for Enterobacteriaceae (contain methylene blue inhibits G+ve)

Differential for E.coli (green metallic sheen in presence of Eosin)
Both Methylene blue and Eosin combine to form metallic green sheen as a result of lactose fermentation in acid pH.



Name of test:- Manitol salt agar

Aim:- Selective media for *Staphylococcus spp.* differential between *Staphylococcus aureus* (golden colony) and *S. epidermidis* (red colony).

Results:-*Staphylococcus aureus* (golden colony) and *S. epidermidis* (red colony).