Culture enrichment and isolation

Culture Media

- Culture is the process of **growing** a bacterial or other biological entity such as fungi in an **artificial medium**.

- **Culture media** are the **food** used to grow and control microbes.

- Culture media can vary in many ingredients allowing the worker to **select for or against** microbes.

- Culture media are usually in the form of a **liquid or gel** designed to support the growth of microorganisms.

- There are **different types** of media suitable for growing different types of microorganisms.

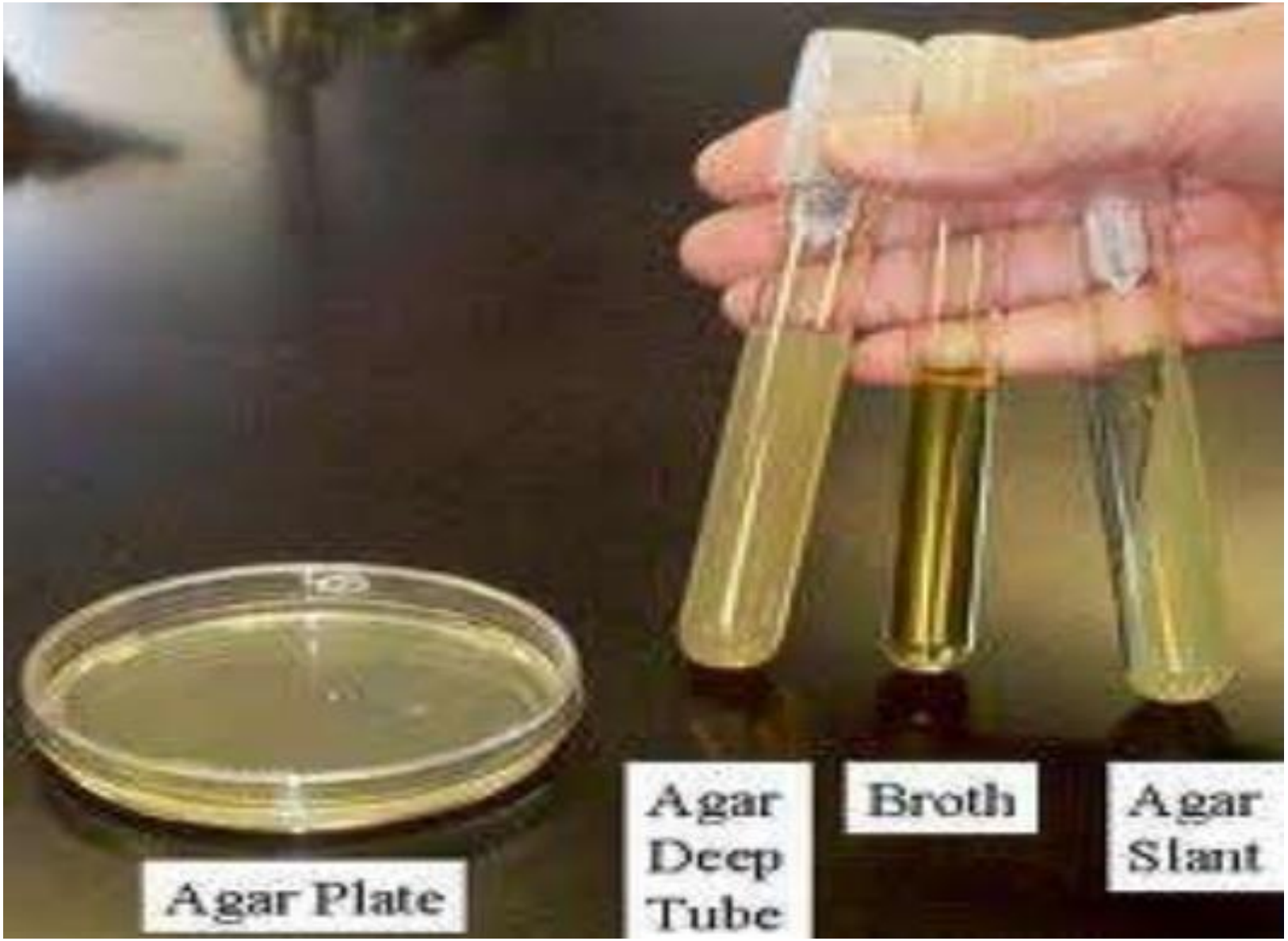
- **Specialized media** are sometimes required for growth of certain microorganisms.

- Some organisms, termed **fastidious organisms**, need specialized environments due to complex nutritional requirements.

- **Viruses**, for example, are obligate intracellular parasites and require a growth medium containing **living cells**.

- Many human microbial pathogens also require the use of **human cells** or **cell lysates** to grow on a medium.

- The **most common** growth media are **nutrient broths** (liquid nutrient medium).
- These are often mixed with **agar** and poured into **Petri dishes** to solidify.
- These agar plates provide a **solid medium** on which microbes may be cultured.
- They remain solid, as **very few bacteria are able to decompose agar.**
- Another advantage of agar is its **high melting point (85°C)** but **low solidification point (40°C)**.


Agar Plate
Agar Deep Tube
Broth
Agar Slant

DEFINED VS UNDEFINED MEDIA

This is an important distinction between growth media types.

Defined media

- A ***defined*** medium will have **known quantities** of all ingredients.
- For microorganisms, it provides **trace elements** and **vitamins** required by the microbe and especially a **defined carbon and nitrogen source.**
- **Glucose or glycerol** are often used as **carbon** sources, and **ammonium salts or nitrates** as **inorganic nitrogen** sources.

- A ***chemically defined*** medium is **entirely free of animal-derived components** (including microbial derived components such as yeast extract).

- It represents the **purest and most consistent** cell culture environment.

- By definition chemically defined media **cannot contain** either fetal bovine serum, bovine serum albumin, or human serum albumin.

- These products are derived from bovine or human sources and contain **complex mixes of albumins and lipids**.

Undefined media

- An ***undefined*** medium has some **complex ingredients**, such as yeast extract, which consists of a mixture of many, many chemical species in unknown proportions.

- Undefined media are sometimes chosen **based on price and sometimes by necessity** – some microorganisms have **never been cultured on defined media.**

- There are many different types of media that can be used to grow specific microbes.

NUTRIENT MEDIA

- They need a **source of amino acids and nitrogen** (e.g., **beef, yeast extract**).
- These are **undefined media** because the amino acid source contains a variety of compounds with **unknown** exact composition.
- These media contain all the elements that most bacteria need for growth and are **non-selective**.
- So they are used for the **general cultivation and maintenance** of bacteria kept in laboratory-culture collections. Examples are Nutrient agar and Nutrient broth.

MINIMAL MEDIA

- These are media that contain the **minimum possible nutrients** for colony growth, generally without the presence of amino acids.

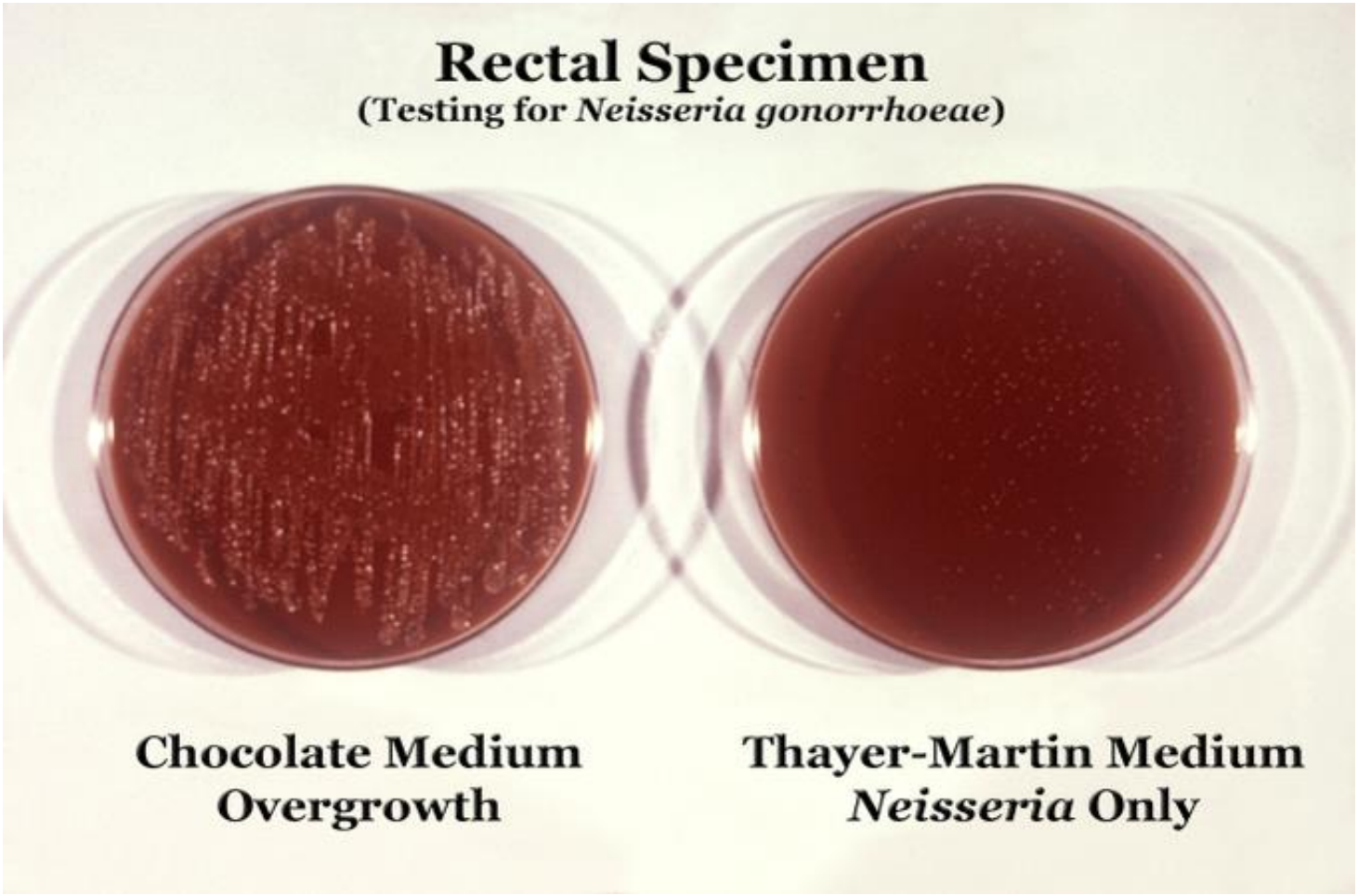
- They are often used by microbiologists and geneticists to grow **“wild type”** (as found in nature) microorganisms.

- These media can also be used to **select for, or against** the growth of specific microbes.

- Usually a fair amount of information must be known about the microbe to determine its minimal media requirements.

SELECTIVE MEDIA

- These are used for the growth of only **selected** microorganisms.
- If a microorganism is known to be **resistant** to a certain antibiotic, that antibiotic can be added to the medium in order to prevent other sensitive bacteria from growing.
- Or some **indicator** can be added to **differentiate** between bacteria which utilize a particular substrate or not.



Thyer-Martin medium is Chocolate agar plus antibiotics

Some examples of selective media include:

- **Eosin methylene blue (EMB)** that contains methylene blue – toxic to Gram-positive bacteria, allowing only the growth of Gram negative bacteria.

- **Sabaroud's agar** which has a low pH and high glucose conc, which favors fungal growth but discourage bacterial growth.

- **MacConkey agar** for Gram-negative bacteria.

- **Mannitol salt agar (MSA)** which is selective for Gram-positive bacteria.

DIFFERENTIAL MEDIA

- Also known as **indicator** media, these are used to distinguish one microorganism type from another growing on the **same medium.**

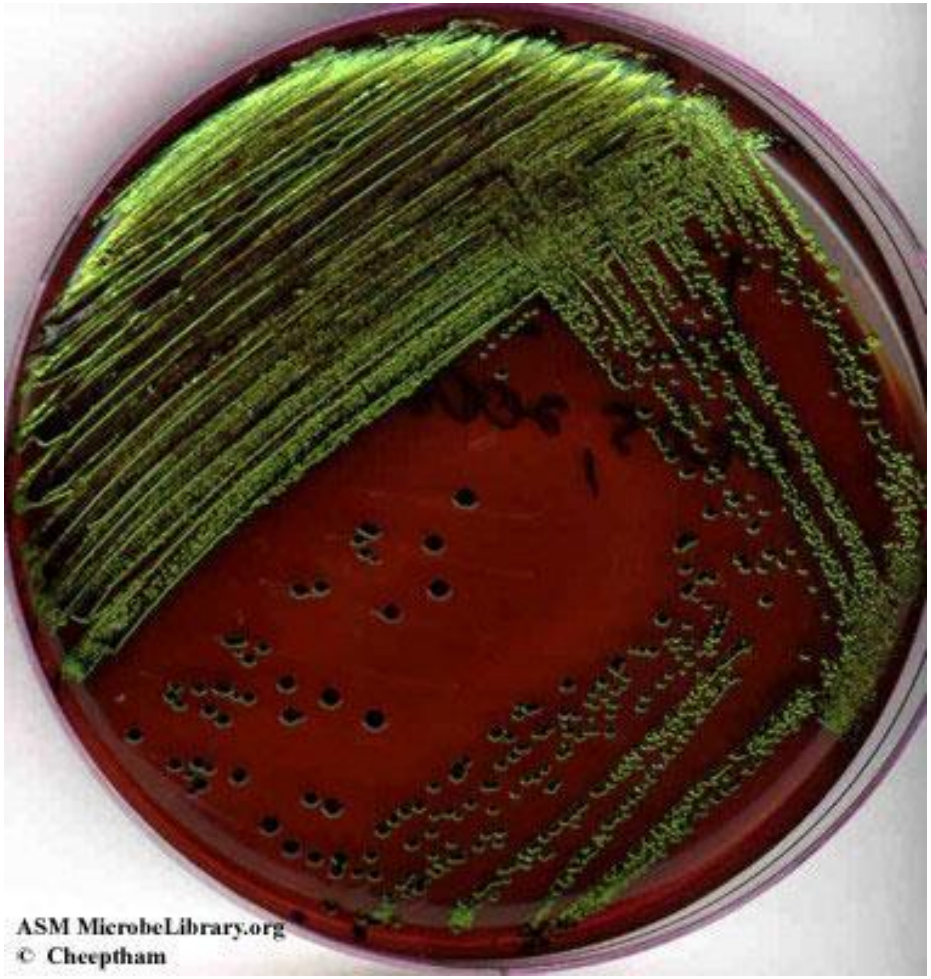
- This type of media uses the **biochemical characteristics** of a microorganism growing in the presence of **specific nutrients or indicators** (such as neutral red, phenol red, eosin y, or methylene blue).

- These indicators are added to the medium to **visibly indicate** the defining characteristics of a microorganism.

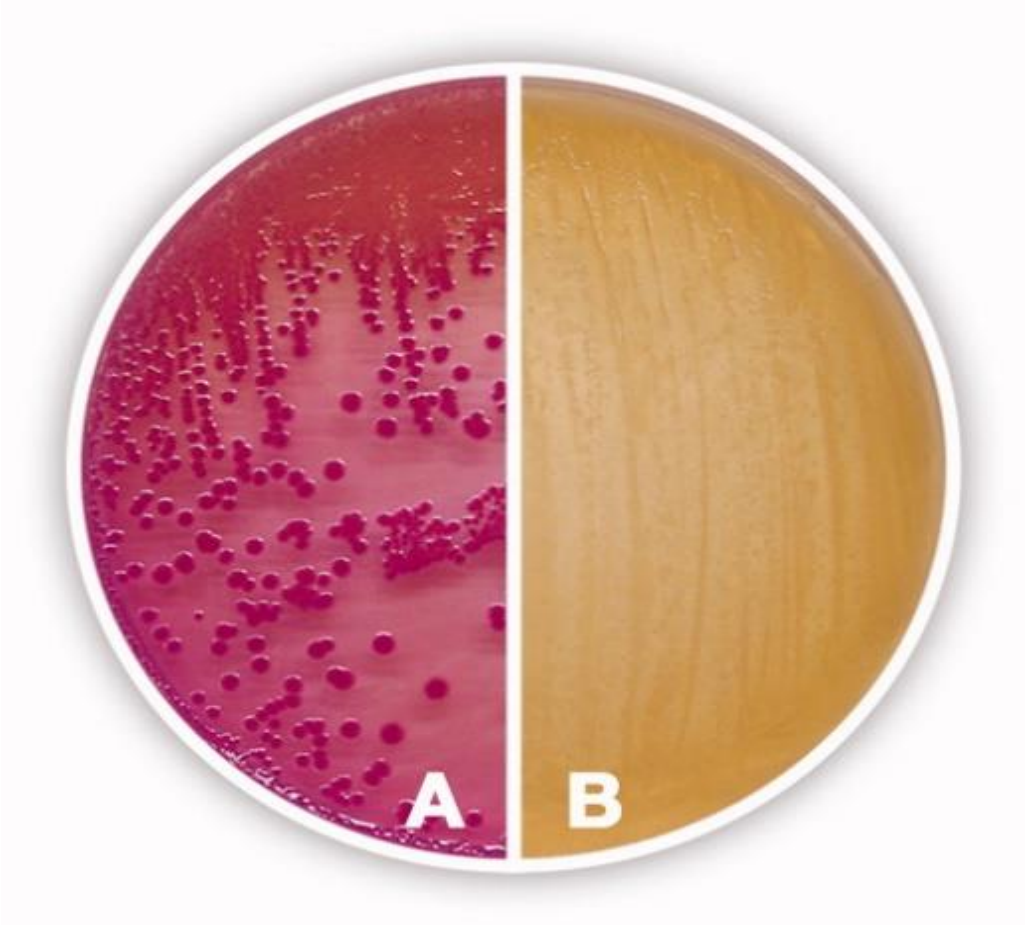
Examples of differential media include:

- **Blood agar**, which contains bovine heart **blood** that becomes transparent in the presence of hemolysis.
- **Eosin methylene blue (EMB)**, which is differential for lactose and sucrose fermentation. Lactose fermenters produce a golden sheen.
- **MacConkey agar** that differentiates between lactose fermenters and non-lactose fermenters. Lactose fermenters produce pink colonies.
- **Mannitol salt agar (MSA)**, which is differential for mannitol fermentation

Blood agar showing three types of hemolysis



Eosin Methylene Blue (EMB) agar
Colonies with golden sheen are lactose fermenters

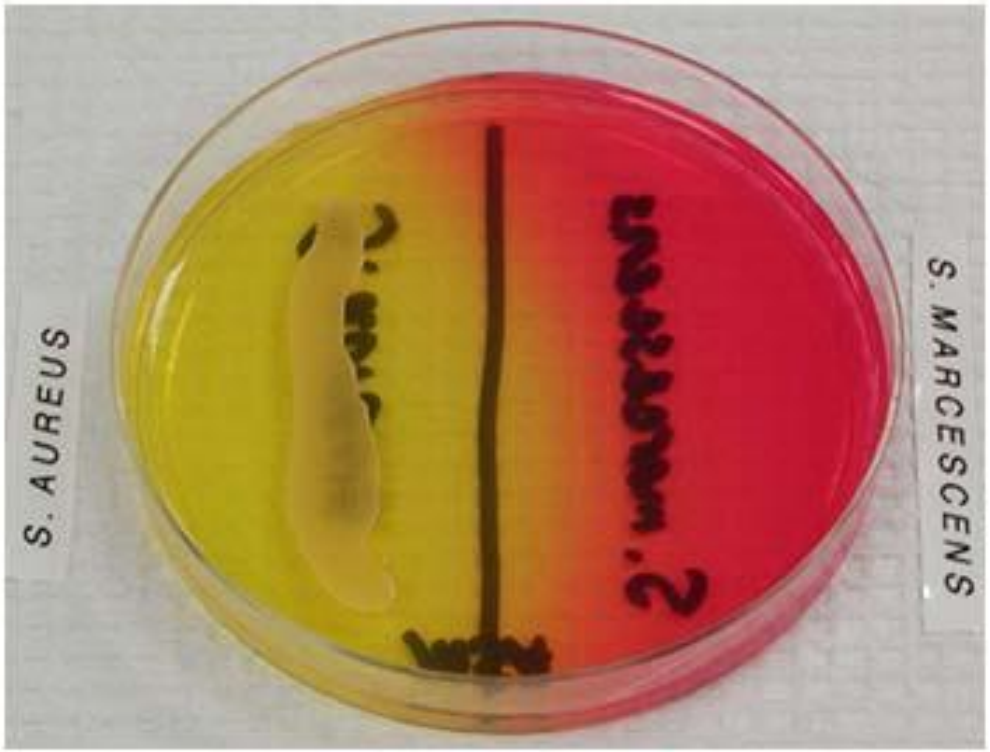


MacConkey agar

A: lactose fermenting rose pink colonies
B: non-lactose fermenting colorless colonies

Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus aureus*

Yellow colonies of *Staphylococcus aureus*



***Staphylococcus aureus* and *Serratia marcescens* on MSA**

Differential media are also used by molecular biologists to detect **recombinant strains** of bacteria.

For example:

X-gal plates, which are differential for ***lac* operon** mutants.

X-gal agar
White colonies indicate recombinants


Thank You!