CULTURE MEDIA & BACTERIAL NUTRITION

• Based on bacterial metabolism
• Culture pH
• Culture oxidation, reduction potential.
• Gaseous requirements: oxygen, carbon dioxide, other gases.
CULTURE MEDIA

- Grow bacteria
- Used to:
  - Detect for certain bacteria
  - Differentiate among different kinds of bacteria
GROWTH PATTERNS ON AGAR

A. Recognizing different bacterial growth morphologies on agar plates is a useful and often crucial step in the identification process.

B. Agar slants are typically used for cultivation of pure cultures.

C. Bacteria also frequently display distinct morphological color and texture on agar slants.
- Eventually a colony appears where the original cell was deposited.
- Color, size, shape, and texture of microbial growth are determined by the genetic makeup of the organism.
- The basic categories of growth include colony shape, margin (edge), elevation, color, and texture.
Blood agar

Chocolate agar
Thiosulfate Citrate Bile Sucrose

Mac Conkey’s medium

TCBS
OXYGEN CONCENTRATION

- Aerobs
- Anaerobs (do not require oxygen)
- Obligate anaerobs (die in the presence of oxygen)
- Facultative anaerobs (E.coli)
- Microaerophilic bacteria
METHODS OF ISOLATION OF PURE CULTURE WITH ..

1. Surface plating
2. Enrichment medium
3. Selective medium
4. Indicator medium
**TYPES OF MEDIA USED**

*General purpose media* will support the growth of many microorganisms.

*Enriched media* are general purpose media supplemented by blood or other special nutrients to encourage the growth of fastidious heterotrophs; *(fastidious = having complicated nutritional requirements)*.
TYPES OF MEDIA USED

Selective media favor the growth of particular microorganisms and inhibits the growth of others.

Differential media distinguish between different groups of bacteria on the basis of their biological characteristics; Causes observable change in medium when biochemical reaction occurs.
THE REQUIREMENTS FOR GROWTH: PHYSICAL REQUIREMENTS

- **pH**
  - Most bacteria grow between pH 6.5 and 7.5
  - Molds and yeasts grow between pH 5 and 6
  - Acidophilic grow in acidic environments
GROWTH PATTERNS IN BROTH

- Purpose

Bacterial genera—and frequently different species within a genus—demonstrate characteristic growth patterns in broth that provide useful information when attempting to identify an organism.
PRINCIPLE

Microorganisms cultivated in broth display a variety of growth characteristics. Some organisms float on top of the medium and produce a type of surface membrane called a pellicle; others sink to the bottom as sediment. Some bacteria produce uniform fine turbidity while others appear to clump in what is called flocculent growth.
FLOCCULENCE IN BROTH
This is a *Streptococcus* species from a throat culture demonstrating floculence in Todd-Hewitt Broth.

PIGMENT IN BROTH
*Rhodospirillum rubrum* has a red color due to carotenoid pigments.
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<th>Obligate Aerobes</th>
<th>Facultative Anaerobes</th>
<th>Obligate Anaerobes</th>
<th>Aerotolerant Anaerobes</th>
<th>Micro-aerophiles</th>
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From left to right, *Clostridium butyricum* (strict anaerobe), *Escherichia coli* (facultative anaerobe, uninoculated control, and *Pseudomonas aeruginosa* (strict aerobe).
METHODS TO ISOLATE THE BACTERIA

- Streak culture
- Stroke
- Stab
- Pour plate
- Liquid culture
- Special methods for anaerobic cultures
HOW TO INOCULATE A CULTURE PLATE

- Plate: provide large surface for isolation and observation of colonies
- Using a sterile loop or a sterile swab streak your sample on the Petri plate
- Important let your sterilized loop cool before you pick up your sample
(a) The direction of streaking is indicated by arrows. Streak series 1 is made from the original bacterial culture. The inoculating loop is sterilized following each streak series. In series 2 and 3, the loop picks up bacteria from the previous series, diluting the number of cells each time. There are numerous variants of such patterns.

(b) In series 3 of this example, notice that well-isolated colonies of bacteria of two different types, red and yellow, have been obtained.
LOWENSTEIN JENSEN MEDIUM - CULTIVATION OF MYCOBACTERIUM TUBERCULOSIS
WORKING WITH MYCOBACTERIUM NEEDS BIOSAFETY CONCERNS
Enrichment media

Liquid media used to isolate pathogens from a mixed culture. Media is incorporated with inhibitory substances to suppress the unwanted organism.

Eg:

- Selenite F Broth – for the isolation of Salmonella, Shigella
- Alkaline Peptone Water – for Vibrio cholerae
Differential media

- A media which has substances incorporated in it enabling it to distinguish between bacteria.

- Eg: Mac Conkey’s medium

- Distinguish between lactose fermenters & non lactose fermenters.
- Lactose fermenters - **Pink** colonies
- Non lactose fermenters - colourless colonies
Sensitivity test or Antimicrobial susceptibility testing
Antimicrobial susceptibility testing is a standardized method that is used to measure the effectiveness of antibiotics and other chemotherapeutic agents on pathogenic microorganisms. In many cases, it is an essential tool in prescribing appropriate treatment.
The Kirby-Bauer test, also called the disk diffusion test:

is a valuable standard tool for measuring the effectiveness of antimicrobics against pathogenic microorganisms.
Antibiotics are natural antimicrobial agents produced by microorganisms. One type of penicillin, for example, is produced by the mold Penicillium notatum. Today, because many agents that are used to treat bacterial infections are synthetic, the terms antimicrobials or antimicrobics are used to describe all substances used for this purpose.
Procedure

In the test, antimicrobial-impregnated paper disks are placed on a plate that is inoculated to form a bacterial lawn. The plates are incubated to allow growth of the bacteria and time for the agent to diffuse into the agar. As the drug moves through the agar, it establishes a concentration gradient. If the organism is susceptible to it, a clear zone around the disk where growth has been inhibited appear.
MEASURING THE ZONE OF INHIBITION
THANK YOU