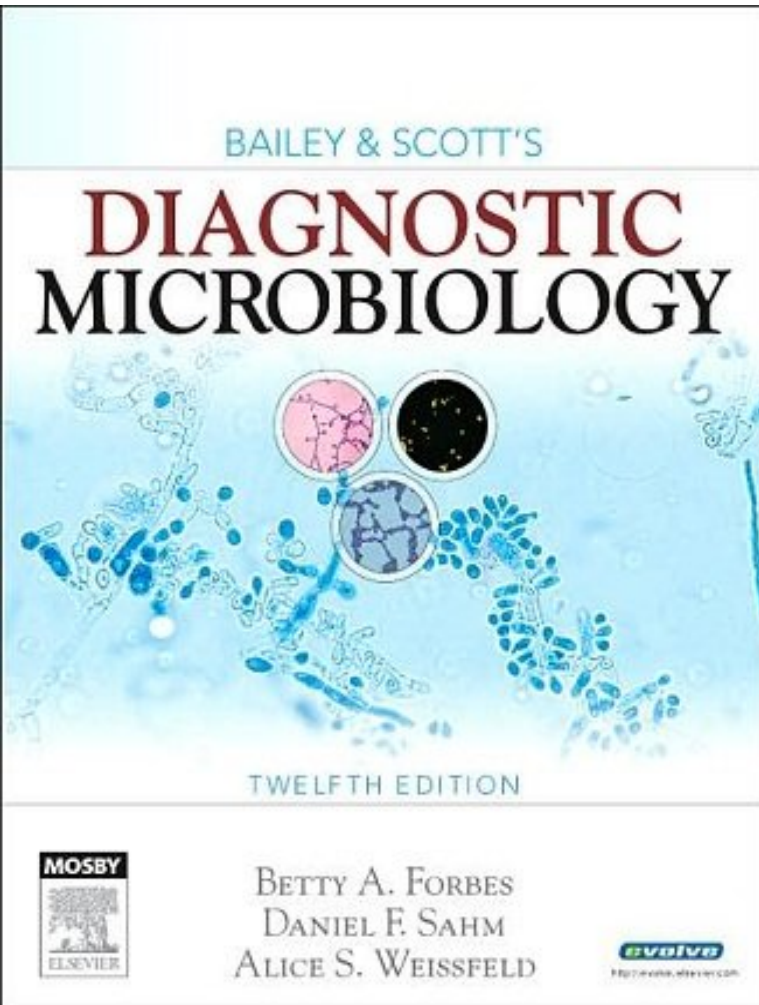


Diagnostic Microbiology

Identification of Microbes

Lecture: 2



2- Culture Techniques

Media: Providing Nutrients in the Laboratory

Culture media are used for:

- Isolation and identification of pathogenic organisms
- Antimicrobial sensitivity tests

*** Types of culture media:**

a- Liquid media:

- Nutrient broth: meat extract and peptone
- Peptone water
- Growth of bacteria detected by turbidity

b- Solid media - advantages:

- isolation of single colony
- Colonial appearance and morphology
- Hemolytic activity
- Pigment production
- colony count



- **Media can be classified according to three properties:**

Three Categories of Media

- **1. Physical state of media**

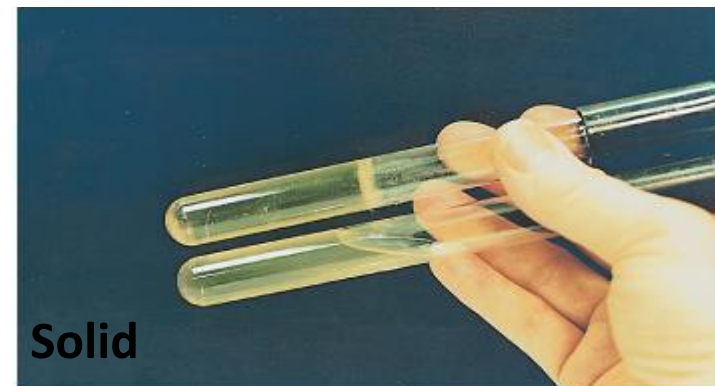
- **Liquid: broth;** does not solidify
- **Semisolid:** contains solidifying agent
- **Solid:** firm surface for colony formation - Contains solidifying agent

- **2. Chemical composition**

- - **synthetic** (chemically defined)
- - **nonsynthetic** (complex)

- **3. Functional type**

- **General purpose**
- **Enriched**
- **Selective**
- **Differential**
- **Anaerobic**
- **Transport**
- **Assay**
- **Enumeration**



Types of solid media

1- Simple media: Nutrient agar

2- Enriched media: media of high nutritive value (Blood agar- Chocolate agar- Loeffler's serum)

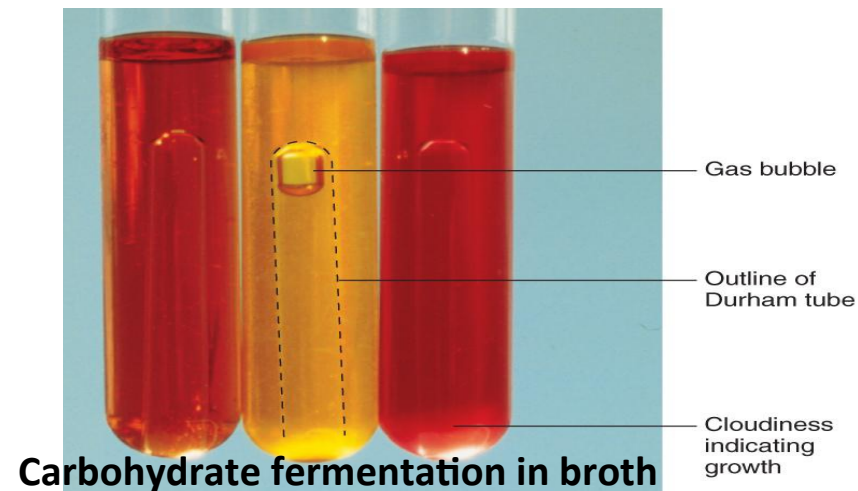
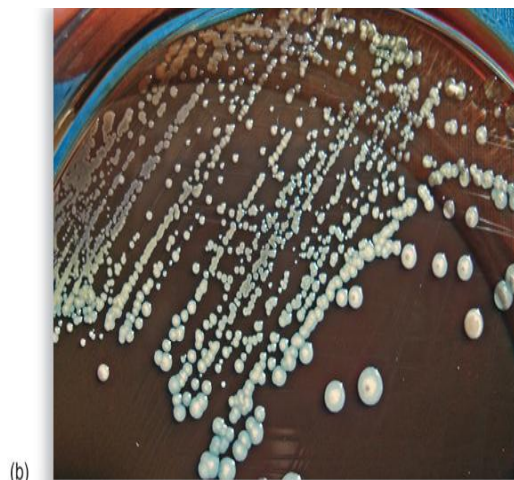
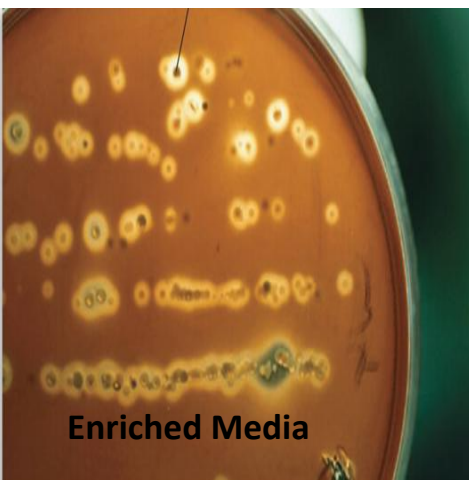
3- Selective media: allow needed bacteria to grow (Lowenstein Jensen medium- MacConkey agar - Mannitol Salt Agar)

4- Indicator media: different lactose & non lactose fermentor (MacConkey- Eosine Methylene blue Agar)

5- Anaerobic media: for anaerobic cultivation (Deep agar- Cooked Meat Medium)

6- Miscellaneous Media: **A-Reducing medium:** contains a substance that absorbs oxygen or slows penetration of oxygen into medium; used for growing anaerobic bacteria

B- Carbohydrate fermentation medium: contains sugars that can be fermented, converted to acids, and a pH indicator to show this reaction



Colonial appearance on culture media

* Colony morphology:

- . Shape
- . Size
- . Edge of colony
- . Color

* Growth pattern in broth:

- . Uniform turbidity
- . Sediment or surface pellicle

* Pigment production:

- . Endopigment production (*Staphylococcus aureus*)
- . Exopigment production (*Pseudomonas aeruginosa*)

* Haemolysis on blood agar:

- . Complete haemolysis (*Streptococcus Pyogenes*)
- . Partial haemolysis (*Streptococcus Viridans*)

* Growth on MacConkey's medium:

- . Rose pink colonies (Lactose fermenters)
- . Pale yellow colonies (Non lactose fermenters)

B- Biochemical Reaction

Most rely on pH indicator or color change when a compound is degraded

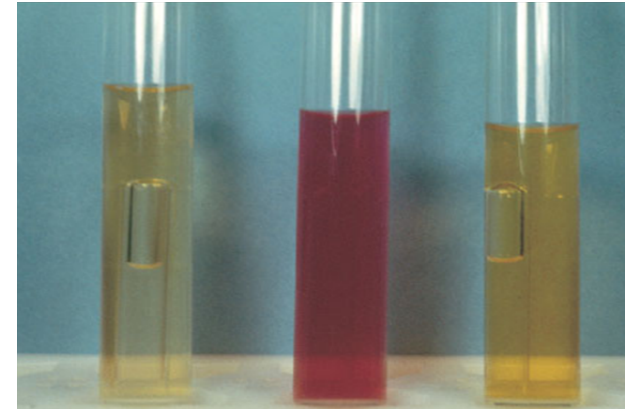
Use of substrates and sugars to identify pathogens:

a- Sugar fermentation: (lactose- sucrose-glucos etc...)

Organisms ferment sugar with production of **acid only**

Organisms ferment sugar with production of **acid and gas**

Organisms do not ferment sugar



b- Production of indole:

Depends on production of indole from amino acid

tryptophanase

Indole is detected by addition of **Kovac's reagent**

Appearance of **red ring** on the surface



C- H₂S production:

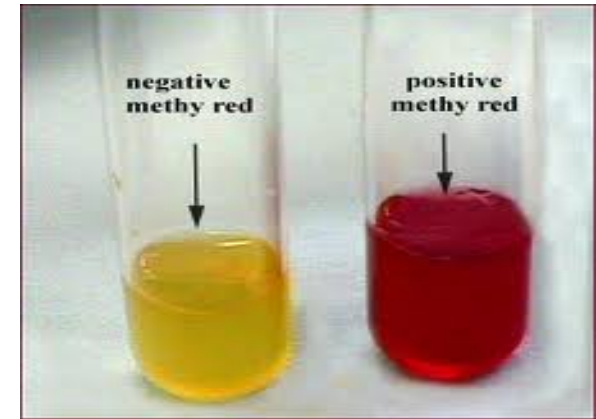
Depends on production H₂S from protein or polypeptides
amino acid (cystine)



Biochemical Reaction (cont.)

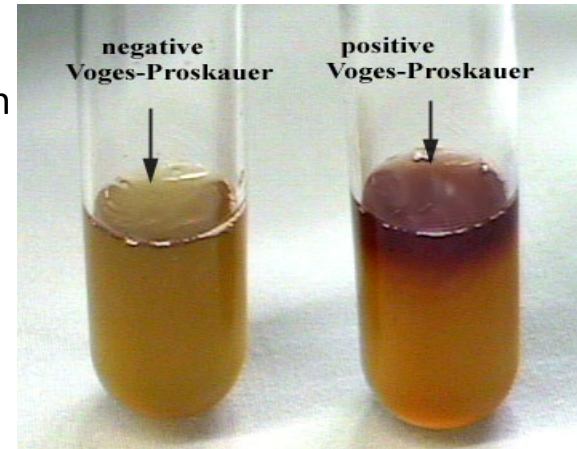
d- Methyl red reaction (MR):

Fermentation of glucose with production of huge amount of acids Lowering pH is detected by **methyl red indicator**



e- Voges proskaur's reaction (VP):

Production of **acetyl methyl carbinol** from glucose fermentation
Acetyl methyl carbinol is detected by addition reagents **Barritt's A (a-naphthol)** and **Barritt's B (KOH)**
Color of medium turns **pink** (positive)



f- Action on milk:

Fermentation of lactose with acid production
Red color if litmus indicator is added



Biochemical Reaction (cont.)

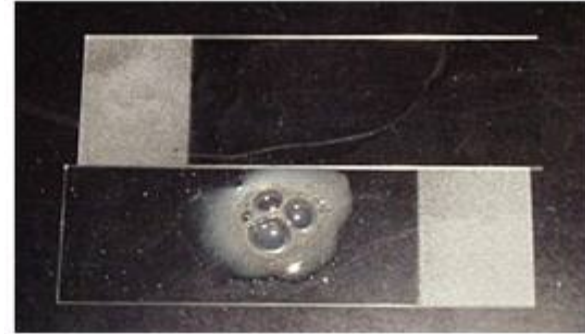
g- Oxidase test:

Some bacteria produce Oxidase enzyme
Detection by adding few drops of colorless **oxidase reagent**
Colonies turn deep **purple in color** (positive)



h- Catalase test:

Some bacteria produce catalase enzyme
Addition of H₂O₂ lead to production of gas bubbles
(O₂ production)



i- Coagulase test:

Some bacteria produce coagulase enzyme
Coagulase enzyme **converts fibrinogen to fibrin (plasma clot)**
Detected by slide or test tube method



j-Urease test:

Some bacteria produce urease enzyme
Urease enzyme hydrolyze urea with production of **NH₃**
Alklnity of media and change color of indicator from **yellow to pink**



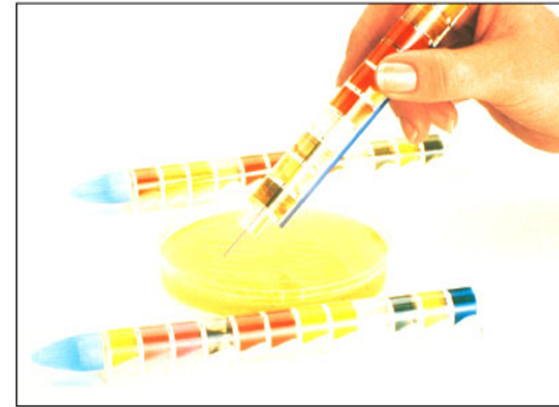
Biochemical Reaction (cont.)

- Commercial modifications of traditional biochemical tests
e.g., API20E system, Vitek system, Enterotube TM, Microscan

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(a)



(b)



Micro scan Machine

Table Characteristics of Some Important Biochemical Tests

Biochemical Test	Principle of the Test	Positive Reaction
Catalase	Detects the activity of the enzyme catalase, which causes the breakdown of hydrogen peroxide to produce O ₂ and water.	Bubbles.
Citrate	Determines whether or not citrate can be used as a sole carbon source.	Growth, which is usually accompanied by the color change of a pH indicator.
Gelatinase	Detects enzymatic breakdown of gelatin to polypeptides.	The solid gelatin is converted to liquid.
Hydrogen Sulfide Production	Detects H ₂ S liberated as a result of the degradation of sulfur-containing amino acids.	A black precipitate forms due to the reaction of H ₂ S with iron salts in the medium.
Indole	Detects the enzymatic removal of the amino group from tryptophan.	The product, indole, reacts with a chemical reagent that is added, turning the reagent a deep red color.
Lysine Decarboxylase	Detects the enzymatic removal of the carboxyl group from lysine.	The medium becomes more alkaline, causing a pH indicator to change color.
Methyl Red	Detects mixed acids, the characteristic end products of a particular fermentation pathway. ■ mixed acids, p. 153	The medium becomes acidic (pH < 4.5); a red color develops upon the addition of a pH indicator.
Oxidase	Detects the activity of cytochrome <i>c</i> oxidase, a component of the electron transport chain of specific organisms. ■ cytochrome <i>c</i> , p. 148	A dark color develops upon the addition of a specific reagent.
Phenylalanine Deaminase	Detects the enzymatic removal of the amino group from phenylalanine.	The product of the reaction, phenylpyruvic acid, reacts with ferric chloride to give the medium a green color.
Sugar Fermentation	Detects the acidity resulting from fermentation of the sugar incorporated into the medium. Also detects gas production.	The color of a pH indicator incorporated into the medium changes if acid is produced. An inverted tube traps any gas that is made.
Urease	Detects the enzymatic degradation of urea to carbon dioxide and ammonia.	The medium becomes alkaline, causing a pH indicator to change color.
Voges-Proskauer	Detects acetoin, an intermediate of the fermentation pathway that leads to the production of a 2, 3-butandiol. ■ 2, 3-butanediol, p. 152	A red color develops upon addition of chemicals that detect acetoin.