MLS 305 - B2

Nutritional factors in relation to growth and reproduction

Δ Cultivation of Bacteria

Δ Nutritional Requirements

- All forms of life: From micro-organisms to human beings
- Chemical are necessary for proper functioning

Δ Great diversity in *Nutritional types*

Δ All living things require some form of energy

ΔΔ Energy generating mechanism ATP,GTP,UTP and CTP.

□ Energy requirement

Δ Green plants utilize radiant energy- phototrophs.

- Other forms of life incapable of using radiant energy.

Δ Animals rely on Oxidation of chemical components for energy—

- Chemotrophs

ΔΔ Both phototrophs and chemotrophs exist in Bacteria.

□ All Living Things Require Carbon in one form or the other.

Δ Organic Carbon e.g Sugars and other Carbohydrate.

Δ Plants use Carbon dioxide and convert it to carbohydrate by photosynthesis.

Δ Bacteria which require CO_2 as carbon source--- Autotrophs;

Δ Photoautotrophs-Energy from light

Δ Chemoautotrophs-Energy by oxidizing chemical compounds.

Δ Heterotrophs-Require Organic form of Carbon.

□ All Living Organisms require Nitrogen.

Δ Plants require inorganic salts potassium nitrates (KNO_3).

Δ Animals-Organic Nitrogen from proteins and degradation of products e.g peptide and Amino acid.

Δ Bacteria thrive on Atmospheric N_2 and Inorganic Nitrogenous Compound.

- Proteins.

□All living Organisms require:

Δ Sulphur and phosphorus

Δ Plants require organic Sulphur components

Δ Bacteria require organic and inorganic Sulphur components.

□ All living organisms require metallic elements

Δ Sodium,potassium,calcium,magnessium,manganese,phosphorus

● Sometimes in traces.

□ All living things require Vitamins and Vitamin like compounds

Δ Bacteria require Vitamins-Synthesize from medium.

Vitamins	Species exhibitting requirement
Thiamine(B1)	*Bacillus anthracis*
Riboflavin	*Clostridium tetani*
Niacin	*Brucella abortus*
Pyridoxine (B8)	*Lactobacillus sp*
Biotin	*Leuconostoc Mesenteriodes*
Bantothonic acid	*Proteus morganii*
Folic acid	*Leuconostoc dextranum*
Cobalamin(B12)	*Lactobacillus sp*
Vitamin K	*Bacteroides melaninogenicus*

□ NUTRITIONAL TYPES OF BACTERIA

Δ Bacteria-Two major groups based;

- Nutritional requirements
- Phototrophs and Chemotrophs
- Subdivision based on:

Δ Energy utilized for growth

- Light or Oxidation of Chemical Compounds.

□ Phototrophs

Δ Bacteria which utilize CO_2 as principle source of carbon.

- Photolithotrophs e.g *Chromatium* Sp.

Δ Others require Organic Compounds

- Photo- organotrophs e.g *Rhodopseudomonas palustris*
- Utilize-Alcohols,fatty acids and Amino acids.

□ Chemotrophs

Δ Bacteria which fix CO_2 for energy and Carbon needs

- Oxidize nitrite from nitrate
- Derives energy from elemental Sulphur and inorganic sulfur compounds.

Δ Chemolithotrophs e.g *Thiobacillus thiopanis*

Δ Chemotrophs which require organic carbon compound by oxidation.

- Chemo-organotrophs.

□ Photolitotrophic and Chemolithotrophic

Δ Bacteria----------Autotrophs

Δ Photoorganotrophic and Chemoorganotrophic

● Bacteria----------Heterotrophs

Δ Autotrophs and Heterotrophs exhibit simple requirements.

● Powered sulphur,$CaCl_2$ etc

ΔΔ see table

Δ The fact that an organism can perform in such combination

ΔΔ Shows it has elaborate capacity for synthesizing carbohydrate,fats,nucleic acid,protein e.t.c.

ΔΔ media for growth are chemically defined synthetic media.

□ Heterotrophs and Autotrophs

Δ Heterotrophs: Cause diseases of human beings and other animals

- Cause plant diseases,found in our immediate environment

Δ Autotrophs: Indispensable in nature

- Recycling of elements through biological systems
- Activities of microbes in soil and water

Δ See table.

□ BACTERIOLOGICAL MEDIA

Δ Group of complex chemically defined composition for cultivation.

- Specific known types of bacteria
- Routine cultivation of bacteria
- Synthetic media; not generally employed

Δ Peptones,meat extract,yeast extract,etc

□ Composition of Basic Raw Material

Δ Beef extracts:Lean meat,tissue concentrated to paste.

Δ Peptone: Digestion of proteinous material e.g Casein,meat,gelatin

Δ Yeast extract: Extract of yeast cells commercially available as powder.

Δ Agar-Complex carbohydratye obtained from certain marine algae.

ΔΔ Ingredients; possible to Compound medium to support growth of *Heterotrophs*

Δ Nutrient Broth and Nutrient Agar.

□ Types of Media

Δ Bacteria grow in varying proportion in media or may not grow.

•• Bacteriologist should have a knowledge

- Media to support growth
- On the basis of application and function

Δ Enriched media

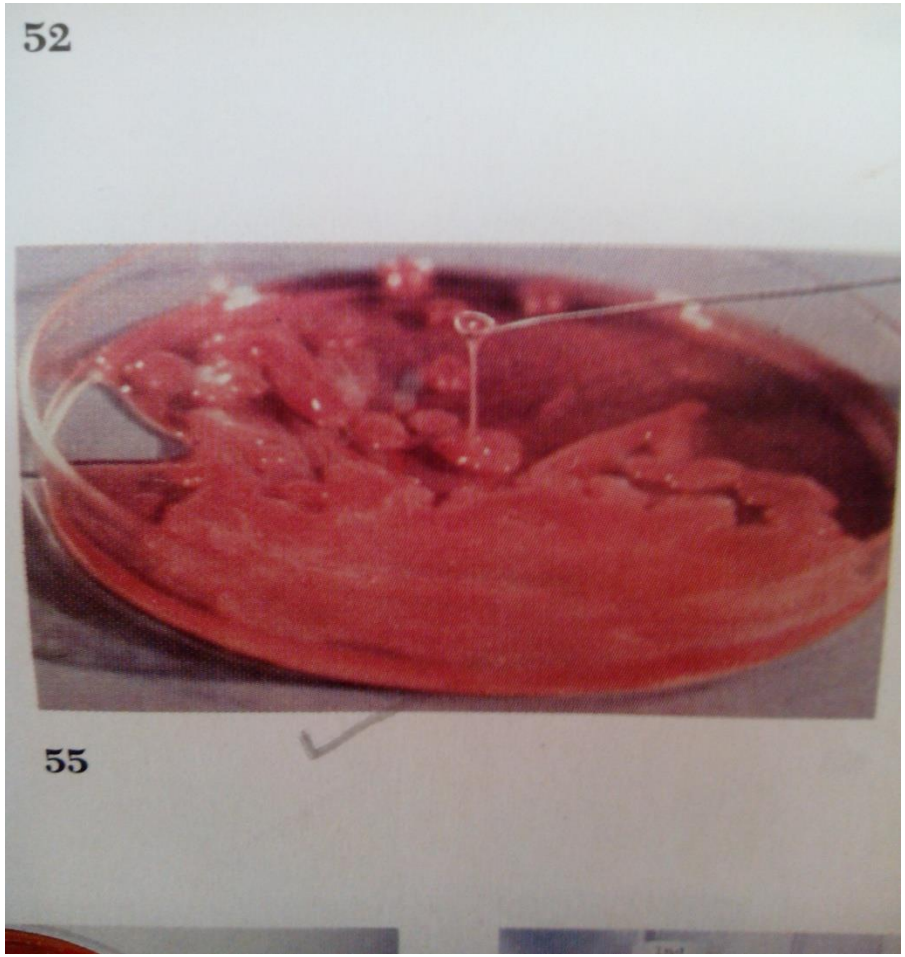
- Addition of components:Blood,Serum,plants or animal extract
- Examples: S S Agar-Salmonella Shigella Agar Selenite- F broth
- TCBS- Thiosulfate Citrate bile salts Sucrose Agar.

  Apw *Vibrio cholera* medium.

Δ Differential media:

- Addition of reagents or chemical
- Result to observed change for differentiating.
- Blood Agar; Haemolytic or non-haemolytic
- MacConkey Agar: Lactose fermentation or Non-lactose fermentation.

MacConkey Agar showing growth of *Klebsiella pneumoniae*



Δ Assay media,

- Various chemical composition
- Assay vitamins,Amino acids,antibiotics e.g testing disinfectants

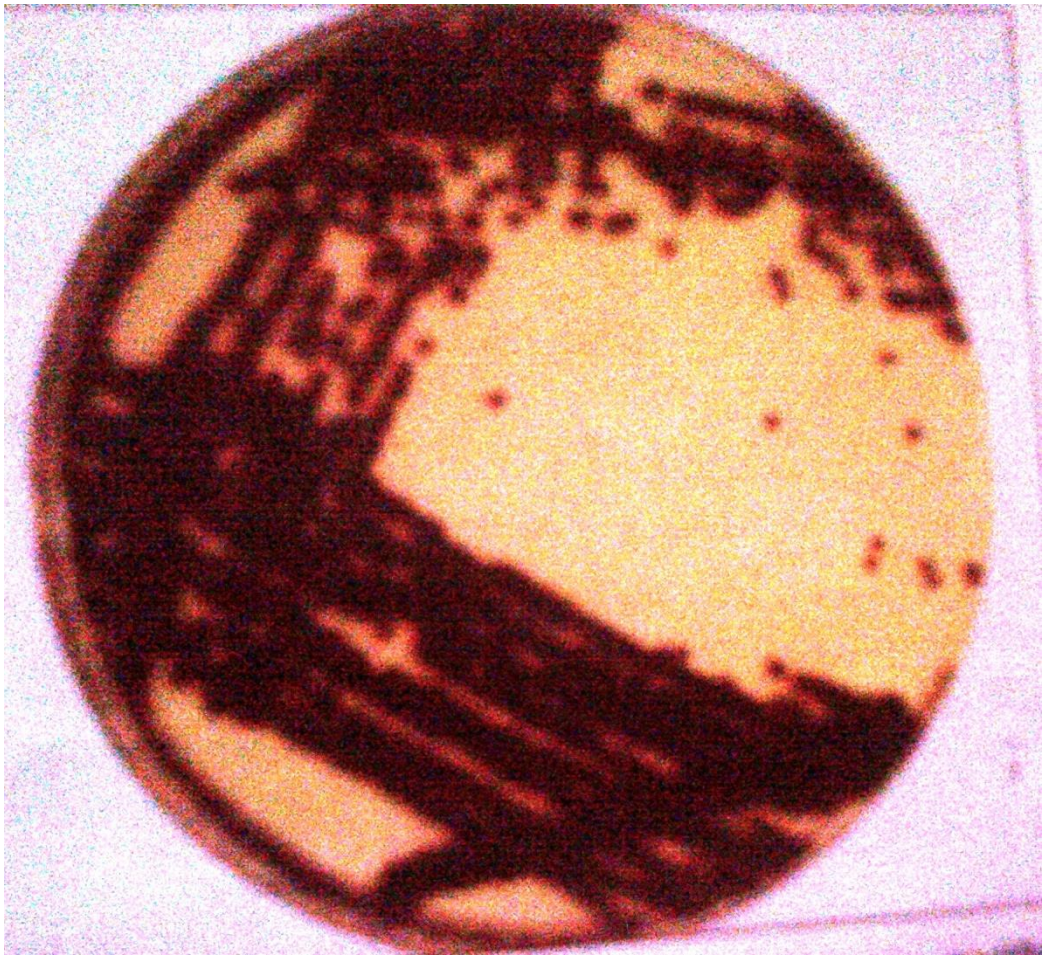
Δ Media for enumeration of Bacteria

- Determining bacterial content
- Milk and water

Δ Media for Characterization of Bacteria

- Type of growth produced
- Type of Chemical change.

Corynebacterium diphtheriae cultured on Tinsdale medium. Brown discolouration-Colonies



Blood Agar Showing Haemolysis

Δ Maintenance media

- Continous growth measurement
- Specific organisms
- Example: Glucose facilitates growth and production of acid
- Omission of Glucose is prefered in maintenance culture.

ΔΔ Media Commercially available.

TYPES OF MEDIA

There are several types and forms of culture media

Liquid media or broth

In liquid media, bacteria can freely move about. The growth and multiplication of bacteria in liquid media is enhanced. Growth is liquid medium is shown by turbidity, though some organisms show surface growth.

Liquid media are used mainly for biochemical testing, blood culture, testing for motility and as enrichment media. The major disadvantage is that purity of the growth cannot be guaranteed.

Solid media

When organisms are grown on solid media, they grow and multiply at the site of inoculation and form visible colonies. Colonial appearance and any changed in the surrounding medium help in the identification of the bacterial species.

A liquid medium is made solid by the incorporation of solidifying agent which does not alter the nutritional content of the medium. The most widely used solidifying agent is the agar (formerly called agar-agar). Agar is an inert carbohydrate extract obtained from a type of seaweed found in Japan, New Zealand, and California (USA). It is available in powder form.

A good brand of agar must:

1. Gel at a concentration of 1%
2. Melt at 98^0C
3. Set on cooling at 42^0C
4. Be easily soluble and maintain clarity in solution

Basal media

Basal media are simple media that will support the growth of most micro organisms that do not need special nutritional requirements. They contain the basic nutrients: peptone, mineral salts and water. They are generally referred to as nutrient broths. Nutrient broths may be in the following forms.

Infusion broth

This is a watery extract of fat-free minced meat which has been infused overnight at 4^0C. The extract is filtered, peptone and salt added and pH adjusted to 8.0

Digest broth

This is an enzyme digested meat broth: fresh fat free minced meat is placed in sodium carbonate which neutralizes any sarcolactic acid in the meat. The mixture is heated and cooled to 45^0C. Trypsin is added to digest the meat. The digestion may last between 4-6 hours. Chloroform is added as a preservative to prevent putrefaction. Trypsin digests the meat protein to peptone, concentrated hydrochloric acid is added to stop the digestion when the end point of digestion is reached.

Enriched media

Enriched media are culture media that are enriched with whole or lysed blood, serum, special extracts or nutrients to support the growth of those bacteria that cannot grow on the basal media. Examples are blood agar, serum agar, chocolate agar:

Nutrient broth + agar = Nutrient agar

Nutrient agar + blood = Blood agar

Blood agar + heat = Chocolate agar

Selective media

Selective media are solid media which contain substances that prevent, slow down or inhibit the growth of micro-organism other than those for which the media are devised. Examples of selective media are:

1. MacConkey agar will differentiate lactose fermenting bacteria from non-lactose fermenting ones
2. Blood agar will differentiate haemolytic bacteria from non-haemolytic ones.

Auxanographic media

These media are deficient in certain nutrient requirements. The auxanographic media will require special growth factors in order to support the growth of some bacteria. When the medium is inoculated with an organism, and certain growth factors are spotted on the surface of the medium, the growth of the organism occurs only in or around the factors. This indicates that the organism relies on the factors for growth. For examples *Haemophilus influenzae* will only grow on nutrient in the vicinity of X+V factors.

Transport media

Transport media as the name indicates, are meant for the transportation of clinical specimens containing delicate micro-organisms from the ward or clinic to the laboratory if there is to be a delay in their delivery to the laboratory or in processing. They contain substances that can prevent the overgrowth of commensals and prevent bacteria from dying as a result of change of pH or enzyme action. They are generally semisolid. Examples are:

Amies transports medium for *Neisseria gonorrhoeae*

Stuart's transport medium for delicate organism including anaerobes