MICROBIAL NUTRITION

Study of microorganisms requires their cultivation under laboratory conditions. For this organisms must be provided with suitable physical conditions and also the substances which they require for the synthesis of their cellular materials and for the generation of energy. These substances are termed as nutrients. Therefore the culture medium must contain all necessary nutrients in appropriate quantities. However the nutritional requirements of microorganisms vary widely.

Basic Nutritional Requirements Of Microorganisms

Microorganisms derive their nutrition from following sources.

- Source of Carbon
- Source of Nitrogen
- Source of Sulfur
- Source of Phosphorus
- Source of Energy
- Metallic Elements
- Additional Growth Factors
- Water

(1) Source of Carbon –

All organisms require carbon in some form. Probably all microorganisms can use or fix the simplest carbon compound CO_2. A few organisms can use CO_2 as their sole or principal source of carbon. On the basis of this property microorganism has been divided into two categories.

(i) Autotrophs – These are the organisms which can use CO_2 as their sole or principal source

of carbon. They have the ability to synthesize all of their cellular carbon compounds from CO_2. A wide variety of microorganisms are autotrophic; many of these are photosynthetic. Autotrophs are self-feeders or self-nourishing organisms.

(ii) Heterotrophs – Many organisms cannot use CO_2 as their principal source of carbon and depends upon the presence of organic compound for the supply of carbon. Such organisms that use organic compounds as a principal source of carbon are called heterotrophs. Most heterotrophs use organic compound as a source of both carbon and energy.

Microorganisms show great variations with respect to both kind and number of organic compounds that they can use as a source of carbon. Some organisms use many more compounds as a source of carbon (for exa. *Pseudomonas cepacia* can use more than 100 compounds as a source of carbon), whereas some uses limited number of compounds as a source of carbon (for exa. Methylotrophic bacteria can use only methane and methanol as a source of carbon).

(2) Source of Nitrogen –

All organisms require nitrogen in some form. Plants utilize nitrogen in the form of inorganic nitrogen salts such as potassium nitrate (KNO3), where as animals require organic nitrogen compounds (proteins, peptides, amino acids etc). Bacteria are extremely versatile in this respect. Some bacteria use atmospheric nitrogen. However most bacteria can not utilize atmospheric nitrogen and obtain this element from inorganic nitrogen compounds (like ammonium chloride NH4Cl, sodium nitrate NaNO3) or from organic nitrogen compounds (peptides, amino acids, aliphatic amides).

Nitrogen is a constituent of many substances such as amino acids, purines, pyrimidines, some carbohydrates and lipids, proteins (both enzymes and structural polymers), enzyme cofactors and other substances. Therefore nitrogen is needed for the synthesis of all above mentioned components within the cell. Hence a utilizable source of nitrogen must be present in the medium.

(3) Source of Sulfur

All organisms require sulfur in some form. Some microorganisms require organic sulfur compound and some are capable of using inorganic sulfur compounds. Still some organisms can satisfy their requirement by using elemental sulfur (S^o).

Element sulfur is usually provided by sulfate salt such as ammonium sulfate [$(NH_4)_2SO_4$], and thiosulfates ($S_2O_3^{2-}$). The element sulfur is a constituent of many substances such as sulfur containing amino acids (cysteine & methionine), coenzymes, growth factors (biotin & thiamine) and other cellular constituents. Thus element sulfur is required for the synthesis of all these mentioned cellular components.

(4) Source of Phosphorus

Allmost all organisms use inorganic phosphorus compounds as their phosphorus source. It is generally supplied to the cell as a salt of phosphoric acid, such as the potassium phosphate KH_2PO_4 & K_2HPO_4. Phosphorus is present in nucleic acid, phospholipids, key molecule of energy metabolism ATP, several cofactors, some proteins and other cell components. Thus element phosphorus is required for the synthesis of all these mentioned cellular components.

(5) Source of Energy –

All the organisms require source of energy. Although the ultimate source of energy on this planet is the sun, only few organisms including algae, plants photosynthetic bacteria and protozoa are able to use directly the energy of sun (light energy). These organisms are called as phototrophs. Majority of organisms including bacteria and animals obtains their energy from chemical compounds either organic or inorganic (chemical energy). These organisms are referred as chemotrophs. Thus there are two groups of organisms depending upon the source of energy.

(6) Metallic Elements –

All living organisms require several metallic elements such as Na, K, Ca, Mg, Fe, Zn, Co, Cu, Mo, Mn etc for their normal growth. These elements can be supplied in the form of inorganic salts. Under laboratory condition, they can generally be supplied as contaminants in tap water.

These elements fall into two classes according to concentrations in which they are required. Na, K, Ca, Mg and Fe are required in relatively large amounts and are termed as macronutrients. Some elements like Zn, Co, Cu, Mo and Mn are required in very small concentrations. It is often difficult to demonstrate their essentiality. These elements are referred as micronutrients or trace elements.

(7) Additional Growth Factors –

These are the substances which can not be synthesized by microorganisms from supplied simpler sources, but they are necessary for the synthesis of certain cellular components required for well-being. Therefore these substances must be added in culture medium as a nutrient and are called as growth factors. There are three major classes of growth factors.

(1) Amino acids – Needed for the synthesis of proteins.

(2) Purines and Pyrimidines – Needed for the synthesis of nucleic acids.

(3) Vitamins - Needed for the synthesis enzyme cofactors. Example – riboflavin, pyridoxine, folic acid, pantothenic acid, nicotinic acid etc.

(8) Water –

Water plays an important role in microbial nutrition and is required for metabolic functions and growth. It is called as master solvent and forms 80 to90 % of the total weight of the cell. It helps the microorganisms in absorption, osmosis, excretion, secretion etc. for bacteria, all nutrients must be in solution form before they can enter the bacterial cell. To a certain extent synthesis of water also takes place within the organism, during various metabolic reactions such as oxidation of hydrogen, polymerization reactions.

MEDIA

Classification of media on the basis of Consistency i.e. their physical state

Culture media may be divided into four categories on the basis of consistency i,e their physical state

(A) Liquid Media (B) Semisolid Media (C) Liquefiable Solid Media (D) Solid Media

(A) Liquid Media –

These media are used in liquid form and do not contain any solidifying agent. For Example – Nutrient Broth, Skimmed Milk, Peptone Solution etc.

(B) Semisolid Media –

These media contains smaller amount (0.5% or less) of agar – agar (solidifying agent), which imparts semisolid consistency. These media are used for the cultivation of microaerophilic bacteria and for demonstration of motility. For Exa. Cystine Trypticase Agar Medium.

(C) Liquefiable Solid Media –

This medium is also called 'Solid Reversible to Liquid Medium'. These media are prepared by adding suitable amount of (1.5 to 2.0 %) agar – agar to the liquid medium. These media becomes solid when cooled but becomes liquid when heated or vice versa. Example – Nutrient Agar, Potato Dextrose Agar etc.

(D) Solid Media –

These media always remains solid. For example – Potato Slices, Coagulated Egg.

Classification of media on the basis of Nature of Ingredients

On the basis of nature of ingredients, culture media are divided into two types.

(A) Synthetic Media (B) Nonsynthetic Media

(A) Synthetic Media –

- These media are also called as 'chemically defined media'.
- Synthetic media are those media which are composed of ingredients of known composition.
- The exact chemical composition of these media is known. In other words, all components and their concentrations are known in synthetic media.
- These media are designed for the cultivation specific known bacteria.
- In synthetic media, since exact chemical composition of all ingredients is known, two batches of same medium can be duplicated to a higher degree of accuracy.
- Synthetic media are not so commonly used for routine purposes because they are often expensive and time consuming to prepare.
- Example of synthetic media –

  Synthetic media used for the cultivation of *Escherichia coli*.

  Glucose ($C_6H_{12}O_6$) ---------- 5.0 g

$NH_4H_2PO_4$	----------	1.0 g
NaCl	----------	5.0 g
$MgSO_4.7H_2O$	----------	0.2 g
K_2HPO_4	----------	1.0 g
WATER	----------	1000 ml

(B) <u>Nonsynthetic Media</u> –

- These media are also called as 'chemically complex media' or 'biological media'.
- Nonsynthetic media are those media which are composed of ingredients of unknown chemical composition. Some of these ingredients are beef extract, yeast extract, peptone, blood, serum, casein hydrolysate etc.
- The exact chemical make up of these media is unknown.
- These media can support the growth of wide variety of bacteria and other microorganisms.
- Therefore, nonsynthetic media are used as routine culture media by bacteriologist.
- In nonsynthetic media, it is practically impossible to prepare two identical lots of the same medium from different batches of the same ingredients. .
- These media are convenient to prepare and inexpensive.
- Examples of commonly used nonsynthetic media are Nutrient Broth, Tryptic Soy Broth, Cooked Meat Medium, Nutrient Agar etc.

<u>Composition of Common Nonsynthetic Media</u>

<u>Nutrient Broth</u>		***<u>Nutrient Agar</u>***	
Peptone ----------	5.0 g	Peptone ----------	5.0 g
Beef extract -------	3.0 g	Beef extract -------	3.0 g
Dist. Water -------	1000 ml	Agar – agar -------	15 g
		Dist. Water -------	1000 ml

Differentiation between Synthetic Media and Nonsynthetic Media

S.N	**Synthetic Media**	**Nonsynthetic Media**
1	Also called as 'chemically defined media'	Also called as 'chemically complex media' or 'biological media'
2	These media are composed of ingredients of known chemical composition.	These media are composed of ingredients of unknown chemical composition such as beef extract, yeast extract, peptone, blood, serum, casein hydrolysate etc.
3	The exact chemical composition of these media is known.	The exact chemical make up of these media is unknown.
4	These media are designed for the cultivation specific known bacteria.	These media can support the growth of wide variety of bacteria and other microorganisms.
5	Two batches of same medium can be duplicated to a higher degree of accuracy.	It is practically impossible to prepare two identical lots of the same medium from different batches of the same ingredients.
6	These media are expensive and time consuming to prepare.	These media are convenient to prepare and inexpensive.
7	Synthetic media are not so commonly used for routine purposes	Nonsynthetic media are used as routine culture media by bacteriologist
8	Example of Synthetic Media – Synthetic media used for the cultivation of *Escherichia coli*. Glucose ($C_6H_{12}O_6$) --------- 5.0 g $NH_4H_2PO_4$ ---------- 1.0 g NaCl --------- 5.0 g $MgSO_4.7H_2O$ ---------- 0.2 g K_2HPO_4 ---------- 1.0 g WATER ---------- 1000 ml	Example Nonsynthetic Media ***Nutrient Broth*** Peptone ---------- 5.0 g Beef extract ------- 3.0 g Dist. Water ------- 1000 ml Other examples are - Tryptic Soy Broth, Cooked Meat Medium, and Nutrient Agar etc.

Composition and Functions of Beef Extract

- Beef extract is the common ingredient of media used for the cultivation of many microorganisms.
- The use of beef extract in culture media was introduced by Loeffler in 1881.
- Beef extract is an aqueous extract of beef tissue concentrated to a paste.
- It is prepared by cutting fresh beef into pieces, placing them in vessel with an appropriate amount of water and then heating for several hours with occasional stirring. Heating extract s certain constituents from beef tissues. After heating, beef tissues are separated and liquid portion is obtained separately. The liquid portion is then filtered and evaporated in vacuum until it becomes a paste.
- Beef extract contains several water soluble substances of beef tissue. These includes —

  ----- Carbohydrates

  ----- Organic nitrogen compounds such as amino acids, peptides etc.

  ----- Organic acids such as lactic acid, succinic acid etc.

  ----- Nucleotides, Purines, Pyrimidine's.

  ----- Water soluble B complex vitamins such as riboflavin, pantothenic acid, nicotinic acid, biotin, pyridoxine, folic acid etc.

  ----- Inorganic salts, Minerals

  ----- Nitrogenous substances such as creatine, xanthine, uric acid, urea, hypoxanthine, glutamine etc.

- Beef extract is added to media to provide above substances that stimulates bacterial growth.

Composition and Functions of Yeast Extract

- Yeast extract is the ingredient used frequently in complex nonsynthetic media.
- It is an aqueous extract of brewer's yeast (*Saccharomyces cerevisiae*), commercially available as a powder.
- It is prepared by heating the yeast cells in distilled water at 45^0C, for a period of about 14 hrs. This process breaks the yeast cells and extracts the intracellular proteins and other cellular constituents into the water. The aqueous extract is filtered to separate the cells and then evaporated in vacuum.
- Yeast extract is excellent stimulator of bacterial growth.
- It is a rich source of B – vitamins, in addition to organic carbon and nitrogen compounds. Yeast extract is added in culture medium to supply these factors to organisms.
- It is superior to meat extract in most culture media.

Composition and Functions of Peptones -

- Peptones are intermediate hydrolysis product of natural proteins of animal (meat, casein, gelatin) or plant (soybean) origin.
- In other words, peptone is a product obtained by partial digestion of proteinaceous material.
- Hydrolysis or digestion of the protein material is brought about by acid or certain proteolytic enzyme like trypsin, pepsin etc.
- As hydrolysis proceeds, the large protein molecules are broken up into

series of smaller fragments which are known as proteoses, peptones, peptides and amino acids, respectively.

- Many different peptones depending upon the protein material used for its manufacture are available.
- The commercial peptones used by bacteriologists are composed of proteoses, peptones, peptides and amino acids.
- The most important function of peptones in culture media is to provide source of nitrogen. Peptone is a principal source of organic nitrogen.
- Since amino acids are amphoteric compounds, peptones are also excellent buffers

Composition and Functions of Solidifying Agents

Gelatin –

Gelatin is a protein obtained by boiling skin, bones, tendons and ligaments with water. It is an incomplete protein, lacking tryptophan amino acid.

Gelatin is not soluble in cold water but soluble in boiling water. On cooling it solidifies to form transparent gel. For first time in 1881, gelatin was used as a solidifying agent to solidify regular liquid medium, by Robert Koch. But gelatin was not found to be an ideal solidifying agent because of following disadvantages.

(1) It is attacked and decomposed by many bacteria. As a result, it looses the property to form gel and gets liquefied.

(2) It melts at 27^0C, therefore medium containing gelatin as a solidifying agent can not be incubated at 37^0C, which is the optimum temperature for the growth

of bacteria.

Therefore gelatin is not used as a solidifying agent in the preparation of solid media.

Agar – Agar

This is the most satisfactory solidifying agent used in the preparation of solid media. If a solid medium is required for surface cultivation of microorganisms, liquid medium can be solidified with the addition of 1.5 % agar.

In 1882, agar was first used as a solidifying agent. The use of agar was suggested by Frau Hesse, wife of one of Koch's assistant.

Agar is the dried mucilaginous substance extracted from red algae *Gelidium corneum* and other related algae. These algae grows chiefly at the coasts of Japan, China, Ceylon and southern California.

Chemically, agar is a complex polysaccharide. More specifically it is the sulfuric acid ester of a linear galactan. It is insoluble in cold water but slowly soluble in hot water. On cooling, it forms solid gel.

Agar is an excellent and ideal solidifying agent because-

(1) It melts at 100^0C, so it remains solid throughout the entire temperature range over which bacteria are grown. However once melted, it remains liquid until temperature falls to about 45^0C and solidifies at 45^0C. It will not melt again until the temperature rises to about 100^0C.

(2) Secondly, it is not degraded by most microorganisms. So the problem of its liquification arises rarely. For these reasons agar rapidly replaced gelatin as the solidifying agent of choice for bacteriological works

Nutritional Classification of Microorganisms

Organisms can be divided into number of groups based on the source of carbon and source of energy.

Classification on the basis of Source of Carbon:

(1) Autotrophs – These are the organisms which can use CO_2 as their sole or principal source of carbon. They have the ability to synthesize all of their cellular carbon compounds from CO_2.

(2) Heterotrophs – These are the organisms which are able to use organic compounds as a principal source of carbon. They can not synthesize all of their cellular carbon compounds from CO_2 but requires some organic compound in the environment.

Classification on the basis of Source of Energy

(1) Phototrophs – These are the organisms which can obtain their energy directly from sunlight (uses light energy as a source of energy).

(2) Chemotrophs – These are the organisms which obtains their energy by the oxidation of chemical compounds either organic or inorganic (uses chemical compounds as a source of energy).

With the combination the combination of these two classification schemes, microorganisms can be distinguished into four major nutritional groups.

1. Photoautotrophs –

These organisms use light as an energy source and CO_2 as their principal source of carbon. This group includes most photosynthetic organisms like higher plants, algae and photosynthetic bacteria belonging to family Chlorobacteriaceae (Green Sulphur Bacteria) and Thiorhodaceae (Purple Sulphur Bacteria).

2. Photoheterotrophs -

These organisms use light as an energy source and organic carbon compounds (like sugars, proteins, peptone, amino acids, fatty acids, organic acids, monohydric alcohols etc) as their source of carbon. They can not utilize CO_2 as a sole source of carbon. This group includes photosynthetic bacteria belonging to family Rhodospirillaceae (Purple non sulphur Bacteria).

3. Chemoautotrophs - These organisms use inorganic compounds as a source of energy and CO_2 as their principal source of carbon. They obtains their energy by the oxidation of inorganic compounds such as ammonium compounds, nitrites, iron compounds, sulfur compounds, CO, H_2S etc. Only the members of the bacteria belong to this nutritional category. They strictly grows in a mineral media (media containing inorganic compounds) and in absence of light, these are sometimes referred as chemolithotrophs.

4. Chemoheterotrophs - These organisms obtains their energy from organic chemical compounds and uses organic compound as a principal source of carbon. Indeed, same organic compound may serve both as the energy and carbon source. Therefore they are also referred as chemoorganotrophs. Chemoorganotrophs includes all metazoan animals, protozoa, fungi and great majority of bacteria.

Concept of Autotrophy

Conventional Concept:

According to the conventional views the essential features of autotrophy are

(1) Autotrophs are that organisms which are able to fix carbon dioxide (CO_2) as their principal source of carbon. (Woods and Lascelles, 1954; Schlegel, 1975)

(2) The fixation of CO_2 occurs through the Calvin – Benson Ribulose diphosphate (Rudp) pathway.

(3) The energy is obtained from the oxidation of inorganic compounds.

Revised Concept:

Whittenbury and Kelly (1977) have examined the concept of autotrophy and have proposed a revised and wider concept of autotrophy. According to the broadened views of autotrophy, important features are as follows.

(1) Autotrophs include all microorganisms which are able to use C1 compounds as their sole or principle source of carbon.

(2) The carbon fixation pathway can either be the Ribulose diphosphate (Rudp) pathway, or the Ribulose monophosphate pathway, or the serine pathway.

(3) The inorganic or organic nature of the energy source is not the distinguishing features for defining autotrophs.

SPECIFIC MEDIA

Bacterial media may be classified on the basis of their function or application, as follows.

Enriched Media –

In these media substances such as blood, serum, egg, or extract of plant or animal tissues are added to nutrient broth or nutrient agar (general purpose media). These specially fortified media are called enriched media. These media

are used to grow pathogenic bacteria (fastidious heterotrophs) which are more specific in their nutritional requirements. Enriched media provides somewhat similar environment in composition and pH, to that of tissue and body fluid in which these bacteria grow. Examples of enriched media are blood agar, egg media, chocolate agar etc.

Selective Media –

Selective media are those media that contains specific chemical that inhibits the growth of unwanted type of bacteria but selectively favors the growth of particular bacteria.

Examples - (1) Diagnosis of typhoid fever involves the isolation of causative organism *Salmonella typhi* from stool. To isolate *Salmonella typhi*, stool sample are inoculated in selenite or tetrathionate broth. Both these media selectively favors the growth of *Salmonella typhi* and inhibits the growth of all other microorganisms normally found in the stool.

(2) Media containing dye like crystal violet and brilliant green favors the growth of Gram – ve bacteria and inhibits the growth of Gram + ve bacteria.

(3) Centrimide agar is a selective medium for *Pseudomonas aeruginosa*.

Differential Media –

Differential media are those media which contains a substance (indicator, chemical or dye) that may distinguish between different groups of bacteria on the basis of their colony characteristics.

For example – (1) MacConkey agar is a differential medium. It distinguishes between Lactose fermenting bacteria and Non-Lactose fermenting bacteria.

Lactose fermenting bacteria (*E.coli*) forms pink coloured colonies and Non-Lactose fermenting bacteria forms colourless colonies on the same medium. (2) Endo agar is both differential and selective media. It contains lactose and a dye. Lactose fermenting bacteria forms pink coloured colonies and are easily distinguished from colonies of Non-Lactose fermenting bacteria.

Maintenance Media –

Media which are required for the satisfactory maintenance of the viability and physiological characteristics of an organism for a longer period of time are called maintenance media. These media are different from that which is required for maximum growth.

Enumeration Media –

These are the media which are used for determining the bacterial content (bacterial number) of materials such as milk and water.

DETERMINATION OF NUTRITIONAL REQUIREMENTS

There are following different techniques to determine the nutritional requirements of particular organism.

(1) Auxonographic Technique

(2) Replica Plating Technique

(1) AUXONOGRAPHIC TECHNIQUE

This technique was developed by Beijerinck to find out the different sources of carbon and nitrogen required for the growth of an organism.

Suppose we want to determine different sources of carbon utilized by given

organism.

- **Preparation of basal medium** – Basal media is that media which contain all the nutrients (sources) for the growth of given organism except which we want to determine. Here we are determining source of carbon, hence basal medium will contain all the nutrients except any type of carbon source. The other nutrients of the medium includes inorganic nitrogenous compounds like ammonium chloride (NH_4Cl) , ammonium sulphate (NH_4SO_4), and various other salts like K_2HPO_4, $FeSO_4$, NaCl, $MgSO_4$, vitamins, agar etc.
- **Sterilization of Basal Media** - The basal media is sterilized and if given organism is inoculated in basal medium; there will be no growth due to the absence of carbon source.
- **Inoculation & Solidification of Basal Media** The sterilized basal medium is then inoculated with 0.1 ml of given culture with the help of sterile pipette. The inoculated basal medium is then poured into sterile Petri plate and rotated to get uniform distribution of an organism. Basal medium then allowed to solidify.
- **Cutting of Holes in Basal Media** - After solidification, holes are cutted in basal medium sufficiently apart to prevent the mixing.
- **Placing of Different Carbon Sources** - The carbon requirement is then determined by placing different carbon sources in the holes.
- **Incubation of Basal Media** - The plate is then incubated at 370c for 24 hrs. Added sources of carbon diffuse into the medium during incubation period. The growth around different sources depends upon the ability of an organism to utilize that particular compound or not. Suppose

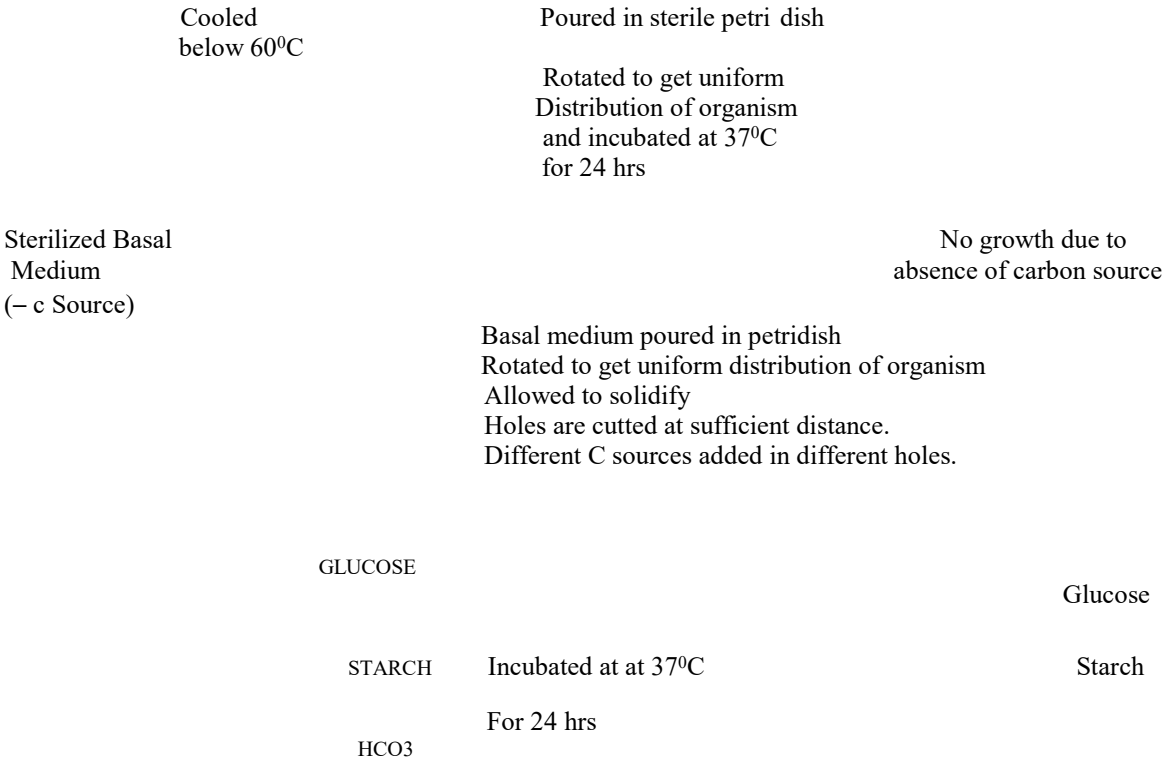
Heavy growth around hole containing Glucose --- means glucose is the excellent source of carbon for the given test organism.

Less growth around hole containing Starch ---- means starch is not the exact source of carbon for the given test organism.

no growth around hole containing Ethanol, Acetate, HCO_3 etc. ---- means these carbon sources are not used by that test organism.

This is how the nutritional requirements of any test organism are determined by auxonographic technique.

In the same way nitrogen requirements can be determined by placing different sources of nitrogen in holes cutted in a basal medium containing all sources except any nitrogen source.



Acetate HCO3

Fig – Auxonographic Technique

REPLICA PLATING TECHNIQUE

This technique was introduced by Lederberg in 1952. This technique can determine nutritional requirements of different organisms in one experiment only. The procedure is as follows.

- Grow the test organism (one or more) in a nutritionally complete medium for example Nutrient Broth.
- Transfer the little amount of growth (0.1 ml) in melted complete medium (Nutrient Agar) and pour in sterilized Petri dish. Rotate the plate to get uniform growth and then incubated. Prepare several plates.
- Select the plate having the well isolated colonies. It is known as the

master plate. The colonies are then numbered.

- Sterilize the cylindrical wooden block with velvitin cloth tied at one end. The diameter of the wooden block should be less than the inside diameter of Petri dish.
- Wooden block is then slightly pressed against the colonies, to get impression of colonies on cloth.
- Suppose, we have to determine source of carbon, then prepare a basal medium and add any one of the carbon source. Prepare different plates containing different sources of carbon.
- The wooden block with impressed colonies is pressed gently against the plate and lifted straight upward to avoid smearing of colonies.
- Plates are then closed and incubated at 37^0C. After the incubation period, observe the plates for growth.
- Compare the plates with original plate (master plate) from which we can determine the nutritional requirements of an organism.
- A well-defined colony will be seen in that Petri dish, in which utilizable carbon source is present.

For example plate no. 2 shows development of colonies. This means that the organism which forms the colony 1, 4, and 6 are able to use glucose as a source of carbon.

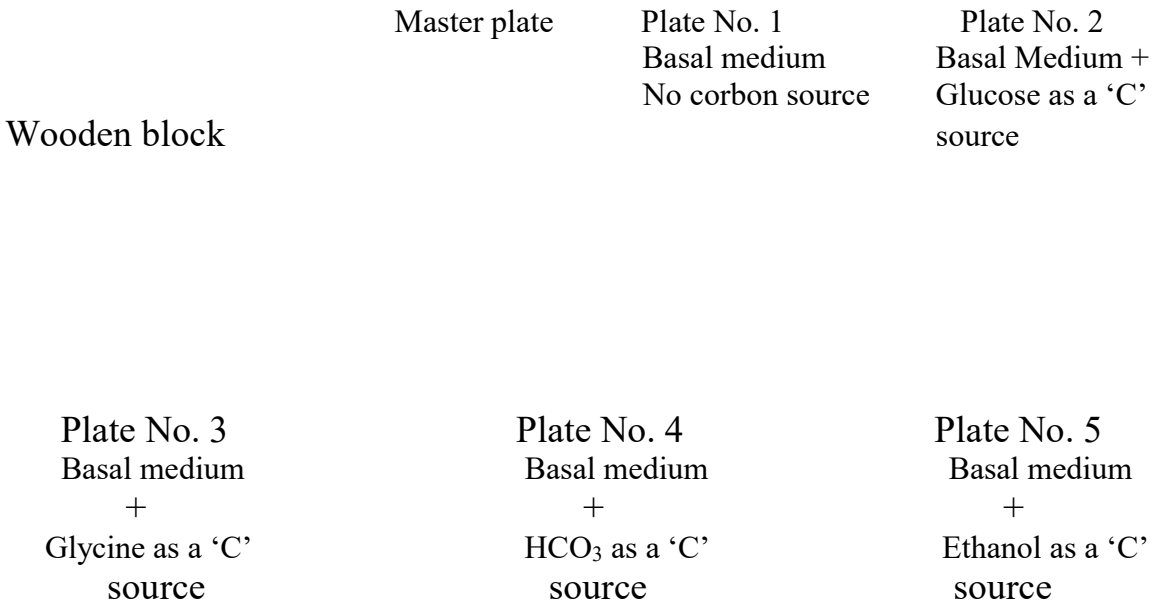


FIG – Replica Plating Technique.

B. <u>Pure Culture Techniques</u>:

i) Definition- Pure and Mixed culture:

ii) Methods of Isolation of Pure culture - Serial dilution, Streak plate, pour plate, spread plate, Enrichment culture, and Single cell isolation method.

iii) Methods of preservation of pure culture- Agar slants, Saline suspension, Overlaying with oil, Freeze Drying.

ii) Methods of Isolation of Pure culture

a number of techniques are available for the isolation of pure culture from natural environment. The methods for isolation of a pure culture include:

- Serial dilution

(ii) Streak plate method
(iii) Spread plate method
(iv) Pour plate method
(v) Enrichment culture technique
(vi) Special isolation methods

- **Serial dilution method** – In this method, a mixed culture is serially diluted in test tubes of sterile medium to the point of extinction in the number of cells, spthat the last tube contains only a single organism. A single viable cell is sufficient to initiate growth in a medium.
- Streak plate method – this is the most widely used method for isolating pure cultures. In this method small amount of mixed culture is streaked over the surface of solid medium in a petridish with the help of inoculating needle.