CULTURE MEDIA

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- The culture media are the nutrients (foods) consisting of chemicals which support the growth of microorganisms.
- Different microorganisms require different materials. Thus culture media vary in form and composition, depending on the species to be cultured.

Characteristics and requirements for a medium:

- A good medium should contain all types nutrients for a particular microbial species in suitable amounts.
- The medium should neither be acidic nor alkaline.
- Most microorganisms require about one per cent of a carbon source in the form of sugar, less than 0.5 per cent of a nitrogen source as salt or yeast extract and small quantities of phosphate, sulphur, potassium, magnesium and traces of calcium, iron, zinc, manganese and molybdenum. These nutrients are supplied to the organisms in different inorganic forms, depending upon their capacity to utilise them.
- The media are used after complete sterilisation.

Types of media: On the basis of chemical composition, the media may be natural, semi-synthetic and synthetic.

Natural media: A natural medias consist of entirely complex natural products of unknown chemical composition. The raw material of natural medium may be of plant or animal origin. The first medium prepared was meat-infusion broth, as most pathogenic microbes require complex food similar in composition to the fluids of the animal body. It was Robert KOCH and his colleagues who used meat infusion and meat extracts as basic ingredients in their culture media for the isolation of pathogenic microbes. For many bacteria, yeast and mold,

fresh extracts of vegetables such as tomato, orange etc. are used. To any of the natural media, different substances may be added for different purposes (as a choice for a specific organism). Then these become special media. For example, blood, which contain a great variety of carbohydrates, esters, alcohols, glucosides etc is added to an infusion broth, which is named as blood-infusion broth. Lactose may be added to broth and known as lactose-broth.

Semi-Synthetic media: These media are so designed that some of their constituents are of known chemical composition, while others are derived from some natural sources with unknown composition. Potato dextrose agar (PDA) is one of such accepted and popular media. Lilly and Barnet (1951) consider all agar-solidified media as semi synthetic ones, because their exact chemical makeup is partly obscured by the addition of agar-agar.

Synthetic media: These are chemically defined media of known chemical composition and concentration, and exclusively composed of pure chemical substances. The formulation and use of these media require an exact knowledge of the nutritional requirements of the microorganism to be cultivated. For example Czapek Dox medium. Such media may be in liquid, solid or semi-solid forms. In order to solidify the liquid medium containing the various ingredients 1-2 per cent agar or 10-20 per cent gelatin is added. For semi-solid media, about half the quantity of agar or gelatin is added.

Nutritional and metabolic studies on fungi as well as microbiological assays are invariably carried on in liquid media. Some of the advantages of liquid media are that they permit the cultures to be aerated, the mycelium to be weighed, and the metabolic products to be analysed easily. Liquid media are, however, have some limitations.

Growth in the media does not manifest the morphological characteristics of an organism. They are also difficult to handle without disturbing the culture. Moreover, liquid media are least helpful in purification of organisms from a mixed culture. For an even distribution of nutrients and for providing uniform aeration to growing fungus, the liquid cultures are sometimes put to constant mechanical shaking.

Preparation of media:

- The liquid medium or broth is prepared by dissolving the known amounts of chemicals in distilled water. The pH is adjusted by adding N/10 HCl or 1N NaOH.

The liquid medium is dissolved and put into either Erlenmeyer flasks or test tubes. In 15 ml capacity of test tube, 5 ml medium should be poured while in flask of 250 ml capacity the amount of the medium should be 100 ml. These are then plugged with non-adsorbent cotton plugs. The plugged tubes or flasks should be wrapped by brown paper and placed for sterilization by autoclaving at a pressure of 15 lbs/inch^2 (at temperature 121°C) for 15 minutes.

- For preparing solid agar medium, half the quantity of water is used for dissolving the chemicals and the remaining half for dissolving the agar shreds or powder. The agar is dissolved in water by slow heating with constant stirring. The melted agar is added to the other half of the medium containing the chemicals. Usually the pH is adjusted before mixing the agar. The mixture is then dispensed into tubes or flasks as desired, the mouths plugged with cotton wool and autoclaved.

Some common Laboratory media; The commonly used laboratory media include Potato Dextrose Agar (PDA), and Czapek's Agar.

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Potato Dextrose Agar (PDA) medium:

The constituents are -

Peeled potato	–	250 gm
Glucose	–	20 gm.
Agar	–	15 gm
water	–	Final volume is made to 1000 ml

pH – 6.0 to 6.5.

Peeled potato is made into thin chips, boiled in 500 ml water and extracted. To the extract the weighed quantity of glucose is added. The agar is melted in the other half of water and mixed in potato glucose solution and the volume made upto a litre (1000 ml.) befor sterilizing.

Czapek's Dox Agar medium:

Sucrose		30 gm.
sodium nitrate	–	2.0 gm.
Dipotassium phosphate	–	1.0 gm.
Magnesium sulphate	–	0.5 gm
potassium chloride	–	0.5 gm
Ferrous sulphate	–	0.01 gm
Agar	–	15.0 gm
Distilled water	–	for 1.0 litre medium.

30 gm of Sucrose is added to 500 ml. of distilled water. All other chemicals are mixed in it and stirred well to make it homogeneous solution. Agar (15 gm) is added to 500 ml. of water and heated gently. The transparent water – agar is then mixed with the solution of chemicals. The prepared medium is sterilized.