

A growth medium or culture medium is a solid or liquid or semi-solid designed to support the growth of microorganisms or cells.

Microbes require nutrients to grow. These are supplied by either solid or liquid culture media. The standard solid medium is nutrient agar, a gelatinous substance derived from seaweed. The basic liquid medium is nutrient broth, typically a mix of water, meat extract peptone, and sodium chloride. The following are the commonly used ingredients in media.

1) Agar:

This is a complex carbohydrate material refined from marine algae and used to produce semi solid media. It solidifies at temperatures between 400C to 450C and it will not remelt until it is boiled. It is not used as nutrient material except by some marine microbes.

2) Beef extract:

Beef is boiled and the resulting broth is concentrated into a paste or dried to a powder. It contains amino acids, vitamins and minerals.

3) Yeast extract:

This is the water soluble portion of autolyzed yeast and contains amino acids, nucleic acids, vitamins and other growth factors.

4) Peptone:

Protein is converted into proteases, peptides and amino acids by means of enzymatic hydrolysis.

Types of culture media:

Classification of culture media used in Microbiology laboratory on the basis of consistency

Solid medium:

- ✓ Solid medium contains agar at a concentration of 1.5-2.0% or some other, mostly inert solidifying agent.
- ✓ Solid medium has physical structure and allows bacteria to grow in physically informative or useful ways (e.g. as colonies or in streaks).
- ✓ Solid medium is useful for isolating bacteria or for determining the colony characteristics of the isolate.

Semisolid media:

- ✓ They are prepared with agar at concentrations of 0.5% or less.

- ✓ They have soft custard like consistency and are useful for the cultivation of microaerophilic bacteria or for determination of bacterial motility.

Liquid (Broth) medium:

- ✓ These media contains specific amounts of nutrients but don't have trace of gelling agents such as gelatin or agar.
- ✓ Broth medium serves various purposes such as propagation of large number of organisms, fermentation studies, and various other tests.
- ✓ E.g. sugar fermentation tests, MR-VR broth.

Classification of culture media based on the basis of composition:

Synthetic or chemically defined medium:

- ✓ A chemically defined medium is one prepared from purified ingredients and therefore whose exact composition is known.
- ✓ Synthetic medium may be simple or complex depending up on the supplement incorporated in it.
- ✓ A simple non-synthetic medium is capable of meeting the nutrient requirements of organisms requiring relatively few growth factors where as complex non-synthetic medium support the growth of more fastidious microorganisms.

Non synthetic or chemically undefined medium:

- ✓ Non-synthetic medium contains at least one component that is neither purified nor completely characterized nor even completely consistent from batch to batch.
- ✓ Often these are partially digested proteins from various organism sources.
- ✓ Nutrient broth, for example, is derived from cultures of yeasts.

Complex media:

Complex media are rich in nutrients, they contain water soluble extracts of plant or animal tissue (e.g., enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar, often glucose is added to serve as the main carbon and energy source. The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called complex.

Many special purpose media are needed to facilitate recognition, enumeration, and isolation of certain types of bacteria. To meet these needs, numerous media are available.

General purpose media/ Basic media:

- ✓ Basal media are basically simple media that supports most non-fastidious bacteria.
- ✓ Peptone water, nutrient broth and nutrient agar are considered as basal medium.
- ✓ These media are generally used for the primary isolation of microorganisms.

Enriched medium (Added growth factors):

- ✓ Addition of extra nutrients in the form of blood, serum, egg yolk etc, to basal medium makes them enriched media.
- ✓ Enriched media are used to grow nutritionally exacting (fastidious) bacteria.
- ✓ Blood agar, chocolate agar, Löffler's serum slope etc are few of the enriched media.
- ✓ Blood agar is prepared by adding 5-10% (by volume) blood to a blood agar base.
- ✓ Chocolate agar is also known as heated blood agar or lysed blood agar.

Selective and enrichment media:

- ✓ Selective and enrichment media designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria.
- ✓ While selective media are agar based, enrichment media are liquid in consistency. Both these media serve the same purpose.
- ✓ Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen of interest.
- ✓ Various approaches to make a medium selective include **addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.**

Selective medium:

- ✓ Selective medium is designed to suppress the growth of some microorganisms while allowing the growth of others.
- ✓ Selective medium are agar based (solid) medium so that individual colonies may be isolated.
- ✓ E.g. **Lowenstein Jensen Medium** used to recover *M.tuberculosis* is made selective by incorporating malachite green and
- ✓ **MacConkey's Agar** used for Enterobacteriaceae members contains bile salt that inhibits most gram positive bacteria.

Enrichment culture medium:

- ✓ Enrichment medium is used to increase the relative concentration of certain microorganisms in the culture prior to plating on solid selective medium.
- ✓ Unlike selective media, enrichment culture is typically used as **broth medium.**

- ✓ Enrichment media are liquid media that also serves to inhibit commensal in the clinical specimen.
- ✓ **Selenite F broth, tetrathionate broth and alkaline peptone water (APW)** are used to recover pathogens from fecal specimens.

Differential/ indicator medium: differential appearance:

- ✓ Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour.
- ✓ Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently colored colonies.
- ✓ Such media are called differential media or indicator media.
- ✓ Differential media allow the growth of more than one microorganism of interest but with morphologically distinguishable colonies.
- ✓ Examples: **Mannitol salts agar** (mannitol fermentation = yellow)
- ✓ **Blood agar** (various kinds of hemolysis i.e. α , β and γ hemolysis)
- ✓ Mac Conkey agar (lactose fermenters, pink colonies whereas non- lactose fermenter produces pale or colorless colonies).

Transport media:

- ✓ Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media.
- ✓ Such media prevent drying (desiccation) of specimen, maintain the pathogen to commensal ratio and inhibit overgrowth of unwanted bacteria.
- ✓ Some of these media (Stuart's & Amie's) are semi-solid in consistency. Addition of charcoal serves to neutralize inhibitory factors.
- ✓ **Example:** Cary Blair transport medium and Venkatraman Ramakrishna (VR) medium are used to transport feces from suspected cholera patients.

Anaerobic media:

- ✓ Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.
- ✓ Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Such media may also have to be reduced by physical or chemical means.

- ✓ Boiling the medium serves to expel any dissolved oxygen. Addition of 1% glucose, 0.1% thioglycollate, 0.1% ascorbic acid, 0.05% cystine or red hot iron filings can render a medium reduced.
- ✓ Before use the medium must be boiled in water bath to expel any dissolved oxygen and then sealed with sterile liquid paraffin.
- ✓ Robertson Cooked Meat (RCM) medium that is commonly used to grow *Clostridium* sps contains a 2.5 cm column of bullock heart meat and 15 ml of nutrient broth. Thioglycollate broth contains sodium thioglycollate, glucose, cystine, yeast extract and casein hydrolysate.
- ✓ Methylene blue or resazurin is an oxidation-reduction potential indicator that is incorporated in the medium. Under reduced condition, Methylene blue is colorless.

Assay media:

These media are used for the assay of vitamins, amino acids and antibiotics. E.g. antibiotic assay media are used for determining antibiotic potency by the microbiological assay technique.

Other types of medium includes Media for enumeration of Bacteria, Media for characterization of Bacteria, Maintenance media etc.

Media	Purpose
Complex	Grow most heterotrophic organisms
Synthetic	Grow specific heterotrophs and are often mandatory for chemoautotrophs, photoautotrophs and for microbiological assays
Selective	Suppress unwanted microbes, or encourage desired microbes
Differential	Distinguish colonies of specific microbes from others
Enrichment	Similar to selective media but designed to increase the numbers of desired microorganisms to a detectable level without stimulating the rest of the bacterial population