CULTURING MICROBES

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Definition of medium (plural: media)

A medium (plural: media) is any solid or liquid preparation made specifically to enable the growth, storage or transport of bacteria. A medium must be sterile (i.e. it must contain no living organisms). In other to make sense of this discussion, there is need for us to adequately understand certain terminologies empoloyed by bacteriologists in communicating about media; I believe this will be best achieved by adopting a simple laboratory procedure.

- To grow an organism such as *Escherichia coili*, the bacteriologist takes an appropriate sterile medium (food) and adds to it a simple amount of material which consists of, or contains, living cells of that species (sample); the 'small amount of material is called an *inoculum* , and the process of adding the inoculum to the medium is *inoculation*. The inoculated medium is then incubated i.e. kept under controlled and appropriate conditions of temperature, humidity etc. for a suitable period of time. The incubation is usually carried out in thermostatically controlled equipment called incubator. During incubation, the bacteria grow and divide –giving rise to a *culture*; thus, a culture is a medium containing organisms which have grown (or still growing) on or within that medium.
- A liquid medium may be used in a test tube (stoppered by a plug of sterile cotton wool, or which has a simple metal cap or in a glass, screw-cap bottle), a *universal bottle* (cylindrical bottle of about 25ml capacity) and or a *bijou* bottle (smaller about 5-7ml).

- Most solid media are jelly-like materials consistisiting solution of nutrients etc. 'solidified' by agar (a complex polysaccharide gelling agent obtained from certain sea weeds other gelling agents include silica gel, gelatin etc.).
- A solid medium is commonly poured/or contained in a plastic *petri dish* (usually about 9cm and 20-25ml volume; although varied sizes are available). The medium in molten (liquid) state is poured into the petri dish and allowed to *set* (solidified); a petri dish containing the solidified medium is called a *plate*.

Note:

a. *Culture media* are solutions containing all of the nutrients and necessary physical growth parameters necessary for microbial growth.

b. Not all microorganisms can grow in any given culture medium and, in fact, many can't grow in any known culture medium (i.e unculturable).

Types of media

Media can be classified on three bases

i. physical form (solid vs. broth),

ii. chemical composition, and

iii. functional type

i. **Physical states of media**

- Liquid media are water-based solutions that do not solidify at temperature above freezing and tend to take the shape of their container as well as flow freely when the container is tilted.
- These media can exist as **broths, milks, or infusions**. They are made by dissolving appropriate solutes into appropriate solvents (e.g. water). Growth occurs throughout the container and can then present a dispersed, cloudy or particulate appearance. Examples are

nutrient broth (which contains beef extract and peptone dissolved in water), methylene blue milk and litmus milk (opaque liquids containing whole milk and dyes) and brain heart infusion.

- At room temperature, **Semisolid media** exhibit a jelly-like consistency because they contain an amount of solidifying agent (agar or gelatin) which is just enough to thicken them but not solidify the substrate. Semisolid media are useful in the determination of motility of bacteria and to localize a reaction at a specific site. They contain a small amount of agar (0.3-0.5 %).

- **Solid media** provide a firm surface on which cells can form discrete and distinct colonies (mounts of daughter cells originating from a mother single cell) and are advantageous for isolating and culturing bacteria and fungi. They are available in two forms:

  i. liquefiable and

  ii. non-liquefiable

  Liquefiable solid media, sometimes called reversible solid media, contain a solidifying agent that changes its physical properties in response to temperature. The most widely used of these agents is **agar** (a complex polysaccharide isolated from the red alga *Gelidium*. The benefits of agar include;

  a. Agar is solid at room temperature, and it melts (liquefies) at the boiling temperature of water of water (100^0C).Once liquefied, agar does not resolidify until it cools to 42^0C, so that it can be inoculated and poured in liquid form at temperature (45^0-50^0C) that will neither harm the microbe nor the handler.
  b. Agar is flexible and mouldable, and it provides a basic framework to hold moisture and nutrients and not attacked by a vast majority of microorganisms.

c. An agar gel does not melt @ 37^0C- a temperature used for the incubation of most bacteria (by contrast, a gelatin can be liquefied by some bacteria, and is molten @ 37 ^oC .

Any medium containing 1- 5 per cent agar substance usually carry the word *agar* in its name. Although gelatin is not nearly as satisfactory as agar, it will create a reasonably solid surface in concentrations of 10 -15 per cent.

Nonliquefiable solid media are less verse in their applications than agar media because they do not melt. They include materials such as rice grains (used to grow fungi), cooked meat media (good for anaerobes), and potato slices; all of these media start out solid and remain solid after heat sterilization. Other solid media containing egg and serum start out liquid and are permanently coagulated or hardened by moist heat.

ii**. Chemical composition (non-synthetic/complex vs. <u>synthetic/chemically defined</u>)**

- Media whose chemical composition is known are termed synthetic. Such media contain pure organic and inorganic compounds that vary little from one source to another. Defined media are used widely in research, as it is often desirable to know metabolizable substrates of organisms of interest. Example is citrate agar.
- Complex, or non-synthetic, media contain at least one ingredient that is not chemically definable; not a simple, pure compound and not representable by an exact chemical formula. Most of these substances are extracts of animals, plants, or yeasts, including such materials as ground-up cells, tissues, and secretions. Examples are blood, serum, and meat extracts or infusions.

iii. Functional type

- General-purpose media are designed to support the growth of a variety of microbes as possible. As a rule, they are non-synthetic and contain a mixture of nutrients that could support the growth of pathogens and nonpathogens alike. Examples include nutrient agar and broth, brain-heart infusion, and trypticase soy agar (TSA).
- Enriched media contain complex organic substances such as blood, serum, haemoglobin, or special **growth factors** (specific vitamins, amino acids) that certain fsatidious species must have in order to grow. Bacteria requiring such growth factors and complex nutrients are termed **fastidious**. Examples of enriched media include blood agar (an agar based medium enriched with 5-10% blood), chocolate agar(an agar made by heating blood agar to 70-80^{0}C until it becomes chocolate brown in colour).
- Enrichment media allow certain species to outgrow others by encouraging the growth of wanted organism(s) and/or by inhibiting the growth of unwanted species. Hence, if an inoculum contains only a few cells of the required species (among a large population of unwanted organisms), growth in a suitable enrichment medium can increase (’enrich’) the proportion of required organisms. Examples include slenite and Rappaport vassiliadis broth used for enrichment of *Salmonella* spp. **Note**-enrichment media occur more often in liquid form.
- Selective media contain one or more agents that inhibit the growth of a certain microbe or microbes (A, B, C) but not others (D) and thereby encourage, or selects, microbe D and allows it grow.

Medium	Selective Agent	Used for

Mannitol salt agar	Nacl 97.5%	*Staphylococcus* spp.
MacConkey agar	Bile, crystal violet	Isolation of gram negative enterics
		Isolation of *Salmonella* and *Shigella* spp.
Salmonella/Shigella ag	Bile, citrate, brilliant green	
Enterococcus faecalis		Isolation of faecal enterococci
broth	Sodium azide	
		Isolation and maintenance of Mycobacter
Lowenstein-Jensen	Malachite green dye	Isolation of fungi-inhibits bacteria
Sabouraud's agar	PH of 5.6 (acid)	

- Differential media grow several types of microorganisms and are designed to display visible differences among those microorganisms. Differentiation shows up as variations in colony size or colour, in media colour changes, or in the formation of gas bubbles and precipitates. These variations come from the type of chemicals these media contain and the ways that microbes react to them. For example, when microbe X metabolizes a certain substance not used by organism Y, then X will cause a visible change in the medium and Y will not. The simplest differential media show two reaction types such as the use or non-use of a particular nutrient or colour change in some colonies but not in others. Some media are sufficiently complex to show three or four different reactions.

- Dyes can be used as differential agents because many of them are pH indicators that change colour in response to the production of an acid or a base. For example, MacConkey agar contains neutral red, a dye that is yellow when neutral and pink or red when acidic. A common intestinal bacterium such as *Escherichia coli* that gives off acid when it metabolizes the lactose in the medium develops red to pink colonies, and one like *Salmonella* that does not give off acid remains its neutral colour(off-white).

Examples of Differential Media			
S/N	**Medium**	**Substances that facilitate differentiation**	**Differentiates Between**
1.	Blood agar	Intact red blood cells	Types of haemolysis
2.	Mannitol salt agar	Mannitol, Phenol red, and 7.5% NaCl	Species of *Staphylococcus* ;NaCl also inhibits the salt sensitive species
3.	Hektoen (HE) enteric	Brom thymol blue, acid fuchsin, sucrose, salicin, thiosulfate, ferric ammonium citrate and bile	*Salmonella, shigella*, othe lactose fermenters from nonfermenters;Dyes and bile also inhibit gram positive bacteria
4.	Urea broth	Urea, phenol red	Bacteria that hydrolize ure to ammonia
5.	Sulphur Indole Motility (SIM)	Thiosulphate, iron	H_2S gas producers from n producers

6.	Tripple-sugar iron agar (TSA)	Triple sugars, iron, and phen red dye	Fermentation of sugars, H production
7.	Xylose-Lysine deoxycholate (XLD) aga	Lysine, xylose, Iron, thiosulphate, phenol red	*Enterobacter*, *Escherichia Proteus*, *Providencia*, Salmonella and *Shigella*

Miscellaneous Media

- Reducing media contain substances (Thioglycollic acid or cystine) that absorb or reduce the presence of oxygen in a medium. Reducing media are important for growing anaerobic bacteria or determining oxygen requirements.
- Transport media are used to maintain and preserve specimens that have to be held for a period of time before clinical analysis or to sustain delicate species that die rapidly if not held under stable conditions. Stuart's and Amies are examples of transport media which contain salts, buffers, and absorbants to prevent cell destruction by enzymes, pH changes, and toxic substances, but will not support growth.
- **Assay media** (e.g Mueller Hington) are used to test the effectiveness of antimicrobial drugs and by drug manufacturers to assess the effect of disinfectants, antiseptics, cosmetics, and preservatives on the growth of microorganisms.
- Enumeration media are used by industrial and environmental microbiologists to quantify the numbers of organisms in milk, water, food, soil, and other samples.