Isolation of Microbial Pure Culture

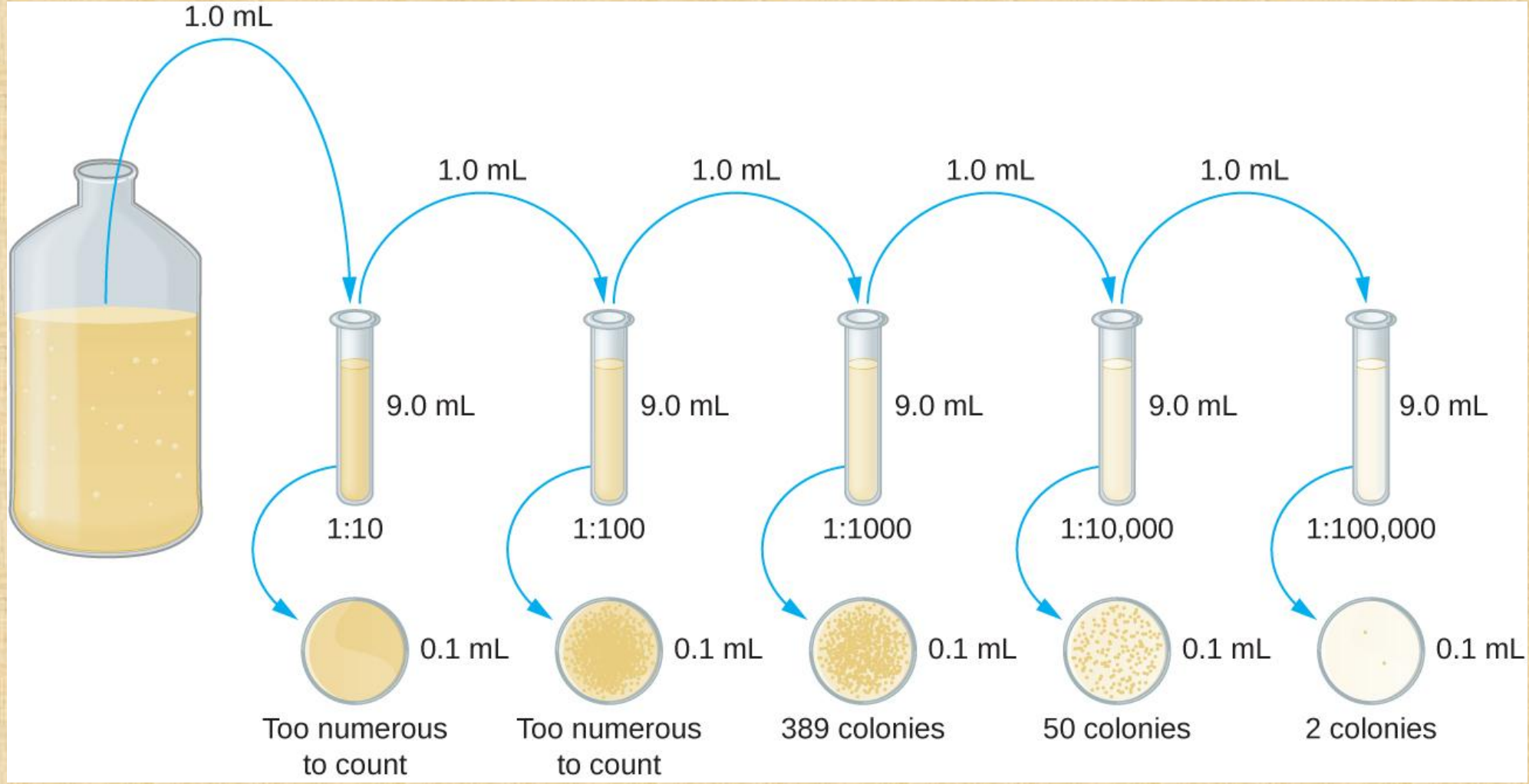
Serial dilution method

- A **serial dilution** is a series of sequential dilutions used to reduce a dense culture of cells to a more usable concentration.
- Each dilution will reduce the concentration of bacteria by a specific amount. So, by calculating the total dilution over the entire series, it is possible to know how many bacteria you started with.

Techniques used to isolate pure culture

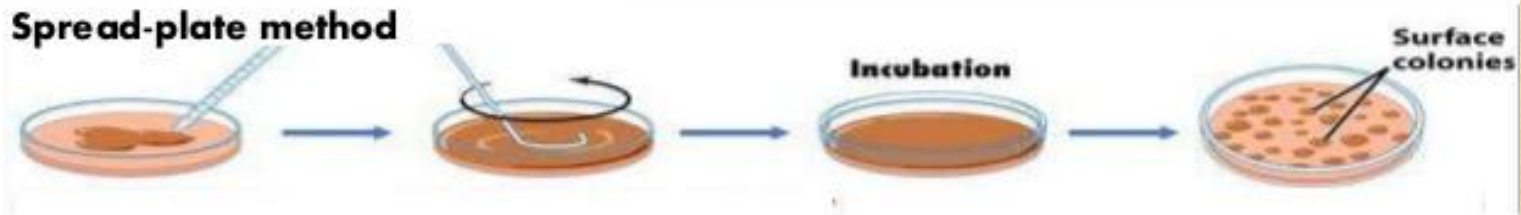
- Streak Plate method
- Spread Plate method
- Pour Plate method

Serial dilution method

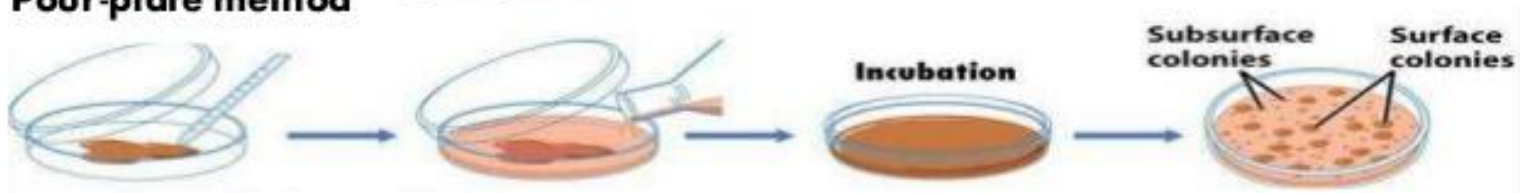


Method of isolating pure cultures

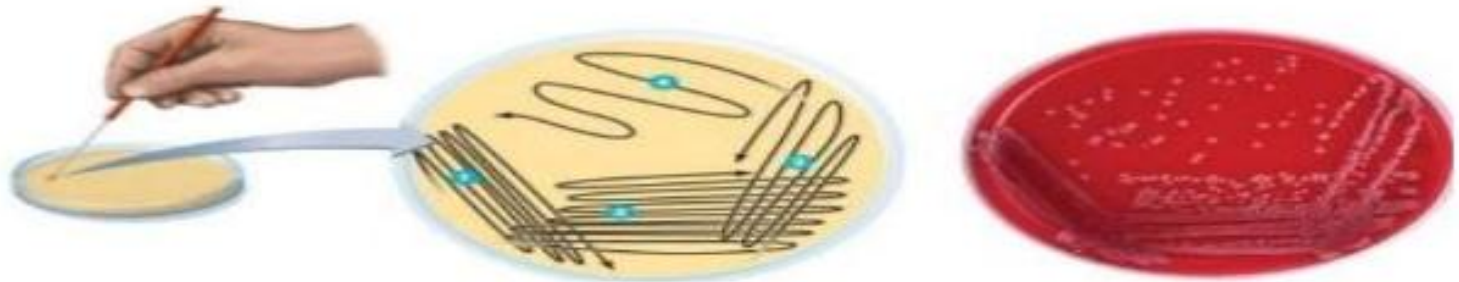
Spread-plate method



Pour-plate method



Streak-plate method



Pure Culture

- Pure culture, in microbiology, a laboratory culture containing a single species of organism.
- A pure culture is usually derived from a mixed culture (one containing many species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample manyfold before inoculating the new medium.

Media Types

- **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**
  Semisolid media 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.

Classification of culture media on the basis of composition

- **Synthetic or chemically defined medium**: A chemically defined medium is one prepared from purified ingredients and therefore its exact composition and concentration is known.
- **Non synthetic or chemically undefined or complex 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.

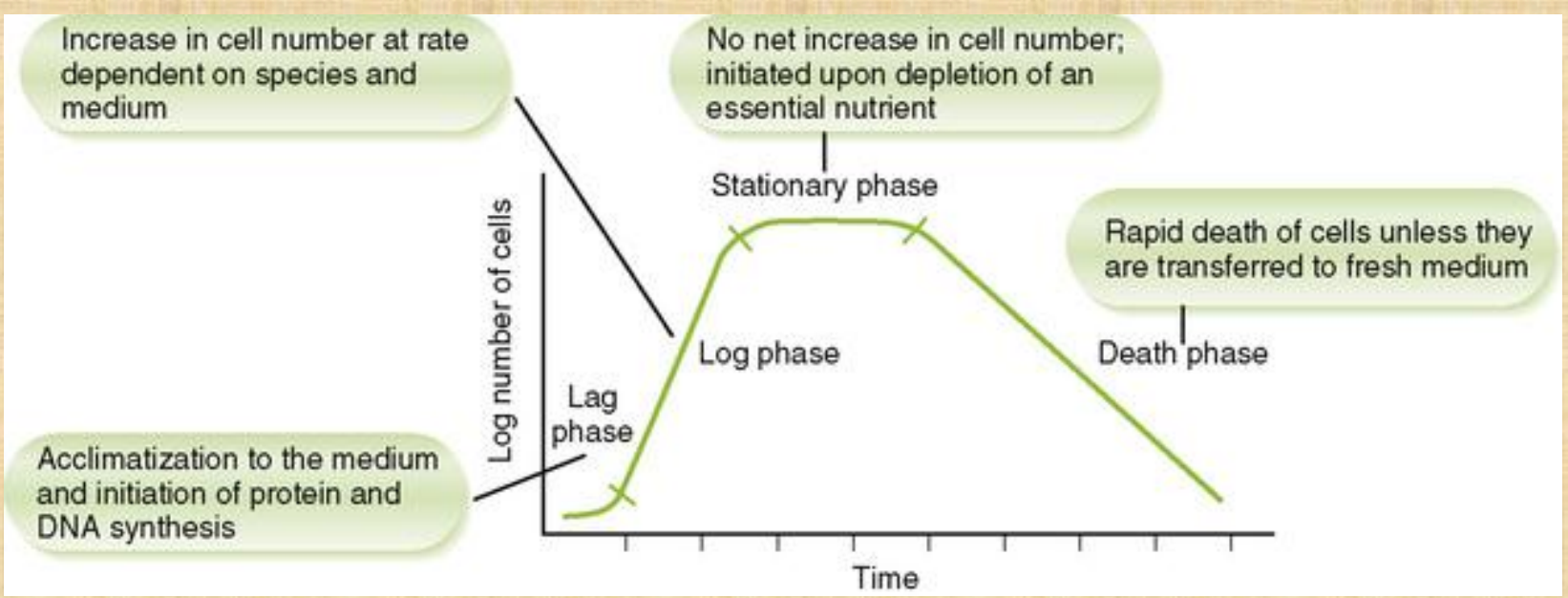
Classification of Bacterial Culture media on the basis of purpose/ functional use/ application

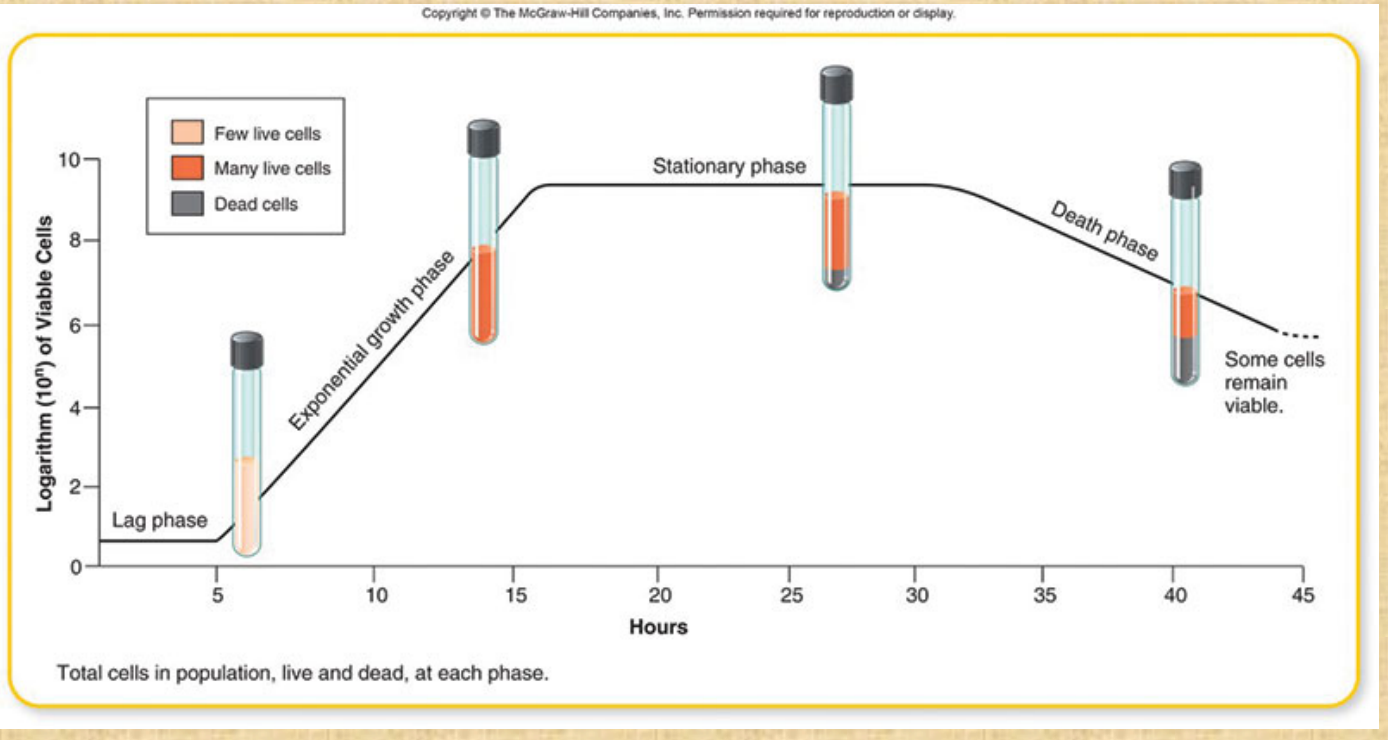
- **General purpose media/ Basic media**
  Basal media are basically simple media that supports most non-fastidious bacteria. **Peptone water**, nutrient broth and **nutrient agar** are considered as basal medium. These media are generally used for the primary isolation of microorganisms.
- **Enriched 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, Loeffler's serum slope etc are few of the enriched media.
- **Selective and enrichment media** are 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. Example: **MacConkey's Agar** used for **Enterobacteriaceae** members contains bile salt that inhibits most gram positive bacteria
- **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 coloured 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. Example: **Mannitol salts agar** (mannitol fermentation = yellow)

•The bacterial population doubles during every generation. They multiply at their maximum rate.

The bacterial growth curve shows the following four distinct phases:

- Lag Phase: In this phase, the inoculated bacteria become acclimatized to the environment, switch on various enzymes, and adjust to the environmental temperature and atmospheric conditions.
- Log Phase: This phase is characterized by rapid exponential cell growth (i.e., 1 to 2 to 4 to 8 and so on). The bacterial population doubles during every generation. They multiply at their maximum rate.
- Stationary Phase: After log phase, the bacterial growth almost stops completely due to lack of essential nutrients, lack of water oxygen, change in pH of the medium, etc. and accumulation of their own toxic metabolic wastes. The culture is at its greatest population density.
- Decline or death phase: During this phase, the bacterial population declines due to death of cells.





Total cells in population, live and dead, at each phase.

References

- https://www.britannica.com/science/pure-culture
- https://microbenotes.com/bacterial-growth-curve-and-its-significance/