Introduction to Microbiology

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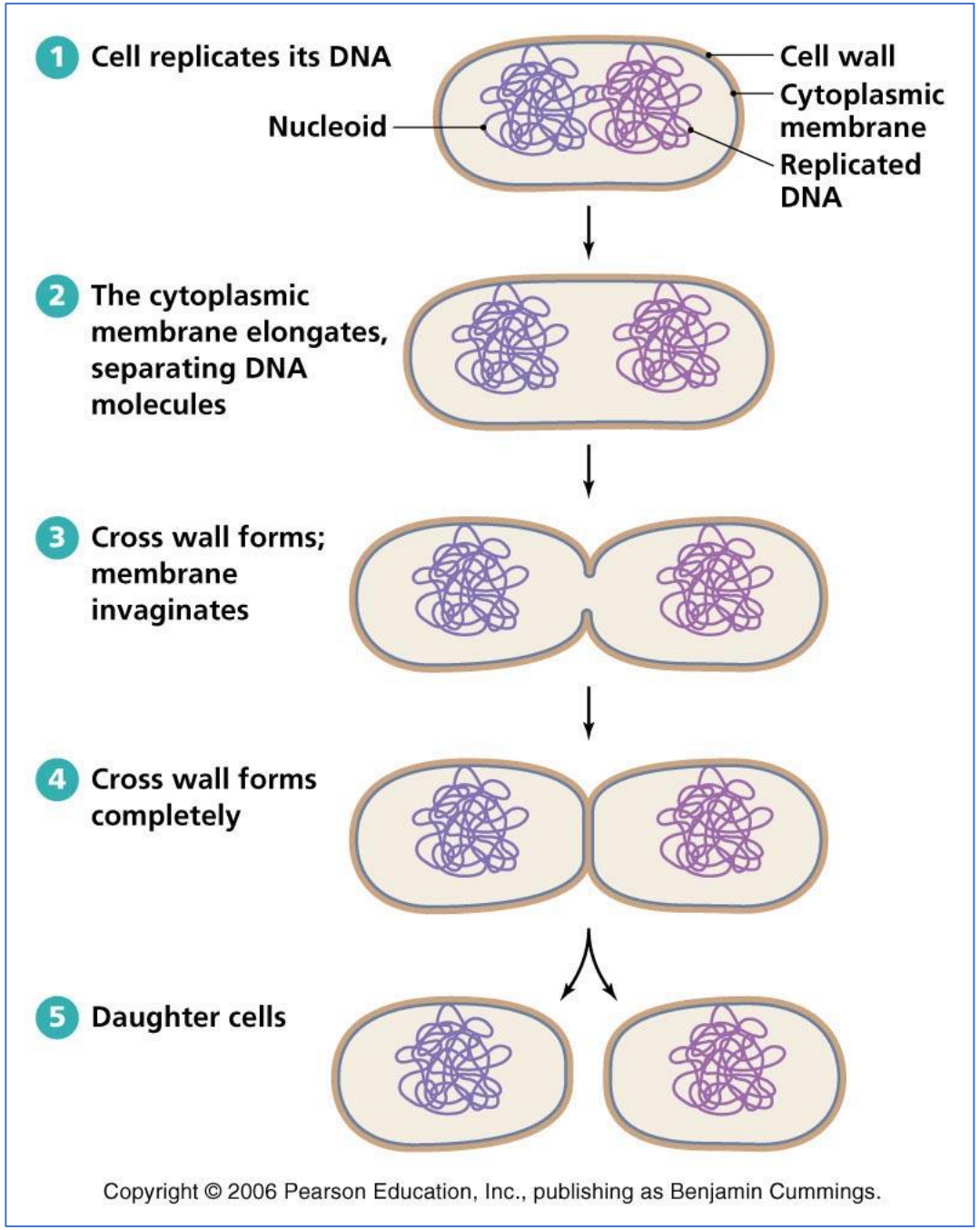
Lecture 3

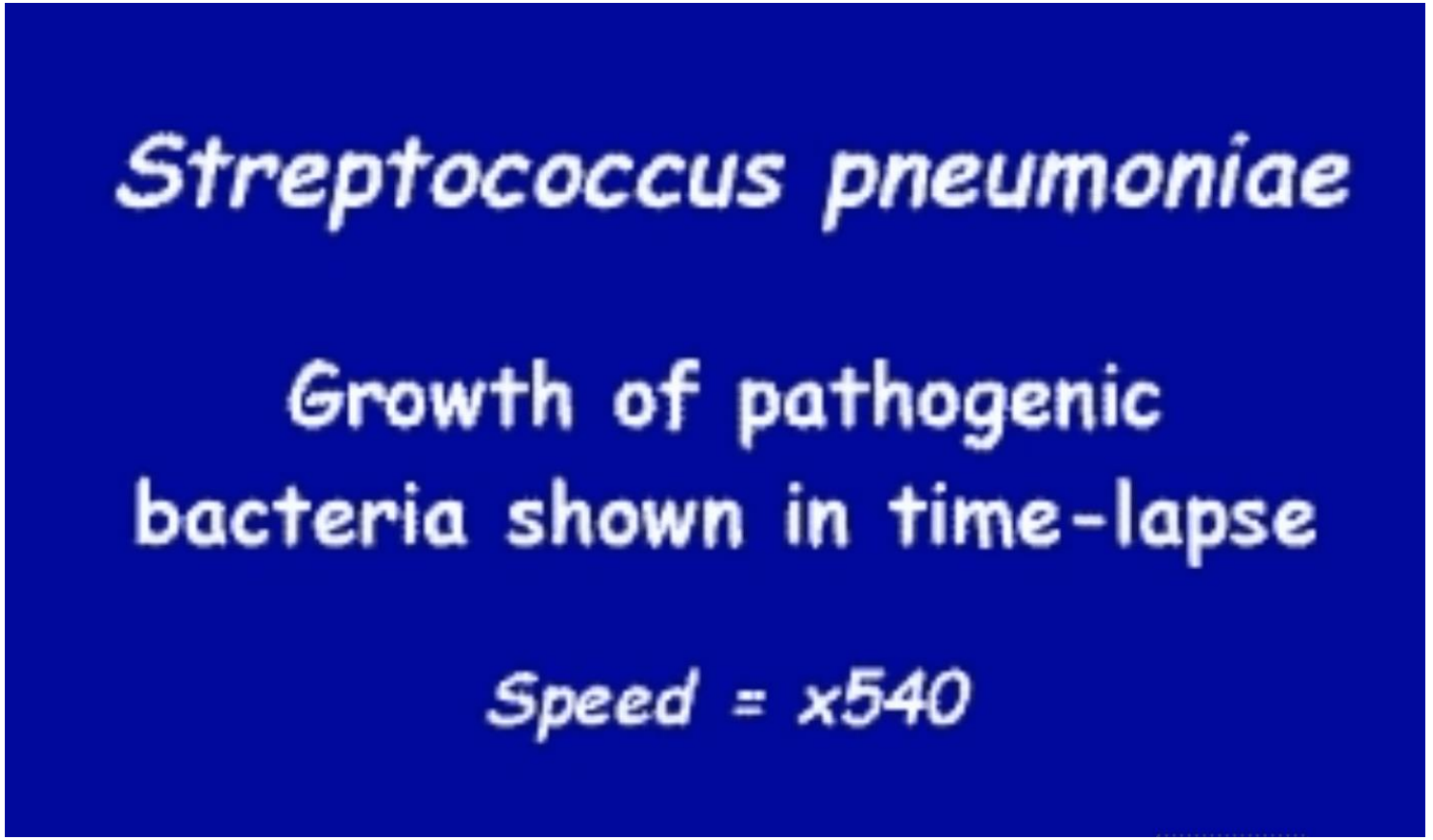
Bacterial survival and growth

- Bacterial survival and growth depend on utilization of nutrients in its niche (environment), it uses these nutrients to synthesize proteins and enzymes to survive .
- Lab cultures provide optimum conditions for **bacterial growth**, defined as **an increase in the sum of all the components of an organism**.
  (we can control the growth of bacteria in vitro)
- Bacteria divide by binary fission producing 2 identical offspring.
  (All prokaryotes divide by binary fission, not just bacteria)

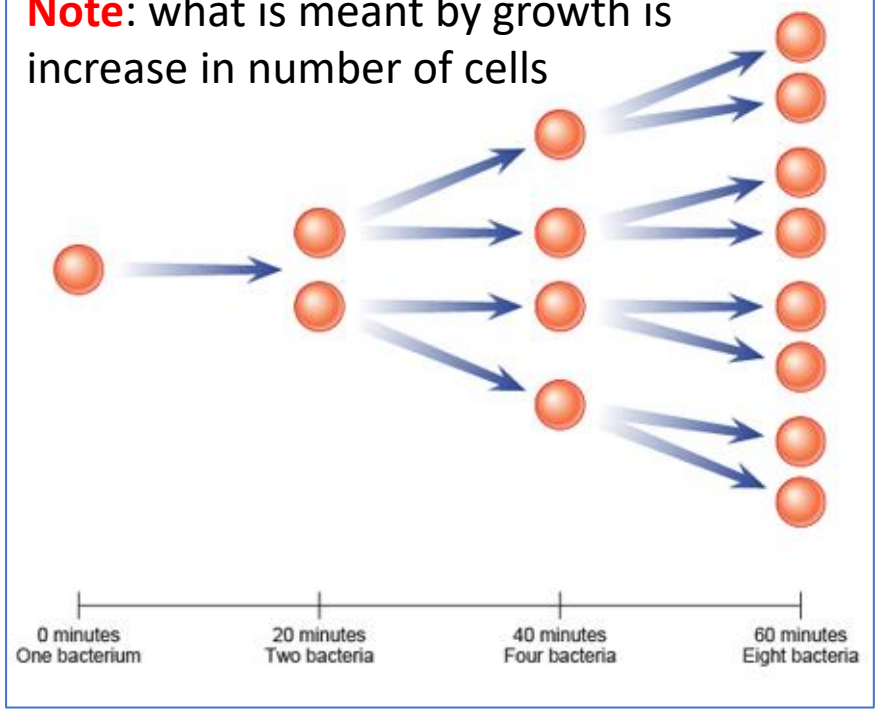
- **Binary fission**: bacteria will replicate its DNA, then cytoplasm will elongate, then DNA seperates, and a wall will form that separate the two Daughter cells.



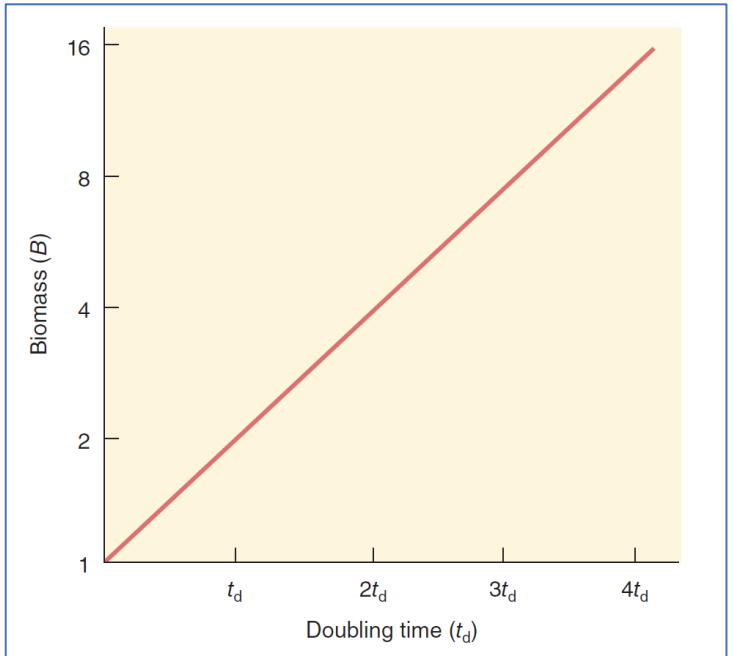
Bacterial survival and growth



Note: what is meant by growth is increase in number of cells

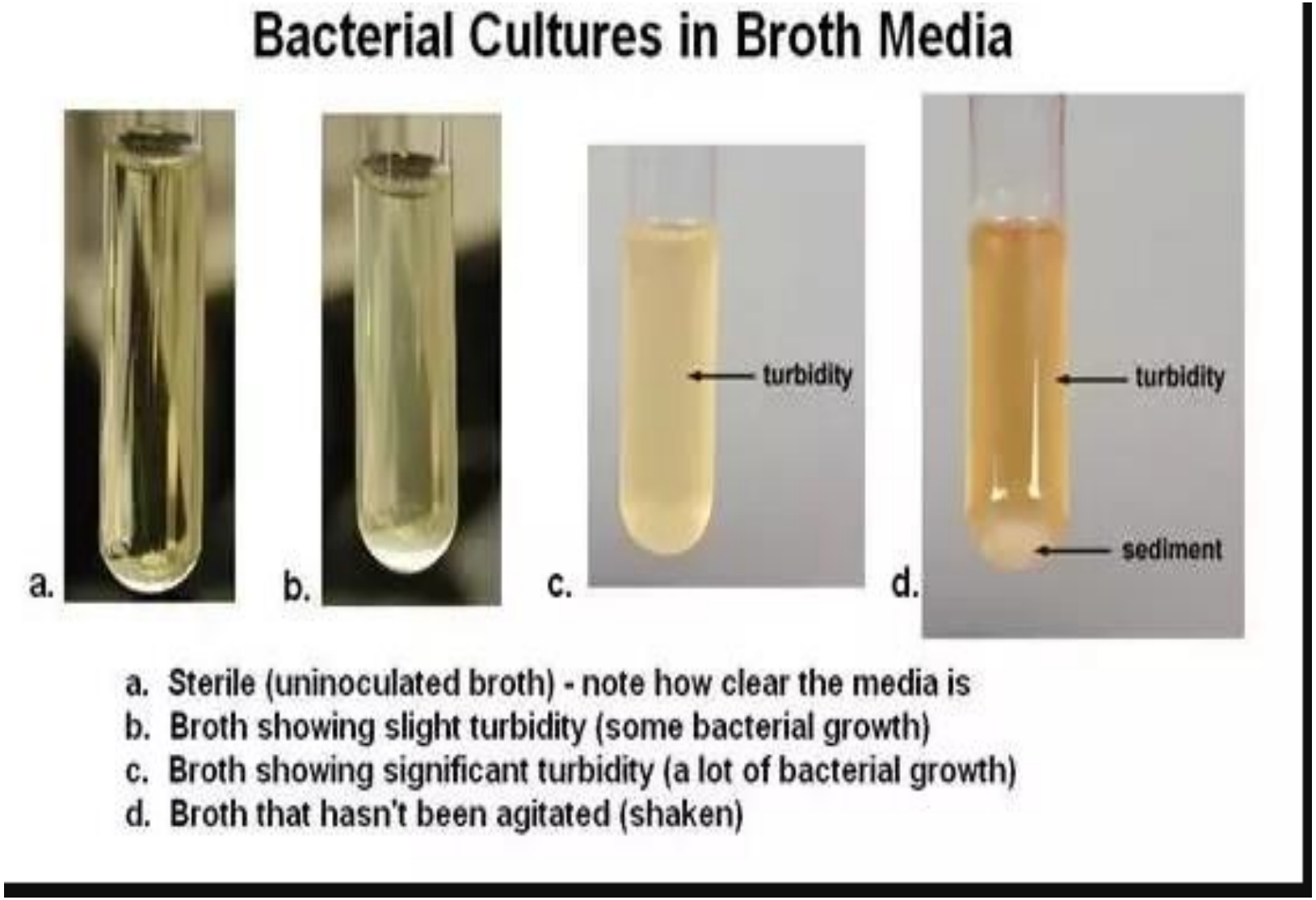


- **Exponential growth**. The biomass (number of bacteria) (B) doubles with each doubling time (td) (the time needed for each bacteria to be doubled).



Measuring bacterial growth

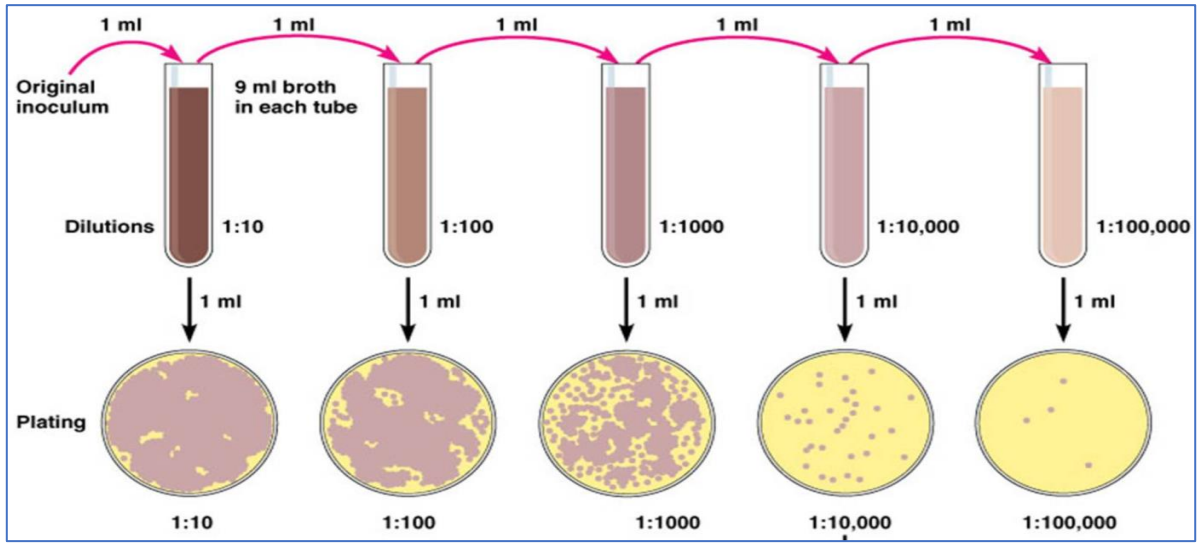
Turbidity: a non-specific measure that indicates how much bacteria in the sample, it can be measured by turbidometer



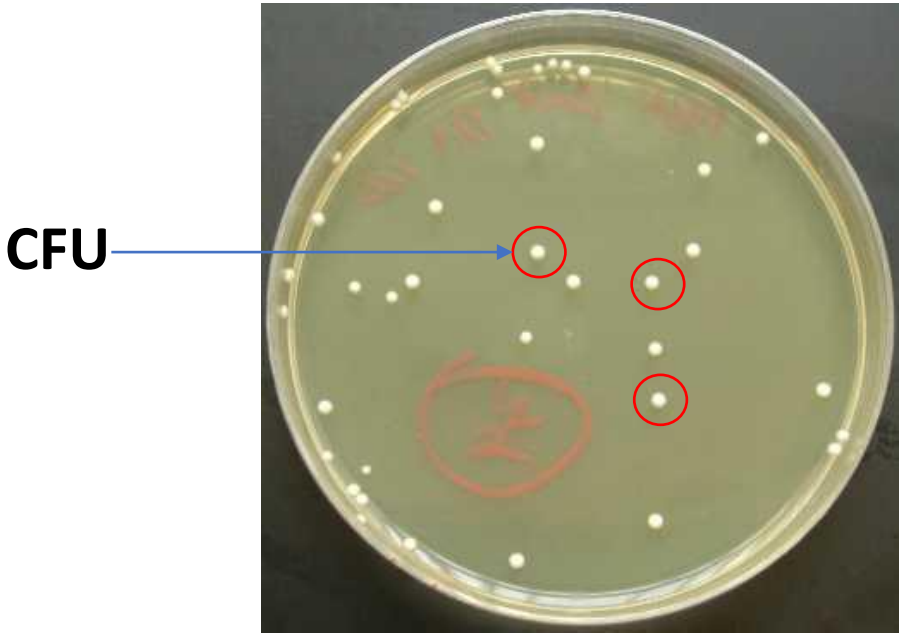
Measuring bacterial growth

- To know how many microorganisms there are in a solution of bacteria or fungi, it's usually too **time-consuming**, even impossible to count every cell individually under the microscope.

- By **diluting** a sample of microbes and **spreading** it across a **petri plate**, microbiologists can instead count groups of microbes, called colonies, **with the naked eye**. Each colony is assumed to have grown from a single **colony-forming unit**, or **CFU**.



Serial dilution: 1 ml of the solution is taken and cultured in a petri dish and diluted several times until we reach certain number of colonies that we can read by naked eye, colony forming units or CFU will appear in the dish, by counting these CFUs, we can estimate the number of bacteria in the original sample as number of bacteria in a colony can be estimated.

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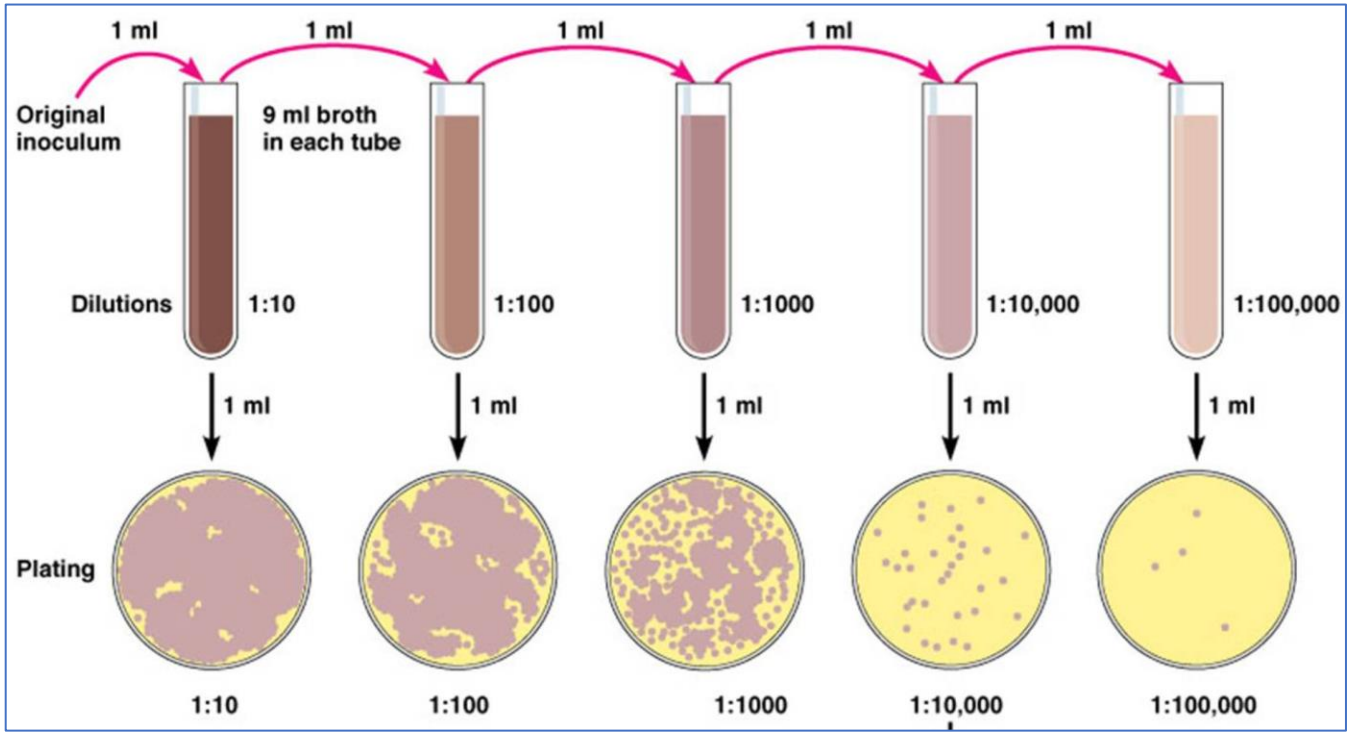
10^{-2}
10^{-3}

Measuring bacterial growth

- Cell concentration by viable counts and turbidometry, both are related through a standard curve.

- Turbidometry (e.g. **measuring the turbidity of a culture using a spectrophotometer**).

- **Biomass density** by dry weight of microbial culture, or through correlations to protein concentration.



Turbidometer measures the density of the biomass (dry weight or the proteins of the bacteria) in the sample, by exposing the tube to light waves. Then, we compare the results with an already existed curve (measured by scientists by viable counts) so we can estimate the number of bacteria in the sample.

Measuring bacterial growth

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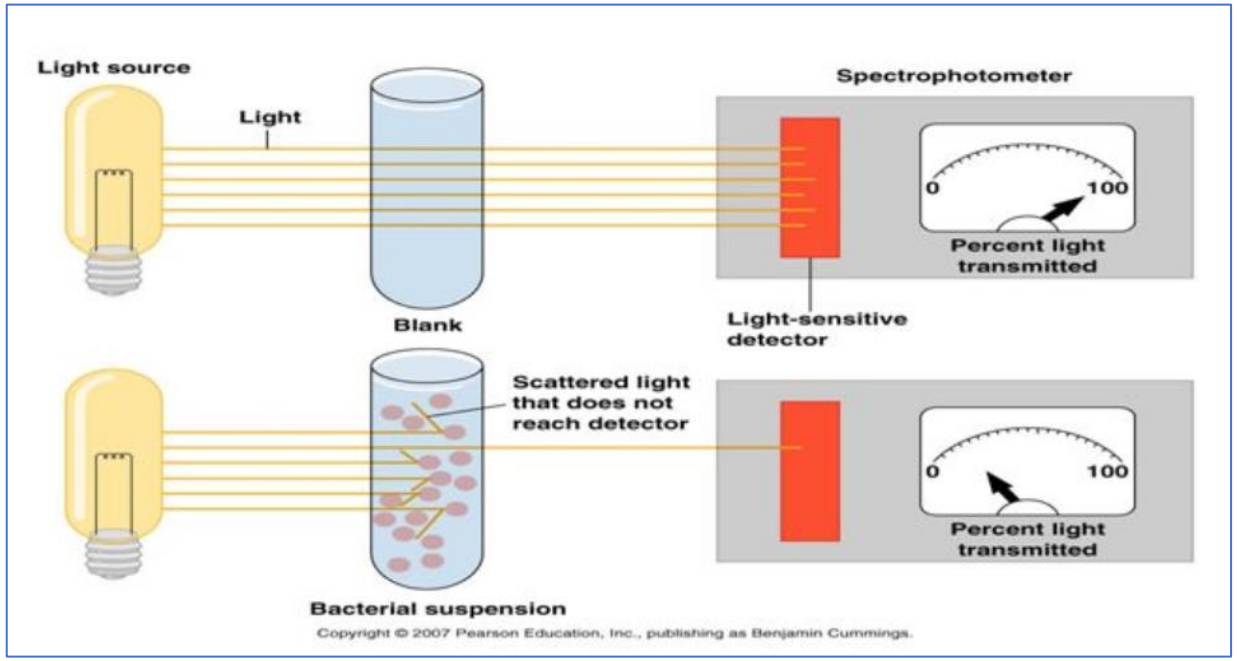
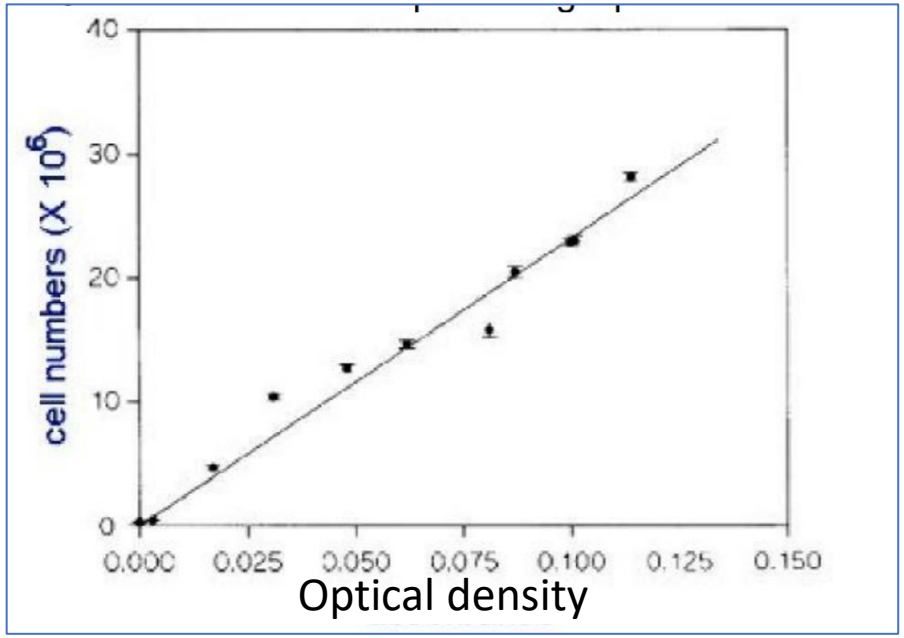


The experiment is repeated many times so we can compare the results with the standard curve



Calculating and predicting bacterial growth

- Bacteria have different doubling times (e.g. ***E.coli* 20 mins**, *M.tuberculosis* 100 hours) .

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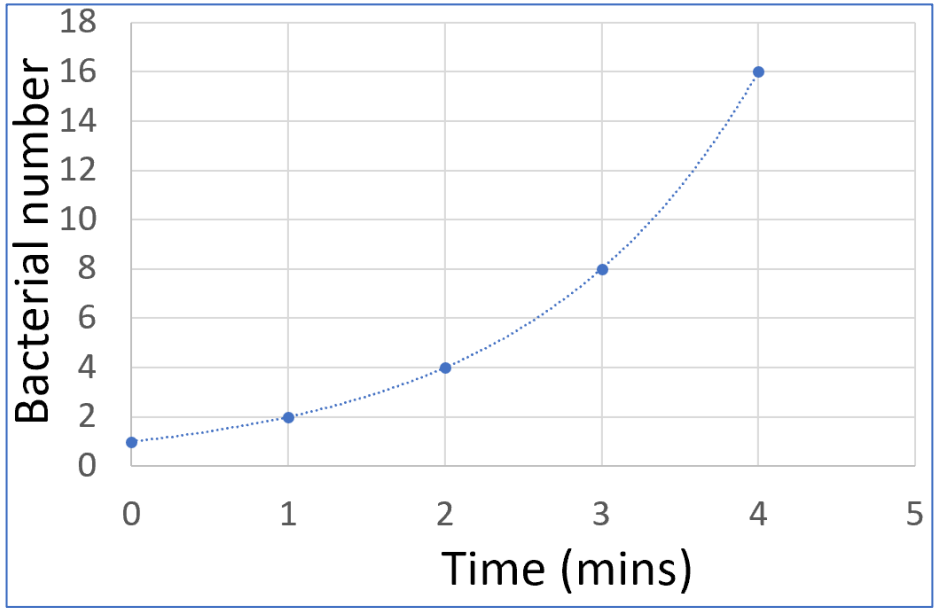
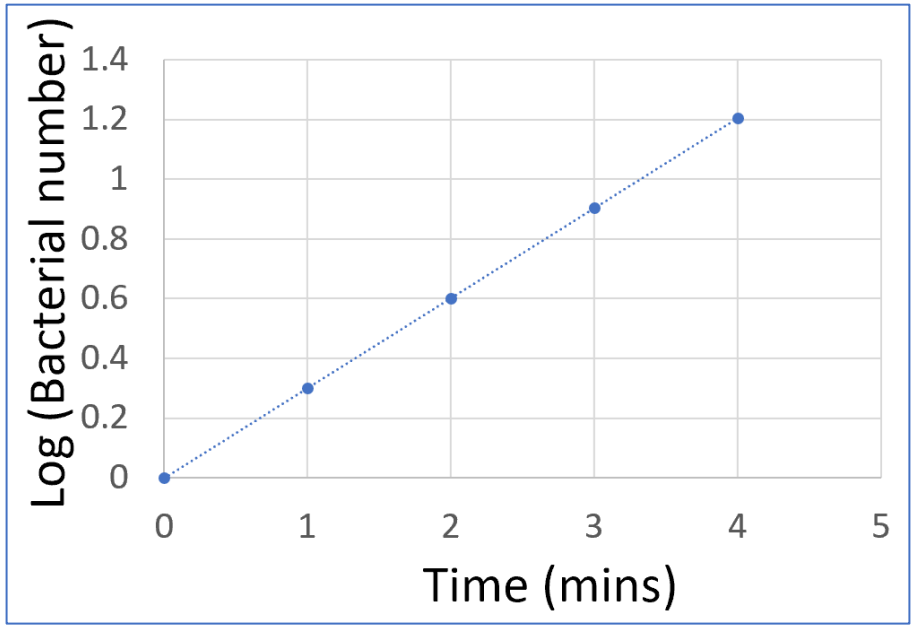
- By measuring bacterial numbers over time, we can calculate the doubling time (specific for each bacterial species under optimal growth conditions).

- We can then calculate number of bacteria following a period of time, or time needed to reach a certain number of bacteria.

Logarithm is used to make the curve more practical and easier for comparasion





Calculating and predicting bacterial growth rate

- If growth would begin with any cell number (N0), after a certain time (t) the number of cells (N) can be calculated by knowing the doubling time (td) as the following : $N = N0 * 2^{t / td}$ t/td is the number of td in a given time

- Example: In a bacterial culture of *E.coli* (doubling time 20 mins) inoculated with 2 bacterial cells, how many bacterial cells are expected to be found in the culture after 2 hours?

- Answer:

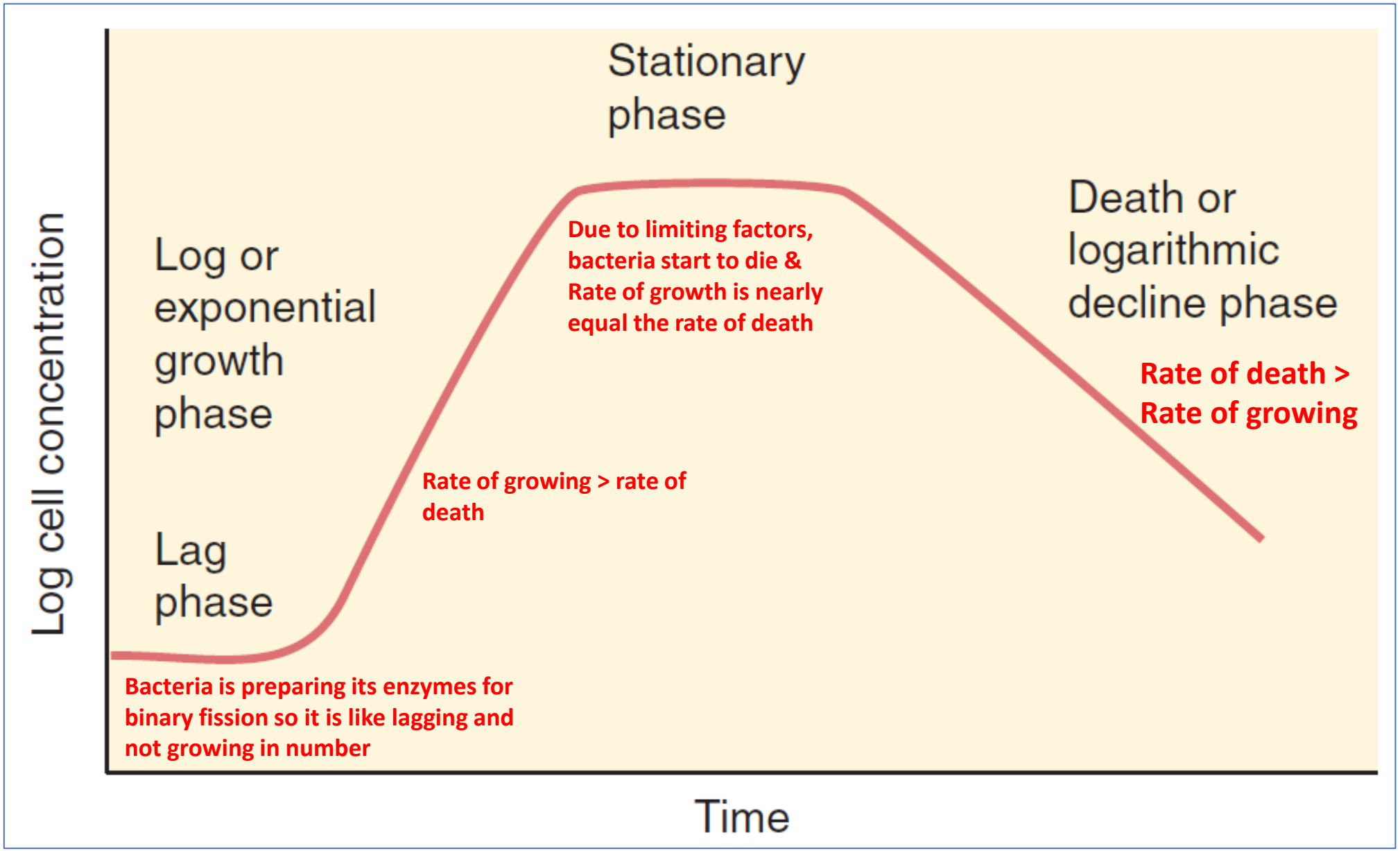
$$N = 2 * 2^{120 / 20}$$

$$N = 2 * 2^{6} \qquad = 2^{7} = 128$$

- After 4 hours = 8192
- After 24 hours = 4,722,366,482,869,645,213,696

This not what really happens because of the lack of nutrients for bacteria so the number will decrease before we get to that number

Bacterial growth curve



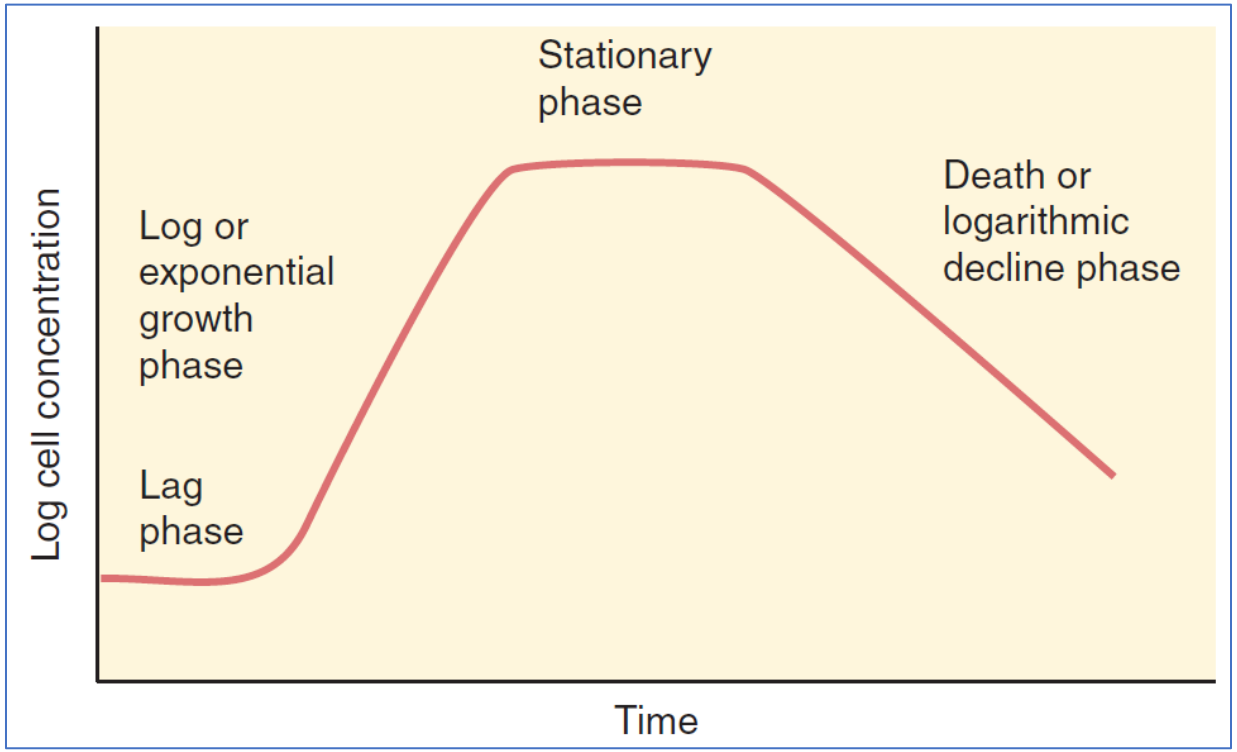
Bacterial growth curve

- Bacteria in culture follow a growth curve related to nutrient supply and waste production.

- The curve varies with the organism and culture medium.

(EX: Log phase will last longer if bacteria use less nutrients)

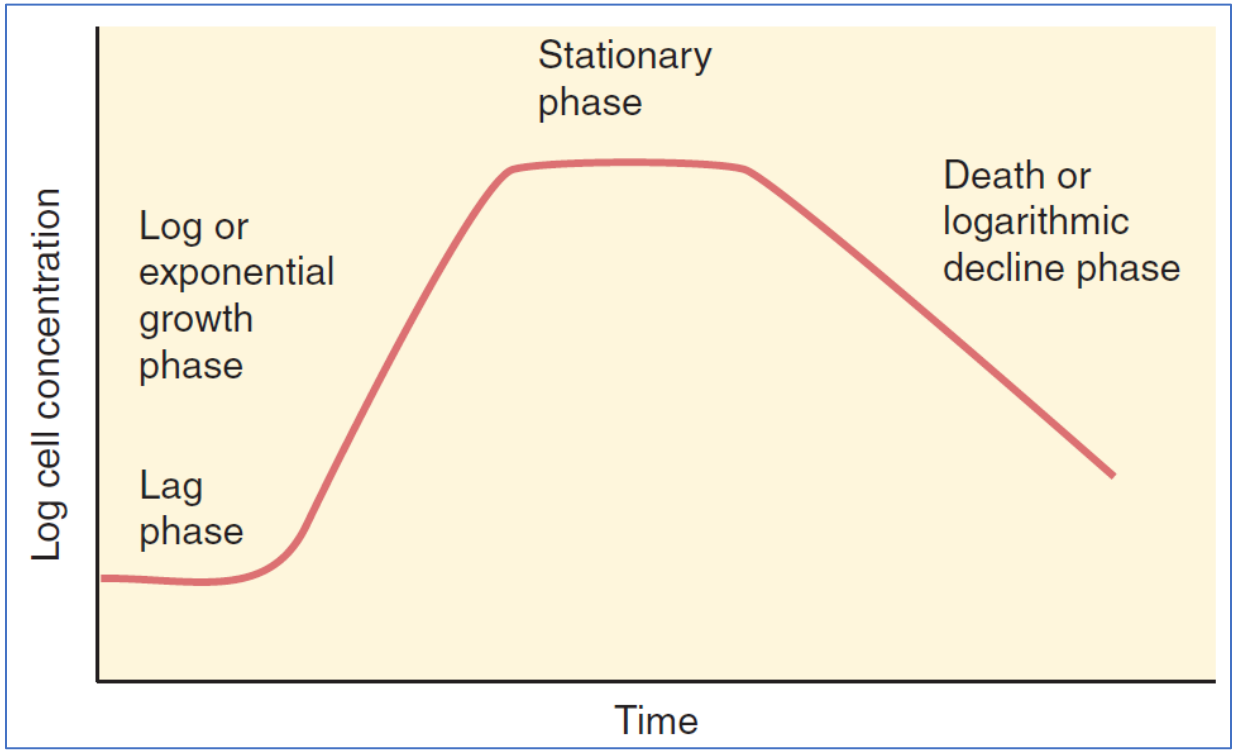
- In the Lag phase, enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume.



Phase	Growth Rate
Lag	Zero
Exponential	Constant
Maximum stationary	Zero
Decline	Negative (death)

Bacterial growth curve

- For aerobic organisms, oxygen limitation usually results in stopping the exponential growth phase.
- In the stationary phase, loss of cells is balanced by cell division.
- Decline phase is much slower than the exponential growth phase, and some bacteria tend to survive for prolonged periods after death of the majority of bacteria.

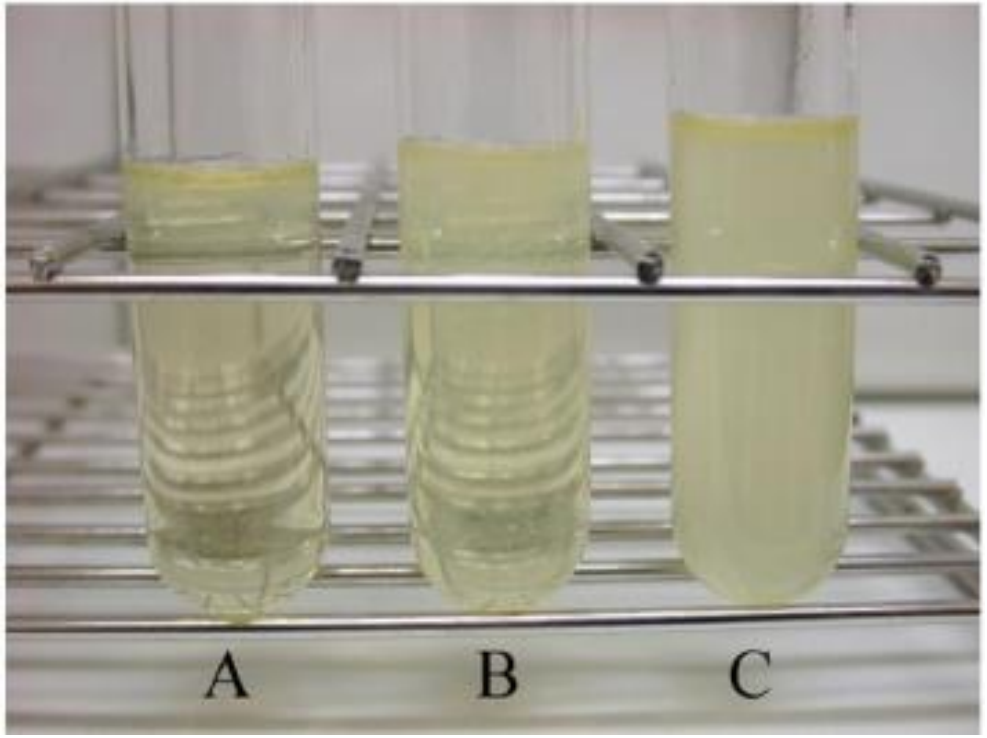


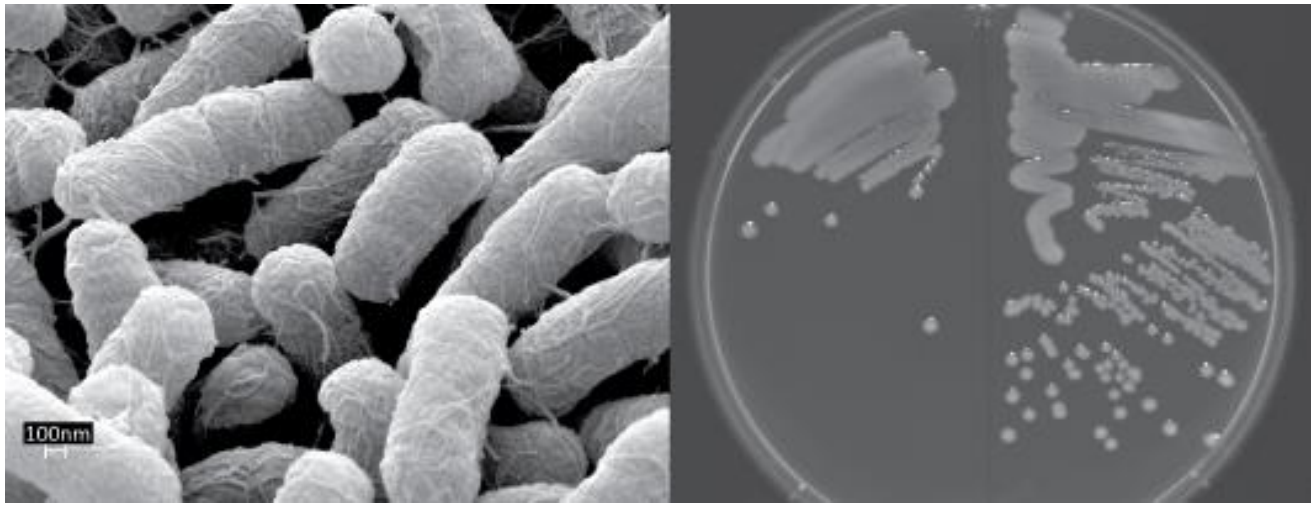
Phase	Growth Rate
Lag	Zero
Exponential	Constant
Maximum stationary	Zero
Decline	Negative (death)

Cultivation of bacteria

- The medium for growth can be either in **liquid** or **gelled medium using Agar**.

Like broth



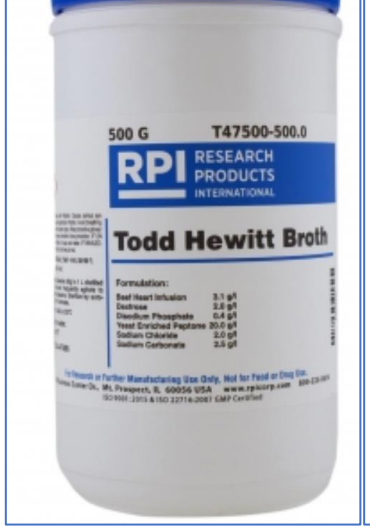


Cultivation of bacteria/ nutrients

- To propagate bacteria in culture, **nutrients in the medium must provide the building blocks as well as energy for growth of the specific bacteria**. (Carbon, sulphur, nitrogen, phosphorus, minerals, growth factors).

- Other than nutrients, several factors affect growth as well, for example: **pH**, **temperature**, **aeration**, **salt concentration** must be controlled.

Making agar plates: A solution just like broth with agarose is added in plates and when the temperature cools down, the liquid medium turns solid in petri dishes



Components

Components (g/L):

Beef Heart Infusion 500.00

Peptic Digest of Animal Tissue 20.00

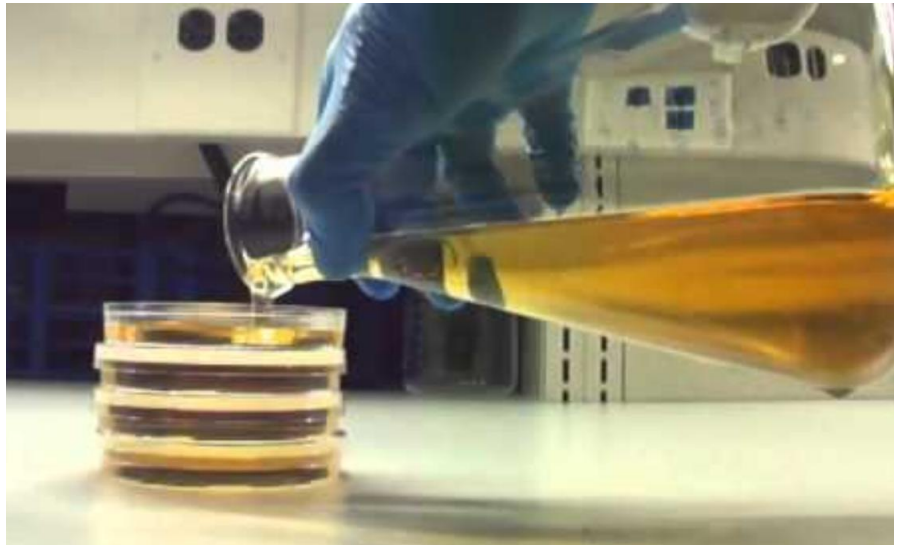
Dextrose 2.00

Sodium Chloride 2.00

Sodium Phosphate 0.40

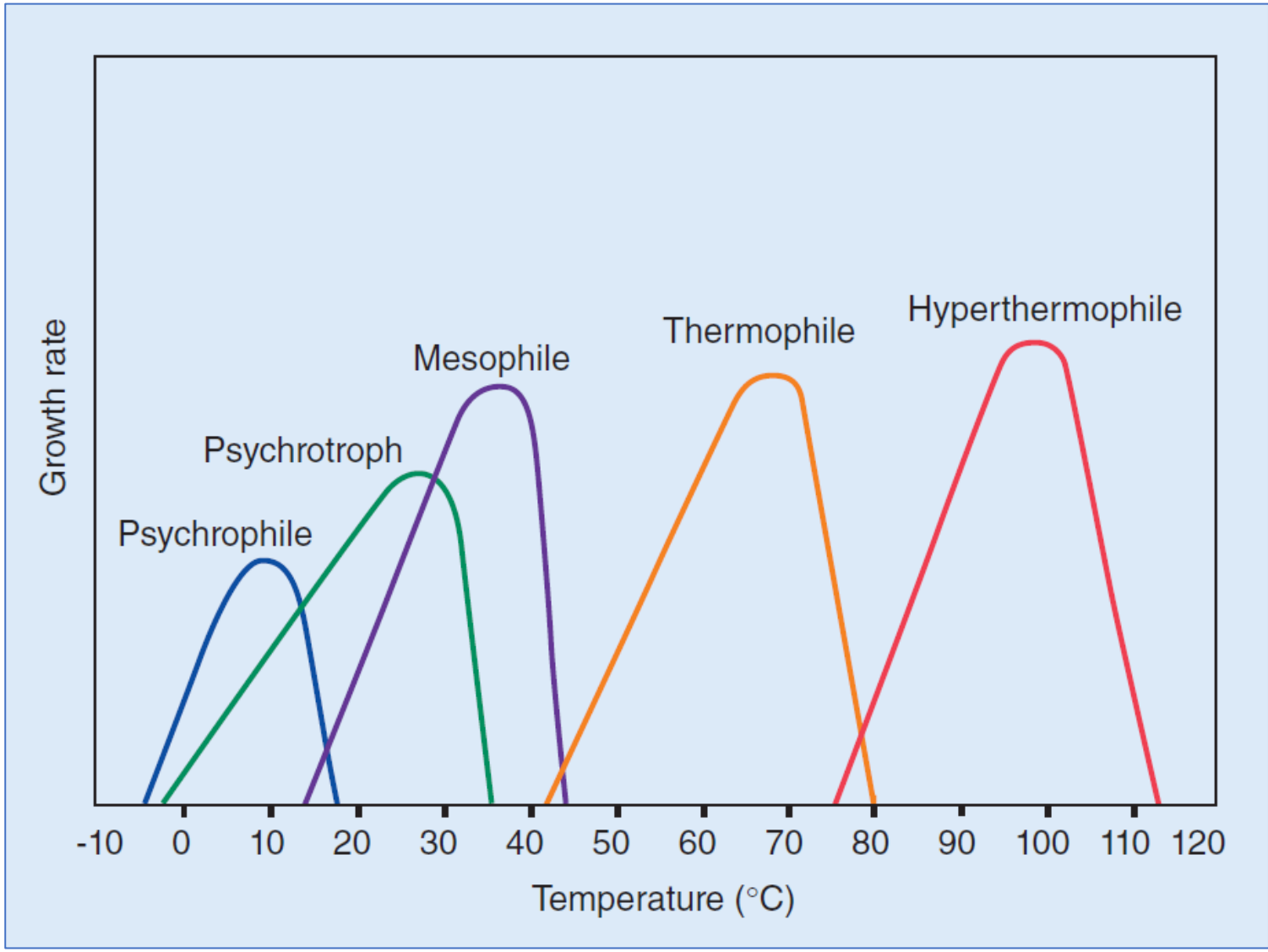
Sodium Carbonate 2.50

An example of components of a media used to grow many types of bacteria.



Cultivation of bacteria/ temperature

- Different temperatures fit growth of different bacteria.
- Most medically relevant bacteria grow in a temperature of 37 degrees celcius, which can be controlled in incubators in the lab.



Cultivation of bacteria/ aeration

- Different O2 and CO2 concentrations fit growth of different bacteria.
- Some bacteria cannot survive **without O2** and are called **obligate aerobes.**
- Others cannot survive in the **presence of O2**, and are called **obligate anaerobes**.
- Some will survive **in the absence or presence of O2**, called **facultative anaerobes**.

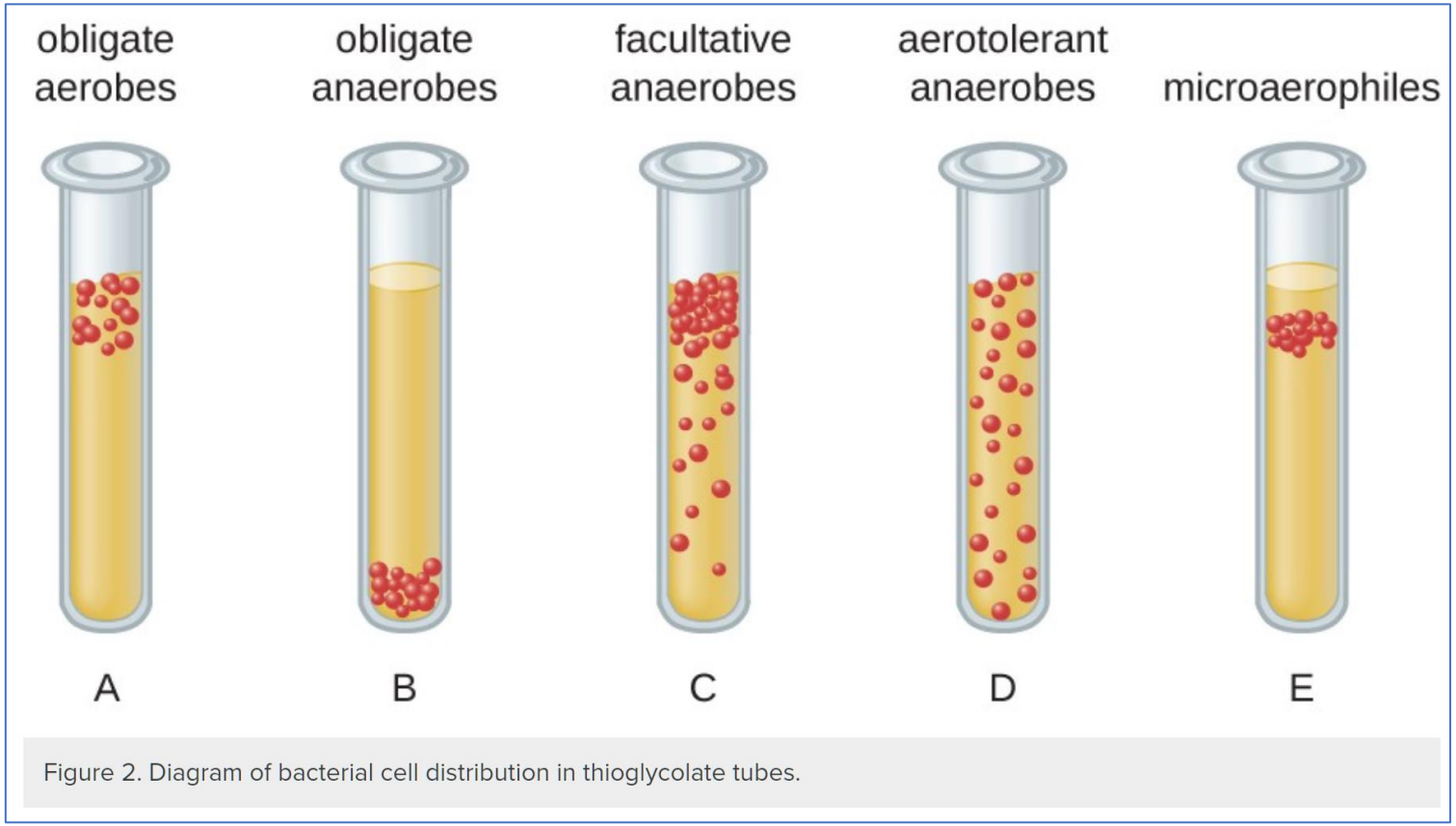


Figure 2. Diagram of bacterial cell distribution in thioglycolate tubes.

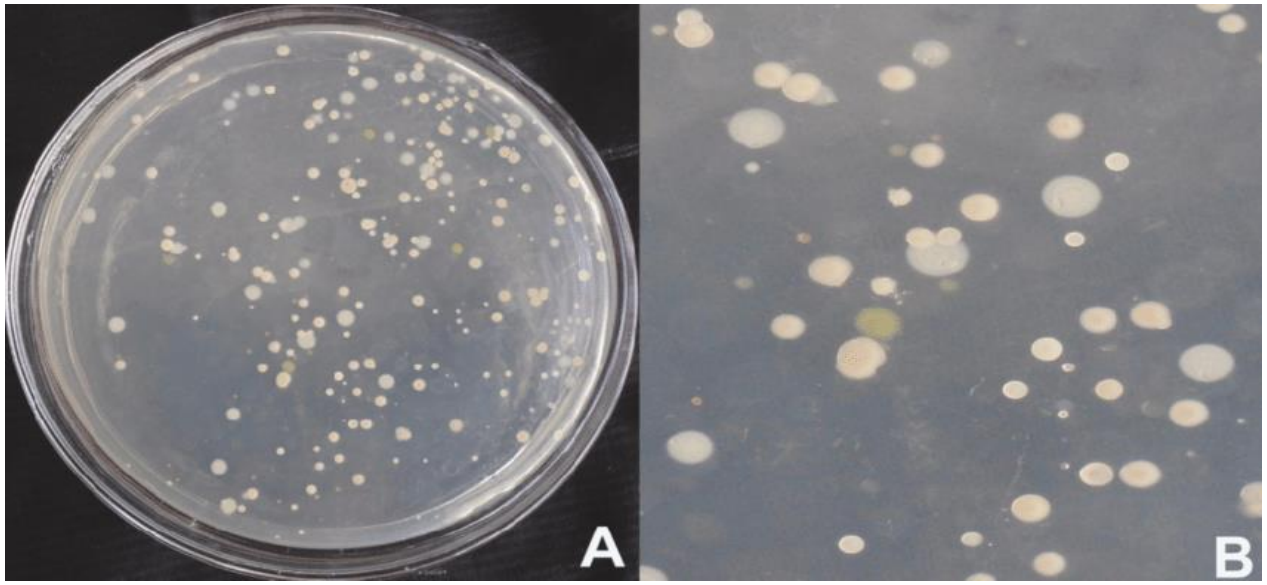
Microaerophiles survive with specific concentration oxygen (not too much and not a little of oxygen)

Isolation of bacteria in pure culture

- Mixed populations of bacteria are commonly found in tested samples.

- In order to study the properties of an organism (e.g. antibiotic sensitivity), **it must be handled separately**. (to study a type of bacteria you should isolate it from the sample and raise it in pure colonies)

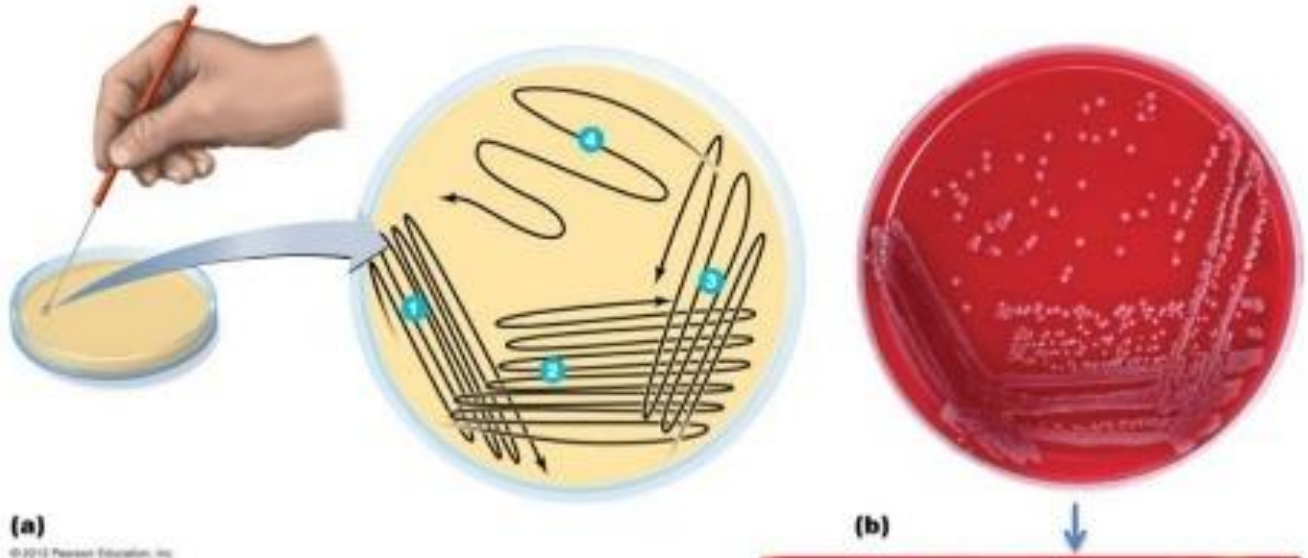
- Several methods (such as pour-plate, and streak-plate) aim to isolate single colonies, which will be used to propagate a pure cultures.



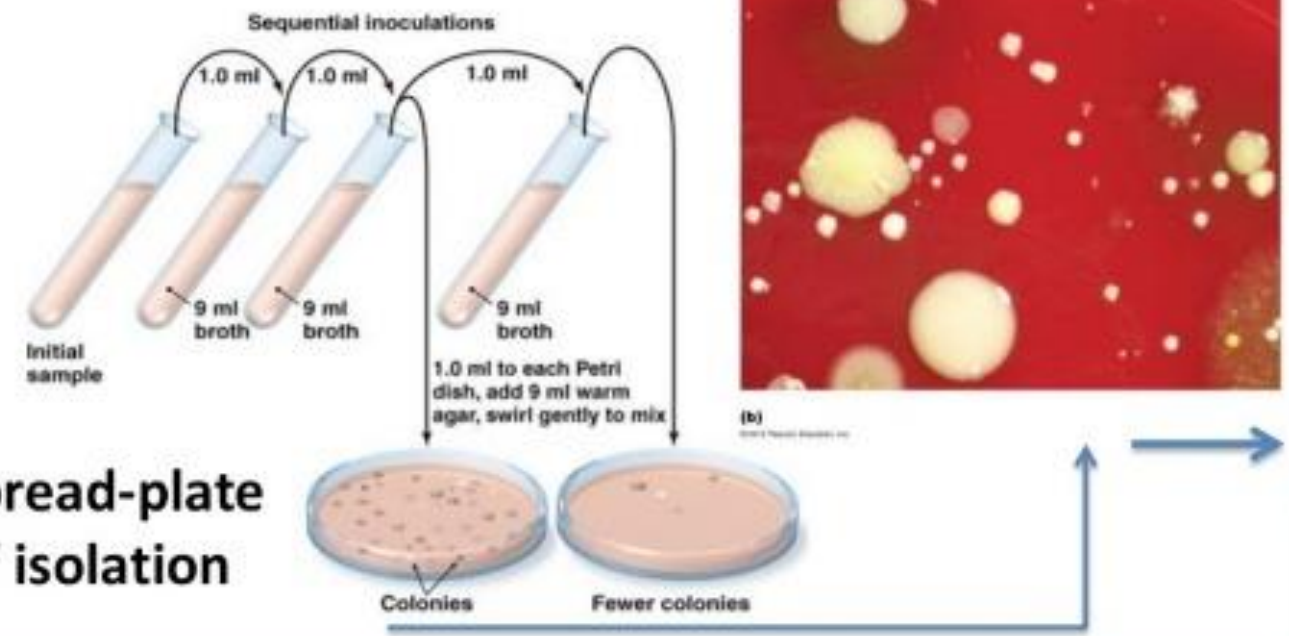
Sample to use for streaking or dilution. E.g. oral swaps, urine, sputum, feces.

Techniques to isolate microorganisms in pure cultures or axenic cultures

- Streak-plate technique of isolation



- The method of serial dilutions



- Pour-plate/spread-plate techniques of isolation

Further reading:

- Jawetz, Melnick & Adelberg's Medical Microbiology, 26th edition-
  Section 1: Fundamentals of Microbiology-
    Chapter 4: The Growth, Survival, and death of Microorganisms
    Chapter 5: Cultivation of Microorganisms

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