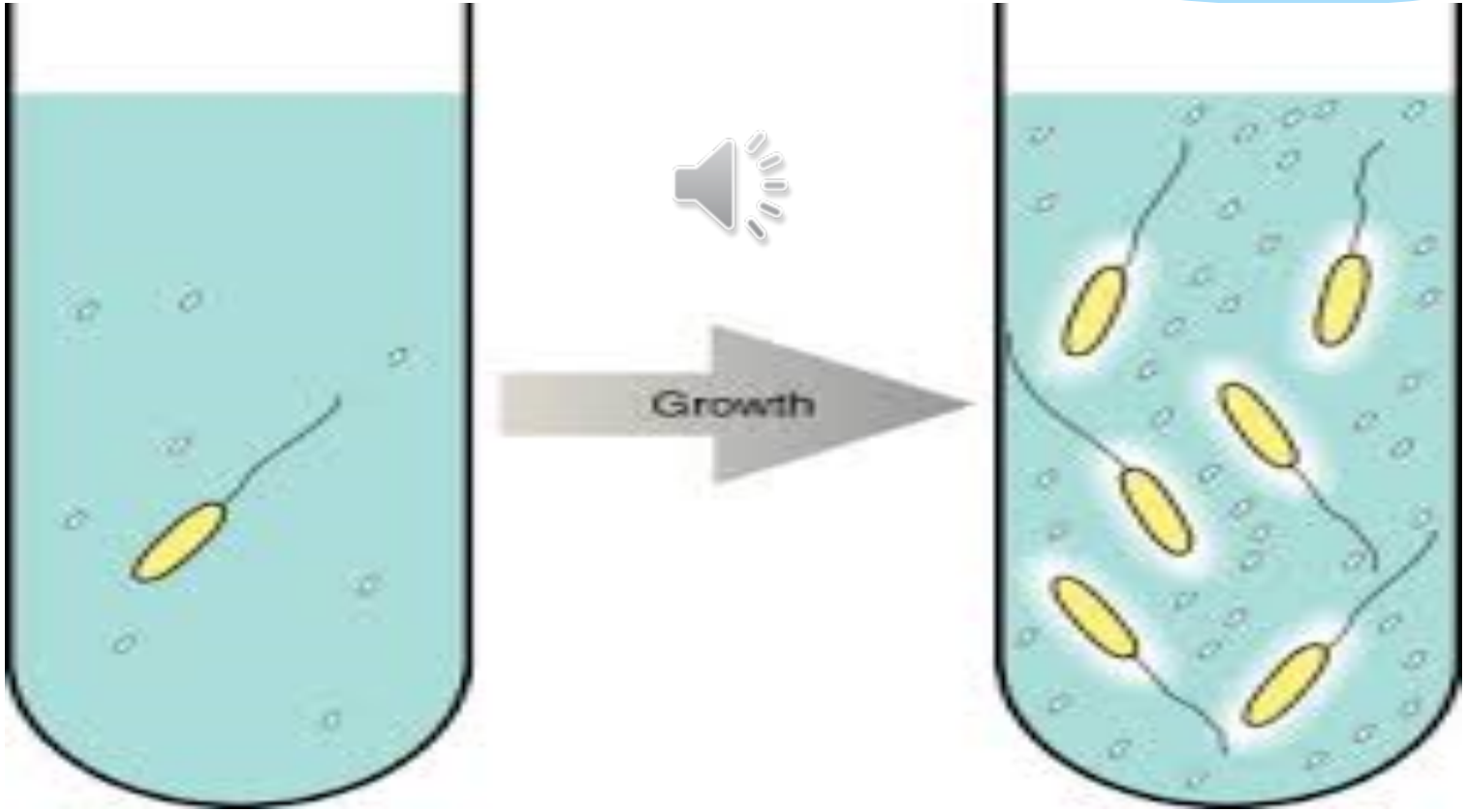
Bacterial growth



Bacterial survival and growth

* Bacterial survival = growth & replication.

Can not just sit around.

*Growth stages include metabolism, regulation & division.

*Fast growing bacteria that divide each 10-30 minutes e.g. Vibrio .

*Slow growing: each 24 hours e.g. Mycobacterium tuberculosis.

*Bacteria consists of many structures & elements e.g. protein, polysaccharides, lipids, nucleic acid & peptidoglycan.

*Growth needs materials (nutrient) & energy/metabolism.

* Bacteria divide by binary fission producing two identical offspring.

Bacterial Growth

- Growth: Increase in the size of organisms and an increase in their number.

Whatever the balance between these two processes, the net effect is an increase in the total mass(biomass) of the culture.

- In the laboratory, growth is used as central technique for detection , identification and for assessment of antibiotic effects.

- The number will be adopted here, as outcome of infections and in the measurement of the effects of antibiotics.

Types of growth in the laboratory

In the laboratory, bacterial growth can be seen in three main forms:

1. By the development of colonies, the macroscopic product of 20–30 cell divisions of a single cell.

2. By the transformation of a clear broth medium to a turbid suspension of 107–109 cells per ml.

3. In biofilm formation, in which growth is spread thinly (300–400 μm thick) over an inert surface.

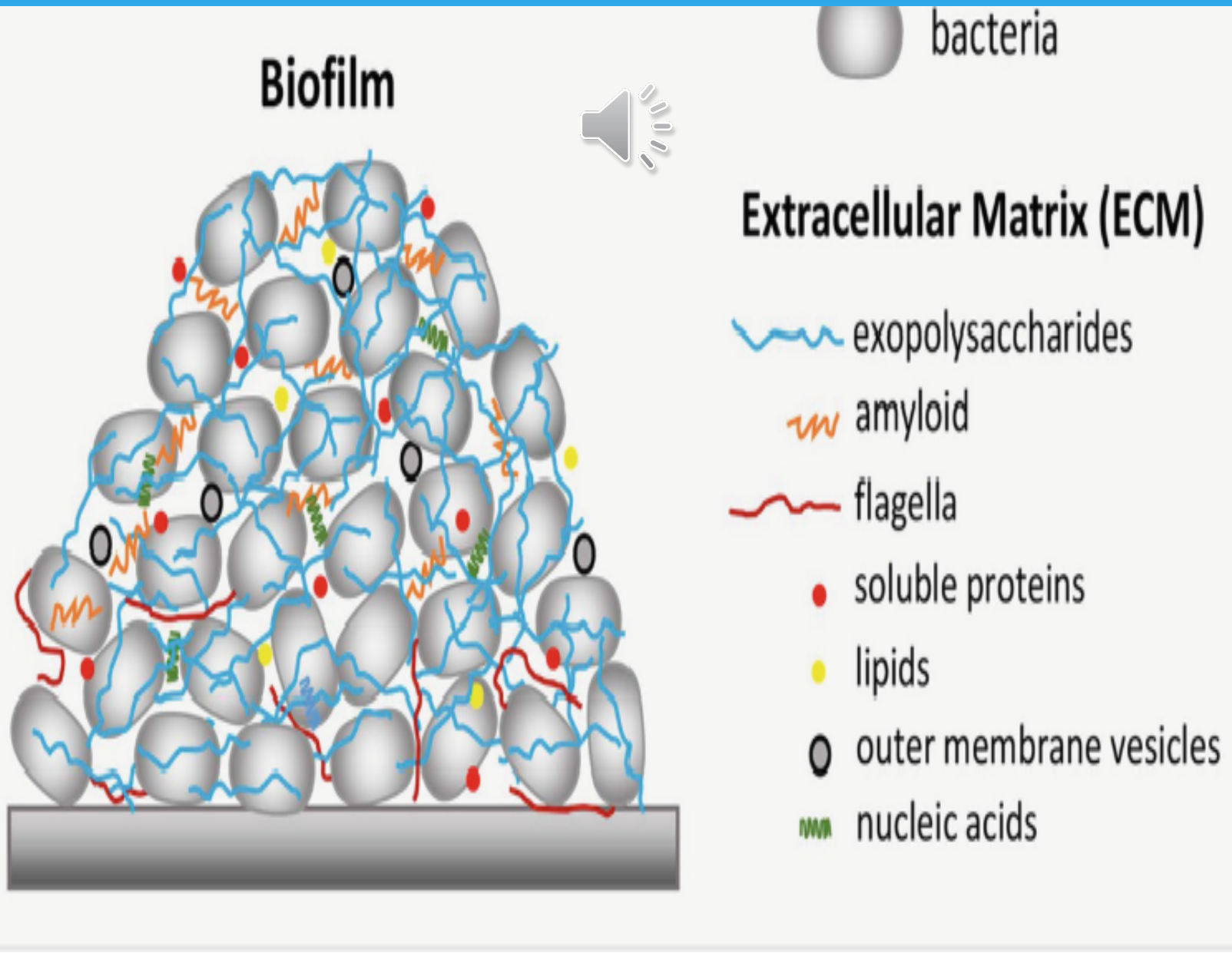
BIOFILM

is a layer of prokaryotic organisms that have aggregated to form a colony. The colony attaches to a surface with a slime layer which aids in protecting the microorganisms.

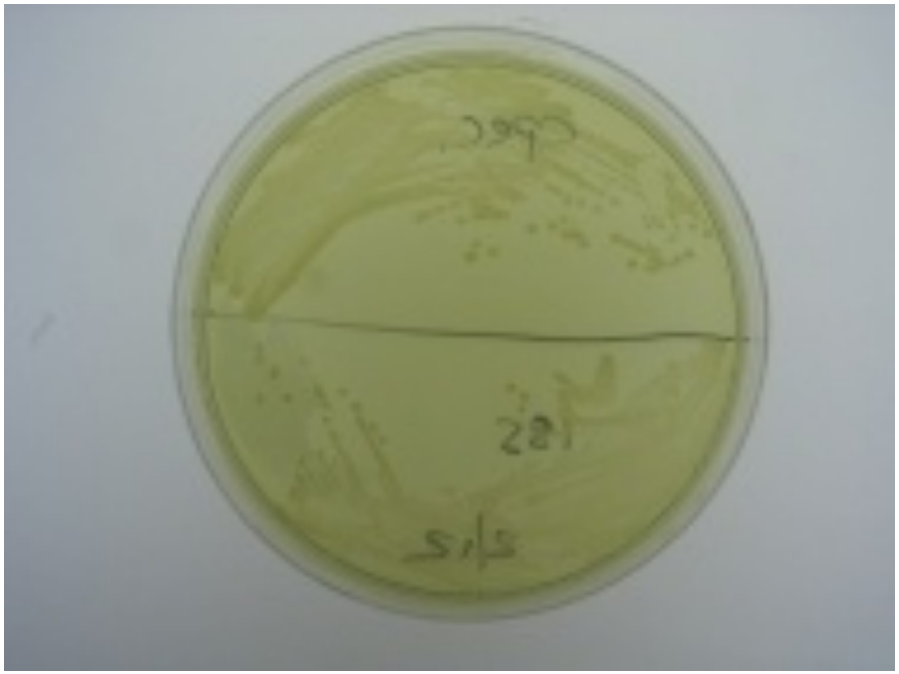
Biofilms often form on the inert surfaces of implanted devices such as catheters, prosthetic, cardiac valves and intrauterine devices.

Biofilm formation

* Biofilm formation begins when free-floating microorganisms such as bacteria come in contact with an appropriate surface and begin to put down roots, so to speak. This first step of attachment occurs when the microorganisms produce a gooey substance known as an extracellular polymeric substance (EPS). An EPS is a network of sugars, proteins and nucleic acids (such as DNA). It enables the microorganisms in a biofilm to stick together.


bacteria
Biofilm
Extracellular Matrix (ECM)
exopolysaccharides
amyloid
flagella
soluble proteins
lipids
outer membrane vesicles
nucleic acids

In the laboratory



In the laboratory

* Growth requirements in the lab.:

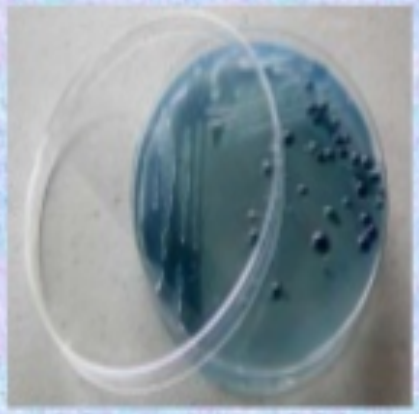
1. Fastidious organisms require many nutrients.
2. Simple requirements can make everything from scratch.
3. selective (enrichment) with indicator.
4. Some bacteria cannot be cultured in vitro (Lab.).

a. Chlamydia and Rickettsia : need tissue culture like viruses

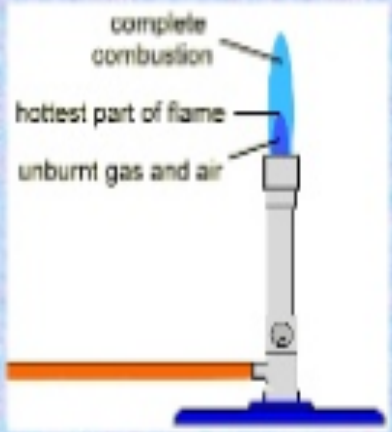
b. Treponema pallidum, Mycobacterium leprae, require animal infection.

EQUIPMENT :

- 1.Petri dish :

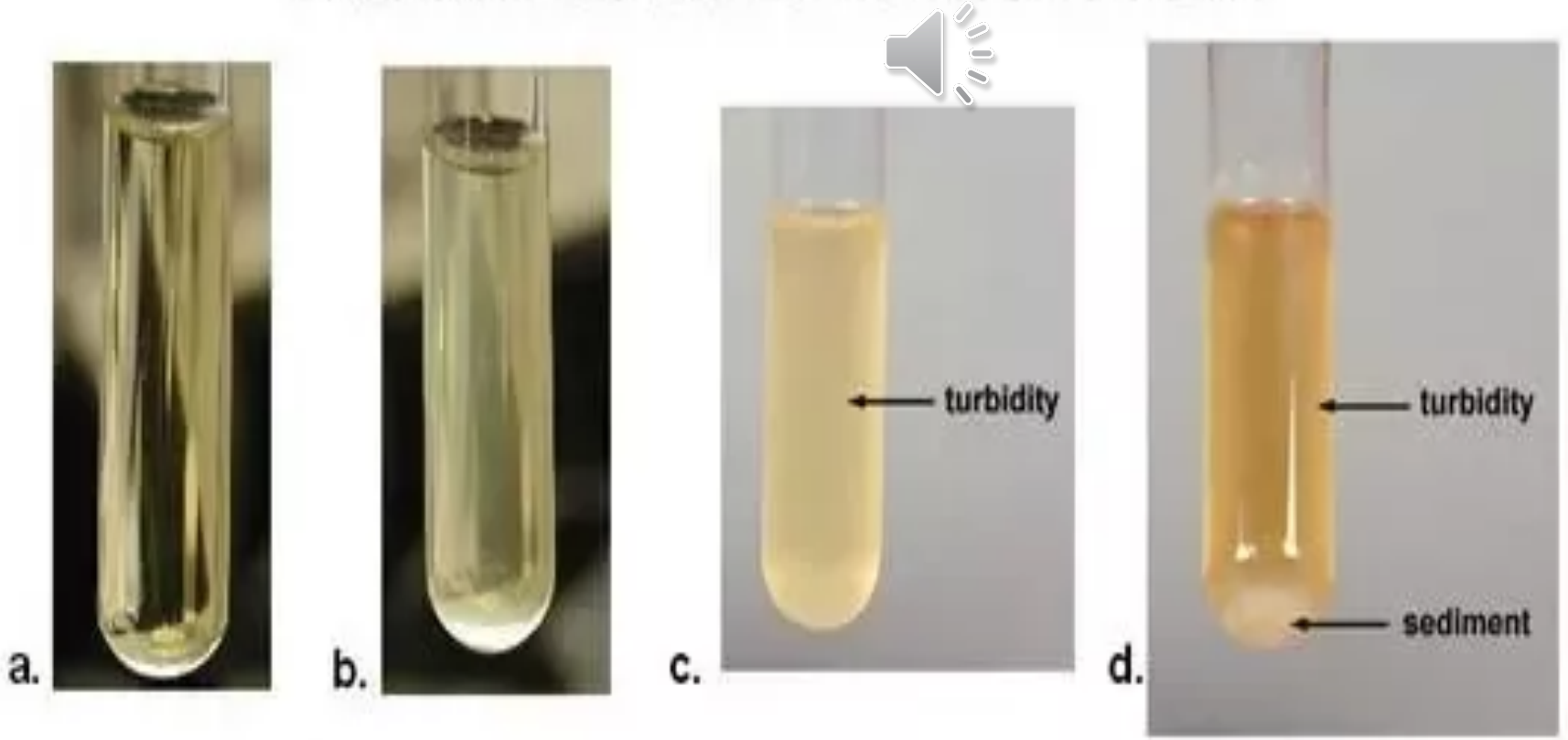


2.Bunsen Burner :



3.Inoculating loop :

Bacterial Cultures in Broth Media



a. Sterile (uninoculated broth) - note how clear the media is
b. Broth showing slight turbidity (some bacterial growth)
c. Broth showing significant turbidity (a lot of bacterial growth)
d. Broth that hasn't been agitated (shaken)

Cultivation of bacteria

Components

Components (g/L):

Beef Heart Infusion 500.00

Peptic Digest of Animal Tissue 20.00

Dextrose 2.00

Sodium Chloride 2.00

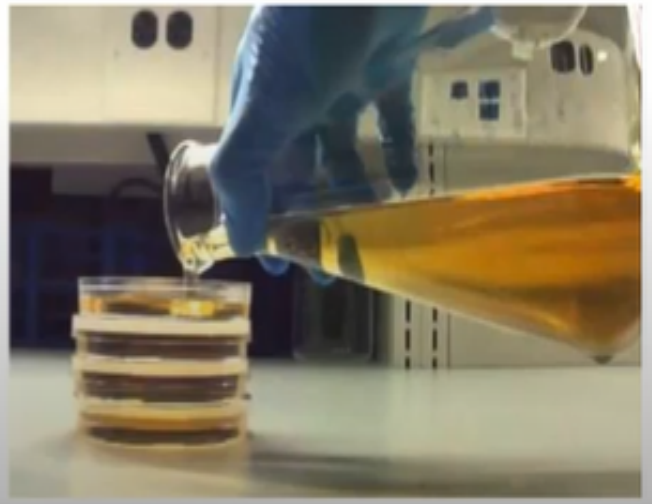
Sodium Phosphate 0.40

Sodium Carbonate 2.50

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

- 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. When the temperature cools down the liquid medium turns solid in petri dishes

Classification of culture media

Classification based on the ingredients

Simple media

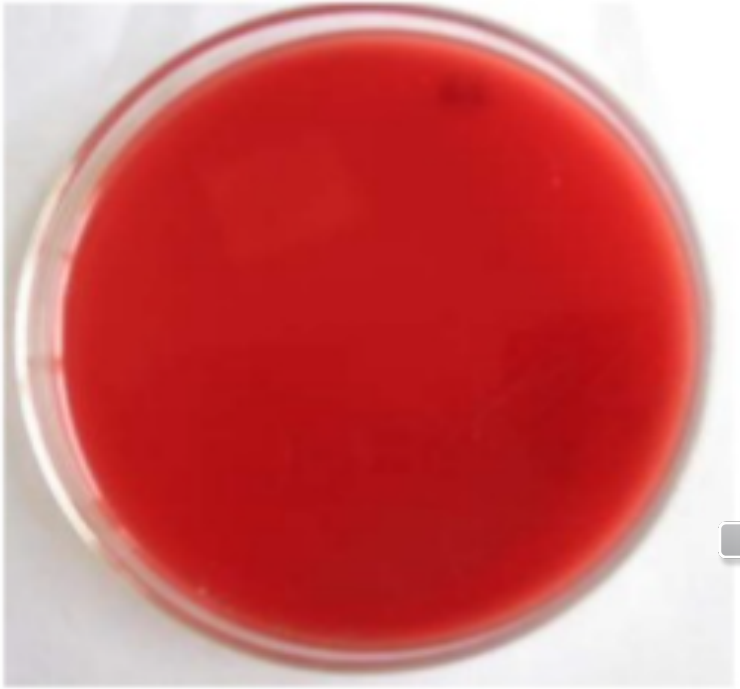
- eg: Nutrient broth, N. agar
- NB consists of peptone, meat extract, NaCl,
- NB + 2% agar = Nutrient agar

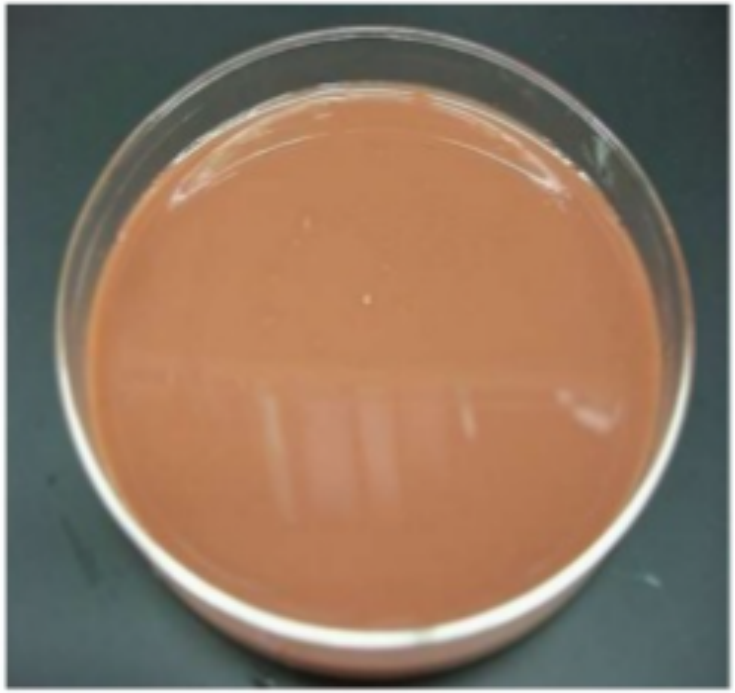
Special media

- Enriched media
- Selective media
- Differential media
- Transport media
- Anaerobic media

Enriched media

- Substances like blood, serum, egg are added to the simple medium.
- Used to grow bacteria that are exacting in their nutritional needs.
- eg: Blood agar, Chocolate agar





Blood agar

BAP contains mammalian blood(usually sheep or horse) typically at a concentration of 5-10%, used to isolate fastidious organisms and detect hemolysis.

Chocolate agar

contain red blood cells that have been lysed by slowly heating to 80 c .and it used for growing fastidious bacteria, such as *Haemophilus influenzae*

Selective media

- The inhibitory substance is added to a solid media to inhibit commensal or contaminating bacteria such as :

- Antibiotics
- Dyes
- Chemicals
- Alteration of pH

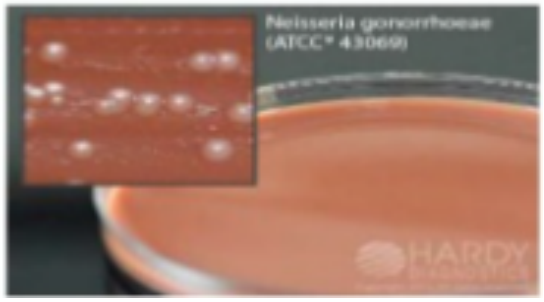
Examples

Thayer Martin medium

selective for *Neisseria gonorrhoeae*

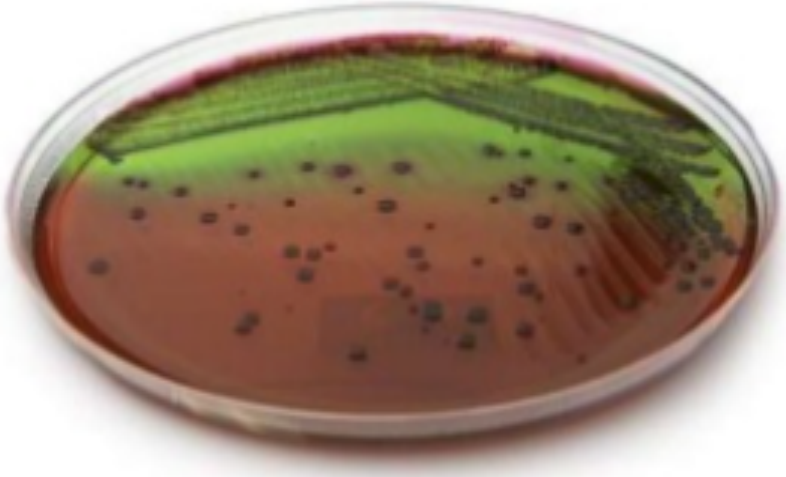
- It usually contains the following combination of antibiotics:

- Vancomycin:
  which is able to kill most **Gram-positive** organisms.
- Colistin,:
  which is added to kill most **Gram-negative** organisms except *Neisseria.*
- Nystatin,:
  which can kill most **fungi**
- Trimethoprim:
  which inhibits Gram-negative organisms, especially swarming ***Proteus.***



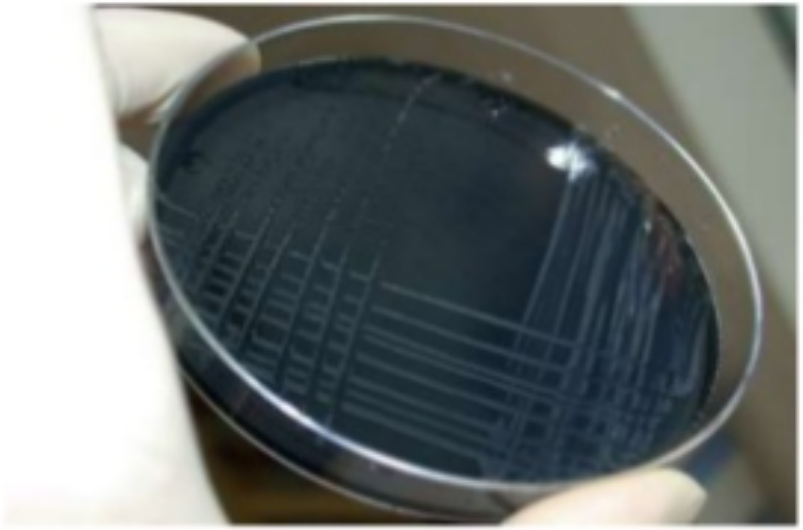
Eosin methylene blue

- selective for gram negative bacteria
- The dye methylene blue in the medium inhibit the growth of gram positive bacteria.



Campylobacter agar

- Is used for isolation of *Campylobacter jejuni* from fecal or rectal swab.
- Contain Bacteriological **charcoal** , **Cefoperazone** and **Amphotericin B**.



Lowenstein –Jenson medium

- is solid medium used for *Mycobacterium tuberculosis.*

- contain **penicillin**, **nalidixic acid** and **malachite green** to inhibit growth of gram positive and gram negative bacteria, in order to limit growth to Mycobacteria species only.

- **Differential media**
- are designed in such a way that different bacteria can be recognized on the basis of their colony color.

- Dyes and metabolic substrates are incorporated so that those bacteria that utilize them appear as differently colored colonies.

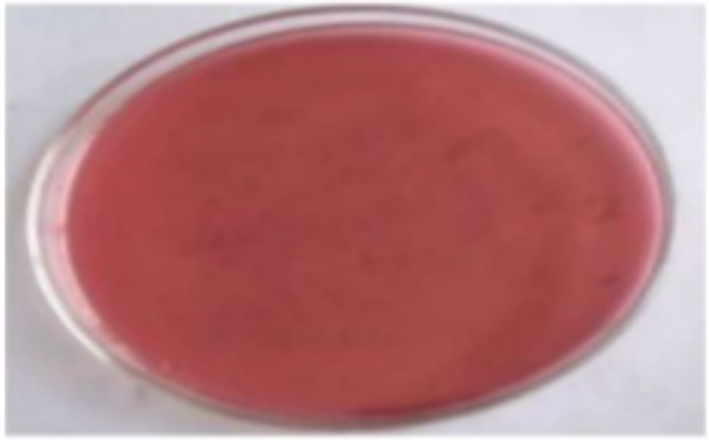
Examples:

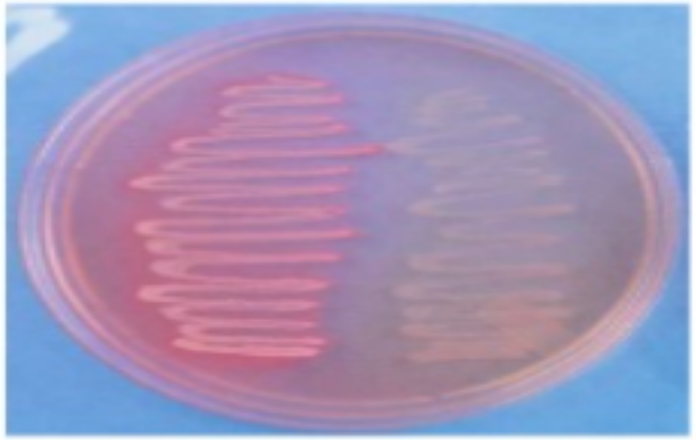
- **MacConkey agar**
- **CLED agar**
- **TCBS agar**
- **XLD agar**

Examples

MacConkey medium

- Distinguish between lactose fermenters & non lactose fermenters.
- Lactose fermenters – **Pink** colonies
- Non lactose fermenters – colorless colonies

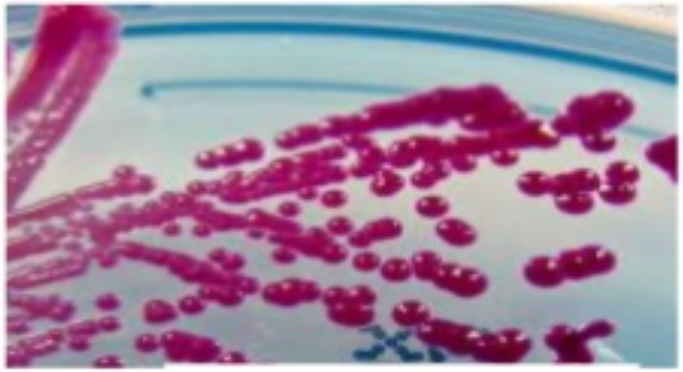




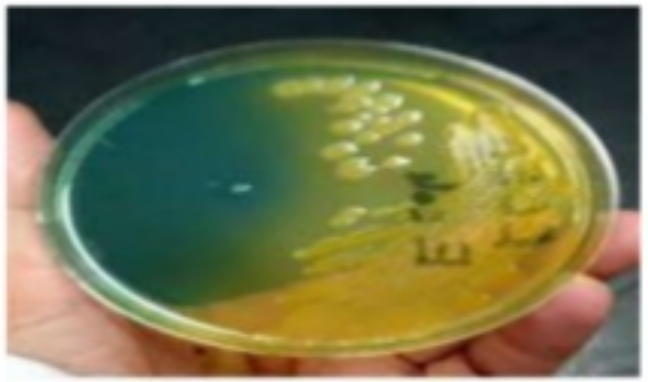
Examples

Cysteine Lactose Electrolyte Deficient Agar(CLED)

- For cultivation of pathogen from urine specimen , inhibit swarming of *proteus sp*.



CLED, serratia

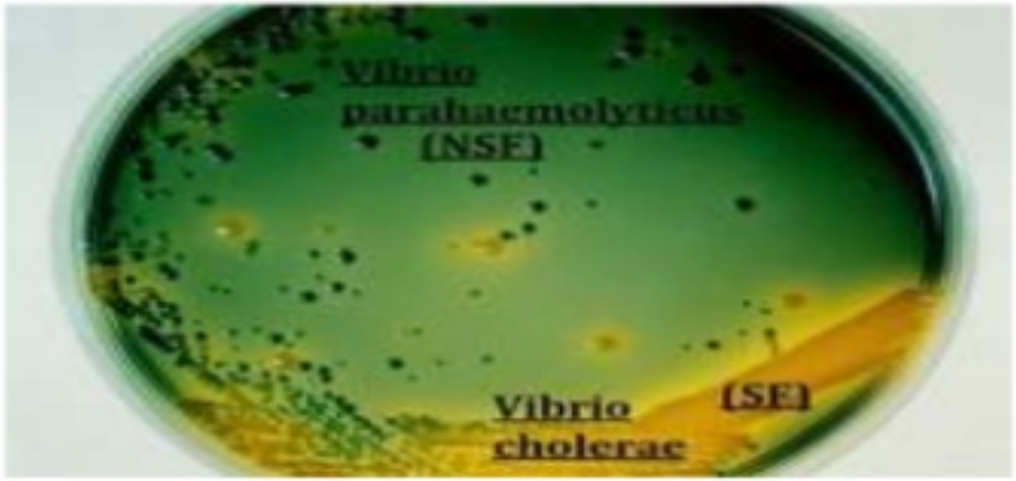


CLED , e-coli

Examples

Thiosulfate-citrate-bile salts-sucrose agar(TCBS)

- highly selective for the isolation of V. cholerae and V. parahaemolyticus

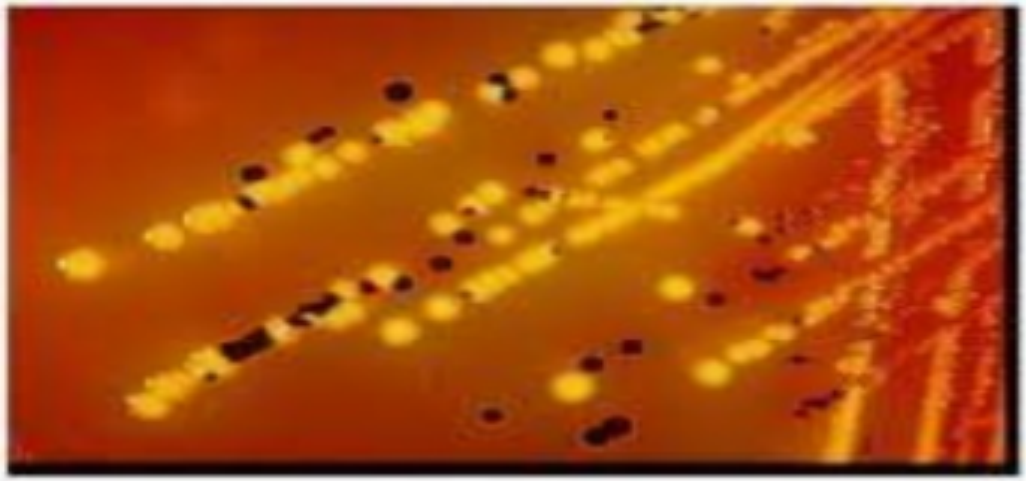


Yellow coloured (sucrose fermenting) colonies of Vibrio cholerae on TCBS agar.

Examples

Xylose Lysine Deoxycholate Agar(XLD)

- Used for the recovery of *Salmonella* and *Shigella* species.



XLD Agar

Escherichia coli **(yellow colonies)**
Salmonella **sp. (black colonies)**

Transport media

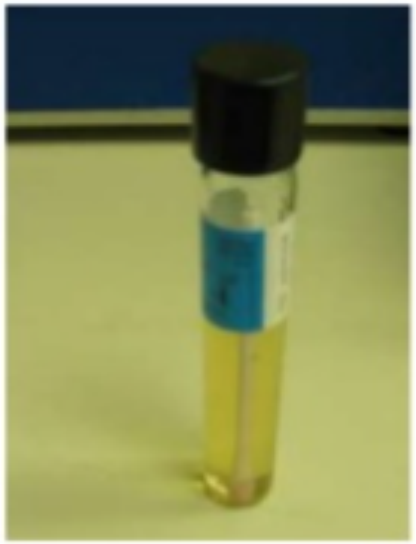
- Media used for transporting the samples.
- Delicate organisms may not survive the time taken for transporting the specimen without a transport media.
- Eg:
  - **Stuart's medium**
  - **Buffered glycerol saline**

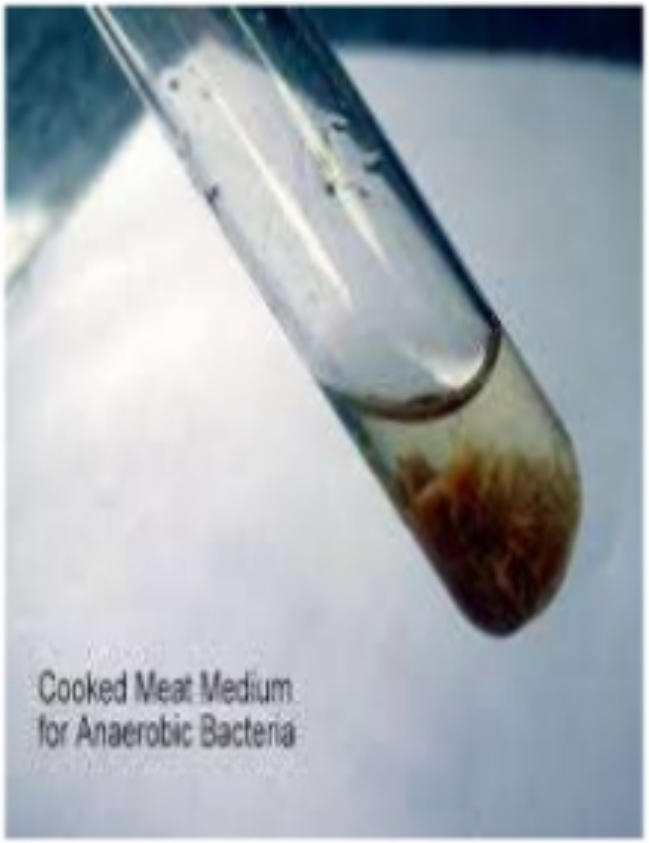
Anaerobic media

- These media are used to grow anaerobic organisms.

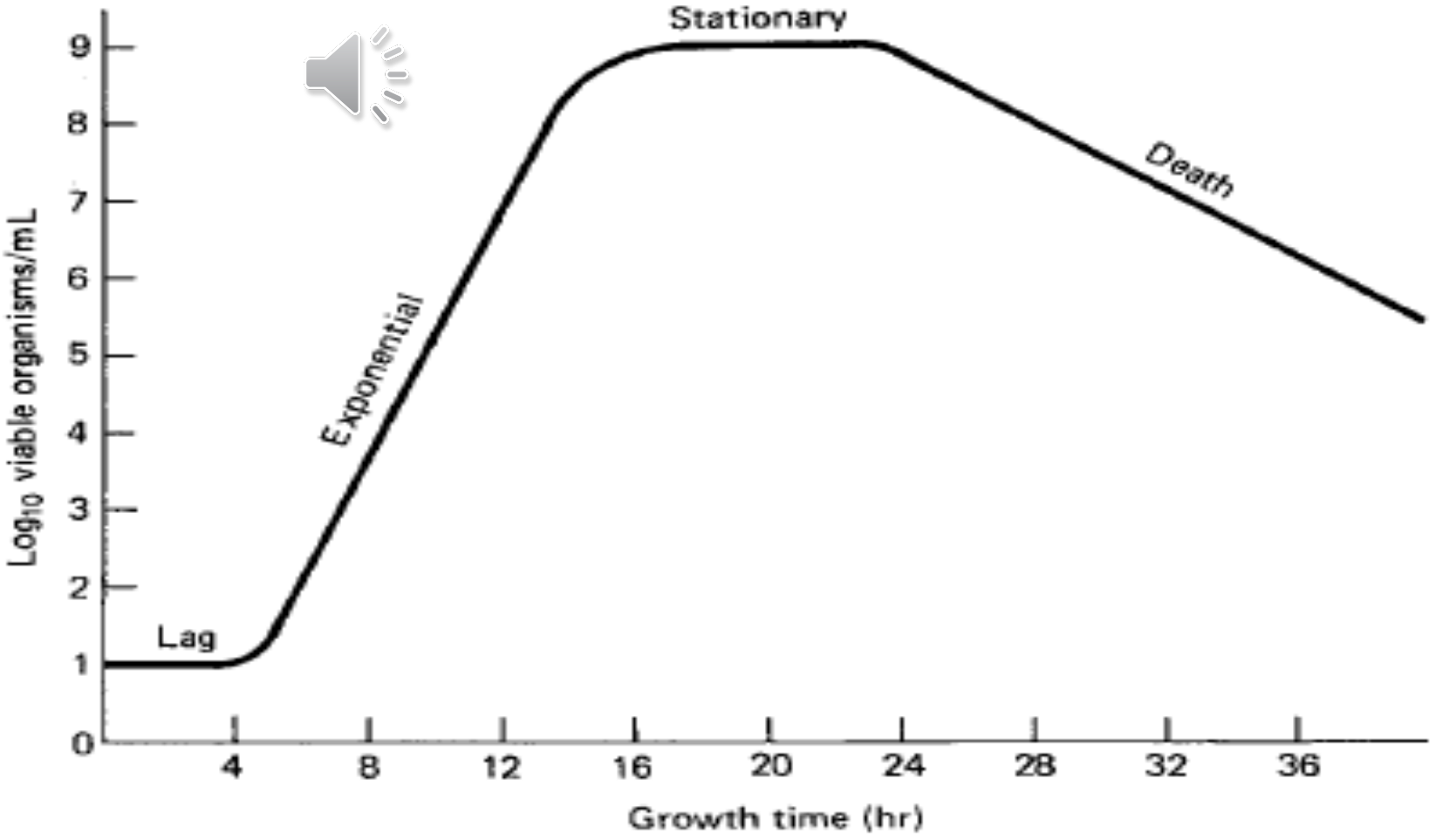
Eg:

- Robertson's cooked meat medium.
- Thioglycolate broth medium.





Growth curve/growth phases occur in medium



Bacterial growth stages

1. Lag phase, there is little or no change in the number of cells (adjustment stage), but metabolic activity is high.
2. Log or exponential phase, the bacteria multiply at the fastest rate possible under the conditions provided. The bacterial population doubling occurs at a constant rate.
3. Stationary phase, there is an equilibrium between cell division and death (nutrients start to deplete & toxic materials start to be produced).
4. Death (decline) phase, the number of deaths exceeds the number of new cells formed.

** The curve varies with the organism and culture medium.

Extending log phase

** Maintenance of bacteria in continuous culture is sometimes necessary in industrial and research purposes

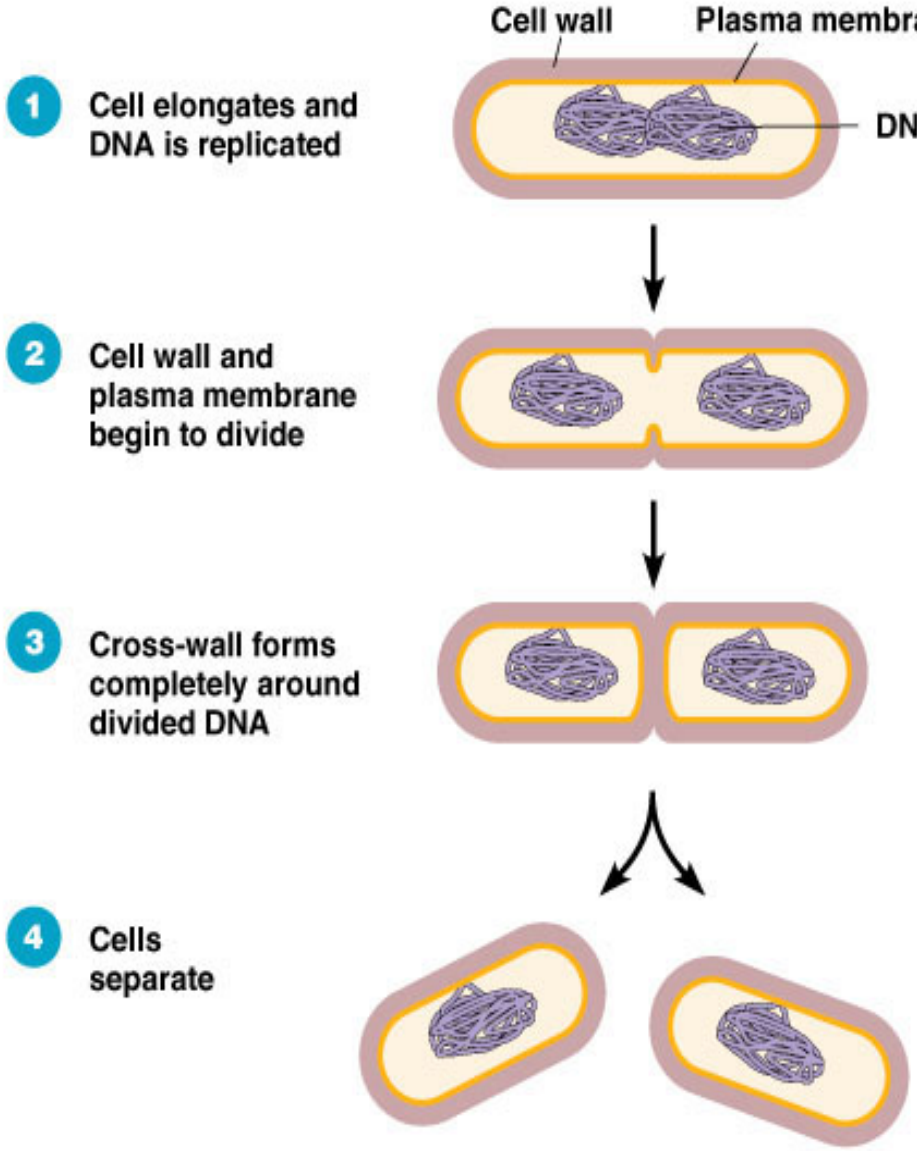
Chemostat: (chemical environment is static) : cells of a growing culture are harvested continuously and nutrients replenished continuously

Bacterial division

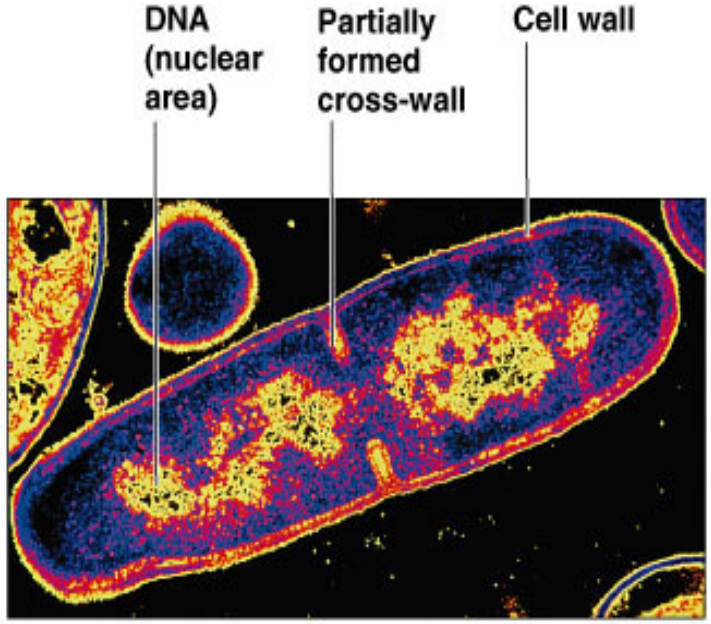
- The reproduction method of bacteria is binary fission, in which a single cell divides into two identical cells. Some organisms reproduce by budding, aerial spore formation or fragmentation.

- Bacterial division:

Replication of the chromosome triggers cell division, a septum forms, which divides the cell into two daughter cells.



(a) A diagram of the sequence of cell division.



(b) A thin section of a cell of *Bacillus licheniformis* starting to divide.

THE END