SHEET NO.

MICROBIOLOGY (Bacteriology)

DOCTOR 2019 | MEDICINE | JU

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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(الانشطار الثنائي(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.

Why do we grow the bacterial cell !??LOVE IT !!! OF COURSE NOT.
After cell culture ,if we find a colonies we will be able to identify and detect the type of bacteria and choose the appropriate antibody.

- 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.(the cell is grown on petri dish in a nutrient medium contain (AGAR)that is semi-solid and put it in incubator)الحاضنة().

2.By the transformation of a clear broth medium to a turbid suspension of 107–109 cells per ml.(the cell is grown on test tube in liquid nutrient medium (be very clear))

3.In biofilm formation, in which growth is spread thinly (300–400 μm

thick) over an inert surface.

So bacterial growth is determined by 1- Colonies 2- Turbidity of the clear liquid broth 3- Biofilms just like dental plaques.

BIOFILM (aggregationof microorganism)

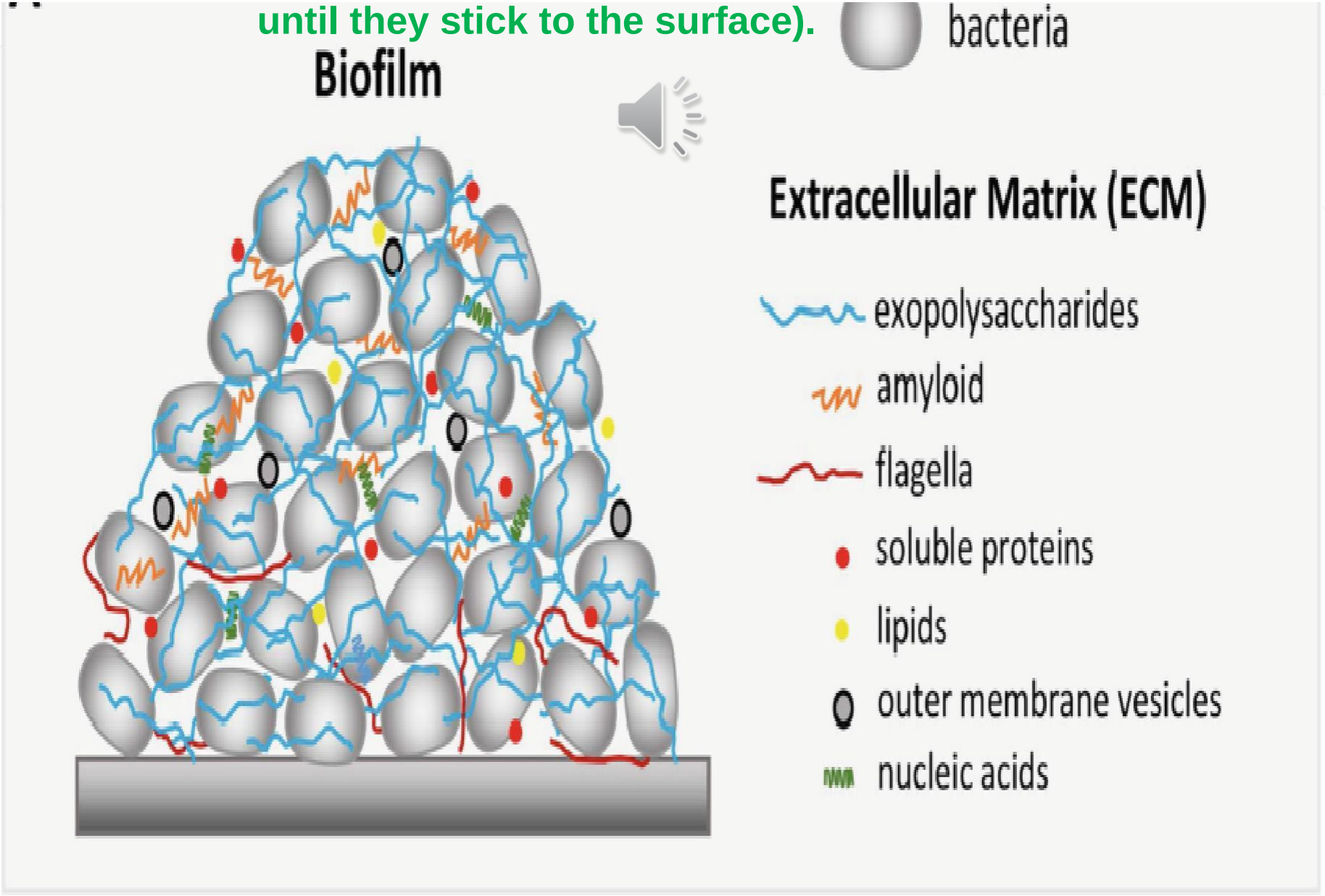
- is a layer of prokaryotic organisms that have aggregated to form a colony. The colony attaches to a surface with a slime(لزج) layer which aids in protecting the microorganisms.
- Biofilms often form on the inert surfaces of implanted devices such as catheters, prosthetic, cardiac valves and intrauterine devices(LIKE IUD) اللولب .

Biofilm formation

- **Biofilm formation begins when freefloating microorganisms such as bacteria (they stick together) thencome 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.

(By of these components it works a slim layer until they stick to the surface).

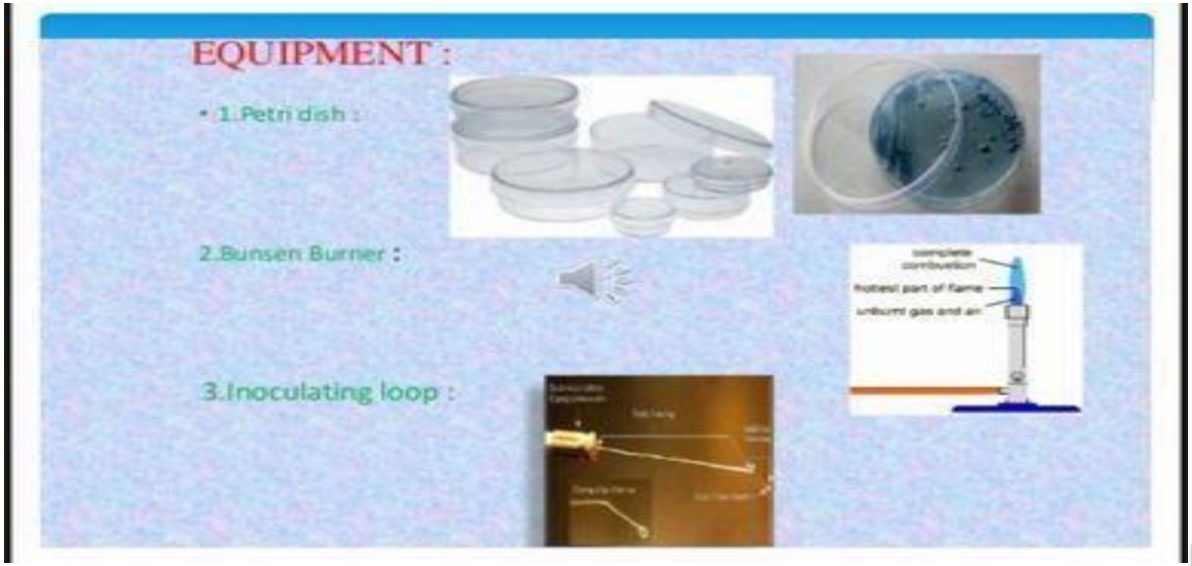


Growth requirements in the lab.:

1. Fastidious (PICKY من الصعب إرضائها oit requires a certain condition) 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.).

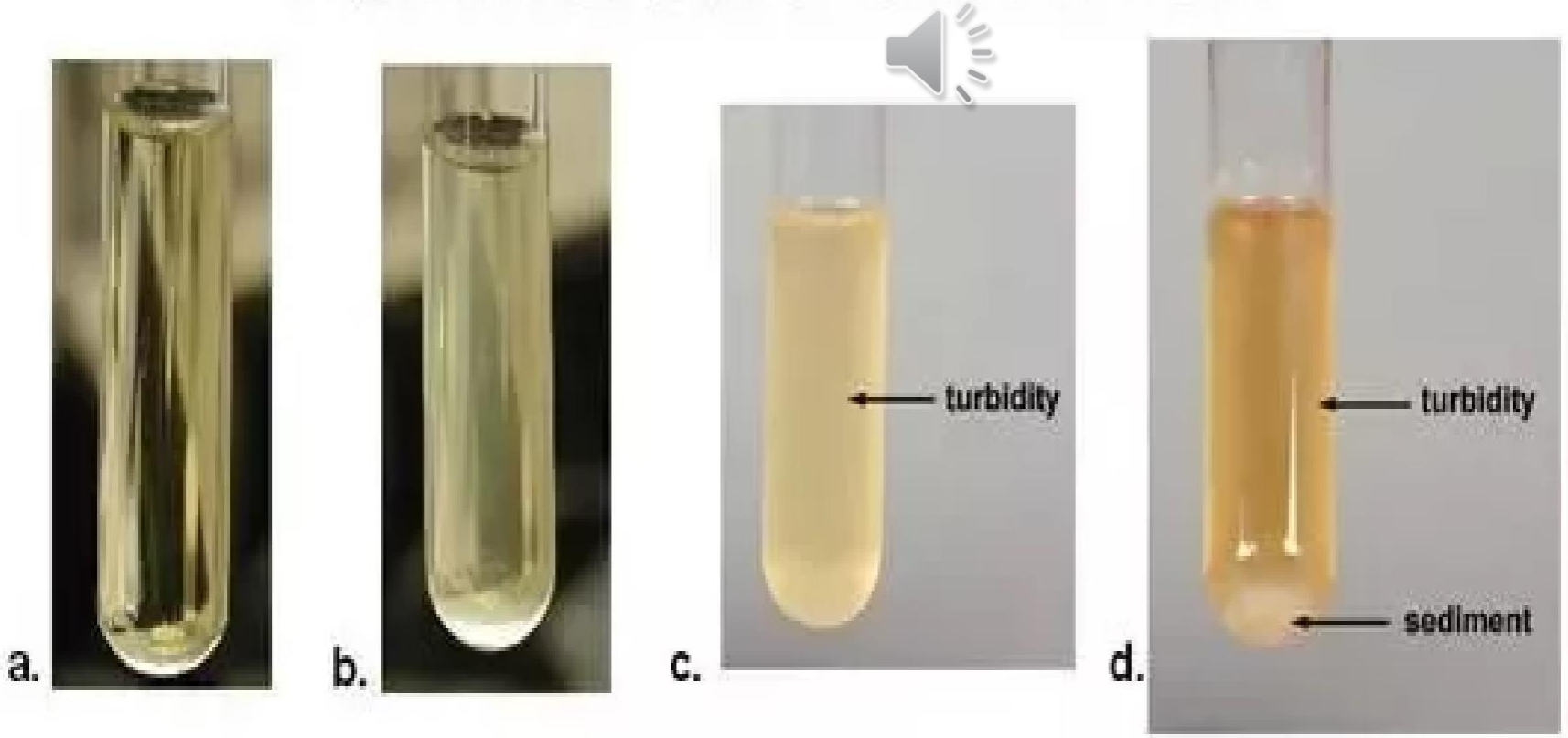
a. Chlamydia and Rickettsia : need tissue culture like viruses

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



Explain growth of bacteria in colonies (petri dish) in diffarnt media.

Bacterial Cultures in Broth Media

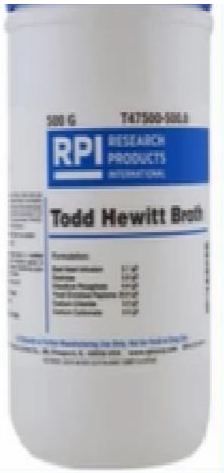


a. Sterile (uninoculated broth) - note how clear the media is
b. Broth showing slight turbidity (some bacterial growth)
c. Broth showing significant turbidity (a lot of bacterial growth)
d. Broth that hasn't been agitated (shaken)

Cultivation of bacteria

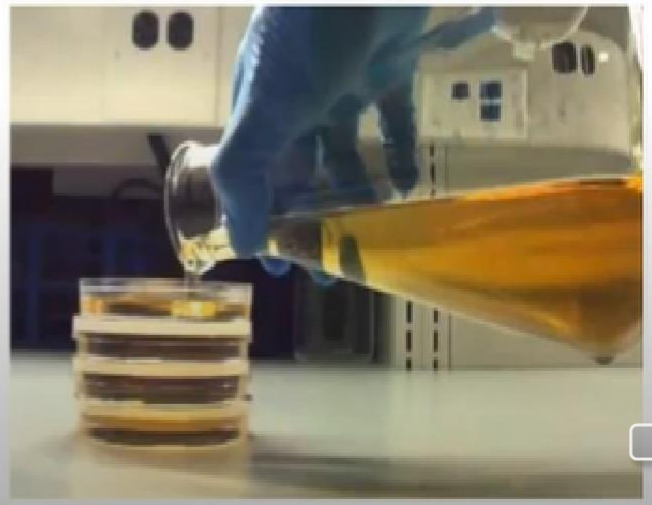
- To propagate bacteria in culture, **nutrients in the medium must provide the building blocks as well as energy for growth of the specific bacteria**. (Carbon, sulphur, nitrogen, phosphorus, minerals, growth factors).

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



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.



Making agar plates. When the temperature cools down the liquid medium turns solid in petri dishes

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

NOTE;

blood agar detect hemolysis Such as alpha ,beta and gama.

Chocolate agar (reason of naming):it is a dark brown color like chocolate.

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

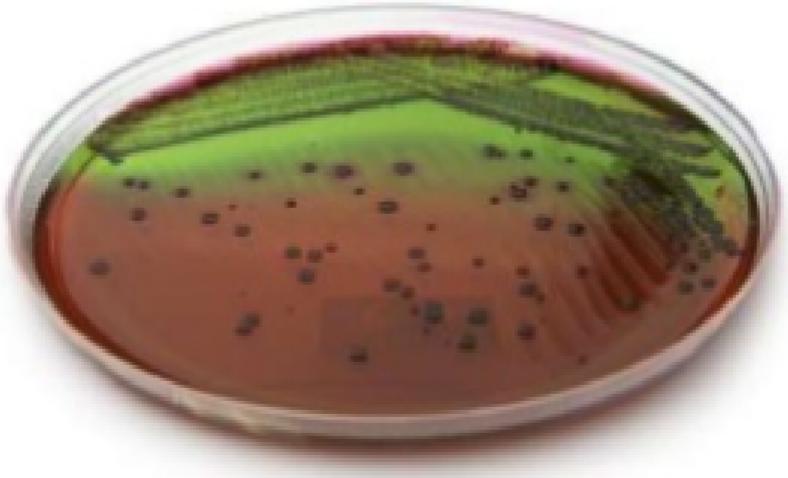
selective for *Neisseria gonorrhoeae*

- 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.***

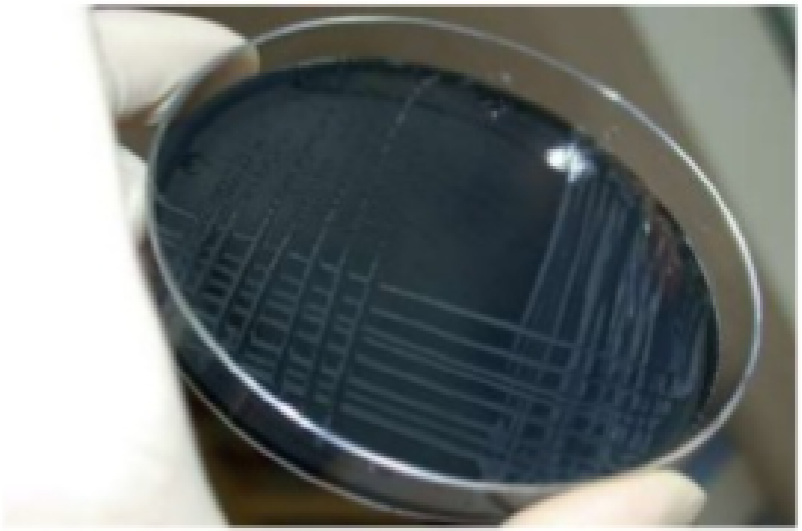
Eosin methylene blue

- 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.

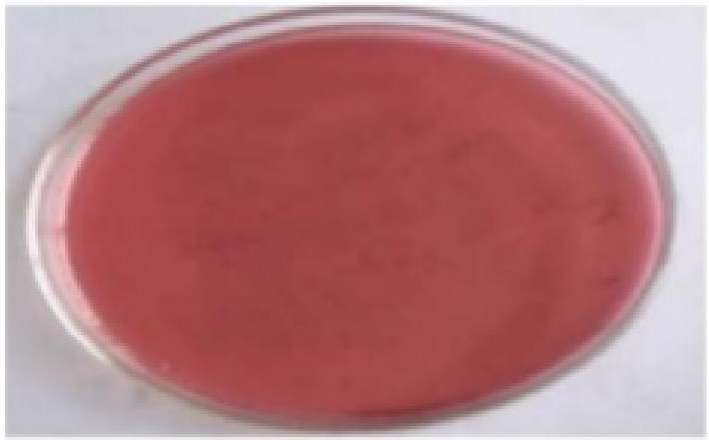
Examples:

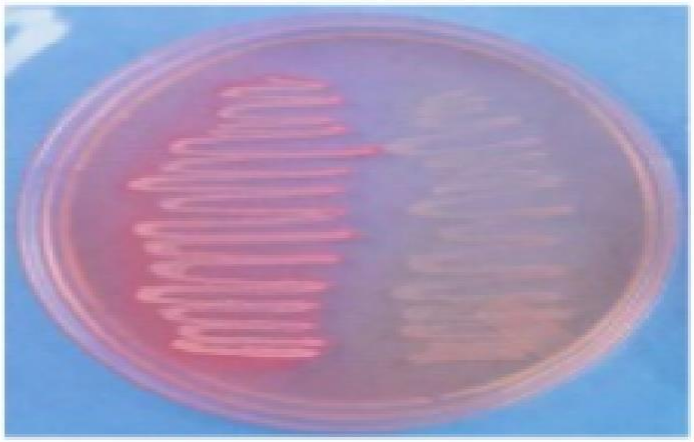
- **MacConkey agar**
- **CLED agar**
- **TCBS agar**
- **XLD agar**

Examples

MacConkey medium

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

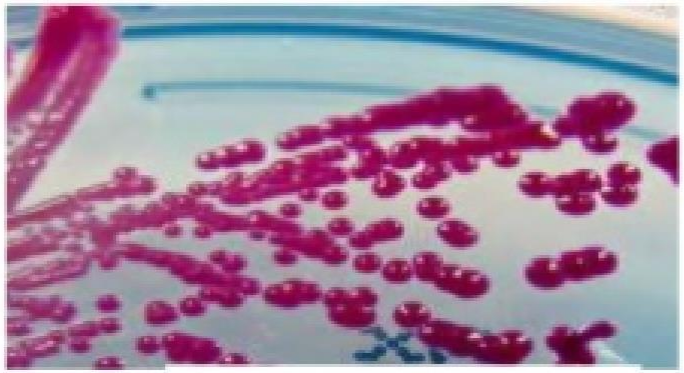




Examples

Cysteine Lactose Electrolyte Deficient Agar(CLED)

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



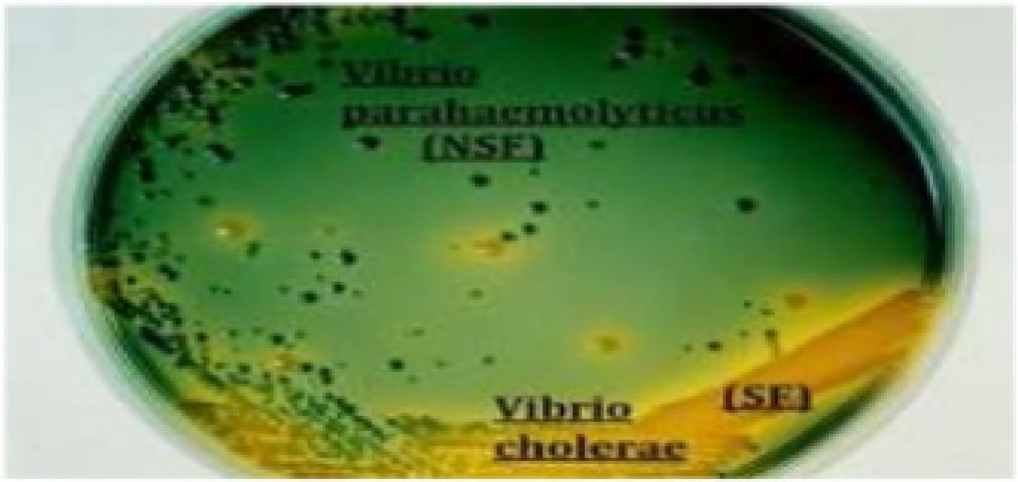
CLED, serratia

CLED , e-coli

Examples

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

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

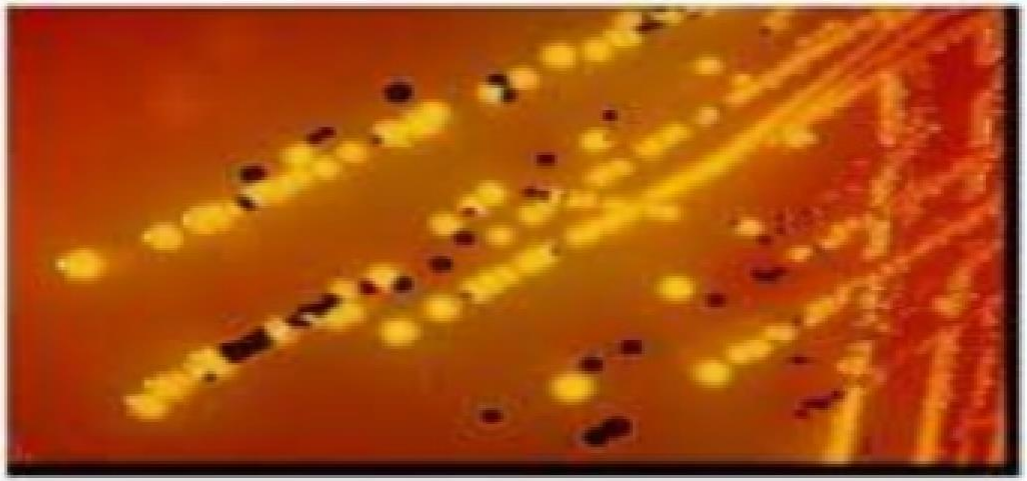


Yellow coloured (sucrose fermenting) colonies of Vibrio cholerae on TCBS agar.

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**

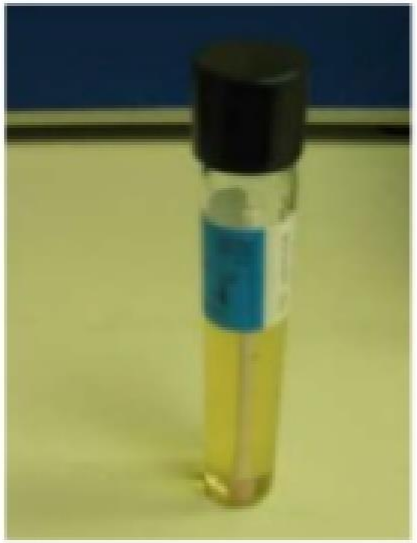
Anaerobic media

- These media are used to grow anaerobic organisms.

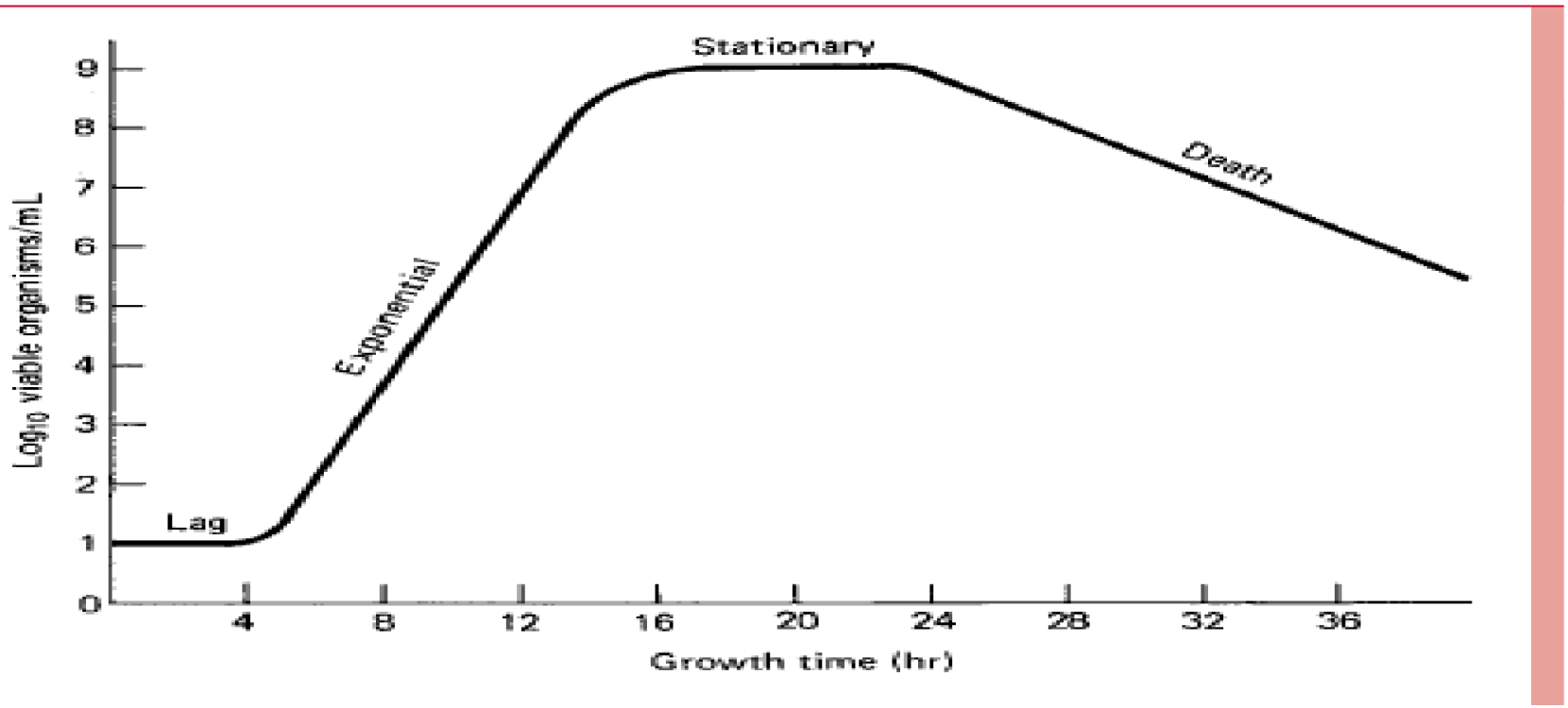
Eg:

- Robertson's cooked meat medium.

- Thioglycolate broth medium.



All bacteria are similar to these stage , but they differ in duration of the stage , and this depends on organism and culture 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.(it prepares itself to produce cell components)

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. (stage of divisions) 4 2 يزداد العدد بمقدار ثابت

8 3. Stationary phase, there is an equilibrium between

cell division and death (nutrients start to deplete & toxic materials start to be produced).(nutriuntis almost consumed)……rate of divisions =rate of death.

4. Death (decline) phase, the number of deaths exceeds the number of new cells formed.(growth is negative because death is more than living)

- **Extending log phase**

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

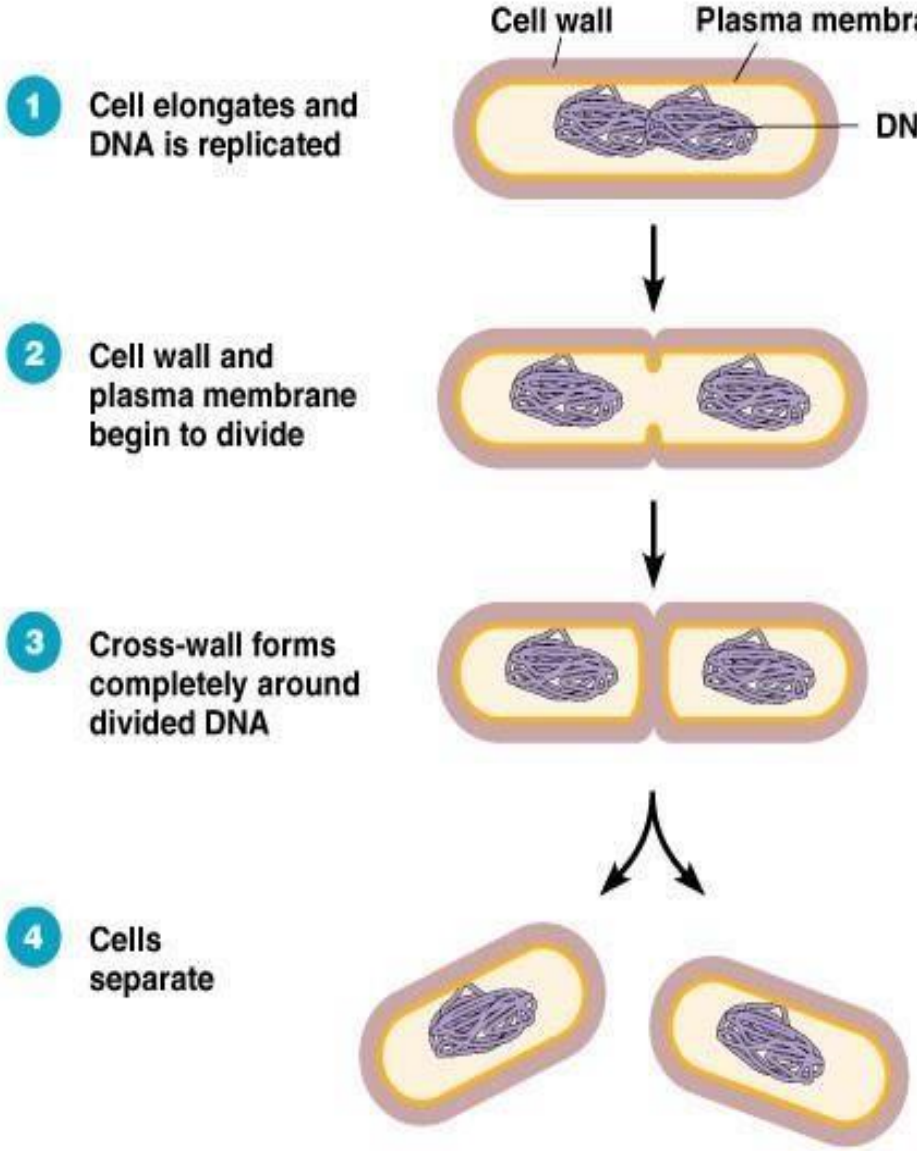
Chemostat: (chemical environment is static) : cells of a growing culture are harvested continuously and nutrients replenished continuously

Bacterial division

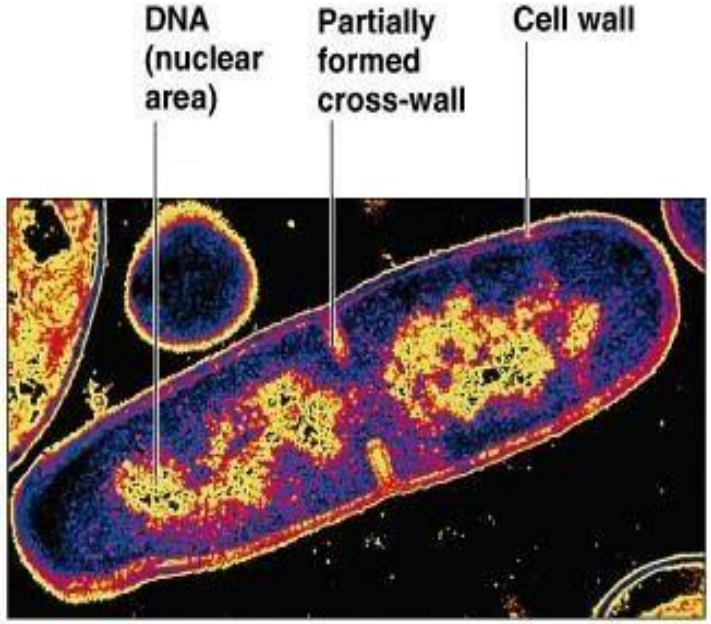
- The reproduction method of bacteria is binary fission, in which a single cell divides into two identical cells. Some organisms reproduce by budding, aerial spore formation or fragmentation.

- 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.