## Bacterial growth

Before we start, we have some good news!

## Growth

Doctor ala'a said that she is going to exclude some of the upcoming weeks material from the exam. Cheer up! :

## 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. To defect if the infection is bacterial lif
colonies grow on the petri dish) or caused by other reasons e.g. viral(colonies don't
grow even after 2-3 days).
In the laboratory, growth is used as central technique for detection
 identification and for assessment of antibiotic effects.
after the detection, if the infection is bacterial, we identify and after identifying the bacteria, we choose recognize the bacteria we are dealing with. the suitable anti-biotic to work against it.
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: (They are visble to the naked eye after culture) 1. By the development of colonies, the macroscopic product of 20-30 cell divisions of a single cell.
-We use a petri dish that contains the bacteria inside a medium which is made semi-solid by adding agarose to make it ready for culture.
2. By the transformation of a clear broth medium to a turbid suspension of 107-109 cells per ml .

- Broth = liquid medium - Turbidity in a test tube indicates bacterial growth.

3. In biofilm formation, in which growth is spread thinly ( $300-400 \mu \mathrm{~m}$ thick) over an inert surface.
(Next slide)
"Chemically inactive"

## BIOFILM

Thin
is allayer 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.' (Next slide)
Biofilms often form on the inert surfaces of implanted devices such as catheters, prosthetic, cardiac valves and intrauterine devices.
(اللولب)


## Biofilm formation

* Biofilm formation begins when free-floating microorganisms such as bacteria come in contact with an appropriate surface and begin to put down
- Using
their
flagella
and pili roots, so to speak. This first step of attachment occurs when the microorganisms produce a gooey "sticky" 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.


## Biofilm



## Extracellular Matrix (ECM)

-u exopolysaccharides ww amyloid
~m flagella

- soluble proteins
- lipids

0 outer membrane vesicles
m nucleic acids

## In the laboratory

## Extra slide



## In the laboratory

1. Fastidious organisms require many nutrients.(Demanding organisms "مدلعة")
2. Simple requirements can make everything from scratch.
3. selective (enrichment) with indicator. -Classes of culture media will be explained
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.Petridish:
Or test tubes
(if it is a broth media)


## 2.Bunsen Burner :

3. Inoculating loop :


These petri-dishes shows that depending on the media inside it, bacteria could appear differently.


## Bacterial Cultures in Broth Media



## Cultivation of bacteria

- We take the specific ingredients we need from the container (1st photo) and then we either add agarose to create the semi-solid medium (agar plate) or add water to make a broth medium .
- 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.
"نسبة الهواء"


```
Components
Componenis (gl):
BeeflHearl Infusion 500.00
Peptic Digest of Animal Tissue 20:00
Dextrose 2.00
Sodum 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.


## 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. The bacteria that require special needs "the demanding bacteria".
- eg: Blood agar, Chocolate agar

-The red colour is gained by ( $X$ factor) Blood agar produced by RBCs. BAP contains mammalian blood (usually sheep or horse) typically at a concentration of 5-10\%, used to isolate fastidious organisms and detect hemolysis.
- Depending on the type of hemolysis ( $\alpha, \beta, \gamma$ ), we can identify and classify some bacteria( e.g. streptococci).


Contains chocolate? NO! 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 Hoemophilus influenzae

- It gains its colour because of the presence of ( $V$ factor) that is produced by the breaking down of RBCs at $80 \mathrm{C}^{\circ}$ alongside ( $X$ factor) which is produced by RBCs.


## Selective media

- The inhibitory substance is added to a solid media to inhibit
commensal or contaminating bacteria such as :
(Bacterial normal flora)
- Antibiotics
- Dyes
- Chemicals
- Alteration of pH


## Examples

## Thayer Martin medium

selective for Neisseria gonorrhoeae (It is a Gram negative bacteria).

- It usually contains the following combination of antibiotics:
-It gets rid of everything but Niesseria Gonorrhoeae using these 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.
-Its a gram negative bacteria that swarms the petri-dish and takes over it. Disturbing the observation and not allowing us to see the microorganism we want to see.



## 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.

$32 x$


## 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 (Colour dependant)
- 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
-Can media be differntial and selective at the same time?
Yes, remember the definitions
A bacteria could be selective (for 1 or more types
of bacteria) and differntial (gives a specific
colonies colour).
It could be enrichment and selective media too.


## Examples

## MacConkey medium (Contains lactose)

- 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.



## Examples

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

- highly selective for the isolation of $\underline{\mathrm{V}}$. cholerae and $\underline{\mathrm{V}}$. parahaemolyticus
- Was used in cholera outbreaks.


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
-For the survival of the bacteria in
transport until it reaches the lab.



## 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. -The goal of spores in fungi differ from that in the bacterial spores. - In bacteria, its for survival.

- Bacterial division: -In fungi, its for reproduction.

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

1. Cell elongates and DNA is replicated


2 Cell wall and plasma membrane begin to divide


3 Cross-wall forms completely around divided DNA

(4) Cells separate

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

## SUI!

