Microbiology

L1

small life science

* medical microbiology :

is a science of studying micro-organisms (too small to be seen by naked eye) which associated with human disease, their activities and their influences on different aspects of life.

* Benefits :

1. food industry : fermentation of products (bread, wine, cheese, yogurt, vinegar)
2. industrial applications : for modern biotechnology such as genetic engineering, insulin, amino acids, vitamins, antibiotics, vaccines
3. sewage treatment : recycling water
4. recycling vital elements in the environment : N_2, C, O_2, S, P, ..

* harmful :

1. food spoilage
2. diseases

* Pathogenic microorganisms : microorganisms that cause a disease.

* Portal of entry :

- respiratory : inhalation
- alimentary (GIT) : ingestion
- Genital tract : sexual contract
- skin : abrasion, bites
- others : conjunctiva, blood transfusion, injections and organ transplants.
- congenital infections (vertical transmission).

Short history :-

① Antony van leeuwenhoek :-

- microscopist
- father of microbiology
- the first to observe live microorganisms in water mud and saliva

② John Hunter :-

- surgeon
- the leading authority on veneral disease
- he belived that syphilis and Gonorrhea were caused by a single pathogen.

③ Edward Jenner :-

- physician and scientist
- pioneered the concept of vaccines
- creating the smallpox (الجدري) vaccines ((the world's first vaccine))

④ John snow :-

- physician
- known for locating source of cholera outbreak in london (establishing the disease as a water-borne)
- one of the founders of modern epidermology.

⑤ Ignaz semmelweis :-

- physician and scientist
- early pioneer of antiseptic procedures
- savior of mothers
- he discovered that the incidence of Puerperal (حمى النفاس) sepsis can be prevented if the attending nurses apply hygienic measures.
- Hand washing stops infections.

6 louis pasteur :—

- biologist , microbiologist and chemist

- discovered the principle of fermentation of alchohol by microorganisms
- Invent a technique of treating milk and wine to stop bacterial contamination , a process called pasteurization.
- Create the first Vaccins of rabies (داء الكلب) , Bacillus anthracis (الجمرة الخبثية)

* louis Pasteur and Germ theory

- he performed numerous experiments to discover why wine and dairy products became sour , and he found that it was the bacteria and he stirred scientists to think that if bacteria could make the wine "sick" then perhaps they could cause human illness . But his attempts to prove the germ theory were unsuccessful: Robert Koch provieded the proof by cultivating anthrax bacteria apart from any other type of organism

7 Robert Koch :—

- developed microbiological medica & streak plates for pure culture

* Germ theory (Koch's postulates)

- microorganisms must be present in every case of disease
- organisms must be grown in pure culture from the diseased host
- Inoculation of above into host must give same disease
- organisme must be recovered from experimentally infected host

8 Alexander fleming :—

- Physician and microbiologist
- discover the world's first broadly effective antibiotic ((Penicillin G)) from the mould penicillium rubens

9 kary mullis :—

- biochemist
- invent Polymerase Chin reaction (PCR).

10 Zur Hausen :—

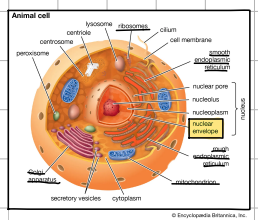
- virologist
- he has done research on Cancer of the cervix , so he discovered the role of papilloma virus
- this research directly made the development of vaccine "HPV" possible .

* the classes of organisms that can cause disease :-

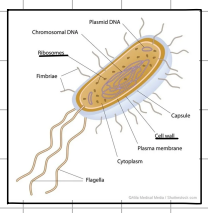
1. viruses
2. Bacteria
3. fungi :-
   a. yeast : unicellular
   b. molds : large multicellular
4. parasite :-
   a. protozoa : unicellular, vary in size, very small → intercecellular infection, large → extracellular infection
   b. Helminthes : multicellular, can reach several meters in length

* the classification of microorganisms :-

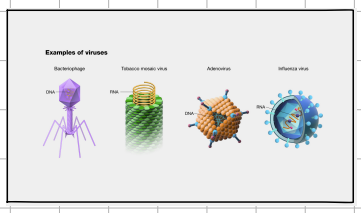
① Eukaryotic (true nucleus)



② prokaryotic (primitive nucleus)



© viruses : the next slide



Characteristic	eukaryotic	Prokaryotic
nucleus	Yes	no
size	16 - 100 μm	0.05 - 10 μm
nuclear membrane	yes (nucleus)	no (nucleoid)
membrane-bound organelles	present	absent
chromosome membrane	multiple (linear)	1 (circular)
ribosomes	80s (40s - 60s)	70s (30s - 50s)
cell wall	absent except fungi (chitin)	present except mycoplasma
cell membrane	has sterols	no sterlos except mycoplasma
division	mitosis	binary fission

* Viruses :—

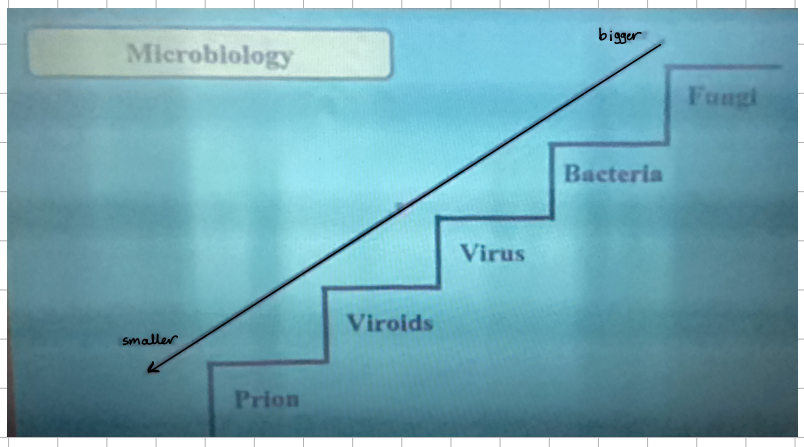
- acellular
- one of the smallest infectious agents
- no cell structure
- has DNA or RNA
- obligate intracellular
- directed host cell for replication

* Viroids :—

- single stranded RNA without protein coat
- only infect the plants
- smaller than virus

* Prion :—

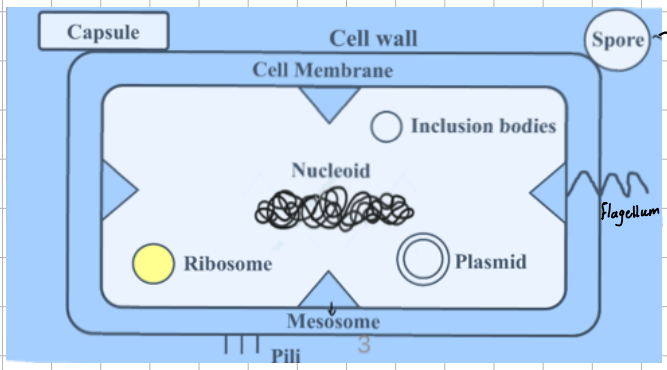
- protein without nucleic acid (infectious)
- the misfolded protein (from α-helix to B-sheet) will aggregate in the in CNS → spongiform in the brain
  → Creutzfeldt-Jakob disease (CJD) in human
- mad cow disease : bovine spongiform encephalpathy (BSE or mad cow disease) seen in cattle



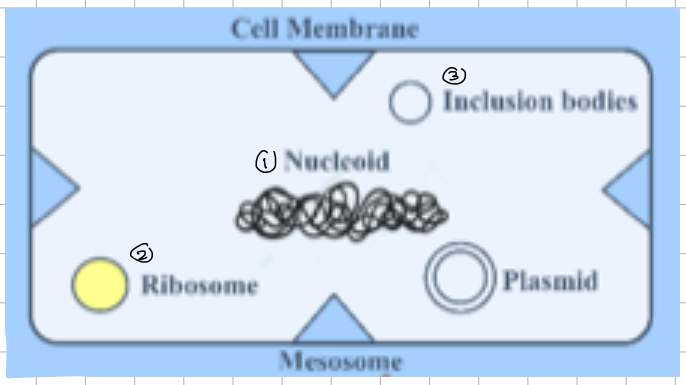
L2

Bacterial structure

* the Bacterial structure s—



* Intracytoplasmic structure



① nucleoid s

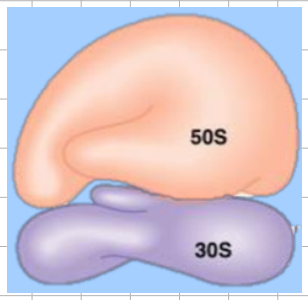
- essential
- single chromosome
- circular
- double stranded DNA
- 1mm in length
- super coiled
- carry genetic information for growth & survival

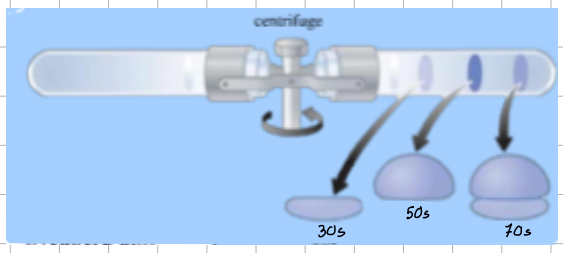
② ribosome :

- essential
- ribo : RNA , some : body
- site of protein sythesis
- bacterial ribosome : 70s → 50s large subunit
  ↳ 30s small subunit

S : svedberg unit .

*note : 70s , 50s , 30s refer to the densities





③ Inclusion granules (body) :

- store of nutrient : Glycogen , starch , phosphate.

* corynebacterium diphtheria stores the phosphate in it's granules that called volutin granule (Metachromatic granule)

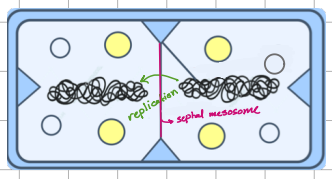
④ Cell membrane :

* definition :—
  - essential
  - thin , fragile membrane
  - located just under the cell wall
* composition :—
  - phopholipid bilayar (head & tail) + Proteins
  - no sterols (except mycoplasma : has a cell membrane with sterol)
* function :—

1 - selective trasport :—
  ① passive : from high conc. to low conc. .
  ② active : from low conc. to high con. , need energy .

2 - mesosome :
  ① has a respiration enzyme that make energy (like the mitochondria)
  ② cell division : seperate DNA , septal mesosome

2.



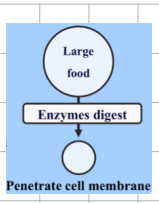
3 - biosythesis of cell wall

4 - excretion of extracellular enzyme (hydrolytic enzyme)

5 - excretion of extracellular enzyme (Penicillinase)

6 - chemotactic system (the substance bind to it's receptor on the bacterial membrane if it is a good subs. the cell membrane send a signal to the flagella to go toward it, and if it is bad the membrane send a signal to the flagella to go away.

4.



⑤ Plasmid :-

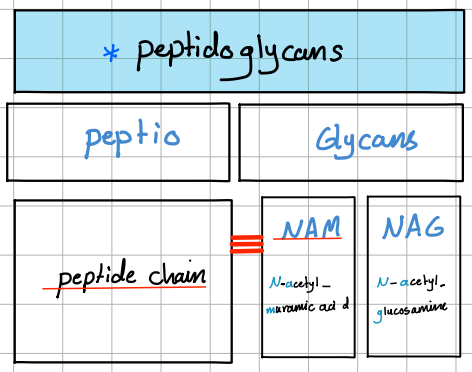
- not essential
- extra chromosal ds DNA
- replicate autonomously (independent of bacterial chromosome)
- toxin production , drug resistance

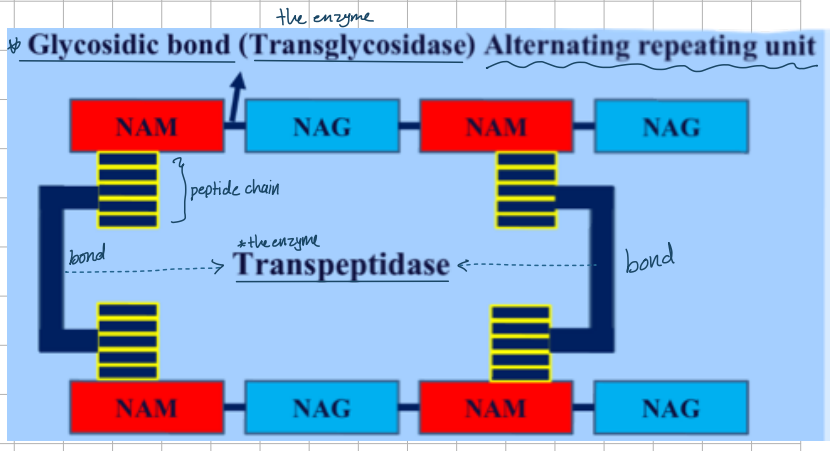
* Cell wall

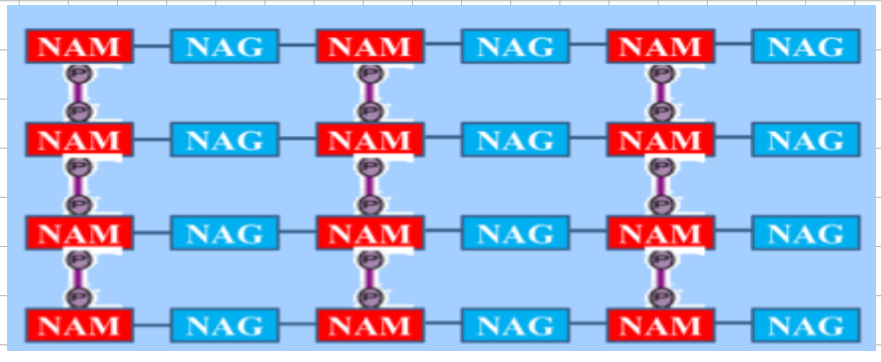
* definition :-

- surrounds the cell membrane (outermost layer) (not always)
- Rigid : comes from peptidoglycans

* compositions-

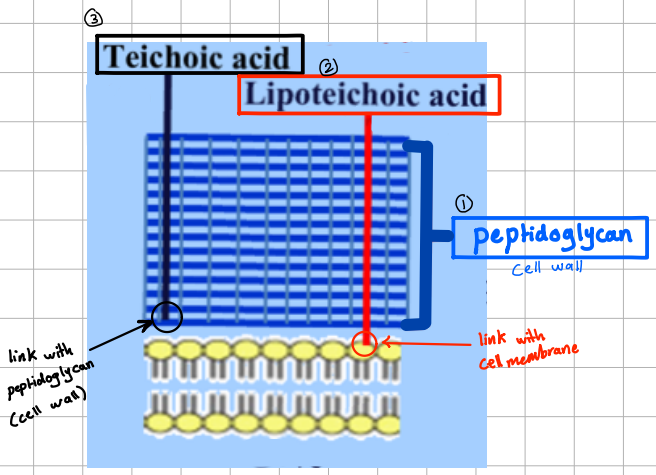






* Gram stain.

- There are 2 types of bacteria :— (depend on the differences of the cell wall)
  - gram positive bacteria
  - gram negative bacteria

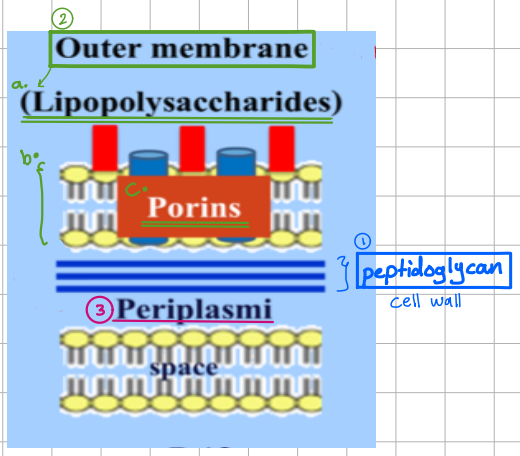


G +ve ⇝ no outer membrane

[1] peptidoglycan :—
- (50%) thick
- (NAM - NAG)
  ↓
  peptide

[2] lipoteichoic acid :—
- link with the cell membrane

[3] teichoic acid :—
- polymers of glycerol or Abitol
- linked with the cell wall
- major surface Ag (antigen) of G+ve
- highly immunogenic (it cause the immune response and release) :
  - ① TNF-α
  - ② IL-1
- responsible for Toxic shock



G -ve ⇝ no teichoic acid & lipoteichoic acid.

[1] peptidoglycan :—
- (5%) thin layer
- 2 sheets of (NAM - NAG)
  ↓
  peptide

[2] Outer membrane :—

a. bilayer phospholipid :

b. lipopolysaccharides :
- ① lipid A (endotoxin)
- ② polysaccharides (somatic O Ag)

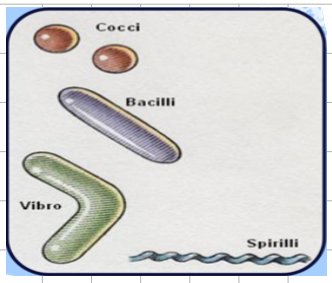
c. porins :
- hydrophobic proteins
- in the outer membrane
- transportation.

[3] periplasmic space :—
- space between cytoplasmic & outer membrane
- composed of peptidoglycan layer & gel-like proteins

* Function :-

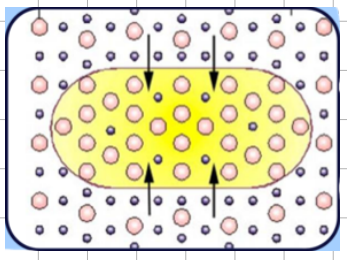
1. maintenance of the shape (rigid)
   - deficient of cell wall → polymorphic → e.g. mycoplasma.

1.



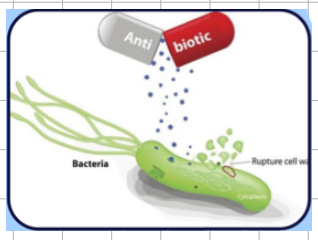
2. protection & Osmosis insensitive.
   - the cell membrane can't hold the osmotic pressure, so there is a cell wall.

2.



3. target site for antibiotics :
   - Penicillin & cephalosporines work on the cell wall.

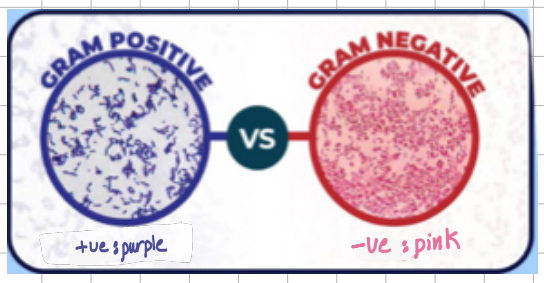
3.

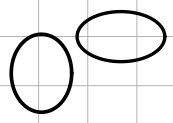
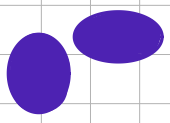
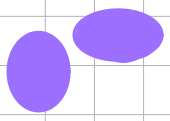
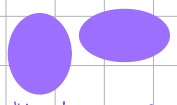
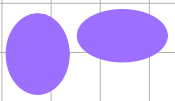
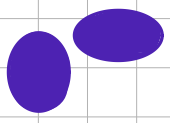
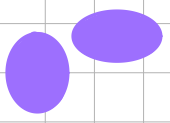
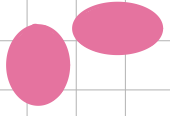


4. role in cell division

5. responsible for staining :

5.



G+ve 			 * Nothing changes cuz G+ve bacteria has a thick cell wall (acetone doesn't enter the cell)	
fixation	① Crystal violet	② Iodine * For color fixation	③ Asetone * For decolorisation	④ Counter stain safranin is pink
G-ve 			 * it's colorless cuz G-ve has a thin cell wall and the outer membrane dissolve in alcohol, so the acetone enters the cell	

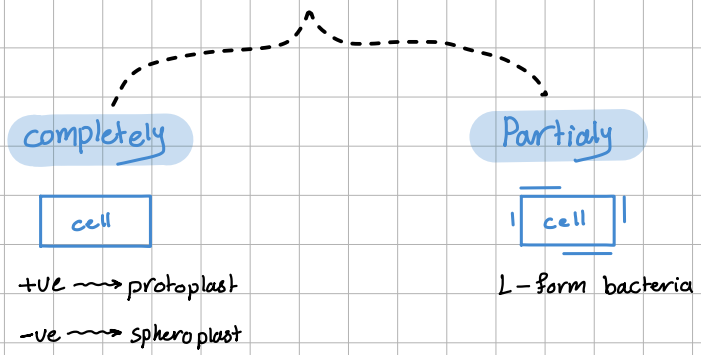
* Bacteria without cell wall :—

① naturely :—

- mycoplasma (has sterol)

② Induced :—

- cell wall inhibitors
- Lysozyme



Q. Do L-form & mycoplasma resist to penicillin & cephalosporines ?!

yes, because we use antibiotics work on the cell membrane and these bacteria don't have cell wall.

* structures outside the cell wall :—
  - capsule.
  - Flagella.
  - Pili.
  - spore formation.

* Capsule :—

- definition :—

- Glyco calyx
  carbohydrates enveloped
- Gelatinous (viscous) layer covering cell wall of some bacteria
- extra layer

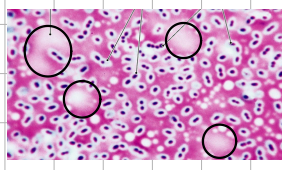
- composition :—

- usually polysaccharides ⇝ exception : B. anthracis it's capsule made of poly peptide
- the variation of capsule ⇝ depend on "different arrangment of polysaccharides"
  ⇝ e.g. (Str. pneumoniae) → has 91 types

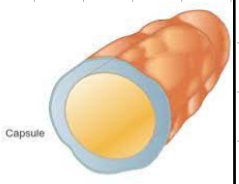
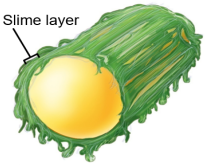
sucrous - mannose - lactose
mannose - sucrose - lactose
mannose - sucrose - mannose

* the different arrangment

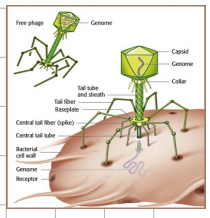
- don't stained by Gram stain
- Quellung reaction (swelling) ⇝ when an antibodies (specific to the capsule) are added to the bacteria and bind with it, it's capsule will swell.
  this reaction is used for identification of bacteria that have a capsule.



* unstained halo around the organisms

	the binding to the cell wall	the Adherence to surface organism	
Capsule	tightly & organized	firmly	
Glycocalyx	loosely & unorganized	firmly * has fibrils extending, it adhere firmly to skin, heart. e.g. strept. mutans	
Slime layer	loosely & unorganized	loosely	

Capsule	Slime layer
Capsule is a glycocalyx layer, consisting of firmly associated polysaccharide molecules with the cell wall	Slime layer is a glycocalyx layer that consists of loosely associated glycoprotein molecules
Composed of polysaccharides	Composed of exopolysaccharides, glycoproteins, and glycolipids
Thicker than the slime layer	A thin glycocalyx layer
Tightly bound to the cell wall	Loosely bound to the cell wall
Well organized layer; difficult to be washed off	Unorganized layer and can be easily washed off
Acts as a virulence factor that helps to evade phagocytosis	Mainly aids in the adherence; also protects the cell from dehydration and nutrient loss

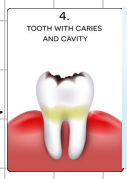


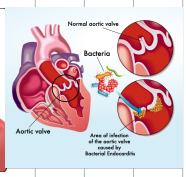
- Functions :-

- protect cell wall from ① bacteriophage : virus can infect the bacteria and it has receptors on the bacterial cell wall , but because of capsule , the virus won't be able to bind with the cell wall
  ② complement : complement components bind to the bacterial cell wall but because of capsule , they won't be able to bind with the cell wall
  ③ lysosomes : breakdown the bacterial cell wall but the capsule will protect it

- Prevent phagocytosis (virulence) اسلحة دفاع

- attachment of Glycocalyx by using it's fibrils leads to fermintation of sugars to acids , like what happens in : ① dental caries ② prosthetic heart valve.





- development of vaccines

the capsule is formed when the bacteria enters the host cell (vivo : living cells)

* Flagella :-

- definition :-

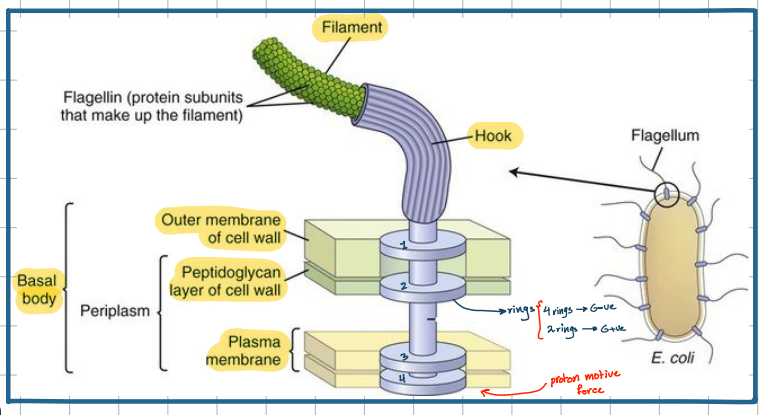
- long thick threads like filamentous formed from protein (flagellin : H antigen)

- seen by EM (20nm)

- distribution of flgella : ① polar spiral → monotrichous / amphitrichous / lophotrichous
  ② peritrichous (around) → e.g. salmonella typhi

- Function :-

- the organ of motility
  - the "proton motive force" provides the rings energy to move , so flagella moves , leads to motility of the bacteria
  - motility of G-ve (4 rings) more active than G+ve (2 rings)



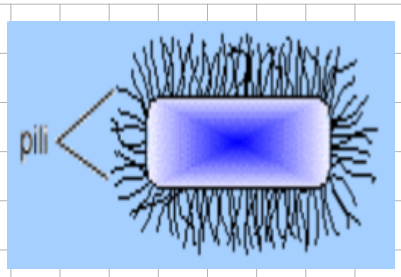
- tactic responce (taxis)

the agents bind to it's receptor on the bacteria , if it was good agent the bacteria will send signals to the flagella to go toward it (+ve) chemotactic response , and if it was harmful agent the the bacteria will send signals to the flagella to go away from it (-ve) chemotactic response .

* if the stimulating agent was chemical → the tactic respone will be chemotaxis
* = = = = light → = = = = phototaxis

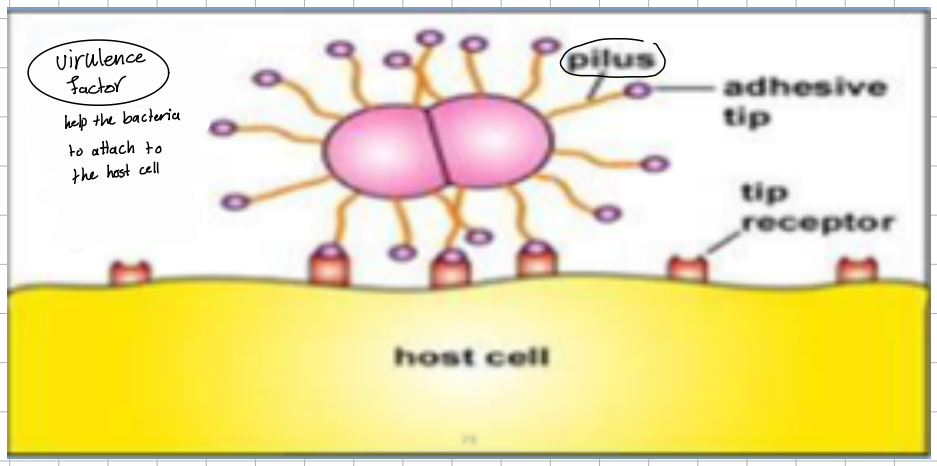
Endoflagella :-
some bacteria has flagella inside them (axial filament)
e.g. spirochetes

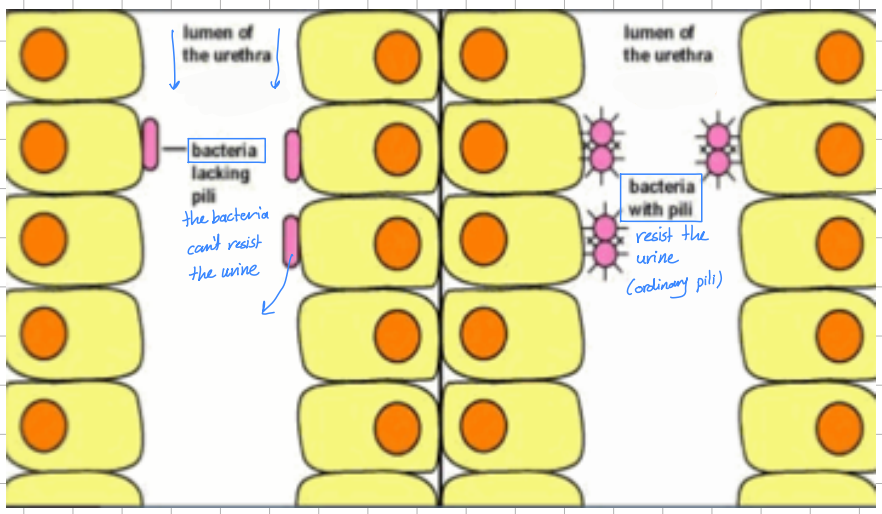
* pili 8



- short and thin
- hair like formed from protein (pilin)
- seen by EM
- 2 types 8 ① ordinary pili (attachment)

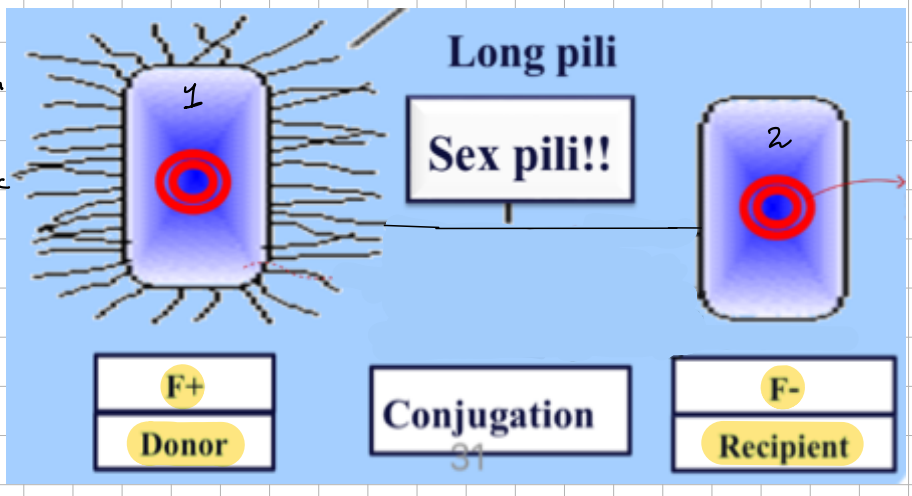
urine





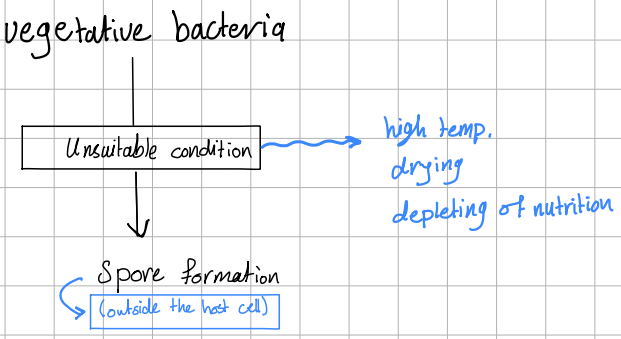
② sex pili (conjugation)

- long pili
- bacteria number 1 has genetic material with a specific properties, make a copy of the genetic material and give it to bacteria number 2 by "sex pili"



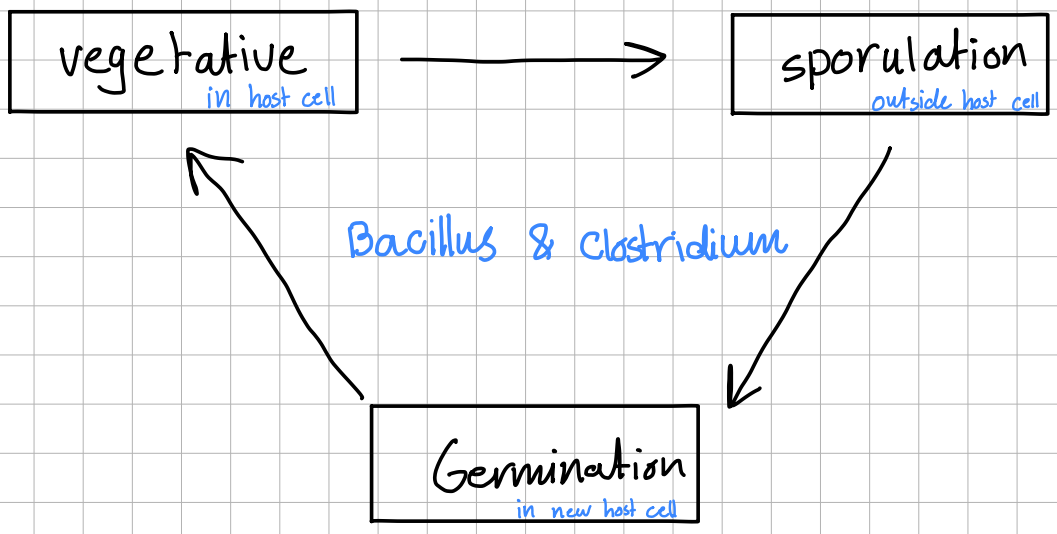
* spore formation :-

- when bacteria enters a host cell, it start to devide,... which called vegetative phase.



spore : highly resistent resting phase (endospores) in VITRO (outside the host cell)
no division, no reproduction,... .
- highly resistent to dryness, heat & disinfectant
e.g. Bacillus & Clostridium

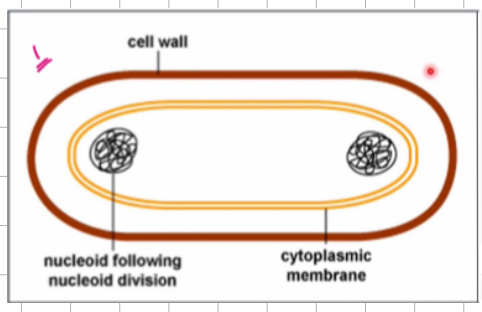
* can't stained by ordinary stain



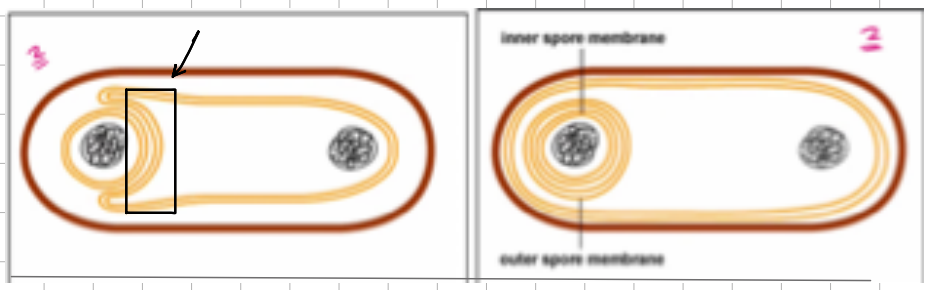
From mariam ayasrah modified slides ☺

* the steps :-

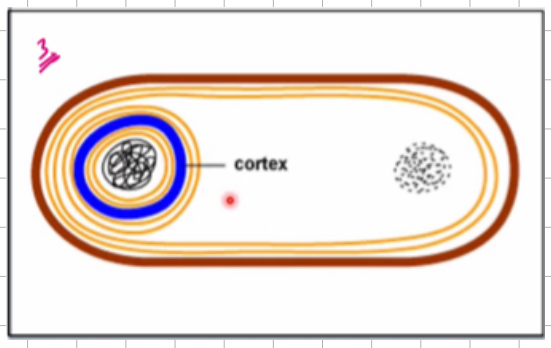
1 DNA replication



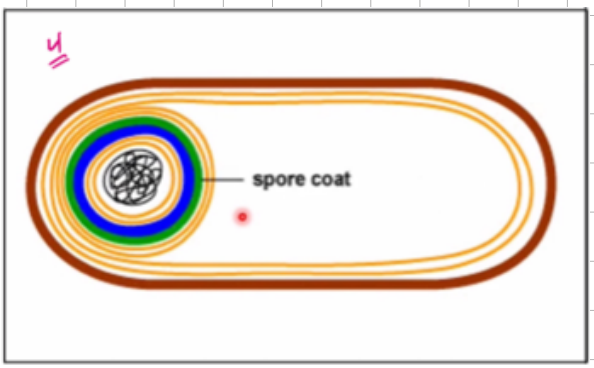
2 formation of multiple layes of cell membrane & peptidoglycans



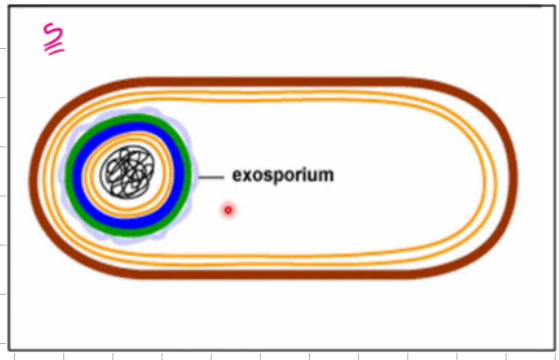
3 produce Ca^{+2} & dipclonic acid forming a hard layer called "cortex"



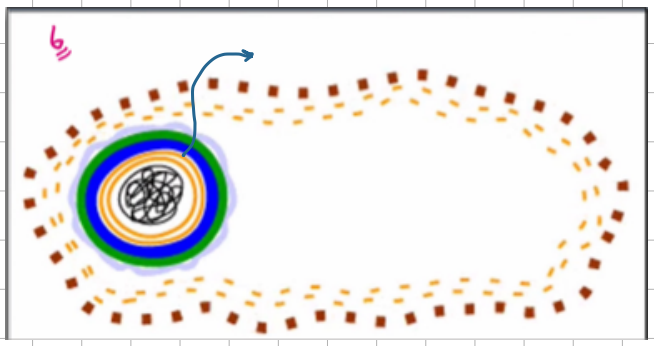
4 formation of spore coat (from more than 80 types of proteins)

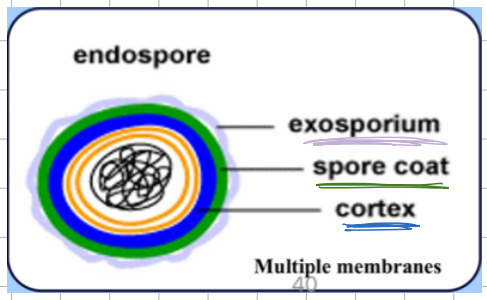


5 formation exosporium
(collagen-like glycoprotein)



6 get out of the cell and live for many years





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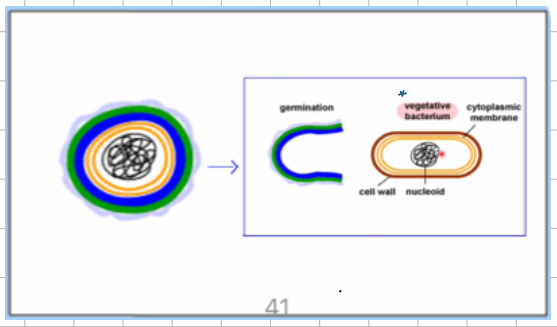
when it enters a new host cell



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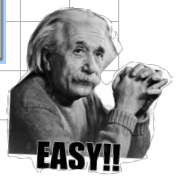

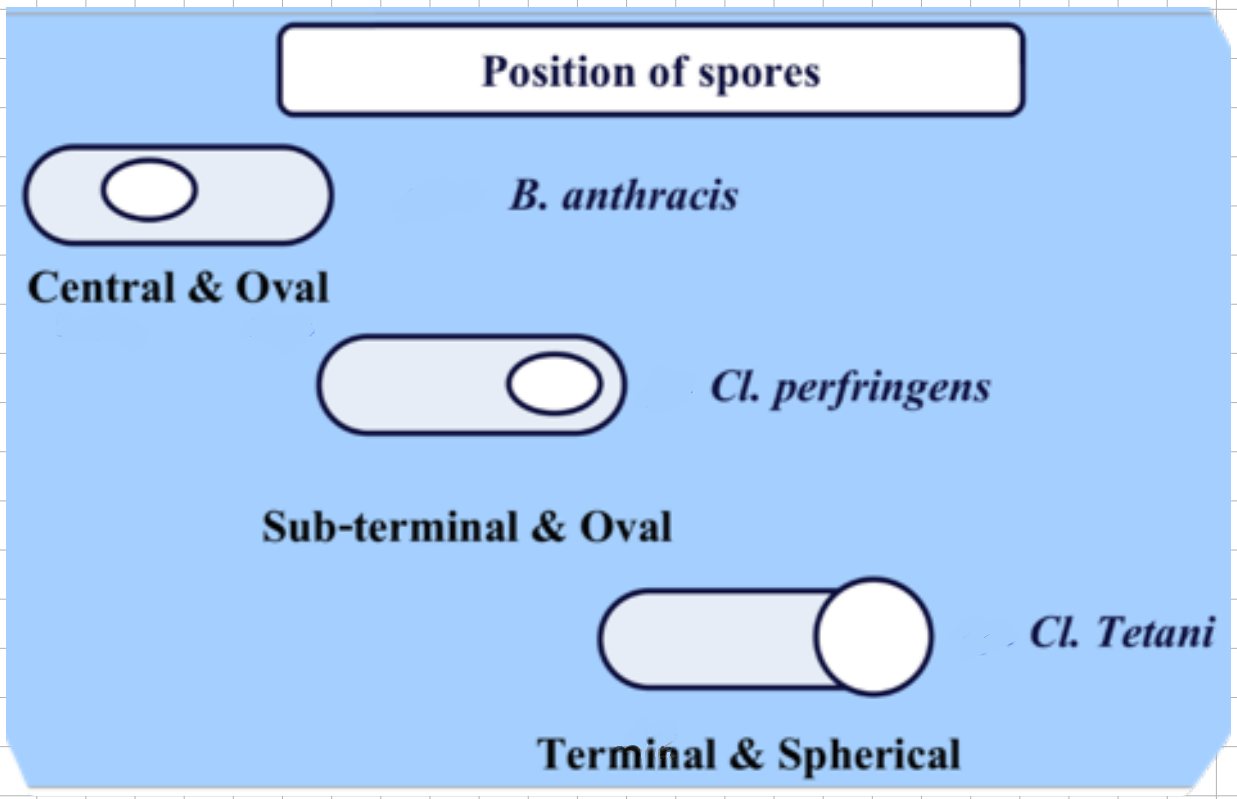

end of Lec 2.

LoSh