General Microbiology

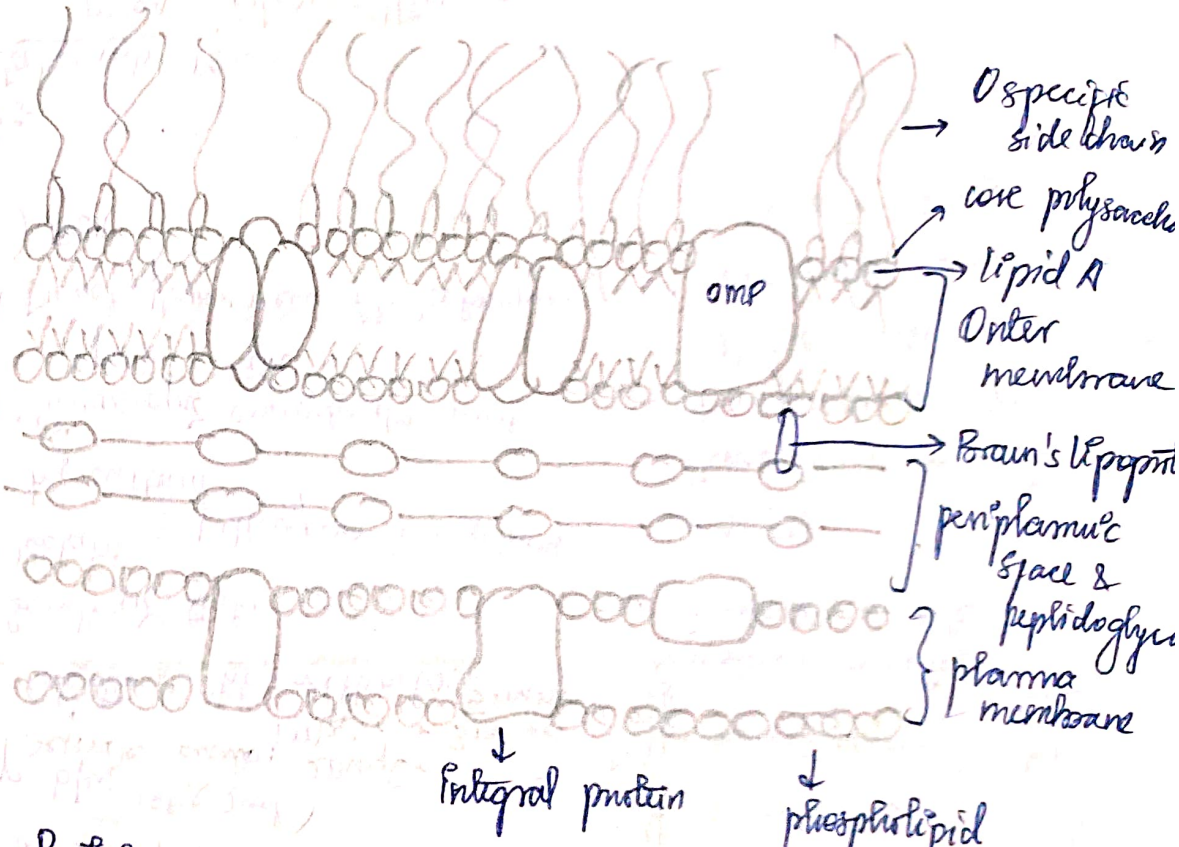
Louis Pasteur -

- Father of Microbiology ✓
① Principles of fermentation ✓
② Sterilization techniques → steam sterilizer → hot air oven → autoclave
③ Pasteurization of milk
④ He disproved the theory of Germ theory of disease

Robert Koch:

→ Solid media - culture of bacteria
→ Agar - solidifying agents.
→ Pure culture - isolation of bacteria
→ hanging drop - motility
→ Discovered bacteria - anthrax bacilli tubercle bacilli & cholera bacilli
→ Staining technique - Aniline dye

GRAM NEGATIVE CELL WALL



Peptidoglycan layer - thin (1-2 layer; 2nm thick)
made peptide chain similar to GPCW with alt. NAM & NAM. Differs from GPCW - (Absence of pentaglycine bridge. Tetrapeptide side chains are directly linked to each other by covalent bridges.)

Outer membrane: Attached to peptidoglycan layer by Braun's lipoprotein. Contains OMP - help in transport of smaller molecules.

LPS - lipid A (endotoxin - endotoxic Ag sites; core polysaccharide; O side chain)

Diff b/w GPCW & GNCW :-

Characters	GPCW	GNCW
Peptidoglycan Layer	Multilayer (16-80nm)	Thinner (2nm)
Pentaglycine bridge	Present	Absent
Lipid Content	Scanty	Present
LPS	Absent	Present (endotoxin)
Teichoic Acid	Present	Absent
Variety of AA	few	several
Aromatic AA	Absent	Present

Bacterial Growth Curve:

When bacterial count of a culture is determined at diff. intervals & plotted in relation with time — a bacterial growth curve is obtained.

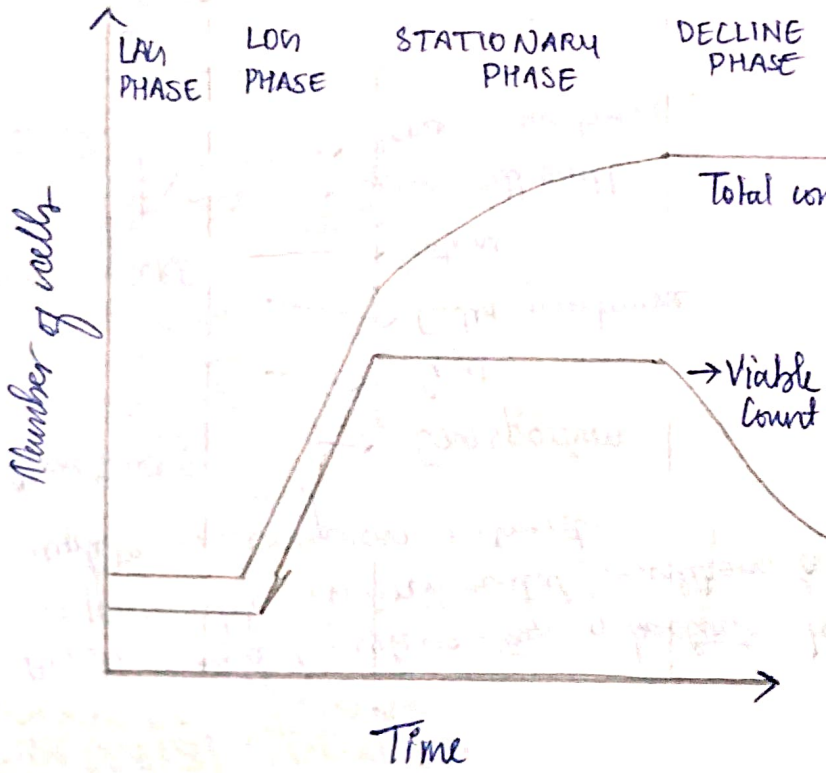
1) LAG PHASE : Period b/w inoculation & beginning of multiplication
- Bacteria ↑ in size due to accumulation of enzymes & metabolites
- Bacteria reach max size at the end of lag phase.

2. LOG PHASE : Bacteria divide exponentially. so that growth curve takes shape of a straight line.
- Bacterium - smaller in size, biochemically active, uniformly stained.

3. STATIONARY PHASE :- Bacterial growth almost ceases completely due to exhaustion of nutrients, accumulation of toxic products & autolytic enzymes.

Total count No. of viable cells = Stationary but total count keeps rising
1. Bacterium - becomes gram-VARIABLE.
2. more storage granules are formed.
3. Sporulation occurs
4. Bacteria - exotoxins, antibiotics & bacteriocins

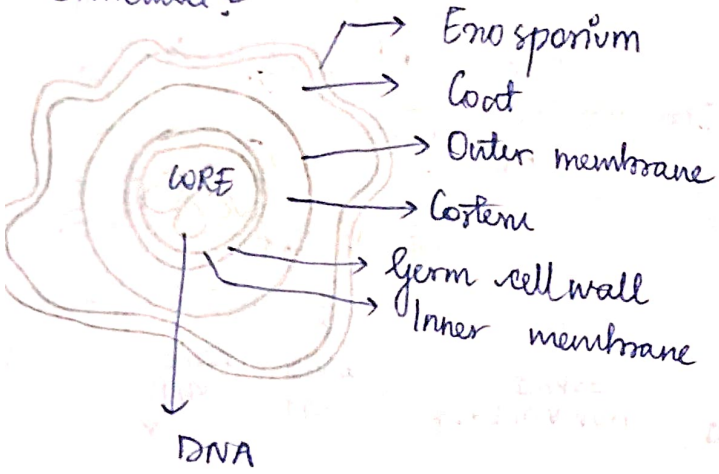
4. DECLINE PHASE - Bacteria stops dividing.
Decline in viable count & not in total count
Involution forms are seen



Bacterial Spores :-

Highly resistant resting stage of bacteria formed in unfavourable environmental conditions as a result of depletion of exogenous nutrients.

Structure :-



Sporulation - process of formation of spores from the vegetative bacteria.

Germination - transformation of dormant spores into active vegetative cells. when grown in nutrient rich medium.

Sporicidal Agents :-

Sterilants :- Autoclave

Ethylene oxide sterilizer

Demonstration -

1) Gram staining - spores appear as unstained refractile bodies within the cell.
2) Modified Ziehl Neelsen staining : 0.25 - 0.5 % sulphuric acid

Capsule

Amorphous viscid material lying outside the cell wall called capsule.

Capsule → unorganised substance loose material

↓

Slime layer

Examples :- pneumococcus, meningococcus, hemophilus influenzae, klebsiella pneumonia

Uses :- (1) Bacterial virulence -

⇒ Capsule protects the bacterium from phagocytosis & from the action of host cell lysozymes.

⇒ helps in biofilm formation (helps in bacterial adherion)

(2) Bacterial identification - Capsular Ags can be used for identification & typing of bacteria.

(3) Used as vaccine : eg pneumococcus, meningococcus, H. influenzae

Demonstration of Capsule :-

1) Negative staining by India Ink & Nigrosin Stain
2). M Fayderan capsule stain
3) Quelling Rxn.

Bacterial Flagella :-

Thread like appendages protruding from the cellwall.

Length : 3-20 µm ; Thickness :- 0.01 - 0.02 µm.

Arrangement :- Peritrichous - E.coli

Monotrichous - Vibrio cholerae

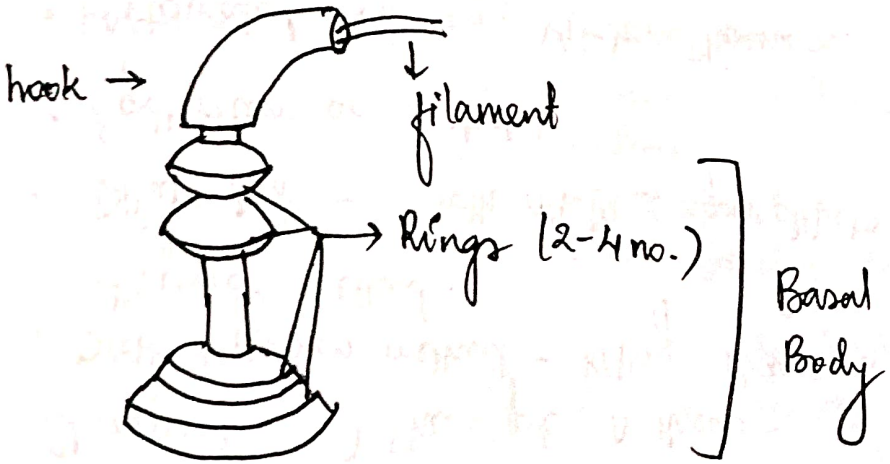
Lophotrichous - Spirillum

Amphitrichous - Alcaligenes faecalis

Ultrastructure :- filament, hook & basal body.

Detection :- Direct :- EM or tannic acid staining (Leifson's method)

Indirect :- motility by hanging drop method, DGM or TCM.



FIMBRIAE

Fimbriae or pili are short fine hair-like appendages that help in bacterial adhesion → ORGAN OF ADHESION.

Types 1) Common pili or fimbriae - Bacterial adhesion to epithelial surface. Helping in colonization. Present in gram negative & some gram positive bacteria.

2.) Sex pili :- bacterial conjugation
↳ Gram negative

Detection :-

Detected by EM or indirectly through formation of surface pellicle.
↓
thin layer formed at the surface of liquid culture.

ACID-FAST STAINING :-

Acid fastness is due to presence of mycolic acid in their cell wall.

Ziehl - Neelsen technique :-

1) 1° stain :- Strong carbol fuschin (1%) - 5 min.
2) Decolorization - 25% H_2SO_4 - 2-4 minutes
3) Counter staining - 0.1% methylene blue - 30 sec.

Antimicrobial Susceptibility testing:

Classificatn:- 1) Phenotypic Methods -

- Disk diffusion method - Kirby Bauer's disk diffusion method.
- Dilutn test - Broth dilutn & agar dilutn method.
- Epsilometer or E tests
- Automated AST eg. Vitek, Phoenix

2). Genotypic Methods -

PCR - drug resistant genes.

DISK - DIFFUSION METHOD -

Procedure - Mueller Hinton Agar

- Bacterial colony suspension (of 0.5 McFarland turbidity) is prepared in saline & then inoculated onto MHA by spreading (lawn culture) with sterile swabs.
- Antimicrobial disks are then placed on the surface of MHA plate → Incubated at 37°C for 16-18 hrs then interpreted.

Interpretatn:- Susceptibility to drug is determined by the zone of inhibitn of bacterial growth.

DIRECT DISK DIFFUSION TEST -

Specimen is directly inoculated into agar plate and the antibiotic disks are applied.

⇒ no use for mixed growth.

DILUTION TESTS -

MIC - lowest conc. of drug that will inhibit the visible growth of an organism

Types - ① Broth dilutn - Mueller - Hinton Broth
② Agar dilutn - Mueller - Hinton Agar

Epsilometer or Etest :-

MIC based method - uses principle of both dilutn & diffusion.

Automated Antimicrobial Susceptibility tests :-

- VITEK-2
- Phoenix System
- Micro scan walk away system

GENETIC ENGINEERING :-

- deliberate modification of an organism's genetic information by altering its nucleic acid genome.
- accomplished by RDNA technology.
  ↓
  gene coding for any desired protein is isolated from the organism & then inserted into suitable vector. which is then cloned in such a way that it can be expressed in the forming specific (desired) protein.

Procedure:-

1) Rx with restriction enzyme -
2) Southern blot - i) Electrophoresis
   ii) transfer to nitrocellulose membrane
   iii) Detection of desired gene - using Specific DNA probe
   iv) Isolation by DNA extraction & then Electrophoresis in different gel
3) Recombination - Annealed by DNA ligase enzyme
4.) Introduction of vector into bacteria - by transduction
5.) Cloning

Applications:-

1.) Production of vaccines :- Subunit vac for HepB & ~~pap~~ papillomavirus
2.) Production of Ag used in diagnostic kits. eg. ELISA
3.) Transgenic animals -
4.) Gene therapy

PLASMID :-

Plasmids are the extrachromosal ds circular DNA molecules that exist in free state in cytoplasm of bacteria.

→ not essential for life.

→ numbers - maybe present singly or in multiple no.s

→ independent replicaτn - behave as replicons possessing an orgin of replicaτn & other genes that help in replicaτn.

→ episome - sometimes, plasmid may integrate with chromosomal DNA of bacteria & such plasmids are called episomes.

→ curing - process of eliminating the plasmids from bacteria.

↳ done with the help of acridine radiation thymine starvaτn growth at higher temp.

Classificaτn :-

Plasmid

- Conjugative
- Non-Conjugative

• Based on function :-

A) Fertility or F-plasmids - tra gene - codes for sex pili

B) Resistance (R) plasmids - contains gene that codes for resistance to various antibiotics.

C) Col plasmids - contains genes that code for bacteriocins.

D) Virulence plasmids - code for virulence factors & toxins that help in bacterial pathogenesis.

E) Metabolic plasmids :- digestn of unusual substances — toluene & salicylate camphor.

MUTATION :-

Random, unidirected, heritable variation caused by Δ in nucleotide sequence of the genome of the cell.

2 types - 1) Spontaneous - naturally w/o mutagen

2) Induced ; exposure of the organism to mutagen.

- Physical agents - UV radiation, cytosine, thymine (are more vulnerable to UV.)
- Chemical agents - alkylating agents
  5-bromouracil
  Acridine dyes.

→ most mutants - unrecognized
  ↳ lethal
  ↳ minor functn which cannot be expressed.

→ Mutation can affect any gene & hence may modify any characteristic eg.
→ sensitivity to bacteriophages
→ loss of ability to produce capsule or flagella
→ loss of virulence

Classification :- 1) Small scale - (point) mutation, addition/deletion of single nucleotide pair
2) Large scale - deletion/addition of several nucleotide pairs.

HORIZONTAL GENE TRANSFER

1) TRANSFORMATION

It is a process of random uptake of free naked DNA fragment from the surrounding medium by a bacterial cell & incorporation of this DNA fragment into its chromosome

Mech :-

When bacteria lyse, they release destroyed DNA into the surrounding environment. which can be taken up by the bacteria present in the surroundings.

- Transformation promoting factor - competence factor

2) TRANSDUCTION :-

Transfer of a portion of DNA from one bacteria to another by bacteriophage.

Mechanism :-

⇒ Bacteriophage is a virus that infects & multiplies inside the bacterium.