Major :Medicine & Dentistry

Lecture :*Microbiology #6*

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* Virion : Virus
→ Complete virus particle
→ Very simple & small
→ size range = 10-300nm
→ Ebola Virus = 1 Km length
→ unable to bee seen until electron microscope invention
→ No HO is safe from Viral infection : viruses infect humans, animals, plants, fungi, protozoa & algae

→ Retroviruses : have reverse transcriptase (RNA → DNA)
→ Bacteriophage →
• viruses that infect bacteria
• can infect diphtheria & cholera
• Contribute to toxicity of bacteria
• gives bacteria tox genes that enable them to produce toxins

** Viruses have specific features
→ Possess either DNA or RNA
→ unable to replicate on their own →
→ don't divide by binary fission, mitosis or meiosis →
→ lack genes & enzymes for energy production
→ depend on ribosomes, enzymes & metabolites of the host for protein & genome productia

Acellular Microbes [viruses]

** Categories of viruses
1- ds DNA viruses
2- ss DNA viruses
3- ds RNA viruses
4- ss RNA viruses

* Viruses are classified by
1- Type of genome
2- shape of capsid
3- Number of capsomeres
4- size of capsid
5- presence of envelope
6- Type of host
7- Type of disease
8- Target cell
9- immunologic / antigenic properties

** Shape & symmetry of capsid
1] Polyhedral (many sides)
→ 20 sides or facets
→ referred to as icosahedrons
2] Helical (coiled tubes)
3] Bullet shaped
4] Spherical (enveloped viruse)
5] Combination

** Envelope :
→ acquired by certain animal viruses as they escape from nucleus of host or cytoplasm by budding
→ Derived from host → cell membrane / Nuclear membrane

** Typical virion
→ Consist of a genome of either DNA or RNA surrounded by a capsid
→ Capsid :
• protein coat
• composed of capsomeres
→ Nucleic acid + Capsid = Nucleocapsid +
→ Enveloped viruses
• have an outer envelope
• envelope = Lipids & polysaccharides
→ Bacterial viruses may have tail, sheath & tail fibers
→ Naked viruses
• can resist environmental factors
• most of viruses that infect GIT
→ Viral genomes are usually circular

* Bacteriophages :

→ Viruses that infect bacteria
→ also called phages
→ categories of phages

1] Icosahedron → spherical in shape
→ 20 triangular facets
→ smallest = 25 nm

2] Filamentous

3] Complex → Icosahedaral heads
→ Helical tails

4] Biosynthesis:
- Virus genes are expressed
- Production of viral DNA & protein

5] Assembly:
- Viral pieces are assembled
- to create complete virion

6] Release:
- The complete virion escapes from the host cell by
  - Budding → become surrounded by pieces of cell m. & enveloped
  - Lysis

* Virulent bacteriophages :

→ always cause lytic cycle
→ ends with lysis of bacterial cell
→ the whole process takes < 1 hour
→ steps in lytic cycle

1] Attachment (adsorption):
- The phage (virus) attaches to a protein or polysaccharide receptor on the surface of bacterial cell

2] Penetration:
- The phage injects into bacterial cell its DNA/RNA
- The capsid remains on the outer surface of cell

3] Biosynthesis:
- Phage genes are expressed
- production of phage DNA & protein

4] Assembly:
- Phage pieces are assembled
- to create complete phage

5] Lysis & Release

* * Animal Viruses

→ infects humans & animals
→ steps of multiplication:

1] Attachment (adsorption)
- The virus attaches to a protein or polysaccharide receptor on the surface of host cell

2] Penetration:
- The entire virus enters the host cell
- Because it was phagocytized by the cell

3] uncoating:
- Viral nucleic acid escapes from capsid

* Temperate (lysogenic) phages :

→ don't immediately initiate lytic cycle
→ DNA remains integrated in bacterial chromosome (viral) generation after generation
→ Causes lysogenic conversion of infected bacteria (acquire new features)
→ Causes emergence of multi-drug resistant bacteria (super bugs)

— * Viral Replication *

** Animal Viruses :

[1] Attachment (Adsorption) → to the host cell surface

[2] Penetration → entire virus enters host cell

[3] uncoating → Viral nucleic acids escape from the capsid

[4] Biosynthesis ⇒ Expression of viral gene
= Transcription & Translation
= Proteins synthesis
& viral DNA replication

[5] Assembly → virus pieces are assembled to create complete virion

[6] Release → by ↗ Lysis or ↘ Budding

** Bacteriophages → uses lysozyme

[1] Attachment (Adsorption) → To the host cell surface

[2] penetration → The phage injects its DNA into the bacterial cell
→ The capsid remains on the outer surface of cell

هنا يحل جينوم الفيروس ريس الفيروس كاملاً فلا حاجة لخطوة الـ uncoating التي تكون متطلب في الـ Animal virus

[3] Bio synthesis

[4] Assembly

[5] Release → Complete phage escapes from the bacterial cell by lysis of the cell.

3

*Latent virus infections:

→ After 1st infection, virus remains latent in the human body for many years
→ Limited by defense systems of human → phagocytes & IFN
→ when immune defense weakened by 1. old age 2. Fever 3. Stress 4. Excess light of sun
viruses take over cells & multiply

1] Cold sores (Fever blister)
- Infected person always harbors virus in nerve calls
- ↓ immunity → Cold sores develop

2] shingles هربس نطاقي
- painful nerve disease by herpesvirus
- causes chickenpox in childhood & remain latent in body
- ↓ immunity in old age the virus resurface as shingles

**oncogenic viruses:

1] → DNA oncoViruses
→ causes cancers
→ Examples
1] Epstein Barr Virus EBV → Nasopharyngeal carcinoma
↳ Burkitt lymphoma
↳ B-cell lymphoma
2] Herpes virus 8 → Kapos sarcoma in AIDS
3] Hepatitis B&C HBV, HCV → Hepatocellular CA
4] Human papilloma (HPV) → Cervical Ca.
5] Human T-lymphotropic virus type 1 (HTLV1)
→ adult T-cell leukemia

* Human Immunodeficiency Virus (HIV):

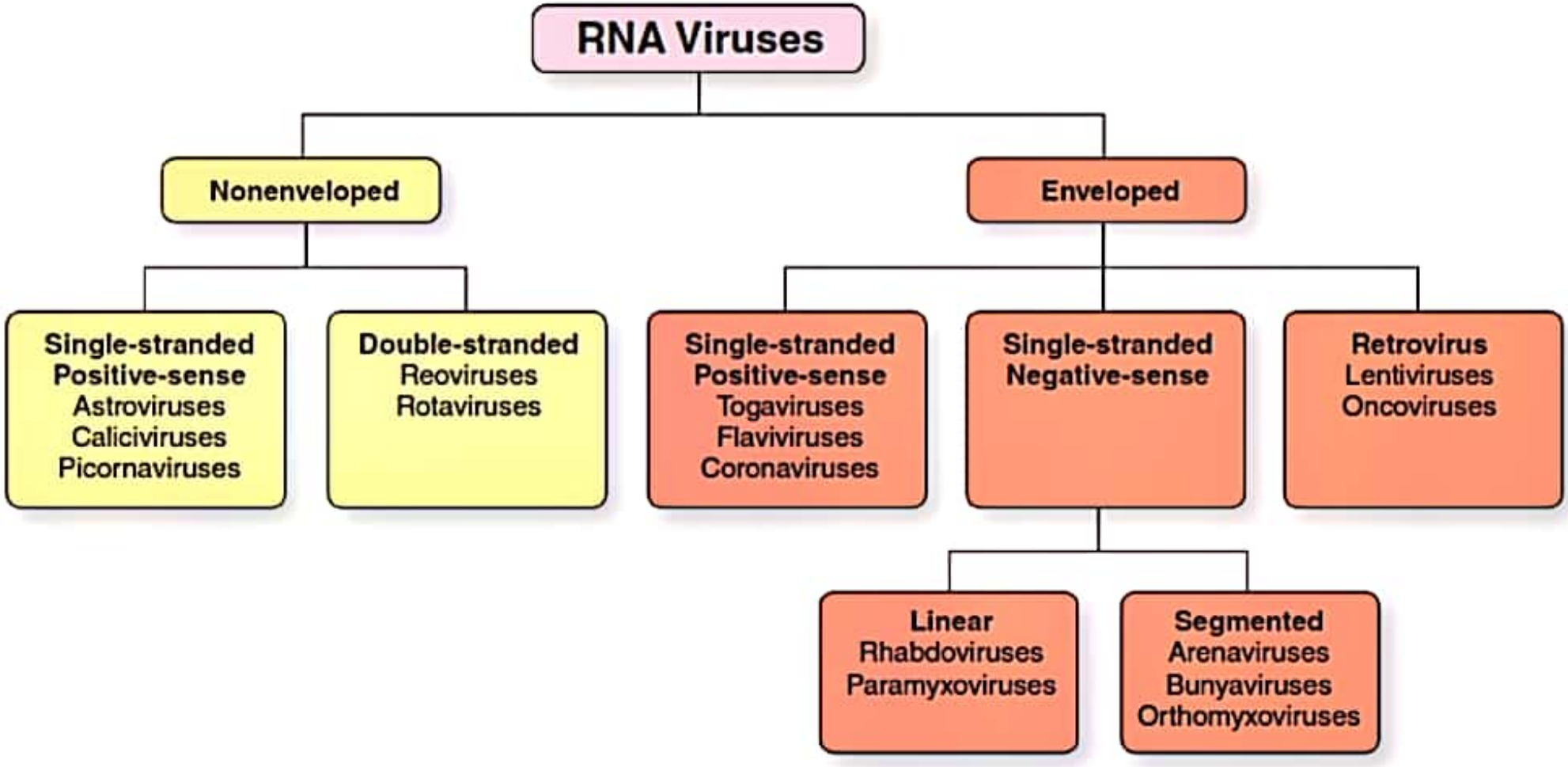
→ The cause of AIDS
→ Enveloped ss RNA virus
→ ~~Genus = Lentiviruses~~
→ ~~Family = Retroviruses~~
→ Infects cells that possess special receptor = CD4+
→ CD4+ = T cells & Macrophages
→ Contain two identical ss RNA molecules
→ Has 72 surface Knobs containing glycoprotein (Gp120) capable of binding CD4 receptors on T-helper
→ The stalk that support the knob is transmembrane Gp41
→ Has reverse transcriptase

**Inclusion bodies:

→ Remnants or collection of viruses seen in infected cells
→ Diagnostic tool
→ Types:
1] cytoplasmic:
→ Negri bodies in Rabies
→ Guarnieri bodies in smallpox
→ AIDS
2] Intranuclear
→ Herpes viruses
→ poliomyelitis virus

**Mimiviruses & Megaviruses:

→ Extremely large ds-DNA virus
→ Mimics bacteria
→ Can be observed by LM
→ Genome: 10x larger than that of large virus smallpox & larger than the genome of smallest bacteria
→ Contains 1,000 genes
→ Contain genes for metabolism of sugar, Lipids & amino acids
→ Contain some RNA molecules
→ May cause human pneumonia
→ Megavirus:-
- Larger ds-DNA viruses
- The largest capsid diameter 440nm
- The most complex viral genome
- > 1000 genes
- Larger than some bacteria
- unknown host



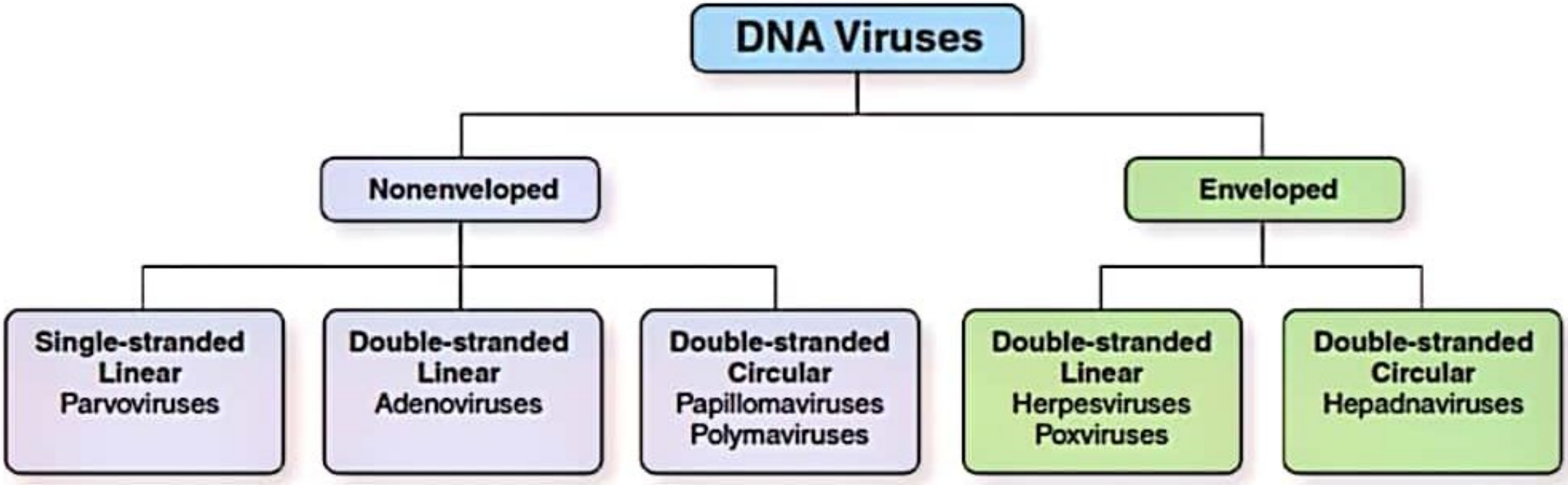


Table 4-2 Selected Important Groups of Viruses and Viral Diseases

Virus Type	Viral Characteristics	Virus	Disease
Poxviruses	Large, brick shape with envelope, dsDNA	Variola	Smallpox
		Vaccinia	Cowpox
Polyoma–papilloma	dsDNA, polyhedral	Papillomavirus	Warts
		Polyomavirus	Some tumors, some cancer
Herpesvirus	Polyhedral with envelope, dsDNA	Herpes simplex I	Cold sores or fever blisters
		Herpes simplex II	Genital herpes
		Herpes zoster	Shingles
		Varicella	Chickenpox
Adenovirus	dsDNA, icosahedral, with envelope		Reepiratory infections, pneumonia, conjunctivitis, some tumors
Picornaviruses (the name means small RNA viruses)	ssRNA, tiny icosahedral, with envelope	Rhinovirus	Colds
		Poliovirus	Poliomyelitis
		Hepatitis types A and B	Hepatitis
		Coxsackie virus	Reepiratory infections, meningitis
Reoviruses	dsRNA, icosahedral with envelope	Enterovirus	Intestinal infections
Myxoviruses	RNA, helical with envelope	Orthomyxoviruses types A and B	Influenza
		Myxovirus parotidis	Mumps
		Paramyxovirus	Measles (rubeola)
		Rhabdovirus	Rabies
Arbovirus	Arthropod-borne RNA, cubic	Mosquito-borne type B	Yellow fever
		Mosquito-borne types A and B	Encephalitis (many types)
		Tick-borne, coronavirus	Colorado tick fever
Retrovirus	dsRNA, helical with envelope	RNA tumor virus	Tumors
		HTLV	Leukemia
		HIV	AIDS

ds, double-stranded; ss, single-stranded.

* Viroids & Prions *

* Viroids *

- Short, naked fragments of ss RNA
- Transmitted between plants
- Causes plants diseases
  1. Potato spindle tuber
  2. Citrus exocortis → stunting of citrus trees

* Prions *

- Infectious protein particles.
- causes fatal human & animal dis
- Long incubation period
- multiply by converting normal proteins into prion proteins

1. scrapie : sheeps & goats
2. Bovine Spongiform Encephalopathy (BSE) = mad-cow disease
3. Kuru : associated with cannibalism.
4. Creutzfeldt-Jakob disease
5. Gerstmann-Sträusler-Scheinker disease
6. Familial insomnia

* Can be classified based on
→ cell shape
→ Morphologic arrangement
→ staining reactions
→ Motility
→ Colony morphology
→ Atmospheric requirements
→ Nutritional requirements
→ Biochemical activities
→ specific enzymes
→ Phathogenicity
→ Genetic composition (GC ratio)

* classes of bacteria based on shape:
1] Coccus / Cocci → spherical, 1 Hm diameter
a) diplococci = pair of cocci = Gonorrhea
b) streptococci = chain of cocci = streptococcus pyogenes
c) staphylococci = cluster of cocci = staphylococcus aureus
d) Tetrads = packet of 4 cocci
e) octads = packet of 8 cocci

d+e → rarely pathogenic
→ part of skin normal flora

2] Bacilli → Rod-like, 3 Hm long & 1 Hm wide
a) single bacillus
b) diplo bacilli
c) strepto bacilli

3] spirilla → helical & curved, spiral in shape
→ Ex: 1 Treponema pallidum (Syphilis)
2 Borrelia hermsii (relapsing fever) 3

4] pleomorphic
→ L-form = cell wall deficient
→ No peptidoglycan
→ Resistant to antibiotics that target CW
→ Ex = Mycoplasma

** The Gram stain:
1] primary stain = Crystal Violet
• both G+ & G- are purple
2] Mordant = Iodine solution
• both G+ & G- are purple
3] Decolorizer (critical step.) = Ethanol
• G+ve = thick = remains purple
• G-ve = thin = colorless
4] Counter-stain (safranin)
• G+ve = remains purple
• G-ve = pink - red

* Gram variable → Neither purple Nor pink.

** Acid-Fast stain:
→ For mycobacteria that have wax (mycolic acid) in CW
→ Differentiates acid-fast which is mycobacteria from non acid-fast

1] Primary stain = Carbol fuchsin (Red), heat is necessary for this step to soften the wax
• Acid-fast = Red
• Non acid-fast = Red

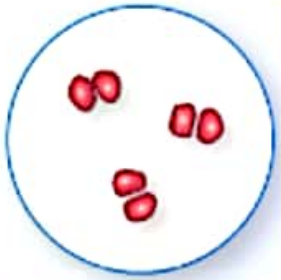
2] Decolorizer = Acid alcohol
• Acid-fast = Red
• Non acid-fast = Colorless

3] Counter-stain → methylene blue or malachite green
• Acid-fast = Red
• Non = blue or green

* Smear Preparation:
1- Bacteria is smeared onto slide
2- Airdry the smear
3- Fixation → purpose → kills the bacteria, Anchors bacteria to slide, Preserve morphology
→ Methods
a) heat fixation → passing over Bunsen burner → Excess distort morphology → in acid-fast staining
b) methanol fix → flooding with absolute 100% methanol → for 30 seconds → more satisfactory → in gram stain

4- staining
a) simple → single stain for shape & arrangement
b) Differential → >1 stain → Gram & acid-fast → for differentiation

5- Rinse with water
6- observe

Arrangement	Description	Appearance	Example	Disease
Diplococci	Cocci in pairs		*Neisseria gonorrhoeae*	Gonorrhea
Streptococci	Cocci in chains		*Streptococcus pyogenes*	Strep throat
Staphylococci	Cocci in clusters		*Staphylococcus aureus*	Boils
Tetrad	A packet of 4 cocci		*Micrococcus luteus*	Rarely pathogenic
Octad	A packet of 8 cocci		*Sarcina ventriculi*	Rarely pathogenic

* Motility :

- → Most spiral-shaped & about 1/2 of bacilli are motile by means of flagella
- → Cocci are generally non-motile
- → some bacteria exhibit a type of gliding motility on slime layer
- → Bacteria never possess cilia
- → Bacteria motility is due to:
  - a) Flagella : observed by flagellar stain
  - b) Axial filaments : endoflagella inside & spiral around the CW

** How to demonstrate motility :

1. Agar stabbing method
   - → Growth in semi-solid agar tubes produces turbidity
   - → non-motile : turbidity along stab line
   - → motile : spread away from stab line
2. Hanging drop technique
   - → Drop of bacteria is placed onto glass coverslip
   - → Coverslip is inverted onto slide
   - → motile bacteria = darting around

* Colony Morphology :

- → Colony : pile of bacteria on surface of solid culture medium . Contains millions of bacteria
- → Colony morphology include
  - . size . Color . shape . elevation . edge or margin of colony
- → size of colonies is determined by organism's rate of growth (generation time)

** Pathogenicity : ability to cause dis

- → pathogenic MO possess
  - . Capsules : antiphagocytic
  - . pili
  - . Endotoxins : Component of G-ve CW
  - . Exotoxins

** Extremophiles :

- Extremely acidic → acidophile
- Extremely alkaline → alkaliphile
- Extremely hot → thermophile
- Extremely cold → Psychrophile
- Extremely salty → Haloplile
- Extremely ↑ pressure → Prezophile (Baroplile)

** Nutritional requirements

- → Bacteria need C, H, O, S, P & N
- → some bacteria need special elements K, Ca, Fe, Mg & Cu
- → Fastidious MO = have specific nutritional requirements & needs enriched media to grow in the lab
- → Some MOs have specific vitamin & organic substances requirements

* Almospheric requirements

1. obligate aerobes → 20-21 % O2 → Room air
2. Micro aerophiles → requires less O2 → 5 % O2
3. Facultative anaerobe → Capable of surviving in presence / absence of O2
4. Aerotolerant anaerobe
   - → doesn't require O2
   - → grows better in absence of O2
   - → Can survive presence of O2
5. obligate anaerobe → Can grow only in an anaerobic environment

** Capnophiles : grow better in ↑ concentration of CO_2

TABLE 4-7 Types of Bacterial Staining Procedures

CATEGORY	EXAMPLE(S)	PURPOSE
Simple staining procedure	Staining with methylene blue	Merely to stain the cells so that their size, shape, and morphologic arrangement can be determined
Structural staining procedures	Capsule stains	To determine whether the organism is encapsulated
	Flagella stains	To determine whether the organism possesses flagella and, if so, their number and location on the cell
	Endospore stains	To determine whether the organism is a spore-former and, if so, to determine whether the spores are terminal or subterminal spores
Differential staining procedures	Gram stain	To differentiate between Gram-positive and Gram-negative bacteria
	Acid-fast stain	To differentiate between acid-fast and non–acid-fast bacteria

* Rickettsias

- G-ve bacteria
- obligate intracellular pathogen
- causes diseases in humans & animals

rr- have no connection to the disease Rickets (Vitamin D deficiency)

- leaky cell membrane
- all diseases are arthropod-borne (transmitted by vectors)
- Diseases ⇒ Typhus & Typus-like
  ⇒ all involve production of Rash

* chlamydias :

- G-ve bacteria
- obligate intracellular pathogens
- causes diseases in humans & animals
- energy-Parasites] - can produce ATP, - prefer ATP of host
- transmitted by ↗ contact, ↘ inhalation] not by arthropod

* Mycoplasma

- smallest cellular Ho
- lacks cell wall
- pleomorphic (many shapes)
- Resistant to penicillin

* Archaea *

- Ancient ~ 3.5 b
- prokaryotic but more related to eukaryotic than bacteria
- lacks peptidoglycans
- not known to cause diseases in human
- Extremophiles
- some live in bottom of ocean near thermal vents

ocean → salty → halophile
bottom → pressure → Barophile or Piezophile
vent → thermal → thermophile

Our Notes

* obligate intracellular pathogens
  - viruses
  - Rickettsia
  - chlamydia
  - Ehrlichia
  - coxiella
  - certain protozoa
  - certain fungi

* Ribosomes

	Large subunit	small subunit	
Eu	60s	40s	] 80s
pro	50s	30s	] 70s

rr * Mercury lamp → Fluorescence Microscope

* spore-Formers → Clostridium Bacillus anthracis

* nm ×10³ → µm ×10³ → mm ÷10 → cm ÷10 → dm ÷10 → m

TABLE 4-8 Human Diseases Caused by Unique Bacteria

GENUS	SPECIES	HUMAN DISEASE(S)
Rickettsia	*R. akari*	Rickettsialpox (a miteborne disease)
	R. prowazekii	Epidemic typhus (a louseborne disease)
	R. rickettsii	Rocky Mountain spotted fever (a tickborne disease)
	R. typhi	Endemic or murine typhus (a fleaborne disease)
Ehrlichia spp.	*E. chaffeensis*	Human monocytic ehrlichiosis
Anaplasma spp.	*Anaplasma phagocytophilum*	Human granulocytic ehrlichiosis
Chlamydia (and *Chlamydia*-like bacteria)	*Chlamydophila pneumoniae*	Pneumonia
	Chlamydophila psittaci	Psittacosis (a respiratory disease; a zoonosis; sometimes called "parrot fever")
	Chlamydia trachomatis	Different serotypes cause different diseases, including trachoma (an eye disease) inclusion conjunctivitis (an eye disease), nongonococcal urethritis (NGU; a sexually transmitted disease), lymphogranuloma venereum (LGV; a sexually transmitted disease)
Mycoplasma	*M. pneumoniae*	Atypical pneumonia
	M. genitalium	Nongonococcal urethritis (NGU)
Orientia	*O. tsutsugamushi*	Scrub typhus (a miteborne disease)
Ureaplasma	*U. urealyticum*	Nongonococcal urethritis (NGU)