Earth is 4.5-4.6 billion years old. Bacteria were found in sedimentary rock formed 3.8 billion years ago.
First 2 billion years- all life was microbial
The size of the microbial cells differ between millimeters and half a micrometer, but viruses are 10 times smaller.

There are three types of microbes
1. Supersized microbial cells
2. Microbial communities
3. Viruses

Bacteria, archaea, and eukarya are the three domains of life, but viruses are non-cellular particles.
Note: A genome is the total genetic information contained in an organism's chromosomal DNA.

Fred Sanger (a biochemist), got the nobel prize in chemistry in 1980 for first method of DNA sequencing to sequence large genomes.
Florence nightingale nurse founded the science of medical statistics, and using this method she figured out that more soldiers were dying because of microbial diseases rather than battle wounds. The chart she created was called the "polar area chart"
Robert hooke built the first microscope to observe mold, and coined the term "cell". It consisted of two or more magnifying glasses but the best power was 30x.
Antonie van leeuwenhoek built the first single lens microscope, and observed single celled microbes. Ahs 5-300x magnification

Francesco Redi (1660's) showed that maggots in decaying meat were the offspring of flies, and Lazzaro Spallanzani showed that a sealed flask of meat broth sterilized by boiling failed to grow microbes.
Louis pasteur - the man behind the swan shaped experiment that proved that cells do not appear spontaneously
He is also the guy who discovered the microbial basis of fermentation, using heat to reduce bacteria, and developing vaccines based on attenuated (weakened) strains.

John tyndall discovered the heat-resistant forms of bacteria - spores.
These four criteria are known as **Koch's postulates**:

1. The microbe is found in all cases of the disease but is absent from healthy individuals.
2. The microbe is isolated from the diseased host and grown in pure culture.
3. When the microbe is introduced into a healthy, suscep- tible host (or animal model), the host shows the same disease.
4. The same strain of microbe is obtained from the newly diseased host. When cultured, the strain shows the same characteristics as before.

He tried isolating the pure cultures on potato slices and gelatin but it did not work, his wife suggested he tries it on agars.

What are the limitations of this method?
Some organisms cannot be grown in pure culture. Using humans to prove this theory is unethical. Molecular and genetic evidence may replace culture based techniques.

Mary montagu introduced the practice of smallpox inoculation to europe in 1717, and edward jenner deliberately infected patients with matter from cowpox lesions.

1847 - ignaz semmelweis ordered doctors to wash their hands with chlorine.
1865 - joseph lister developed carbolic acid to treat wounds and clean surgical instruments. And by the end of the 20th century, the aseptic surgery was developed.

Alexander fleming discovered that penicillium mold generated a substance that kills bacteria.
Howard florey and ernst chain purified penicillin, which was the first antibiotic.

Discovery of viruses.
1892 - **dmitri ivanovsky** was studying the tobacco mosaic disease when the agent of tobacco mosaic disease could pass through a filter that could retain bacteria, and that's how viruses were discovered. Tobacco mosaic ***virus.***

Less than 0.1% of all microbial species can be cultured in the laboratory, and the rest make up the earth's biosphere.

Sergei winogradsky - discovered lithotrophs, enrichment cultures, and built the winogradsky column (a wetland model ecosystem containing regions of enrichment for microbes of diverse metabolism.)
He also proved the importance of bacteria in geochemical cycling.

Endosymbionts - microbes living symbiotically inside a larger organism.
Rhizobia - endosymbiotic microbe - induces the roots of legumes to form special nodules to facilitate bacterial nitrogen fixation

In general - endosymbiotic bacteria make essential nutritional contributions to host animals.

Binomial system of nomenclature
Carolus linnaeus - each organism has two names: a genus name and a species name. For bacteria - bacterium and archaea - archeon
System of classification: kingdom - phylum - class - order - family - genus - species.

What were the two challenges faced early on?
Low resolution of the light microscopy, which was overcame by advances in biochemistry and microscopy.
Microbial species are hard to define, which was overcame by finding 95% similarity between DNA sequences of microbes.

5 kingdom system of classification
Ernst haeckel - microbes are neither plant nor animal - third kingdom called monera
Herbert copeland - monera is divided into two groups. The eukaryotic protists (protozoa and algae) and prokaryotic bacteria
Robert whittaker - fungi is the fifth kingdom of eukaryotic microbes.

How do archaea differ from bacteria and eukaryotes?

Archaea do not have a nucleus or membrane bound organelles. And the composition of the cell walls are different between archaea and bacteria

16S rRNA is always analyzed since it is easier to manage than 23S and phylogeny is formed.

The electron microscope and the ultracentrifuge were exceptional helps on the study of cell structure
The first one was discovered by ernst ruska and got the nobel prize in physics. Revealed the internal structure of the cell.
The second one was developed by theodor svedberg and it enables the separation of subcellular parts.

Microbial genetics
1928, Frederick Griffith, a bacteriologist, discovered transformation in bacteria.
In 1944, Oswald Avery, a physician, and colleagues showed that the transforming substance is DNA.
In 1953, Rosalind Franklin, a chemist and X-ray crystallographer, used X-ray crystallography to determine that DNA is a double helix.
Later that year, James Watson, a molecular biologist, and Francis Crick, a British molecular biologist, discovered the complementary bases and antiparallel nature of DNA.

How did life function in the RNA world?
- We hypothesize that cells used RNA for all the functions of DNA and protein, including information storage and replication and biochemical catalysis: RNA: replicates and has catalytic activity.

Thomas cech and sidney altman discovered the catalytic properties of RNA, Hennifer doudna uncovered the structure and biological function of RNA enzymes and co-developed the cas9/CRISPR

<u>Chapter 2</u>
Eukaryotic microbes - protozoa, algae, fungi.
10-100 micrometer. 10^-6 meter

Prokaryotes - bacteria, archaea
0.4-10 micrometer
Certain shapes of bacteria are common to many taxonomic groups for example rods(bacilli), spheres (cocci) and spirals (spirochetes)

Range of resolution (worst to best)
Human eye naked eye > Light microscopy > SEM - scanning electron microscopy > TEM - transmission electron microscopy > AFM - atomic force microscopy > X-Ray crystallography

Resolution - Smallest distance between two points, at which they can still be distinguished from each other:

- Resolution of the eye - perceived by the fovea (portion of the retina where the photoreceptors are packed at the highest density)

Detection - ability to determine the presence of an object
Magnification - increase in size of an object
Properties of light
For electromagnetic radiation to resolve an object, certain conditions must exist
Contrast - between the object and its surroundings. So that the object and its surroundings do not absorb or reflect radiation equally.
Wavelength - needs to be smaller than object or else it will pass through the object.
Magnification - human retina absorbs 400-750 nm but smallest distance we can resolve is 150 mm which is about 300x wavelength of light.
Speed of light = wavelength of the radiation * frequency (hertz)

Interaction of light with matter
Absorption - photon's energy is acquired by the absorbing object as heat
Reflection - wavefront bounces off the surface of an object at an angle equal to its incident angle
Refraction - bending of light as it enters an object that slows down its speed. The substance has higher refractive index than air.
Scattering - a small fraction of the incident light is scattered in all directions.

Magnification by a lens - required the bending of light rays - refraction
Higher refractive index - light bent towards the first normal
Lower refractive index - light bent away from the second normal.
Parabolic curvature - bends light rays to intersect at a focal point
Focal length is the distance between the centre of the parabolic curvature and the focal point. And strength is related to it. Short focal length = more magnification.
Our eyes cannot focus on objects closer than about 25 cm.

Resolution equation = R = 0.5 sigma (wavelength of ligth) / n (refractive index) sin theta
If we minimize R, then we increase resolution. But how?
R decreases when wavelength decreases and n sin theta increases.
Increase contrast, use wider lens closer to specimen.

Why do we use immersion oil in microscopy? Prevents lights from bending away from the objective lens and therefore there will be a higher resolution and numerical aperture. N of air = 1.00 n of water = 1.33

Bright field microscopy - generates a dark image of an object over a light background
Compound microscope had multiple lenses.
Ocular lens - within the eyepiece
Objective lense - usually 4 different magnifications
Condenser - collects beams of light and directs it onto the specimen to increase contrast

Preparing a specimen for microscopy
Wet mount - microbes present in a drop of water
Advantages - observation of cells in natural state
Disadvantages - sample may dry out and there is little contrast between the cell and background

Fixation - cells adhere to a slide in a fixed position but it also denatures proteins and exposes side chains that bind to glass.
Staining - cells are given a distinct colour.

Simple stain - adds dark colour to cells but not the external medium
Differential stain - stains one kind of cell but not the rest. For example the gram stain, acid-fast stain, spore stain and negative stain.
Gram-positive : retain colour because of thick cell wall
Gram-negative : do not, instead counter stained with safranin

Confocal laser scanning microscopy: a form of fluorescence microscopy that uses laser beams to construct 3D images.
Dark-field microscopy: microbes as halos of bright light against darkness
Phase contrast microscopy: difference between cytoplasm and surrounding medium. Can be used to view live cells and cellular organelles
Differential interference contrast microscopy: can observe organelles better
Electron microscopy: allows study of microbial morphology in great detail
Two types: transmission electron microscopy (electrons pass through the specimen and reveals internal structures)
Scanning electron microscopy (electrons scan the specimen surface and reveals external features in 3D)

Chapter 3
Bacterial cells have:
Cytoplasm - gel like network
Nucleoid - non membrane bound area of the cytoplasm that contains chromosome in the form of looped coils
Cell membrane - encloses the cytoplasm
Cell wall - covers the cell membrane
Flagellum - external helical filament whose rotary motor propels the cell

Chemical components of a cell :
70% water
Essential ions (k-, Mg- phosphate ions)
Small organic molecules (enzyme cofactors, phospholipids)
Macromolecules (DNA, RNA, proteins)

Bacterial cell membrane

All bacteria have it. It is absolutely necessary. Some bacteria also have an internal one (for example, photosynthetic bacteria). It is selectively permeable. Interacts with the outer environment - receptors for detection of and response to chemicals in surroundings, transport systems, metabolic processes like respiration, photosynthesis.
What does the cell membrane consist of?
A phospholipid bilayer, hydrophobic fatty acid chains directed inward, away from water.(non polar) and hydrophilic, charged phosphoryl heads (polar). The two layers are called leaflets
There are also planar molecules that fill gaps between hydrocarbon chains,
Eukaryotic - sterols like cholesterol
Bacteria - hopanoids, or hopanes. (adds strength to the membrane)
Peripheral proteins - loosely connected to the membrane and soluble in water
Integral proteins - hydrophobic - carry out important functions like transportation, energy)

Archaea have the most extreme variations in phospholipid side chain structures. Every fourth carbon has a methyl branch. The ends are fused. Rings stiffen the membrane under stress.

Cell membrane and osmosis
Movement of water across a selectively permeable membrane from dilute to more concentrated.
Hypotonic environment: water moves into cell and cell swells
solute concentration outside of cell < solute concentration inside the cell

Hypertonic environments: water leaves the cell and plasmolysis occurs
solute concentration outside of cell > solute concentration inside the cell

Cells with no cell wall can survive in isotonic environments.
Weak acids and bases can also diffuse across the membrane in their uncharged form to increase or decrease the H+ concentration within the cell.
BUT if molecules with a fixed charge try to pass - that will not happen.
Instead, they will create an ion gradient across the membrane (energy storgage)

The cell wall confers shape and rigidity and helps the cell withstand turgor pressure.
Sacculus - the bacterial cell wall - consists of a single interlinked molecule.
They're made of peptidoglycan (which is unique to bacteria)
The enzymes responsible for its biosynthesis make excellent targets for antibiotics.
Penicillin inhibits vancomycin prevents cross bridge formation

Peptidoglycan structure.
Long strands of two disaccharides, NAG, NAM -> crosslinked with peptides, amino acids (D and L form).
The crosslinked peptides create a dense, porous, elastic and stretchable structure.

<u>Cell envelope of bacteria</u>
Gram positive bacteria have thick cell walls
- Multiple layers of peptidoglycan and contains teichoic acids (-ve charge)
- Some have an additional layer called the capsule

The capsule is made up of polysaccharide and glycoprotein (both are not easily removed from the cell). They also protect the cell from phagocytosis and desiccation
- S-layer additional protective layer found in free living bacteria and archaea.

Consists of protein or glycoprotein. Contributes to cell shape, adhesion and help cells protect from osmotic stress.

Periplasmic space:
- Between plasma and cell wall
- Few proteins
- Exoenzymes aid in the degradation of large nutrients.

Gram negative bacteria have thin walls
More complex
Thin layer of peptidoglycan surrounded by an outer membrane.
Outer membrane composed of lipids, lipoproteins and lipopolysaccharide.
NO TEOCHOIC ACIDS

Periplasmic space:
20-40% of the cell volume
Many enzymes present including hydrolytic enzymes, transport proteins, etc..

Mycobacteria have complex multilayered cell walls
Peptidoglycan layer linked to a chain of galactose polymer and arabinose polymer which form lins to mycolic acids, which form an outer bilayer with phenolic glycolipids.

Side note: importance of LPS - lipopolysaccharide
Negative charge on cell surface
Helps stabilize outer membrane structure
May contribute to attachment to surfaces and biofilm formation creates a permeability barrier.
Protection from host defenses (O antigen)
Can act as an endotoxin (overstimulates host defences lethal shock)

Bacterial cytoskeleton
The bacterial cytoskeletal proteins are revealed by gene defects that drastically alter the cell shape
Shape determining proteins:
ftsZ- forms a Z-ring in spherical cells
MreB forms a coil inside rode shaped cells and maintains shape
CreS forms a polymer along the inner side of crescent-shaped bacteria

Ribosomes: complex RNA structures
Sites of protein synthesis.
Two subunits: 30S (16S rRna and SSU proteins) and 50S (23S and 5S rRNA. LSU proteins) When joint together, 70S
Eukaryotic ribosomes are of 80S

Plasmids: found in bacteria, archaea, and fungi. Closed circular DNA molecules. Exist and replicate independently of the chromosome.

Cell division:
Prokaryotes synthesize RNA and proteins continually while the cell's DNA undergoes replication.
Septum - dividing partition
The orientation of the septation has a key role in determining the shape and arrangement of ccci
Parallel planes - streptococci
Random planes - staphylococci
Perpendicular planes - tetrads and sarcinae

Specialized structures
Storage granules: for energy or metabolic processes
Pili or fimbriae: filaments of pilin protein, and are used in attachment or in conjugatition
Stalks or nanotubes: membrane embedded extensions of cytoplasm. And their tips secrete adhesion factors and are called holdfasts
Rotary flagella:
- Peritrichous have flagella randomly distributed around cell
- Lophotrichous have flagella at the end
- Monotrichous have single flagellum

Chemotaxis:
Movement of bacterium in response to chemical gradients. Detected by chemoreceptors.
Attractants cause ccw rotation. Flagellar bundle together push the cell forward.
Repellent cause cw rotation. Flagellar bundle falls apart.

Polar aging
The poles of each daughter cell differ chemically from each other.

Chapter 4
Microbial nutrition
Macronutrients: major elements in cell macromolecules: C, O, H, N, P, S
Cations necessary for protein function: Mg, Ca, Fe, K
Micronutrients: elements necessary for enzyme function: Co, Cu, Mn, Zn

Nutritional types of organisms:

1. Autotrophs: they make their own food from CO2
2. Photoautotrophs: energy by photolysis of H2, H2S. light.
3. Chemolithoautotrophs: oxidation of organic and inorganic compounds
4. Heterotrophs: breakdown compounds produced by other organisms
5. Photoheterotrophs: break down organic compounds using light.
6. Chemoheterotrophs: energy from organic molecules

Energy is stored for later use
Chemical energy - contained in high energy phosphate bonds in ATP
Electrochemical energy - stored in the form of an electrical potential generated between compartments separated by a membrane and it is called the membrane potential. Generated when chemical or light energy is used to pump protons outside of the cell.
The proton motive force (electrochemical potential)
This stored energy can be used to transport nutrients, drive flagellar rotation, and make ATP.

The Nitrogen Cycle

The nitrogen fixing bacteria convert N2 to ammonia (take place in nodules). Lithotrophic nitrifiers oxidize ammonia to NO+3 for example. Denitrifiers return N2 to atmosphere by denitrification. In this case, nitrate is used as a terminal electron acceptor.

Nutrient uptake
Selective permeability is achieved in three ways:

- Substrate-specific carrier proteins or permeases
- Dedicated nutrient-binding proteins that patrol the periplasmic space.
- Membrane-spanning protein channels or pores

Facilitated diffusion
If solutes are too polar or too large to move by simple diffusion, facilitated diffusion comes into play. It helps solutes move across a membrane from a region of high concentration to one of lower concentration

It does not move against the gradient nor use energy. High concentration to low concentration.

Active transport
Coupled transport systems - the transportation of two substances across a cell membrane.
Symport - If they move in the same direction (both in or both out of the cell)
Red will be moving along its concentration gradient and blue will be against
Antiport - if they move in opposite directions (one in and one out of the cell)
Red will be moving along its concentration gradient. A leaves and B enters the channel. Blue is moved against its gradient in this case.

ABC transporters are powered by ATP
Two main types:
- Uptake ABC transporters, transport nutrients
- Efflux ABC transporters are used as multidrug efflux pumps

Note: Siderophores are special molecules that bind to ferric ion

Group translocation
Uses energy to chemically alter the substrate during its transport.
The pts system - uses energy from PET to attach a phosphate to specific sugars.

Culturing and counting bacteria
To be studied in detail, microbes need to be grown separately in pure culture.
Two types of culture media:
1. Liquid or broth: to study the growth characteristics of a pure culture
2. Solid: to separate mixed cultures from clinical specimen or natural environments.

Pure colonies are isolated by two techniques:
1. Dilution streaking
2. Spread plate

Types of media:
Complex media: nutrient rich but the chemical composition is unknown

Minimal defined media: contains nutrients that are essential for the growth of a given microbe
Enriched media: complex media to which specific blood components are added.
Selective media: supports the growth of one organism over another
Differential media: supports growth of many but distinguishes them in appearance
Example: MacConkey medium, EMB (methylene blue), and Mannitol salt agar - both selective and differential
Blood agar - enriched and differential

Growth factor: a specific nutrient not required by other species
Unculturable microbes: sometimes their growth factors depend on factors provided by other species that cohabit their niche
Obligate intracellular bacteria: unculturable. Parasites. Cannot live outside of host cell.

Techniques for counting bacteria:
Direct counting of living and dead cells: organisms in several squares are counted and averaged.
live/dead stain:
dead bacterial cells - fluoresce orange or yellow
Live cells - fluoresce green
FACS (fluorescence-activated cell sorter):
Fluorescent cells are passed through a small orifice and past a laser.
Detectors measure light scatter in the forward direction (measure of particle size)
And to the side (particle shape)
Pour plate method: viable cells can be counted
Optical density: counted indirectly via cell mass, protein content, and metabolic rate.

Growth cycle:
Bacteria divide by binary fission.
2^n (n= number of generations)
What is generation time? Time it takes for a generation to double.
(final cell number) Nt = N0 * 2^n (n= number of generations)
(original cell #)

Log Nt = log N0 + n* log 2
K = log Nt - log N0 / 0.301 t

If a population doubles
Nt = 2N0
K = log(2N0) - log N0 / 0.301g k = 1/g
Batch culture - simple way to model the effects of a changing environment
It is a liquid medium with a closed system

Logarithm of cell number vs time
Lag phase:
Cell synthesizing new components
Varies in length

Exponential phase (log phase):
Doubling occurs at a constant rate
Population is most uniform in terms of chemical and physical properties during this phase

Stationary phase:
Total number of viable cells remains constant
Reproductive rate is balanced by death rate
Why does this happen?
Nutrient limitation, limited oxygen, toxic waste accumulation, and critical population density reached.

Death phase:
Number of viable cells declines
Two alternative hypothesis

- Cells are viable but not culturable (alive but dormant)
- Programmed cell death

Long term stationary phase
Population continually and dying cells provide nutrients for other cells

Slide #12
It is estimated that only 1% of bacteria are responsible for diseases.

Tyndallization
Sterilization of a fluid by heating it repeatedly, separated by intervals of incubation at a temperature favorable for bacterial growth. With each heating the bacteria which have developed from the more resistant spores are destroyed; when finally no undeveloped spores remain the fluid is sterile.

Common electron microscopes used for research: transmission electron microscopy, scanning electron microscope

Refraction of a surface is higher than the refraction of air, then refraction happens

100x from oil is 100x10 magnitude
Only know the eyepiece, objectives and magnifications
Go over gram positive and gram negative concepts.
And different types of stains
Mitobacteria: They don’t posses gram positive and gram negative, instead theypossess

Microbiology Chapter 4

Growth and development

Suppose that one cell out of a million has a mutant gene blocking s-layer synthesis and suppose that the mutant strain can grow twice as fast as the s-layered parent. How many generations would it take for the mutant strain to grow 90% of the population?