



General-microbiology-notes

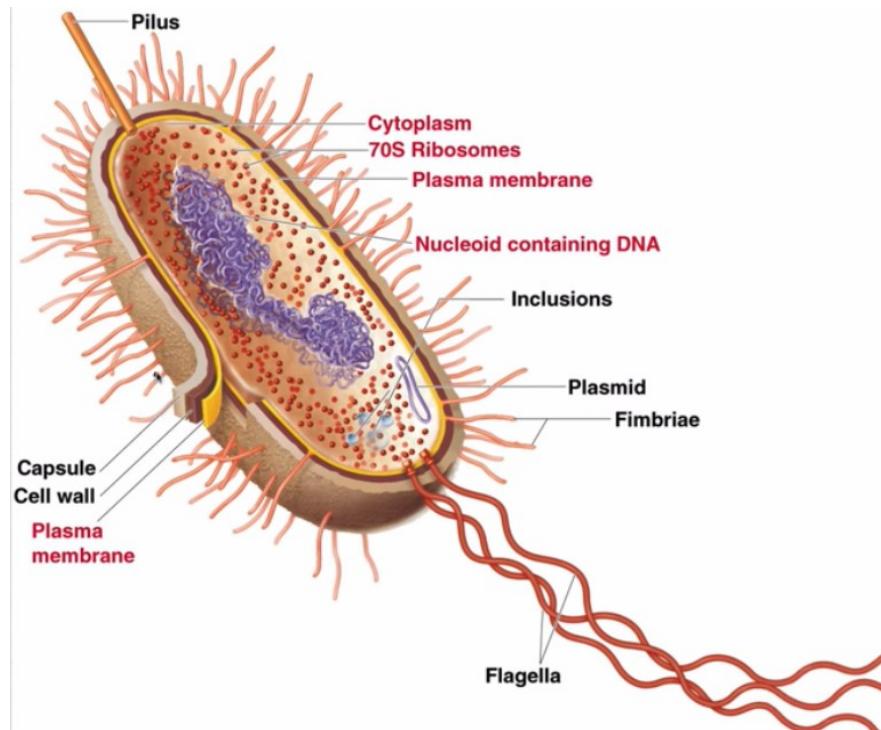
General Microbiology (University of Technology Sydney)



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General Microbiology notes:

Week 2: Bacterial Structure → the cell surface

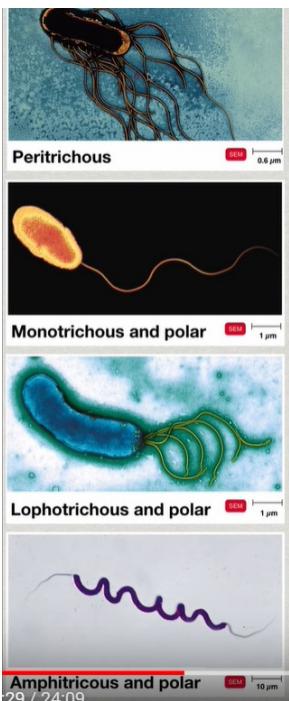


Red = Found in all bacteria

<p>capsules and slime layers</p> <ul style="list-style-type: none">- Outside of the cell surface- Made of polysaccharides (sometimes embedded with proteins)- Sticky and allows cells to adhere to things <p>Capsules</p> <ul style="list-style-type: none">• Layer of material outside of the bacterial cell• Well organised• Not easily washed off as it is attached to the cells surfaced <p>Slime layers</p> <ul style="list-style-type: none">• Layer of material outside of the bacterial cell• Poorly organised e.g. diffuse• Easily washed off not attached to the cell	<p>Advantages</p> <ul style="list-style-type: none">➢ Inhibit phagocytosis➢ Resistance to desiccation (polysaccharide consists of mostly water)➢ Can exclude bacterial viruses. Viruses need to bind to the protein, and they cannot➢ Can aid in attachment both inanimate and animate surface <p>e.g. dental plaque → stick and form biofilm.</p> <p>Ink cannot penetrate the cell due to the capsule</p>
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Flagella

- Allows the cell to be motile
- Thread like appendage approx. 20nm thick and 15-20um long
- Depending on the number and position of the flagella gives the names



Across or around the cell

Polar end of the cell

Multiple and at the polar end

At both ends

Rotation of the flagella in the counter clockwise directions allows to move. To stop the flagella rotates in the clockwise direction and the flagella unbundles and the cell tumbles in a different direction

Chemoreceptors in the plasma membrane that detect nutrients and the cells will swim in that direction.

Axil filaments

- Some cells don't have flagella but can still be motile
- This is internalised in the cell
- Found in spirochetes – cork screw motion
- Drill through thick gummy environments (mucus)

e.g. *Treponema Pallidum* – Syphilis
borrelia burgdorferi – lymes disease

Fimbriae

- Short hair like appendages
- 3-10nm diameter
- Few to hundreds of cells
- Slender tube of helically arranged protein subunits
- Protein at the tip that is a receptor to allow binding
- Mediate attachment to surfaces e.g. bladder cells
- Specificity towards cells – what tissues they infect e.g. cornea cells

Pilli

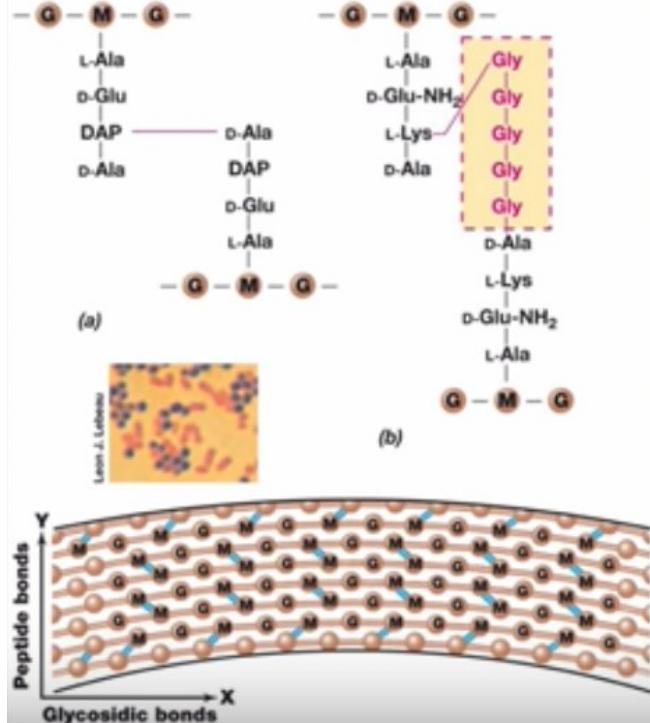
- Thicker and longer appendages than fimbriae
- 9-10nm usually 1-2/cell
- Mediate to surfaces e.g. bladder cells
- Twitching motility – bacterium attached to a surface then retracts the pili and glides over the surfaces
- DNA transfer (sex Pili)
 ➔ Pillis connect, retract, pull the cells together and DNA transfer (conjugation – cell to cell contact allowing DNA transfer)

Cell wall

- Rigid structure lying just outside the bacterial plasma membrane
- Comprised of Peptidoglycan consisting of two modified sugars. NAM (M) and NAG (G)

from carbohydrate strands

- The cell wall provides characteristic shape and protects from osmotic stress different between gram positive and negative bacteria
- Gram positive and gram negative both have a cell wall – thicker in gram positive
- Gram positive – glycine peptide interbridge cross linking strands
- Gram negative – direct link between strands
- Side chains have different compositions in neg and pos
- Positive have a five gly interbridge



- It is thought that the peptidoglycan is synthesised in “cables” about 50nm wide, with each cable containing several cross-linked carbohydrate strands
- The cables become cross-linked to form a strong rigid cell wall

→ The area between the plasma membrane and cell wall is the periplasmic space

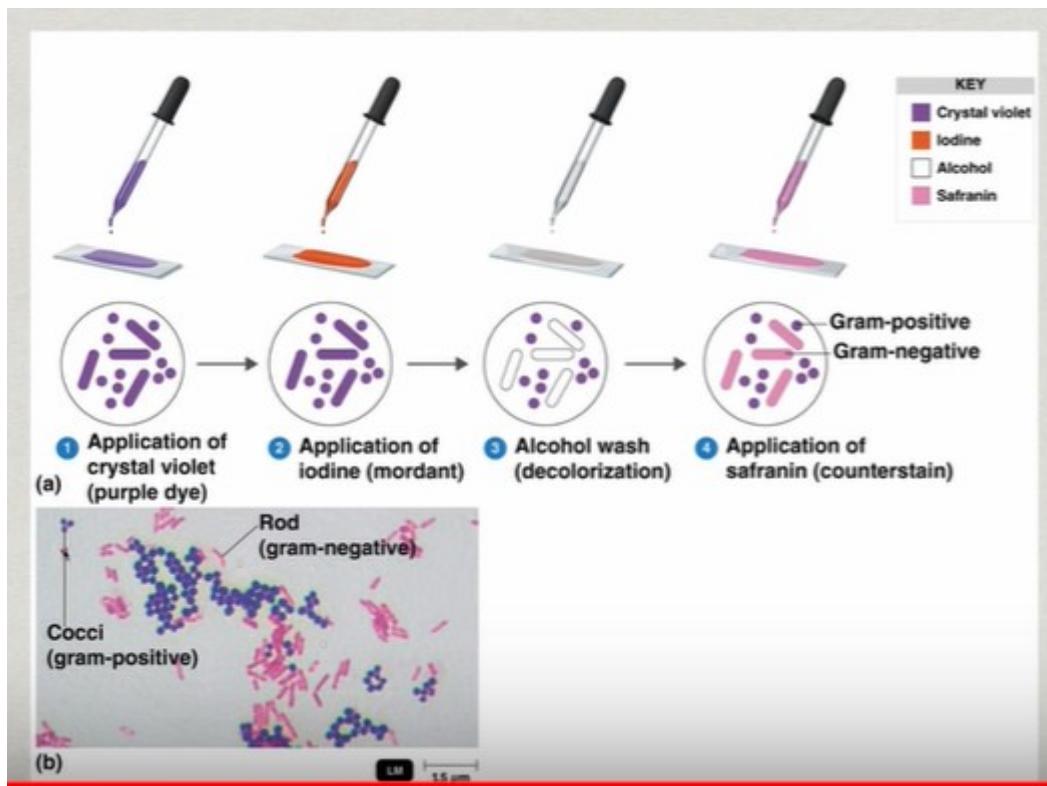
Gram positive

- Thick peptidoglycan -20-80nm
- Lipoteichoic and teichoic acids. Negatively charged and antigenic specificity

Gram negative

- Thin peptidoglycan – 2-7nm
- Lipopolysaccharide

The Gram stain



- Flood smear with crystal violet which goes into both cells making them both purple
- Iodine sticks to crystal violet
- Alcohol dehydrates cell walls both positive and negative cell and removes outer membrane of gram neg bacteria
- Cell wall shrinks and pores become smaller (gram pos has larger cell walls with smaller pores)
- Alcohol washes away the crystal violet from gram negative because the pores are larger
- Counter stain carbol fuchsin to stain the cells that have become clear and gram pos appears pink

Lipopolysaccharide:

Only in gram negatives

LPS contains

- Lipid A - Structure Embedded in outer membrane
- core polysaccharide - Conserved
- O side chain - variable from bacterium and antigenic

LPS functions:

- Sugars - bacteria negative charge
- Enables bacterial attachment
- Important biofilms
- Creates permeability (e.g. detergents and bile salts)
- Lipid A - endotoxin(attached to the cell) - when released into blood stream can cause fever, shock and blood clotting)

Bacterial structure – internal cell structures

Cytoplasm

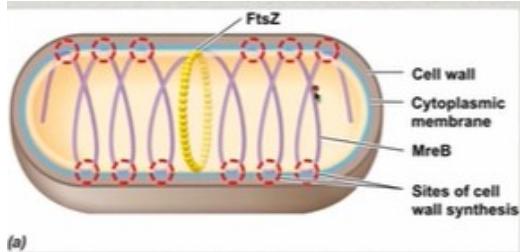
- Substance in which the nucleoid, ribosomes and inclusion bodies are suspended

Cytoskeleton

- Proteins that help organise structures in the cytoplasm. Help direct and act as a scaffold for localisation of proteins. (like scaffolding of a building)

Examples

- FtsZ (eukaryotic homologue tubulin)
- mreB (eukaryotic homologue actin)
- cell shape n rod shaped cells
- site for positioning proteins for cell wall synthesis



(a)

- crescentin (CreS, intermediate filament protein)
- gives cell curve shape
- found in Caulobacter crescentin

FtsZ-GFP in Escherichia coli

- forms are ring in the centre of the cell
- site for recruitment of cell division proteins
- required for formation of the septum



70S ribosomes

- the site of protein synthesis (mrna translation in to amino acid polypeptides)
- composed of both RNA and protein
- bacterial and archaea ribosomes 70S (archaea more similar to eukarya)
- eukaryotic cell ribosomes 80s (s=Svedberg unit)

70s smaller

Plasma Membrane

- Selectively permeable barrier
- Hydrophobic and hydrophilic → phospholipid by-layer
- Peripheral (loosely attached to the cell surface) and integral proteins (embedded in the membrane)
- Nutrient and waste transfer
- Secretion of toxins enzymes e.t.c
- Location of many metabolic processes (electron transport channel) e.g. respiration,

- photosynthesis
- Detection of environmental cues for chemotaxis (detects the environment and allows movement to/from chemicals and nutrients) uses proteins
- H₂O, O₂ and CO₂ can penetrate the layer

Nucleoid containing DNA

- E. coli chromosomes = 1400 nm (230-700 times longer than cell)

Supercoiling (allow long chromosomes to fit inside cells)

- Nucleoid-associated proteins bend and fold DNA and help stop it from unravelling
- DNA packaged to reduce overall size

Plasmid

- Small, closed circular ds(DNA)
- Replicated independently of the bacterial chromosome
- Not required for bacterial cell growth or reproduction
- Can carry gene that confer selective trait (e.g. Antibiotic resistance)
- Can move between different bacteria

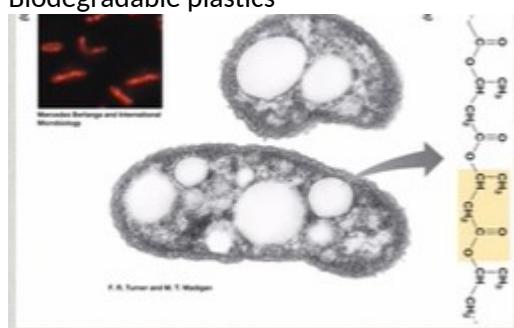


Inclusions

- Granules of organic or inorganic material within the cytoplasmic matrix
- Some are storage of nutrients (e.g. PHB, sulfur granules) e.g. in starvation mode they can access granules for energy
- Some are important function (e.g. gas vacuoles, magnetosomes)

Poly-beta-Hydroxybutyrate granules in *Bacillus megaterium*

- Carbon + energy stores
- Biodegradable plastics



Magnetosomes in *Magnetospirillum magnetotacticum*

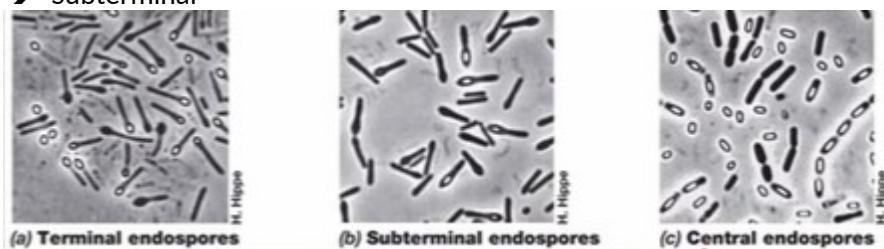
- Contains Fe₃O₄ particles and the iron acts as a tiny magnet
- These inclusion bodies help determine north or south (orientation)
- Swim towards nutrient rich sediments

Polyphosphate granules in *Corynebacterium*

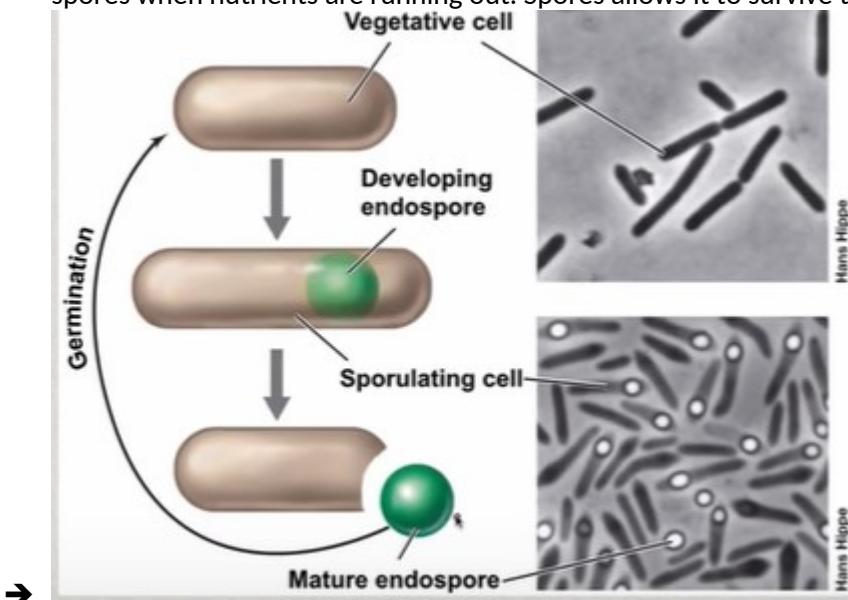
- Phosphate storage

Endospores

- Dormant structures in some Gram positive bacteria e.g. *Bacillus*, *Clostridium*
- Develop within vegetative cells (growing cells)
- Can be positioned in different places within the bacterium
 - Central
 - Terminal
 - Subterminal



- Highly resistant to environmental stress (heat, UV, Gamma radiation, chemical disinfectants, and desiccation)
- Heat resistant to environmental stress (heat, UV, gamma radiation, chemical disinfectants and desiccation)
- Heat resistant (>100C) compared to vegetative cells (approx. 55)
- They are metabolically inactive
- "sporulation" is a complex process which can take hours, which usually commences when growth ceases because of a lack of nutrients (survival). Organisms produce spores when nutrients are running out. Spores allow it to survive tough times.



Importance of spores

- Food Pathogens produce spores and can contaminate vegetable food items. Need to kill the spores (if tin is bloated means they were not killed) and can cause illness such as paralysis e.g. *Clostridium botulinum*
- Botox
- Disease from *Bacillus anthracis* and can cause blisters and gastro.

Week 3 – Microscopy in Microbiology & Bacterial Transport and Secretion

Microscopy in Microbiology

The Microscope (optical = use light)

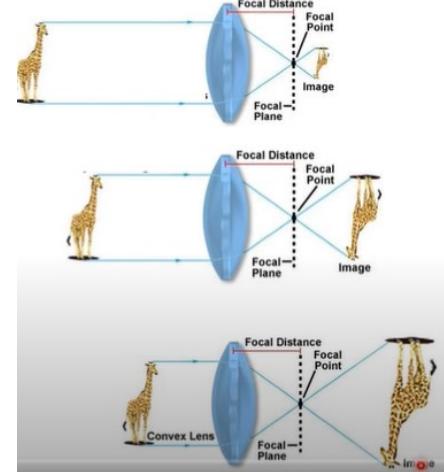
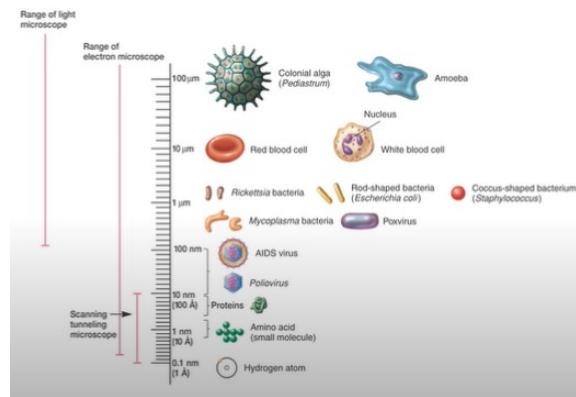
1. Light microscope
 - Can resolve structures down to 100nm
2. Phase contrast microscope
3. Fluorescent microscope
4. Transmission electron microscope

Simple bi-convex lens (magnifying glass)

- Real images just inverted
- 2 focal distance between lens and actual object = 1:1

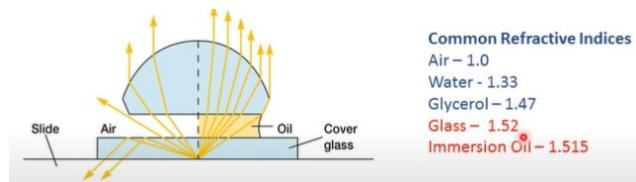
Refraction

- Light is bent = refracted when it passes through one medium to another
- Refractive index = measure of how greatly a substance slows the velocity of light
- The direction and magnitude of bending is determined by the refractive index (RI) of the two media
- Lenses focus light at a specific point called "focal point" (F)
- "focal length" = distance between centre of the lens and the focal point
- The strength of the lens is related to the focal length; short focal length = more magnification



How to manipulate refraction for imaging

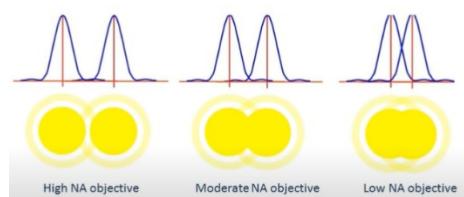
- More light collected = greater resolution
- The RI of immersion oil is the same as RI of glass = is used to increase the resolution in microscopy due to the minimisation of light being refracted away



Resolution (δ)

- = minimum separation distance between 2 points that is needed to distinguish them as two points (or – amount of detail collected in a microscope system = more detail)
- Dependant on the wavelength of light (λ) and the Numerical Aperture (NA) of the objective
- Light resolution of a light microscope is approx. 200nm
- E.g. 2 beads being observed, High NA allows separation

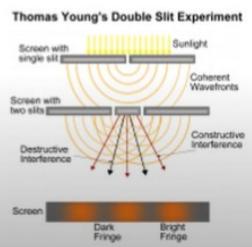
$$\delta = \frac{1.22 \times \lambda}{2 \times NA}$$



Optical Microscopy

- Light is a wave
- Can constructively interfere (create a bright fringe)

- Or destructively interfere (Dark fringe)
- Light we can see is between 400nm and 700nm
- Ultraviolet = hardest to see



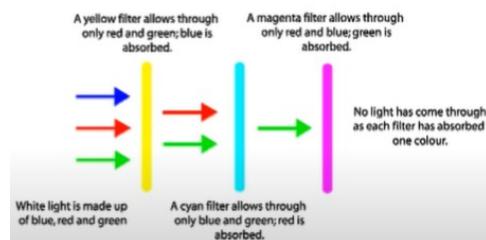
Light Microscopy – Transmitted Light Path

- Transmitted light microscopy relies on light waves passing through the specimen being observed
- Image is magnified by the objective lens (magnified by more lenses, such as ocular lens)
- Compound microscopes have >2 lenses

Contrast – How to achieve

Most cells being observed are made up of water and are therefore transparent

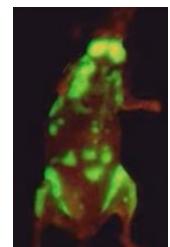
- Stains and Dyes – Absorption of wavelengths of light
 - Based on colourmetric system.
 - Different colours are used as filters as they filter out different light
 - E.g. gram staining
- Bright field and Dark Field – Rejection of reflected or refracted light
 - Dark = dark background with light (bright) object
 - Bright field = light background and dark object.
- Phase Contrast – Destructive Interference of Light Waves
 - Dark cell on light-ish background
- Fluorescence - Absorption of light energy
 - A high energy light (e.g. blue) is shot into a molecule, exciting it so that it releases a lower, longer wavelength (green)



Why use fluorescence microscopy

- Use for High sensitivity and specificity it can provide, and its contrast
- Gives a better insight to details of intracellular/subcellular structures and processes than in light microscopy.

Fluorescent Proteins



- Osamu Shimamura, Marty Chalfie and Roger Tsien discovered the green fluorescent protein (GFP) – called a **chromophore**
- This allows scientists to study interactions within cells while the cells are still alive
- Opened up new fields in Live-Cell imaging

Transmission Electron Microscope (TEM) (down to the nm range – can see viruses...)

- Uses electrons to illuminate, not light!!
- Wavelength on electrons = 100,000 X shorter than light photons = resolution is far greater
- Electrons scatter when they pass through thin sections of a specimen
- Transmitted electrons (those that do not scatter) produce image clearly showing intracellular morphology...
- However, the specimen must be cut super thin (nm's) and it uses harsh chemicals

Bacterial Transport and Secretion

Transport; Gram Positive & Gram Negative

- Gram positive = thick cell wall, cytoplasmic membrane
 - Cell wall = porous, so solutes can surpass and collect in the periplasmic space
- Gram negative = outer membrane, thin cell wall, then cytoplasmic membrane
 - Membrane is selectively permeable = Porins = allow solutes to go in and out
- Periplasm in both, contain a lot of activities. Lots of proteins that prepare solutes for transport and enzymes that modify the solutes for transport or breaking them down ready for full use

	Gram positive	Gram negative
Cell wall	thick (20-80nm)	thin (2-7 nm)
Peptidoglycan cross-link	glycine interbridge	direct link
Teichoic acids	Yes	No
LPS	No	Yes
Membranes	cytoplasmic	cytoplasmic and outer

Uptake of Nutrients into Cells

How do substance get into microbial cells?

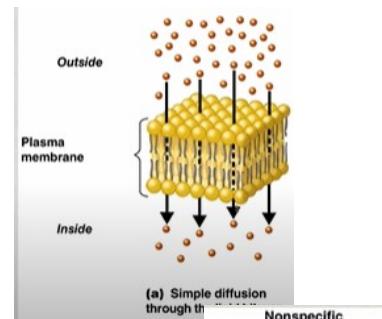
- Must be specific = only substances that are necessary are obtained
- Often up taking nutrients from dilute solutions against concentration gradient = energy required
- Nutrient must pass through selectively permeable membrane (only oxygen, water and CO₂, everything else requires a transport protein)

Therefore most microorganisms use several different transport mechanisms to obtain nutrient

1. Passive diffusion
2. Facilitated diffusion
3. Active transport
4. Group translocation

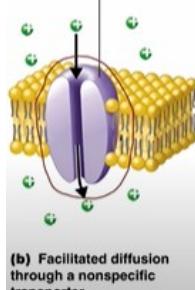
Passive Diffusion (no energy required)

- Movement of things from a high concentration to a low concentration
- How oxygen, carbon dioxide and water get through
- Diffusion rate is dependent on size of concentration gradient



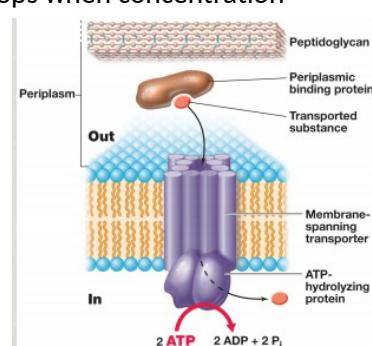
Facilitated diffusion (not energy dependent)

- Diffusion but using transport proteins to do so
- Size of concentration gradient impacts on uptake rate (number of substances on outside influences how much goes in)
- Can be specific or non-specific
- Transport proteins increase uptake rate which plateaus as the carrier protein saturates
- Cannot concentrate molecules in cell relative to outside (stops when concentration outside is = to concentration inside)



Active transport(ABC transporters) (energy dependent – ATP or proton force)

- Moves molecules against a concentration gradient
- This is important because nutrients can be very dilute
- Concentrates molecules inside the cell
- Involves carrier proteins (permeases)

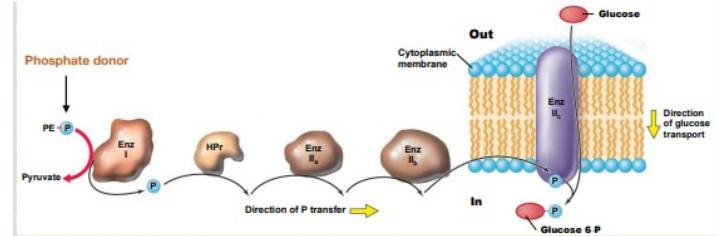


- Proton gradient produced by chemiosmosis – energy required. (inside is negatively charged and protons are pumped outside to create positive outside = like a battery)
- Artificially produced ion gradients allow for transport of other molecules.
- **Antiport:** When ion and other substance move in opposite directions.
- **Symport:** When ion and other substance both move in the same direction

Group translocation

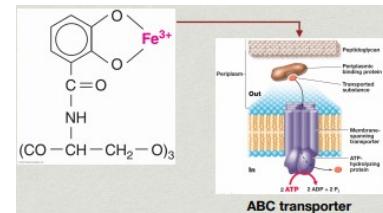
= Chemically modifies molecules as they are brought into the cell (good for transporting sugars e.g. glucose)

- Best known system is Phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS)
- • Transports a variety of sugars while phosphorylating them using phosphoenolpyruvate (PEP)
- Modification of sugar maintains concentration gradient.
- Enters directly into biochemical pathway for energy extraction. (by keeping the gradient it is allowed to pass straight into the glycolytic pathway = efficient)



Iron Uptake

- Ferric ion is very insoluble so uptake is difficult
- Microorganisms use **siderophores** (low molecular weight organic molecules) to aid uptake of iron
- Process:
 1. Siderophores complex with ferric iron and the complex is transported into the cell
 2. The iron-siderophore complex reaches the cell surface & binds to a siderophore receptor protein
 3. The iron is released to enter the cell directly or
 4. The entire complex enters the cell via ABC transporters



Why Secrete?

- Proteins must be delivered to inner or outer membrane
- Proteins are secreted outside to:
 - Breakdown large compounds on the outside so smaller compounds can be brought in for nutrition
 - Secrete toxins in pathogenesis e.g. haemolysins

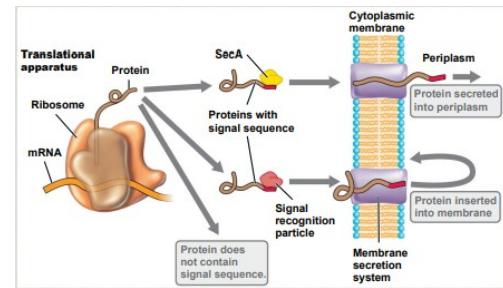
Secretion - Inside To Outside

- **Gram positive** – protein must cross the plasma membrane then through the relatively porous cell wall.
- **Gram negative** – proteins must cross the plasma membrane, then the relatively porous cell wall and then across the outer membrane
- Sec and Tat pathways used for secretion through cytoplasm membrane.
- Most proteins are secreted via the Sec-dependent secretion pathway.

- Gram negative bacteria either bypass this pathway and use several different secretion pathways or use Sec/Tat in combination with other pathways.

Sec-Dependent Pathway (ATP driven process)

- Proteins to be transported across the membrane exist as **"pre-secretory" proteins (pre-proteins)**
- The pre-protein has a signal peptide (N-terminus), recognized by SecA or signal recognition particle (SRP).
- Typically secA-exported proteins secreted to periplasm whereas SRP-exported go to cytoplasm membrane
- When the pre-protein emerges outside the membrane a **signal peptidase** removes the signal peptide and the protein folds into its normal form.



Protein Secretion Systems Of Gram Negative Bacteria

- Type I-VI secretion - delivery to outside
- Type I, III, IV and VI - form pore through both membranes
- Type II and V - Sec-dependent or Tat-dependent pathway used to secrete to periplasm then proteins secrete outside (use sec or tat to secrete)

Week 4 - BACTERIAL GROWTH & THE GROWTH CURVE

Bacterial Growth

The concept of cell growth:

- Growth can be defined as an increase in cell constituents that may result in:
 - increase in cell number (organisms reproduce by budding or binary fission)
 - increase in cell size
- Microbiologists usually study population growth (individual organisms are too small)
 - An individual bacterial cell can give rise to a colony (solid or semi-solid agar)
 - or it can grow in a broth culture (containing millions of cells in suspension)

Culture growth:

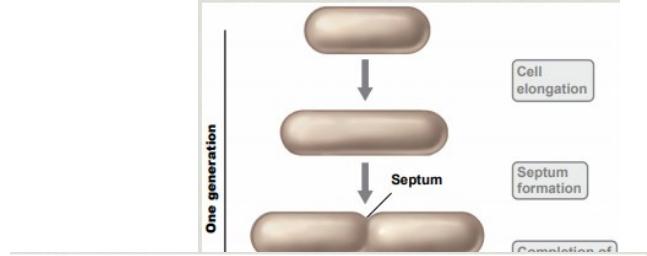
- With shaking, nutrients are evenly distributed so cells in a population are uniformly growing at the same time.

Colony growth:

- most rapid at the edge (oxygen and nutrients are more available at the edge)
- slowest at the centre
- the oldest part of a colony is at its centre, youngest part at the leading edge

Binary Fission

- The cell cycle is the complete sequence of events extending from the formation of a new cell through the next division.
- Most prokaryotes – binary fission
- Yeasts – budding (instead of splitting in half it grows a bud that falls off)



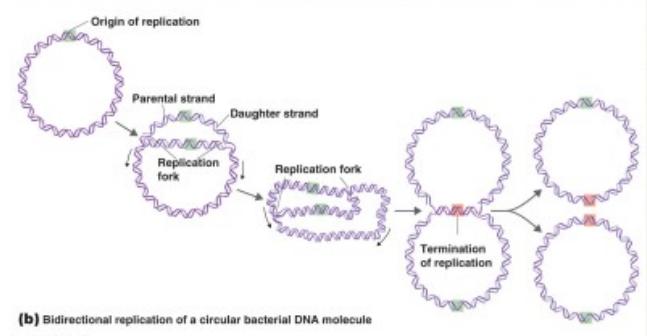
Binary Fission: DNA replication

There are 2 pathways needed:

1. DNA replication
2. Cell division (the formation of 2 daughter cells)

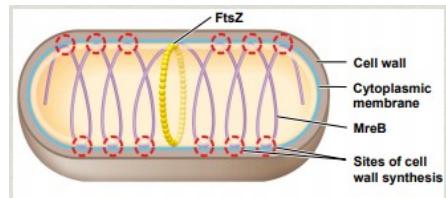
Most bacterial chromosomes are circular:

- **Origin of replication** is the site at which replication begins (where DNA replication begins)
- **The terminus** is the site at which replication is terminated
- **The replisome** is a group of proteins needed for DNA synthesis



Binary Fission: Cell Division

- Cell Division = process by which 2 daughter cells occur
 - Not completely understood
 - It involves cytoskeletal proteins
- Cytoskeleton:
 - **MreB** (actin homologue) determines cell shape and helps moves DNA to either end of dividing cell.
 - **FtsZ** (tubulin homologue) creates the Z-ring (middle of the cell) these proteins participate in cell division, localize proteins to positions within the cell, and determine cell shape. (allows for eventual cell division to occur)
- How does it know where the centre of the cell is for division? A couple of proteins:
 - MinD-GFP fusion protein oscillating from one end of an E. coli cell to the other
 - MinC oscillates with MinD – blocks septum formation
 - MinC concentration highest at poles, meaning that there is less in the centre = allows for septum to form in the centre



Cell Division – Separation

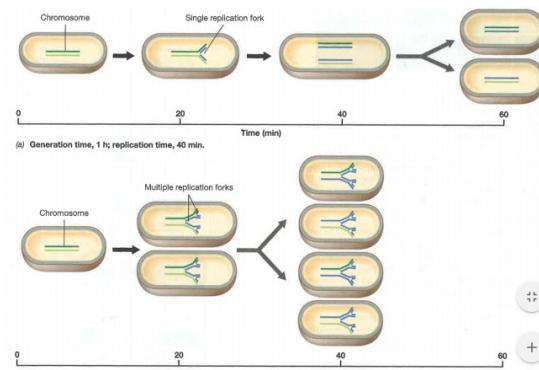
Multiple steps (summary of above)

1. Selection of the site where the septum will be formed – role for MinCD
2. Assembly of a specialized structure called the “Z ring” (it divides the cell into 2 by constriction)
3. Assembly of cell wall synthetising machinery, and
4. Construction of the Z ring and septum formation

DNA Replication and rapidly growing cells

- Bacterial cell division occurs as just described in slow growing cells
- BUT how can cells replicate so fast

The process usually takes about an hour, but E. coli takes 20 mins to prepare for division and 40 mins to fully replicate



- This is because E. coli doesn't have wait for the full chromosome to replicate before division occurs. (bottom division)
- Chromosome begins to form fork, which is passed onto two daughter cells

Pt II - THE GROWTH CURVE

- Cell division increases the number of cells in a population
- Population growth is studied by analysing the growth curve
- In liquid medium cells are cultured in a closed system or batch culture with no new fresh medium. Over time:
 - nutrient concentrations decline
 - waste components increase
- The growth of organisms dividing by binary fission is plotted as the logarithm of the number of viable cells versus incubation time. = plotting log no. of cells or optical density over time

Lag phase:

- Cells synthesising new components (need to adapt to new medium)
- Lag phase (time) can vary in length

Exponential (log) phase:

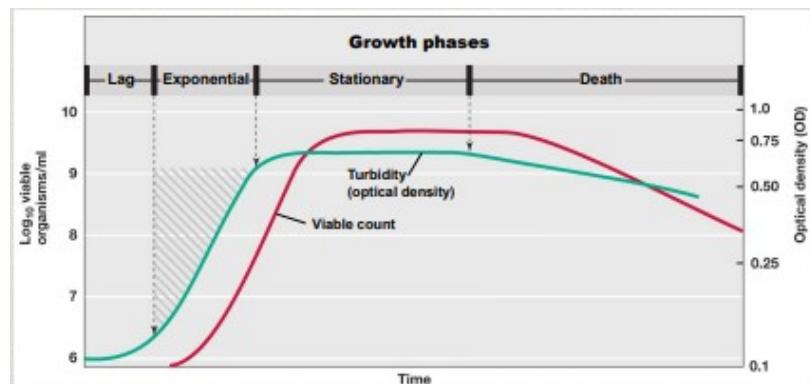
- The rate of growth is constant
- The population is most uniform (physiologically – each cell is essentially the same)
- It represents “balanced” growth i.e. cells constituents are manufactured at a constant rate relative to each other
- If a nutrient or environmental condition changes, “unbalanced” growth results. (would need another lag phase)

Stationary phase:

- total number of viable cells is constant
- occurs because metabolically active cells stop reproducing or because the reproductive rate is equal to death rate.
- Occurs because nutrients are limited, oxygen availability is limited, or toxic wastes have accumulated.
- A starvation response occurs resulting in morphological changes (e.g. endospore formation or decrease in cell size)

Death phase:

- No cell growth (synchronous culture)
- • Possibly cells are “viable but non-culturable” (VBNC) = some cells die to allow other to survive
- Possible that cells die from apoptotic cell death (suicide)



Mathematics Of Microbial Cell Growth

Generation Time (or doubling time)

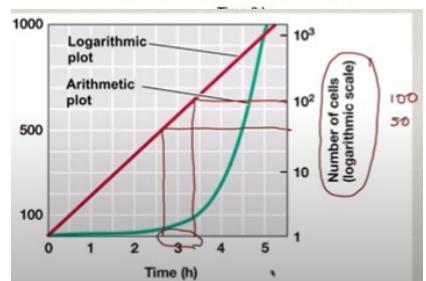
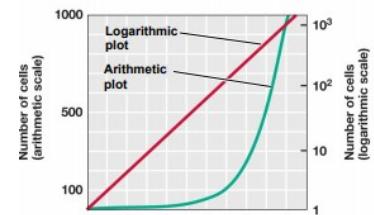
- = time required for a population to double in size
- Varies depending on bacterial species and environmental conditions
- Some bacteria double in 10 mins and others take much longer
- Some eukaryotes double in several days

How To Determine “Generation Time”

- Population data are plotted for number of cells (log axis)
 - time to double the population is extrapolated from the plot
 - Generation times vary markedly with microbial species
 - Environmental conditions can change the doubling time
1. Find a period of doubling (e.g. 50 – 100 cells)
 2. Draw lines to the logarithmic plot
 3. Calculate the difference in time
 4. Can only be calculated in exponential phase (where doubling occurs)

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 (2^8)
0.5	2	4.5	512 (2^9)
1	4	5	1,024 (2^{10})
1.5	8	5.5	2,048 (2^{11})
2	16	6	4,096 (2^{12})
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 (2^{20})

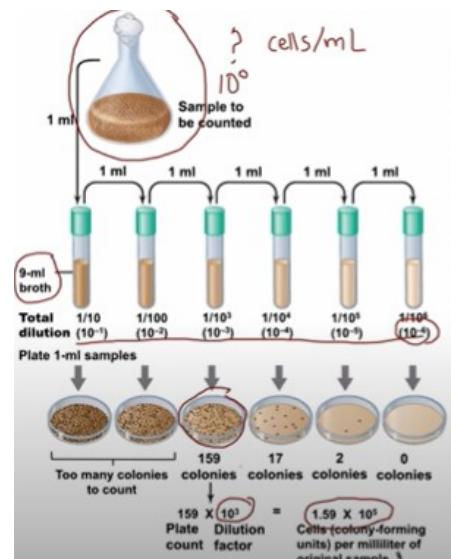
(a)



Measurement Of Microbial Growth

Measurement of cell numbers

1. Direct counting (use a microscope and counting chamber)
 - Grid to count cells within a grid
 - Disadvantages: =
 - Time consuming
 - Can't differentiate dead from live cells although can use stains to solve this
2. Electronic counting (Coulter counter or flow cytometer)
 - Cells run through a laser, which detects and counts the cell (flow cytometer)
 - Cells run through a chamber, which changes current (Coulter counter)
3. Culture techniques →
 - Start off with flask (unknown cells/mL)
 - Use a dilution series with tubes of 9mL of broth and add 1 mL to the first tube (1:10)
 - Then u continue adding 1 mL of the diluted tube into a second tube of 9mL broth.
 - Then plate them out and see colonies and count those between 30-300 cells.
4. Cell mass
 - Increases in the total cell mass
 - Take the total dry weight (take culture, spin out cells, remove liquid then dry and measure the weight)
 - Or use spectrophotometry (light measuring number of cells)



Week 5 - ENVIRONMENTAL FACTORS AFFECTING MICROBIAL GROWTH & TAXONOMY AND CLASSIFICATION

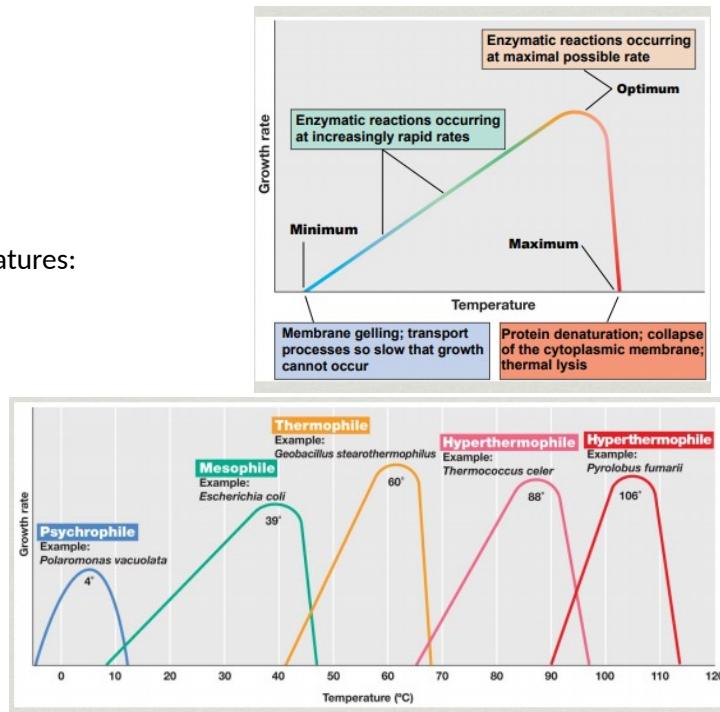
ENVIRONMENTAL FACTORS AFFECTING MICROBIAL GROWTH

- Microbes are affected by changes in physical and chemical nature of their environments and often adapting to severe environmental conditions (extremophiles)
- main environmental influences are:
 1. Temperature
 2. pH
 3. Osmolarity and water activity
 4. Oxygen level
 5. UV Radiation

Temperature

- Microorganisms have distinct cardinal temperatures:
 - Minimal
 - Maximal (optimum)
 - Optimal
- Psychrophiles grow between 0-15°C
- Mesophiles prefer 20-45°C (most microbes)
- Thermophiles grow in extreme temps >55°C. They often grow at 45°C, but often higher
- Hyperthermophiles some even grow at up to 95°C+

High Temperature - How Do They Do It?



Thermophiles & hyperthermophiles manage by:

- Stabilizing proteins through H bonds/prolines
- Express heat shock protein (molecular chaperones)
- Histone-like proteins to stabilize DNA

pH

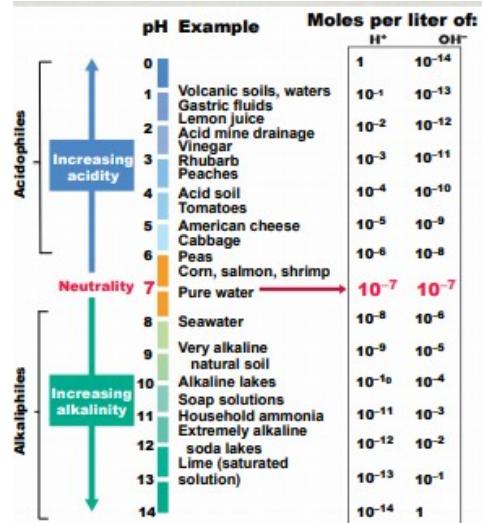
- Acidophiles grow between pH 0 - 5.5
- Neutrophiles grow between pH 5.5 - 8.0
- Alkaliphiles prefer pH 8.0 - 11.5

pH dramatically affects microbial growth by:

- disrupting the plasma membrane
- altering the activity of enzymes
- altering the activity of membrane transport proteins

pH - how to respond?

- By using mechanisms that maintain neutral cytoplasmic pH (but the plasma membrane is impermeable to protons)



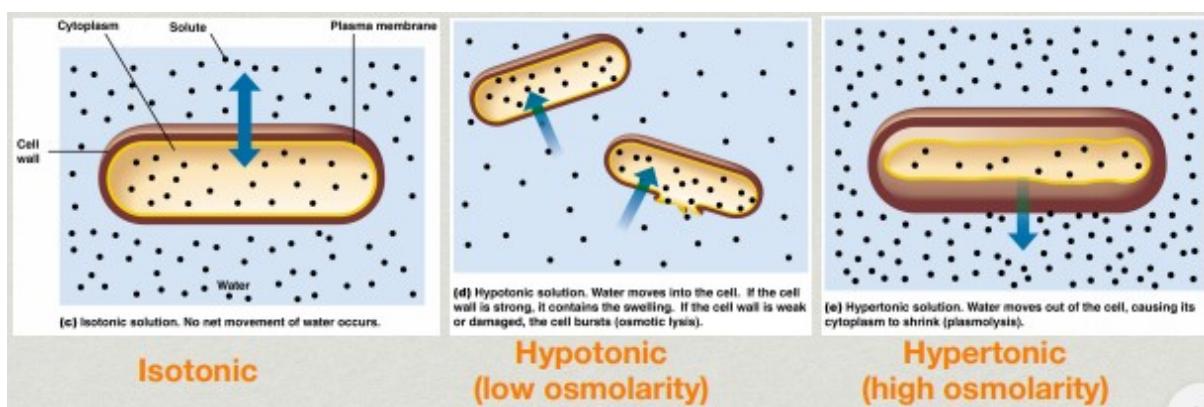
- e.g. Neutrophiles can exchange K for protons using an antiport transport system or use an proton translocating ATPase (= it pumps out H⁺ and replaces with K⁺ to keep inside neutral)

Osmolarity = water activity

- Water activity is the amount of water available to an organism
- Water availability decreases with saltier or sugarier substances

Water activity	Material
1.000	Pure water
0.995	Human blood
0.980	Seawater
0.950	Bread
0.900	Maple syrup, ham
0.850	Salami
0.800	Fruit cake, jams
0.750	Salt lakes, salad fish
0.700	Cereals, candy, dried fruit

- Isotonic = amount of solutes on the outside and inside of a cell are similar = no net movement = water amount is equal on inside and outside of cell
- Hypotonic (low osmolarity) = cell is salty (lots of solutes on inside) e.g. come from salt water marine environment to a fresh water one.
 - Solutes can't pass the membrane but water can (selectively permeable) so water goes in to equilibrate the salt concentration inside and outside the cell. If the cell wall isn't strong enough, it can pop open and lyse.
- Hypertonic (high osmolarity) e.g. organism moves from fresh water to marine = more solutes outside than inside = water from inside the cell rushes out to try equilibrate concentrations.
 - Causes retraction of the cell membrane from the cell wall (shrinking)
 - Can cause cell death (not as dangerous as lyse) but does stop growth

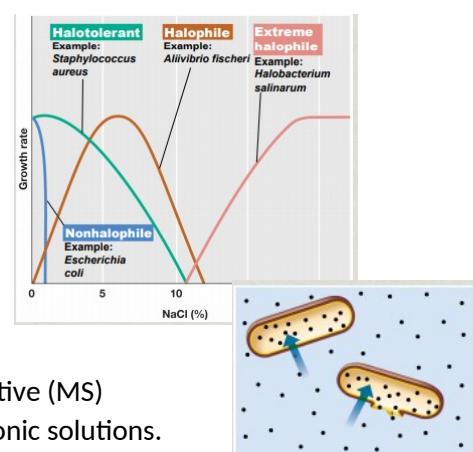


Osmolarity Cont.

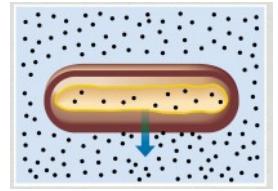
- Non-halophile organisms cannot tolerate high salt
- Halotolerant organisms can grow over a wide range of (Aw)
- Halophiles optimum growth in the presence of high salt >0.4M salt
- Extreme Halophiles optimum growth in the presence of very high salt >2M salt

Osmolarity - how to respond?

- **Hypotonic** e.g. from salt to fresh water= Use of mechano-sensitive (MS) channels – allow solutes out when organism is placed in hypotonic solutions.



- Open channels to allow solutes to rush out in the hope it doesn't burst
- **Hypertonic** e.g. fresh to salt water = Increase internal osmotic concentration through the synthesis or acquisition of choline, betaine, proline, glutamic acid and other amino acids or high K⁺ ions. These compounds balance out salts in the cells without affecting metabolism.



Oxygen Level

- The importance of Oxygen to the growth of an organisms correlates with its metabolism = those that can tolerate oxygen use oxygen (aerobe)
- **Aerobe** - an organism able to grow in oxygen (atmospheric O₂)
- **Anaerobe** - an organism that can grow in the absence of oxygen

Experiment →

Obligate aerobe = need oxygen (at top of tube)

Strict anaerobe = no oxygen (bottom of tube)

Facultative anaerobe = grow anywhere (can grow with or without O₂)

Microaerophile = can deal with some oxygen

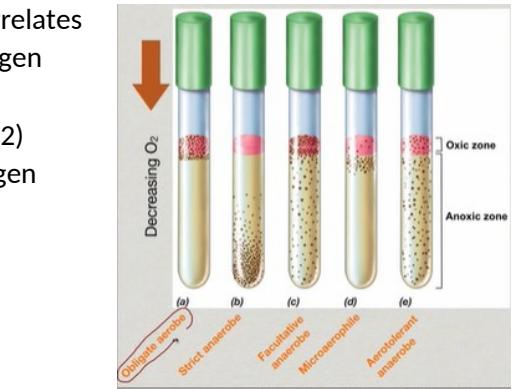
Aerotolerant anaerobe = can grow with or without O₂ but there is no difference in growth

Oxygen is toxic - how to respond?

Why is it toxic? Because it can easily be reduced to toxic products:

- superoxide radical
- hydrogen peroxide
- hydroxyl radical

Which: Damages proteins and DNA.



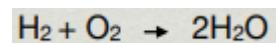
Reactants	Products
$O_2 + e^- \rightarrow O_2^-$	(superoxide)
$O_2^- + e^- + 2 H^+ \rightarrow H_2O_2$	(hydrogen peroxide)
$H_2O_2 + e^- + H^+ \rightarrow H_2O + OH^*$	(hydroxyl radical)
$OH^* + e^- + H^+ \rightarrow H_2O$	(water)
Outcome:	
$O_2 + 4 e^- + 4 H^+ \rightarrow 2 H_2O$	

Aerobes (oxygen tolerating) produce protective enzymes to do so:

- Super oxide dismutase
- Catalase

Growing Anaerobic Microbes

- Some important anaerobic organisms that you can't culture in aerobic environments, so we use anaerobic tools:
 - Anaerobic Chamber – uses a gas pack that produces Hydrogen gas (H₂), which reacts with any oxygen to produce water to ensure there is no oxygen



UV radiation

- Can cause DNA mutation = death (e.g. skin cancer)
- Causes formation of thymine dimers in DNA
- DNA damage can be repaired (if not it becomes cancerous)

How to respond to UV?

- Bacterial pigments

Week 5 pt 2 - TAXONOMY AND CLASSIFICATION

Microbial Taxonomy: Giving Order To Diversity

How do we classify microorganisms?

- We use the science of taxonomy (always changing)

How do we name them?

- System called Natural Classification developed by Swedish botanist Carolus Linnaeus

Why is taxonomy important?

- If we cannot identify and classify microorganisms, we cannot control them. e.g. Quarantine or in diagnosis for administering treatments.
- So we can ascribe characteristics learned from one microbe to others that are related.

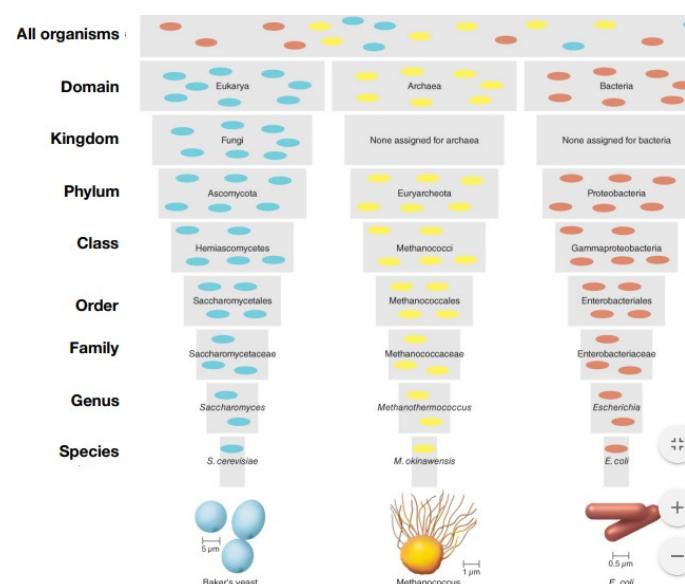
Three ways microbiologists classify microorganisms:

1. **Phenetic classification** = Organisms grouped according to phenotypic characteristics. (e.g. Flagella type or metabolic type).
 - Does not necessarily reflect evolutionary history
 - Still useful for some applications (e.g. hospital identification of infectious bacteria)
2. **Phylogenetic classification** (how we classify 3 domains of life = bacteria, archaea and Eukaryotes) = Groups of organisms are classed based on their evolutionary relationship. i.e. they have a common ancestor: "monophyletic"
 - Based on the DNA sequence of genes encoding ribosome subunits. e.g. 16S SSU rRNA
 - ribosomes are present in all cells, they are essential and stable markers of divergence, and their sequences are readily analysed.
3. **Genotypic** = considers other genetic similarities at a genomic scale e.g. The order of genes along a chromosome, whole genome sequences, genomic hybridization

- What shape is the microorganism?
- Can it grow using alanine as a carbon source?
- Where was it isolated from?
- Can it survive above 45°C?
- Is it motile?

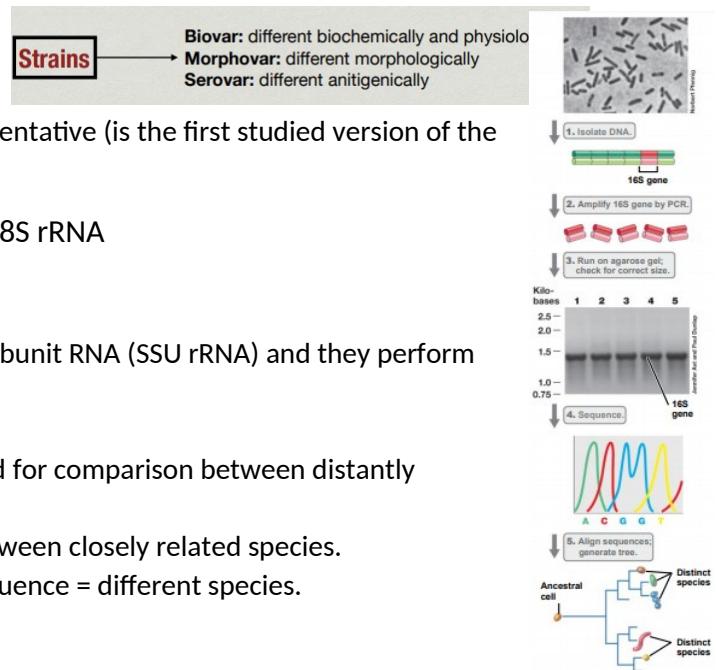
Binomial System For Naming Microorganisms

- When we name microorganisms, they consist of 2 parts
 - Generic name – the Genus (e.g Escherichia)
 - Specific name – the Species (coli)
 - Put together = (e.g. *E. coli*)
- Note: must italicise when referring to biological name. Once said once in a report it, the genus name can be abbreviated (e.g. *E. coli*)



Defining A Prokaryotic Species And Strain

- Strain = descendent from a single pure microbial culture (given to differentiate within a species)
 - prokaryotic species = a collection of organisms that share the same sequences in their core "housekeeping" genes (>98%). If they share 98% they are the same species.
 - Species defined as - collection of strains that share many stable properties and differ significantly from other groups of strains
 - Type strain = First strain of a species to be studied but not necessarily the most representative (is the first studied version of the species)



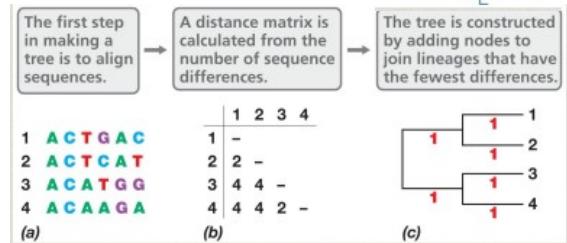
SSU rRNA (single subunit RNA): 16S rRNA and 18S rRNA

Why use 16S rDNA or 18S rDNA in phylogeny?

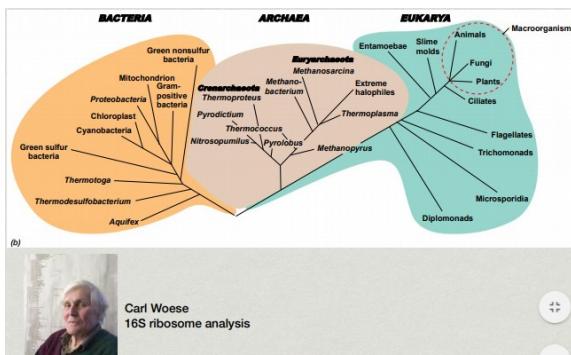
- All life has ribosomes – all life have small subunit RNA (SSU rRNA) and they perform the same role in all life.
 - Ribosomal RNA have regions that are:
 - conserved - allow for alignment and for comparison between distantly related species.
 - Variable - allow for comparison between closely related species.
 - Using this method, a 2-3% difference in sequence = different species.

Cladogram/ Phylogenetic Tree

- Basic phylogenetics
 - Microbes with more related SSU rRNA have diverged more recently and thus will have more characteristics in common than those more distant

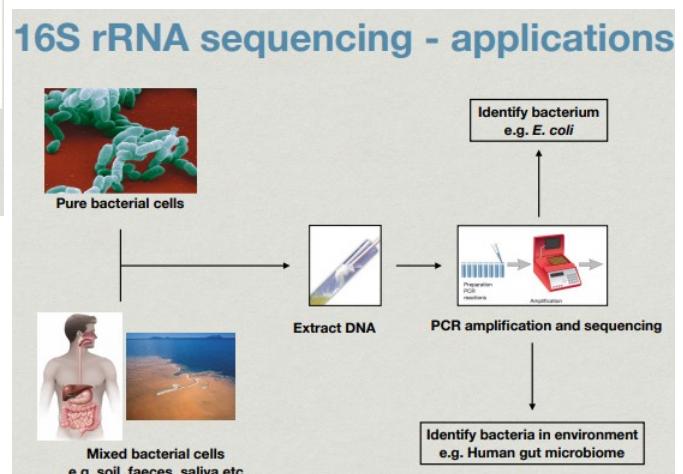


The Universal Phylogenetic Tree - The Three Domains Of Life



16S rRNA sequencing – applications

- Can use: pure bacterial cell or mixed bacterial cells



Week 6 - BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIAL PATHOGENS

Identification Of Bacteria

There are multiple ways of identifying bacteria:

1. Sequencing a common gene (e.g. 16SrDNA or housekeeping gene - rpoB)
 2. Sequencing the genome (takes a lot of time – not effective for quick treatment)
 3. Physical, Growth and Biochemical characteristics (can take more time but there are automated processes that make it faster)
3. Physical, Growth and Biochemical characteristics.

- Relies on the ability to grow organism in culture
- Must have a pure isolate (pure culture)
- Used a lot in hospital-based identification

Identification of bacteria is based on; Done in order:

- Gram stain reaction: Gram positive (purple) and Gram negative (pink)
- Shape and arrangement: cocci, or rods, singularly, clusters, chains, etc
- Culture characteristics: colony morphology and growth conditions e.g. aerobic/anaerobic
 - On blood agar, colonies often surrounded by haemolysis (lysed red cells) 3 types:
 1. α-haemolysis: the colonies are surrounded by a greenish zone of unhaemolysed and haemolysed red blood cells
 2. β-haemolysis: the colonies are surrounded by a wide zone of clear haemolysis of haemolysed red blood cells
 3. γ-haemolysis: there is no haemolysis surrounding the colony
- Biochemical metabolism: There are many tests e.g. Oxidase test, Catalase test...

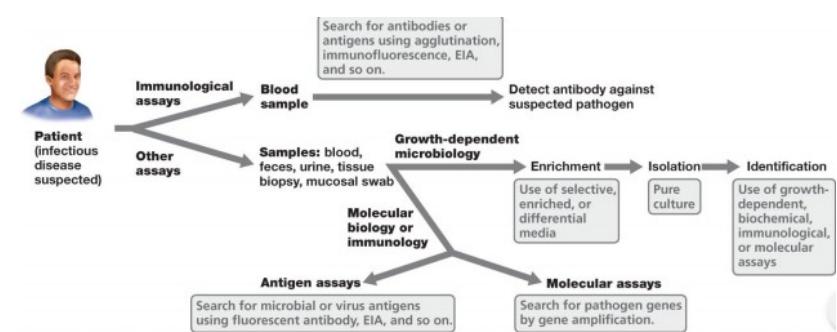
Identification Of Bacteria

First clues:

- Symptoms
- Site of infection
- Is there an outbreak?

Samples:

- Urine
- Blood
- Throat swab
- Wound site
- Faecal sample etc
 - The trick is to identify and isolate infectious agent amongst background “noise” of the normal microbiota. (differentiate the pathogen from the normal bacteria)



Identification Of Bacteria Cont.

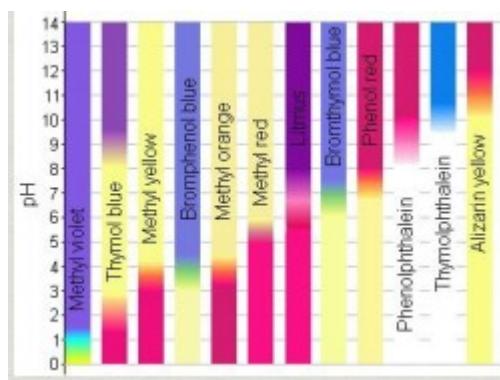
- Growth and nutritional characteristics are often used together with “Dichotomous Keys”.
- **Dichotomous Keys** = a series of “either/or” or “mutually exclusive” statements that leads to an ID.

- Gram positive have different Dichotomous keys. Work down the DK (tree)

Examples of Biochemical tests

pH Indicators

- Dyes that change colour when pH changes
- Can use these to determine whether an organism is using a certain metabolite and producing an end product that changes the pH
- E.g. 



Carbohydrate Fermentation

- There are a range of fermentable carbohydrates e.g. glucose, lactose, mannitol...
- Can only use some of these carbs, so it can help with identification
- Carbohydrate fermentation causes formation of diverse acidic end products which changes colour of dye. Usually use purple as indicator and if the microorganism uses the sugar, it turns yellow.
- Can also show if the microorganism produces gas

Examples of Carbohydrate Fermentation

MacConkey Agar

- medium contains lactose, peptone, bile salts, sodium chloride, and neutral red.
- Bile salts make it a selective medium (for intestinal flora)
- Lactose (carbohydrate source) and neutral red (pH indicator) allows the differentiation of lactose and non-lactose fermenting organisms.
- Results:
 - Lactose fermenter - red colonies e.g. *E. coli*
 - Non-lactose fermenter - creamy pale colonies e.g. *Proteus*, *Salmonella*, *Shigella*, *Pseudomonas*, etc.

Mannitol Salt Agar

- selective and differential media.
- Salt (selective agent is NaCl) allows *Staphylococcus* spp. to grow, inhibits most other organisms.
- Mannitol (carbon source) fermentation differentiates between *S. aureus* (pathogen) and other *Staphylococcus* spp.
- Results:
 - Mannitol fermenter - yellow colonies e.g. *S. aureus* (golden staph) – changes colour pink to yellow
 - Non-mannitol fermenter - red colonies e.g. *S. epidermidis*



Oxidation/Fermentation Test

- single tube test with soft agar containing glucose and peptone
- Simply stab through the agar (inoculate) and let it incubate for 2-5 days
- If less than upper half of medium is yellow the organism has oxidative metabolism (O)

- If yellow colour extends into bottom half of tube, the organism has a fermentative metabolism (F)

Gelatin Liquefaction

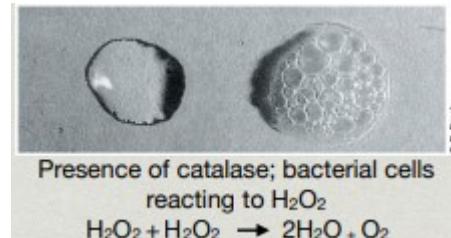
- Gelatinases are enzymes complexes that can act in concert to successively degrade the gelatin, initially to polypeptides and eventually to free amino acids. = ability to degrade gelatine and liquify it
- After incubation place the agar on ice for 30 mins, and then check for liquefaction



Catalase And Oxidase Tests

Catalase

- Most aerobic and facultative anaerobic bacteria contain the enzyme catalase
- Take Hydrogen peroxide and put cells on it. If catalase is present it will cause bubbling = Oxygen



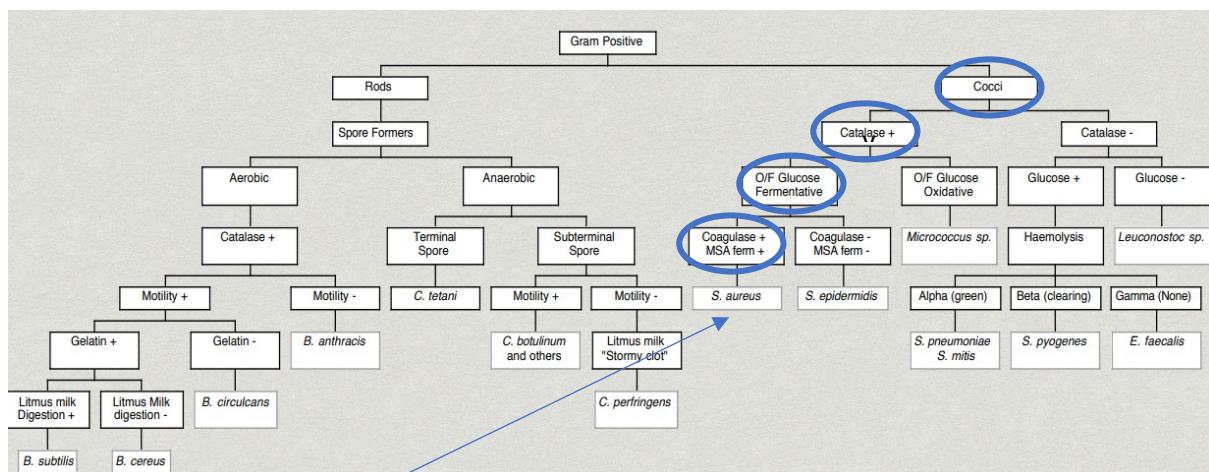
Oxidase

- tests for the presence of cytochrome C. (found in electron transport chain)
- Helps differentiate: Pseudomonas from Enterics.
- Take oxidase reagent and put it on filter paper, then add some colony. If it changes purple = oxidase positive

Example of Using the Dichotomous Key

Results:

1. Gram positive cocci
2. Catalase positive
3. O/F Glucose fermentative
4. Yellow colonies on Mannitol Salt Agar



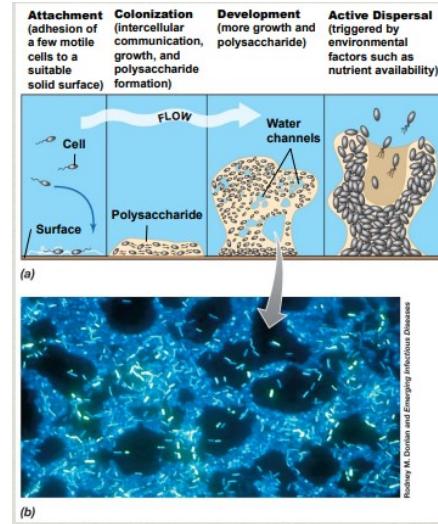
Organism = *S. aureus*

Week 9 – Biofilms What Are Biofilms?

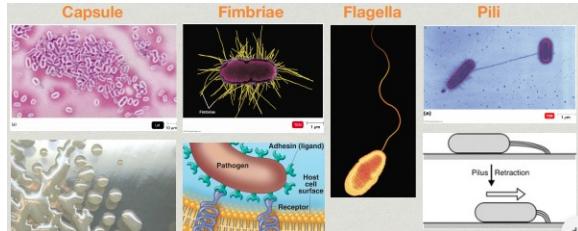
- = a collection of microorganisms surrounded by the slime they secrete, attached to an inert or living surface
- Usually in mixed microbial species (e.g. dental plaque has >700 species of bacteria and archaea. Produce acid that degrades tooth enamel, through fermentation)
- Biofilms are the default mode of growth for microbes
- Biofilms are more resistant than single cell (individuals) so can harder to remove

How Do Biofilms Form? 5 Steps

1. **Reversible adhesion** = become associated (adhere to a surface) but can dis-attach
2. **Irreversible adhesion** = upregulation of (e.g. pili, fimbriae, polysaccharide) causes permanent attachment
3. **Colonisation** = growth of cells and production of slime that helps them stick
4. **Development** = can develop unique structures (e.g. have holes that allow water and nutrient to flow in = helps keep it hydrated and getting nutrient)
5. **Active dispersal** = release bacterial cells to colonise new place (restart cycle)



Linking Bacterial Structures To Biofilm



conjugation (sex pili to attach two cells)

- Capsule = allow bacteria to stick and grow
- Fimbriae = important in making reversible to irreversible adhesion (attachment)
- Flagella = migration and help attachment
- Pili = movement across a surface of biofilm & for conjugation (sex pili to attach two cells)

Why Form Biofilms?

1. Resist phagocytosis by protozoa and immune cells (e.g. too big for cells to consume)
2. Collect nutrients from flowing liquid
3. Nutrient exchange (waste products can be a source of nutrient for other bacterium)
4. Resist penetration of toxic molecules such as antibiotics (may only affect outer surface of biofilm)
5. Genetic exchange (multispecies biofilms can interchange genes from transformation, transduction or conjugation)

Affects of Biofilms →

System	Impact of biofilm
Swimming pools	Pathogen survival & cosmetic degradation
Drinking water pipes	Pathogen survival & pipe corrosion
Food processing	Pathogen survival & contamination
Dental plaque	Acid production = caries &/or gingivitis
Toilet bowls	Cosmetic degradation
Medical devices	Failure of device - source of pathogen
Infection	Failure of treatment (e.g. antibiotics)

P. aeruginosa biofilm contaminated lung

Biofilm on voice prosthesis implant

Week 10 - LATERAL GENE TRANSFER

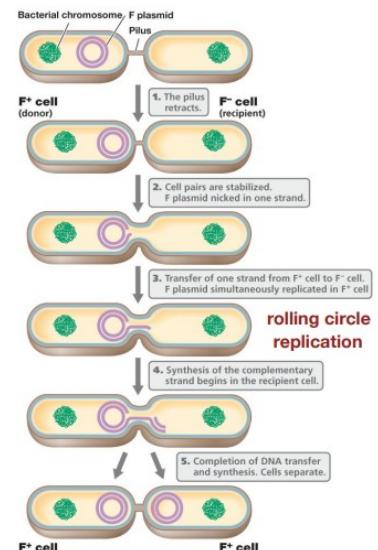
Gene Transfer

- Vertical Gene Transfer
 - DNA passed from mother cell to daughter cells during normal cell growth.
- Horizontal (or lateral) Gene Transfer
 - DNA can be transferred between mature cells of the same or different species.
 - **Conjugation:** direct transfer of chromosomal or plasmid DNA from one bacterium to another (requires cell contact)
 - **Transformation:** uptake of extracellular DNA (no direct cell contact required)
 - **Transduction:** transfer of bacterial genes by a bacteriophage (bacterial virus)

Conjugation

= transfer of DNA from one bacterium to another requiring cell-to-cell contact. (Transfers Plasmids)

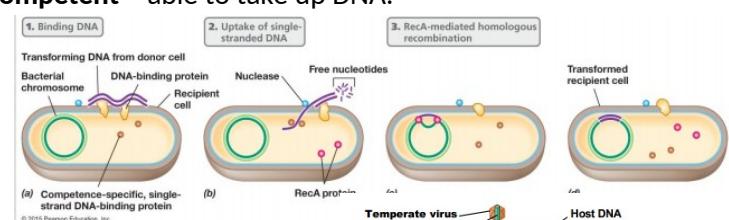
- **Plasmids** - small, circular double-stranded DNA molecules that replicate independently from the chromosome
- Fertility plasmids (F+) – contain genes for conjugations, including F-pilus (allows for DNA to go from one cell to another)
- Can encode important functions:
 - e.g.1. Antibiotic resistance genes (resistance plasmids) – major reason for the spread of antibiotic resistance genes in pathogens.
 - e.g.2. Virulence factors (virulence plasmids)



Transformation

= uptake of extracellular DNA (no cell contact required) e.g. fragments of chromosomal DNA

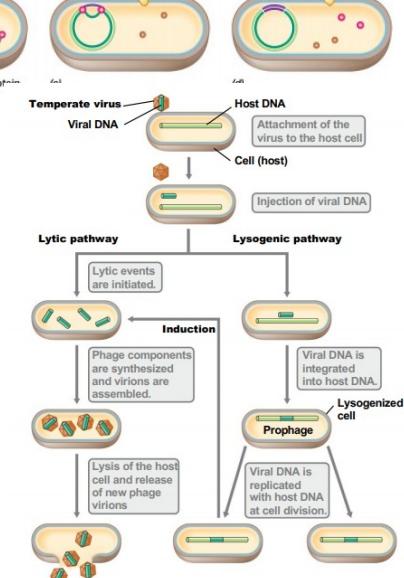
- The recipient cell in transformation must be **competent** = able to take up DNA.
- Some bacteria are naturally competent. e.g. *Bacillus subtilis* - late stationary phase of growth
- Integration of transferred DNA into chromosome mostly by **homologous recombination**



Transduction

=the transfer of bacterial genes by viruses.

- Bacterial viruses (known as bacteriophages) infect bacterial cells. Two cycles:
- 1. Lytic cycle
 - Injected DNA takes over cell machinery to make copies of DNA and copies of capsids. It assembles then causes cell to lyse (burst) to release copies of it.
- 2. Lysogenic cycle (when integrated, virus is called **prophage**)
 - Bacteria phage binds to cell and injects DNA into cell. The DNA incorporates itself into a specific site (same every time if the same bacterium DNA).



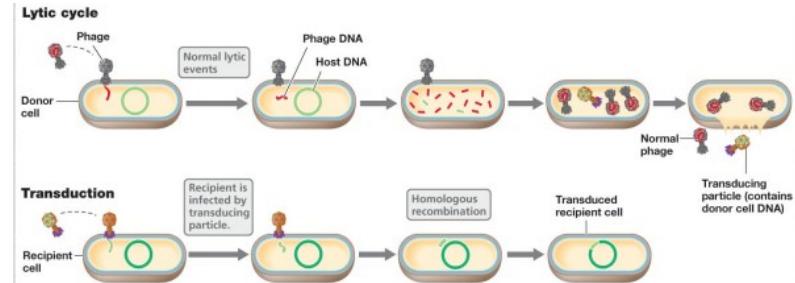
It replicates with the cell as copies are made, becoming part of the genome. The incorporated part = **Prophage**

- Some bacteriophage are lytic only (virulent phages) where others are both lytic and lysogenic (temperate phages).

2 parts of Transduction:

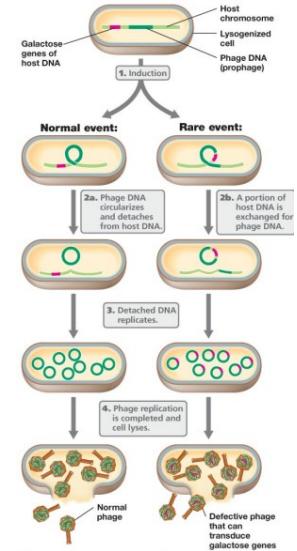
1. Generalised Transduction (takes advantage of lytic cycle)

- Bacteriophage injects DNA in cell. Degrades chromosomal DNA (host DNA) and uses proteins inside the cell to make copies of its DNA. Some leftover host DNA remains and can be incorporated into to phage being ejected (during protein packaging) = **Transducing phage**
- The Transducing phage can then infect another cell and inject its DNA into another cell and be incorporated into the hosts DNA.
- = Any part of the recipient genome can be transferred



2. Specialised Transduction (relies on lysogenic pathway)

- Injects DNA and becomes incorporated (called prophage)
- When lysogenic phage is forced into the lytic cycle. A normal event occurs where the phage DNA circularises out, copies itself, is packaged and released.
- However, on rare occasions of this process, it can circularise and copy other parts of the DNA and leaving a bit of the phage DNA. The DNA with phage and other DNA is packaged and released to infect other cells, passing on the donor (host) DNA.



Phage Conversion

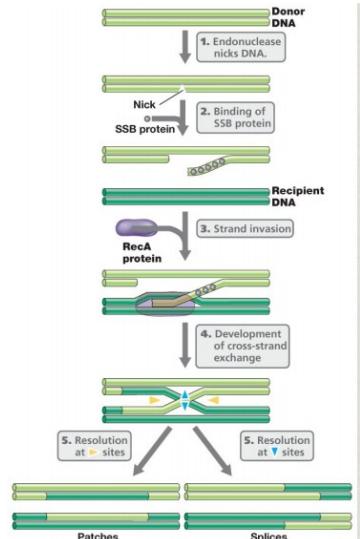
- = a Lysogenic phage carries genes that confer a phenotype to host.
 - e.g. ctx genes in *V. cholerae*. A phage with ctx gene in DNA can infect *Vibrio cholerae* (*V. cholerae*), adding the toxic gene to *V. cholerae* genome, converting it into something different (different phenotype). In this case it causes diarrhea.

DNA integration

- For Lateral gene transfer to be successful it needs:
- 1. Physical transfer of DNA (via conjugation, transduction or transformation) (as talked above)
 - Only exception, that requires only step 1 = plasmids (no integration needed).
- 2. Integration into the genome (needed for transformed and transduction)
 - Done through multiple methods but focus is **Recombination**

Homologous Recombination (needed for lateral gene transfer in transformed or transduction)

- Process by which one or more nucleic acid molecules are rearranged or combined with existing DNA to produce a new nucleotide sequence.
- Homologous recombination requires a high degree of sequence homology.
 - Which means – if they share 80% nucleotide identity, RecA protein allows them to swap bits of DNA.
 - This is one way that DNA that is transferred by lateral gene transfer can be integrated into a chromosome or a plasmid



Mobile Genetic Elements

- A variety of MGEs help carry genes that can integrate into bacterial genomes:
 - Transposons, Genomic/Pathogenicity Islands, Phage

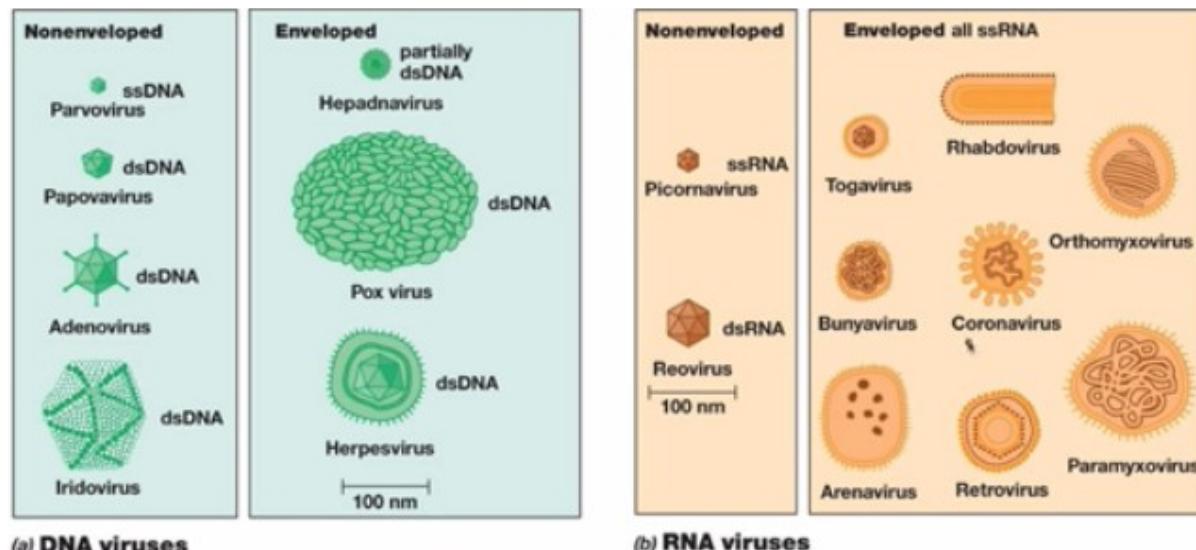
Microbiology week 11 videos

Animal Viruses that cause harm to humans

Learning objective: Compare and contrast the processes of bacterial and animal viral infections

Eukaryotic Viruses

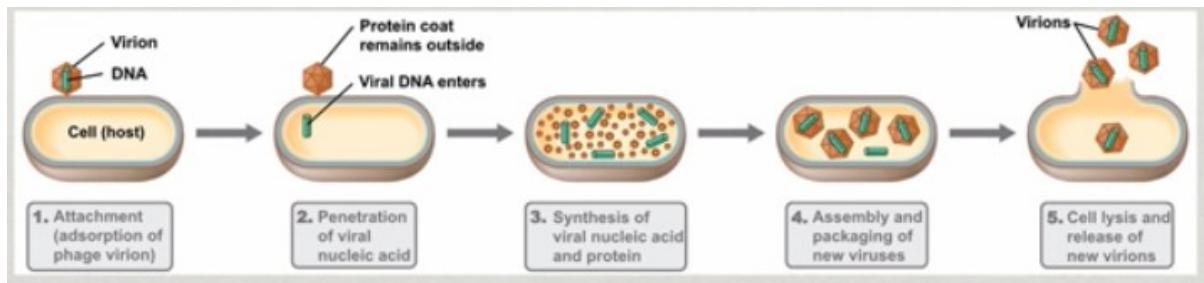
- ➔ Animal Viruses – all types of Nucleic acid (DNA or RNA)
- ➔ Most are enveloped – when they exit the cell they take a bit of the membrane of the cell



Generalised cycles of viral multiplication

1. Attachment
 - ➔ Interaction between specific receptors (e.g. pili, flagella, proteins, LPS etc)
 - ➔ If receptors common – wide host range (e.g. rabies)

- If receptors rare – narrow host range (e.g. HIV)
2. Entry of viral nucleocapsid of nucleic acid
 - Injection of nucleic acid into the cell (e.g. most bacteriophage)
 - Nucleocapsid penetration of plasma membrane to get inside the cell (e.g. most eukaryotic viruses)
 3. Synthesis of viral proteins and nucleic acids
 - Viral genome replication strategy depending on nucleic acid (DNA double stranded or RNA single stranded)
 - mRNA synthesis strategy dependent on nucleic acid
 - If DNA is injected into a cell they can handle DNA replication and make mRNA because DNA does that anyway
 - If RNA is injected cells don't have the proteins to replicate RNA or produce mRNA from RNA so viruses have to synthesise it themselves – bring it into the cell with them
 - Viral proteins synthesised by host systems
 - Early proteins: takeover of host cells and prepare the use of the cell
 - Late proteins: capsid, self-assembly and release proteins and virus
 4. Self-assembly of virions
 - Can be complex. Where the nucleic acid is packed into the capsid
 5. Release of progeny virions
 - By cell lysis (host cell dies and bursts)
 - Budding (host cell may survive)



Attachment phase:

- Viruses attach to host cells bearing specific receptor molecules (usually proteins). If the receptor is not present they cannot enter a host cell
- **A common receptor** infect multiple cell types (e.g. Ebola and TIM1 protein commonly found on epithelia cells)
- **Rare Receptor** – infect specific cell types (e.g. HIV and CD4 protein on T-helper cells)
- **Interspecies Receptors** – animals share the same e.g. rabies can attach to humans and animals.

Examples:

Virus	Cell surface receptor
Adenovirus	Coxsackie adenovirus receptor protein
Influenza A virus	Silica acid-containing glycoprotein
Measles virus	CD46 complement regulator protein
Rabies virus	Acetylcholine receptor on neurone

How they enter cells → penetration and uncoating

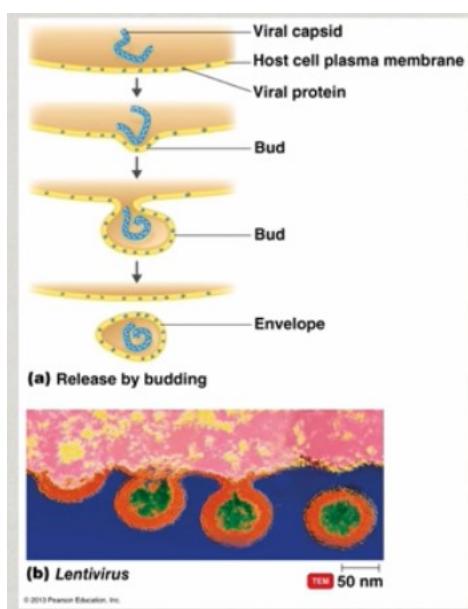
1. **Endocytosis** → used when there is a nuclear capsule no envelope. A virus manipulates the cell to enter (attach to host cell surface and enters vesicle). Exits by disrupting or injecting nucleic acid into the cell.
2. **By fusing with plasma membrane** → envelope. Membrane of the virus fuses with the membrane of the host cell and the capsid is released into the cytoplasm of the host.

Synthesis of Viral proteins and nucleic acid

- Method of viral proteins and nucleic acid is dependent on start nucleic acid (DN/RNA etc)
- The enzyme needed is called **RNA-dependant RNA polymerase** (using RNA as the template)
- Host cells don't have the enzymes for making RNA from DNA so the virus brings it in it is called. **Enzyme reverse transcriptase**

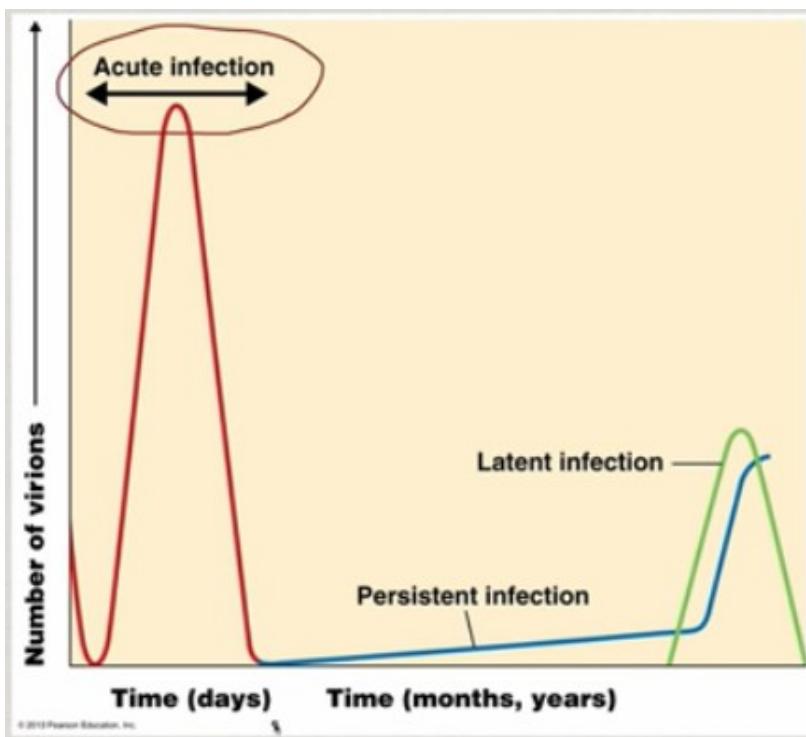
Eukaryotic Virus Release

- Many viruses, especially non-envelope viruses, lyse their host cells at the end of the intracellular phase
- Enveloped viruses commonly use budding to escape
 - Host cell may die or survive this process
 - Envelope is derived from host cell plasma membrane

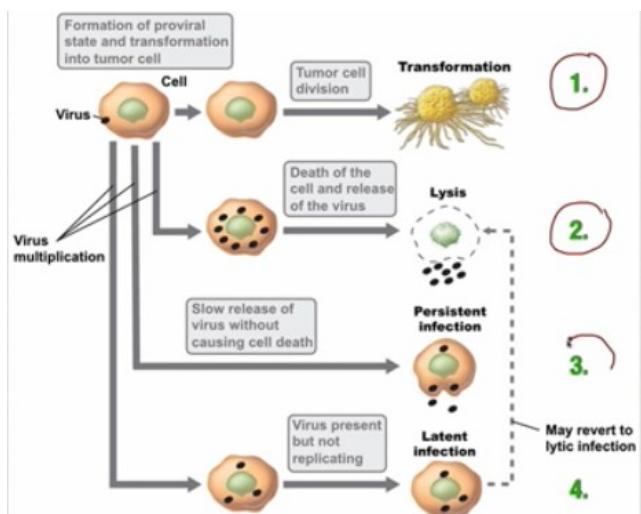


Week 11 pt 2 - Types of Animal Virus Infections

Kinds of infection: 3 kinds:



- 1. Acute:** flu for example. Presents itself quickly. Cells are being lysed
- 2. Persistent:** usually begins with an acute infection and your immune system begins to take hold of it but it lingers (make you feel sickly) and then can come back later e.g HIV.
- 3. Latent:** e.g. Virus that cause cold sores. Lies dormant then presents when under stress. No symptoms at the beginning and suddenly it spikes

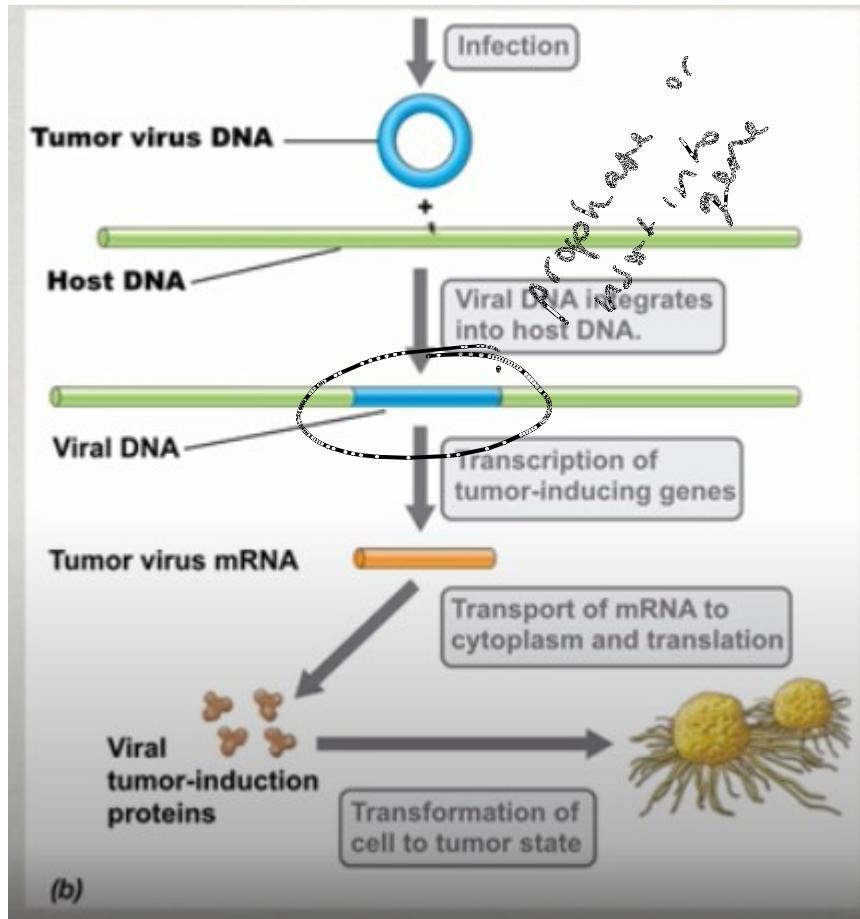


Eukaryotic viruses: type of infections

- 1. Viral infection leading to cancer:**
Process of viral infection leads to cancer
Human papilloma virus (HPV)
- 2. Acute infection:** e.g. Ebola/influenza
- 3. Persistent infection:** Ebstein-Barr virus.
Glandular fever. Budding not lyse
- 4. Latent:** Herpesvirus

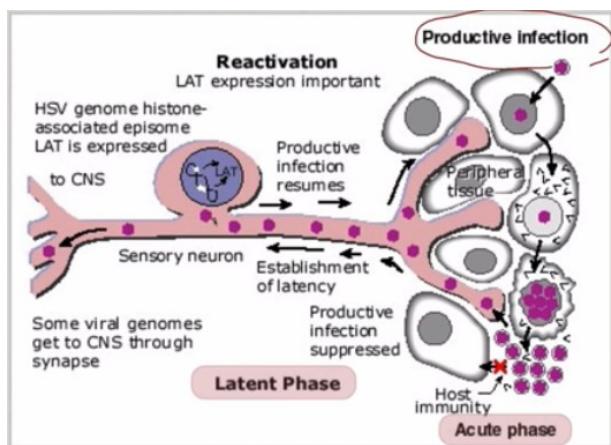
Oncogenic virus – Example of virus than can cause a tumour:

- Polyomavirus and herpesvirus
- DNA integrates (provirus) and encodes genes that produce tumour inducing proteins resulting in cell transformation and uncontrolled cell development



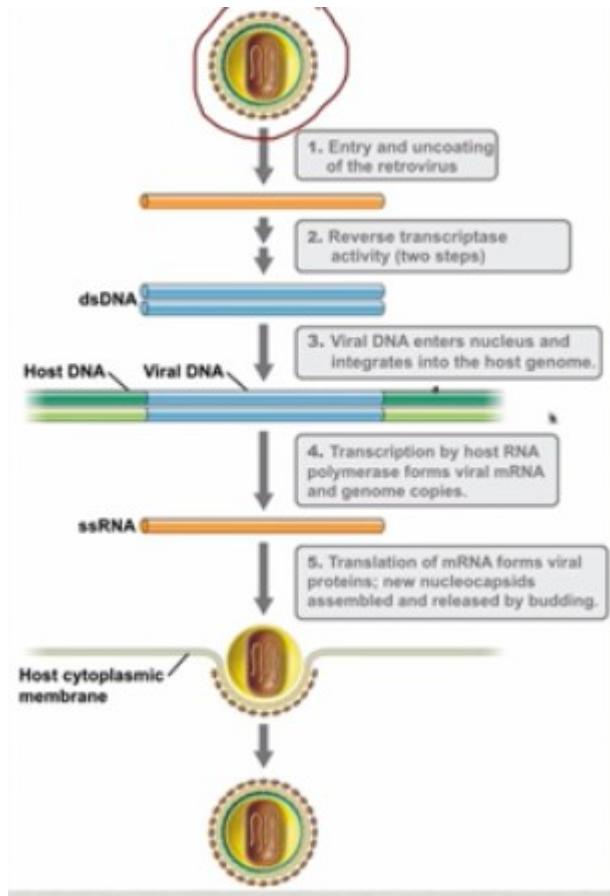
Herpes Simplex Virus – Example of a latent infection

- Induced by stress, other viruses, fatigue or injury and possibly sunlight
- Weakens immune system and cannot control the infection



HIV – Example of a persistent infection

- + ssRNA converted in dsDNA using reverse transcriptase
- Enters nucleus and integrates in host genome (**provirus**)
- Difficult to treat because the DNA cannot be removed from host cells – it is in the genome, even after it replicates and buds out

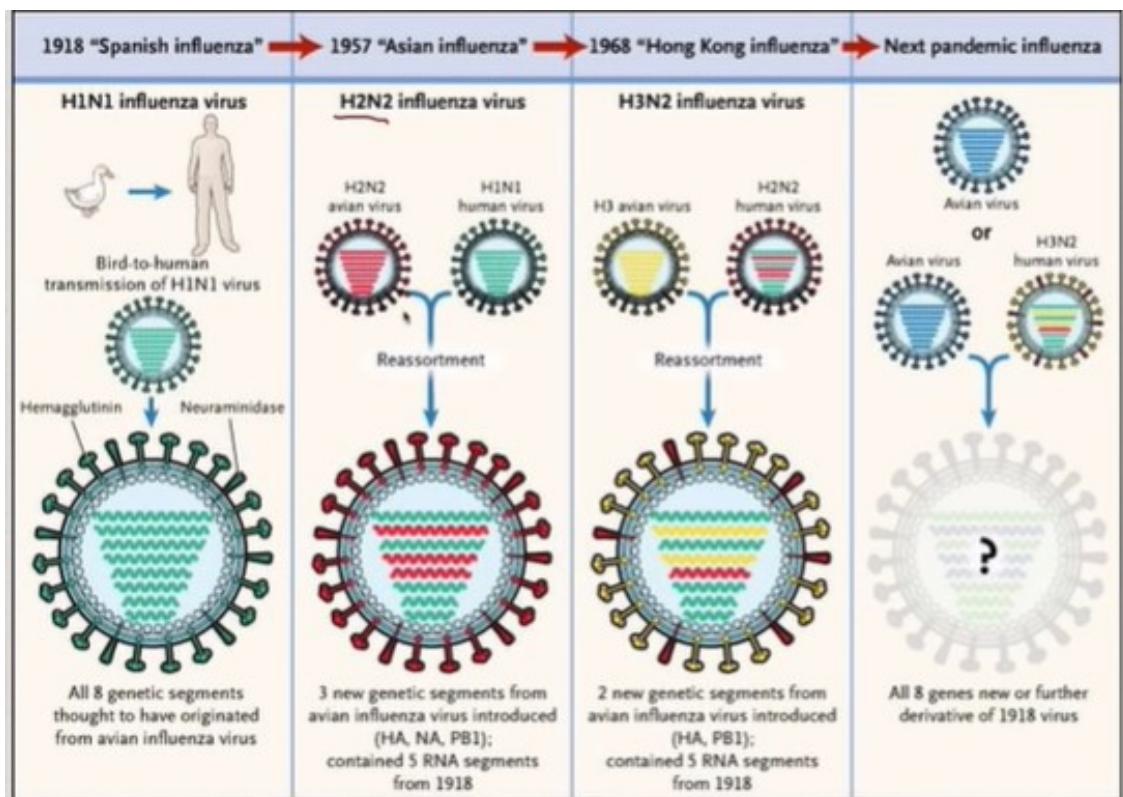


Influenza – Example of an acute infection

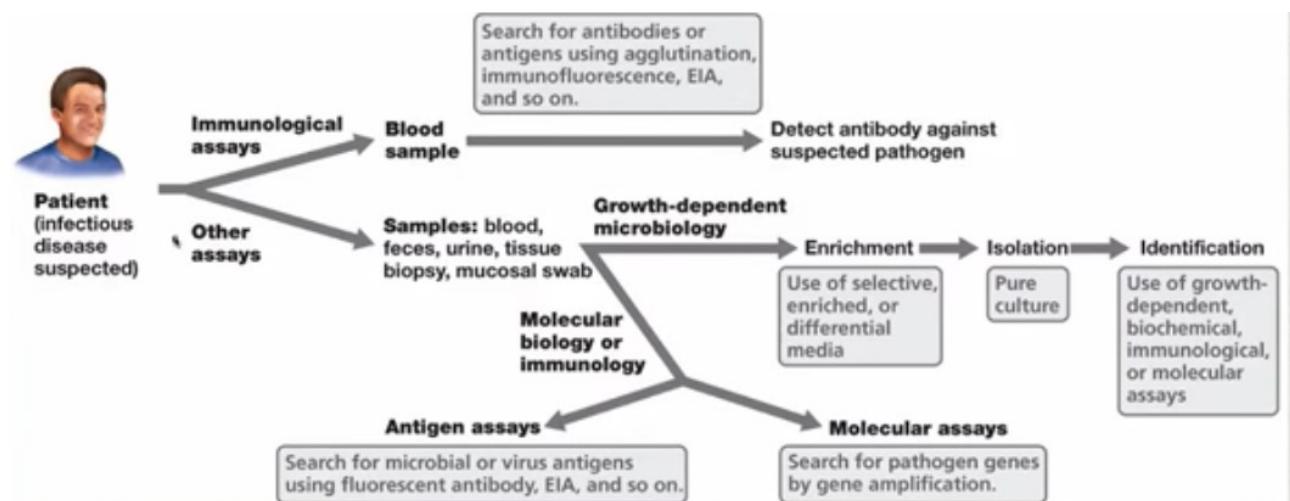
- Enveloped virus
- ssRNA strand
- 7-8 segments of linear RNA
- Acquired by inhalation of virus-infected respiratory secretions
- Two viral proteins present in envelope as spikes. Spikes called:
 - **Hemagglutinin (HA)** – 16 antigenic forms (H1-H16). Antibodies can only recognise the antigenic form its associated with = new antibody needed for new infection.
 - **Neuraminidase (NA)** – 9 antigenic forms (N1-N9)
 - Combinations of these Different variants categorised on HA and NA types e.g. H1N1 or H3N2 etc

Influenza – reassortment

- Reassortment usually occurs in pigs infected with bird and human flu strains
- Antigenic drift – mutations in HA or NA = change → seasonal flu
- Antigenic shift – of more concern pandemic flu. new type A influenza can emerge through genetic reassortment of human strains with wild bird or pig strains resulting in new HA/NA combinations. Worse form of the virus and can lead to death



Week 11 pt 3 - Detection of viral infections



Multiple methods of detection

1. Polymerase chain reaction (PCR)
2. Enzyme-linked immunosorbent assay (ELISA)

PCR: (= used for molecular assay)

- A method for amplifying DNA (to detect it)
- Run to find if a specific DNA sequence is present in a sample

What we need in the tube:

1. Template DNA (e.g. DNA from infected cells) e.g. blood sample with the DNA sample
2. DNA primers (short 18-20 nucleotide long ssDNA fragments artificially synthesised) - The primers target specific gene in viral DNA (e.g. Reverse transcriptase)
3. Heat Stable DNA polymerase (enzyme purified from hyperthermophile)
4. DNA nucleotides

Run the reaction to amplify DNA fragment

PCR Assays: detect what you are looking for primers specific for individuals viral orf/genes

Example: RT-PCR

1. PCR for HIV gene from DNA purified for blood
2. Gel electrophoresis of amplified DNA



Results: patient 1 +ve of HIV and patient 2 -ve for HIV

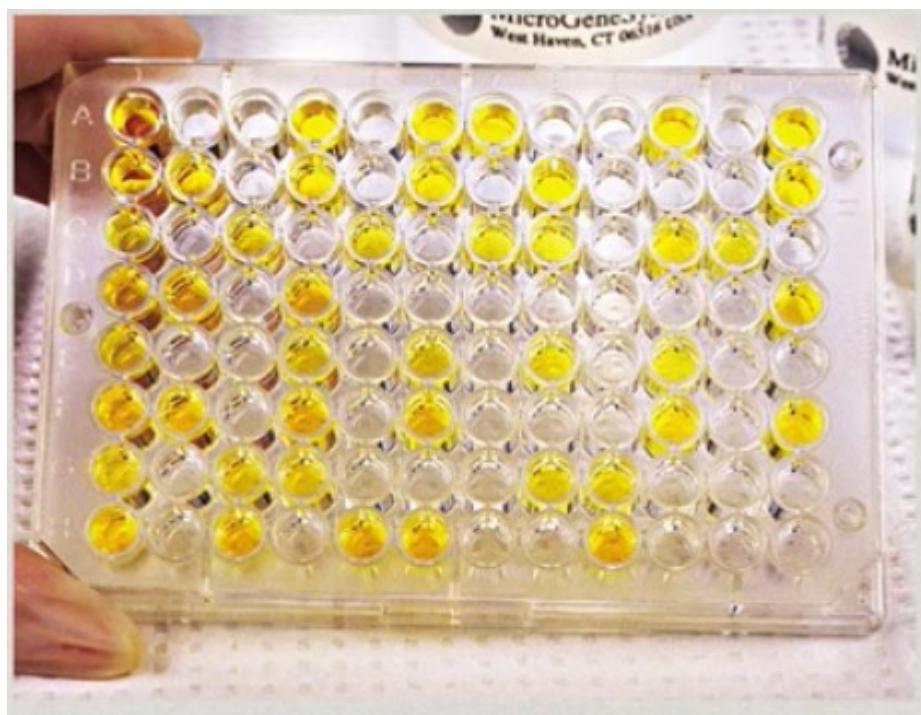
Immunoassays:

1. fluorescent antibodies methods for detection of microbial surface antigens

2. ELISA

- Add a capture antibody (for virus). Then add a sample to the antibody (the antibody will bind to the virus. It is washed. Then a second antibody = detection antibody (with an enzyme) which will bind if the virus is there, if not it will wash away. A substrate is added (e.g. which will change colour if present.)

Example of ELISA: yellow walls are positive i.e. β -galactosidase linked to indicator antibody degrades ONPG to release yellow substrate (p-nitrophenol)



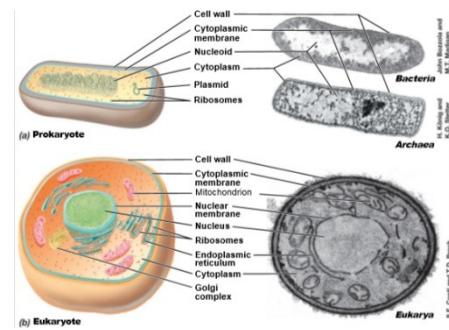
Microbial evolution

- for the first billion years or so life on Earth consisted entirely of simple single cells like Bacteria and Archaea
- around 2 billion years ago complex cells appeared kind of out of the blue
- It all started with the marriage of two simple cells by one cell getting inside another cell really
- the host cell was probably an Archaea and the cell that got inside. It was a bacterium
- So in Archaea like the ones in the hot springs in Iceland swallowed a cell of that other type of microbial life that was around 2 billion years ago
- As a result complex life evolved → green algae, plants, animals
- The Bacteria that got swallowed evolved into mitochondria
- The hosts now had the means to get bigger and maintain bigger genomes

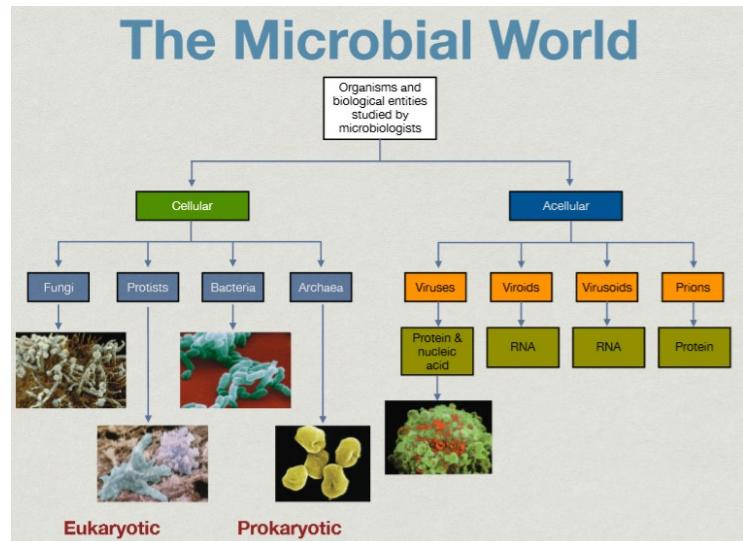
What Is Microbiology

Microbiology is the study of organisms too small to be seen with the naked eye ("micro"organisms).

- Eukaryotes: have a membrane-bound nucleus, more complex and larger than prokaryotes. 10 micrometers in diameter
- Prokaryotes: cells that lack a membrane-bound nucleus. 1 micrometer in diameter



- Microbes can grow or survive as single celled individuals or form multicellular biofilms



History of microbiology

First to observe

- Francesco Stelluti (1577 – 1652), Robert Hooke (1635-1703), Antony van Leeuwenhoek (1632-1723)

Thoughts were that they generated spontaneously – living organisms could develop from non-living matter.

Proof against:

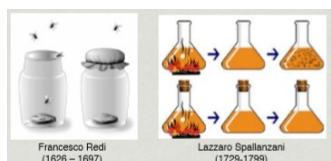
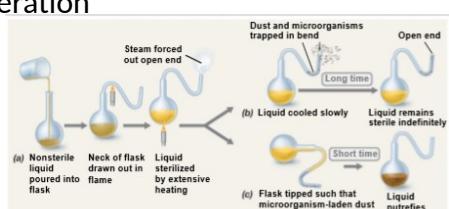


Image 1: meat left out, let flies in which laid maggots and meat was disturbed but covered meat was not.

Image 2: boiled broth

Louis Pasteur (1822-1895) - Disproving the theory of spontaneous generation

- Open flask showed that air could get in and out but that no spontaneous generation happened as liquid stayed sterile



Germ Theory

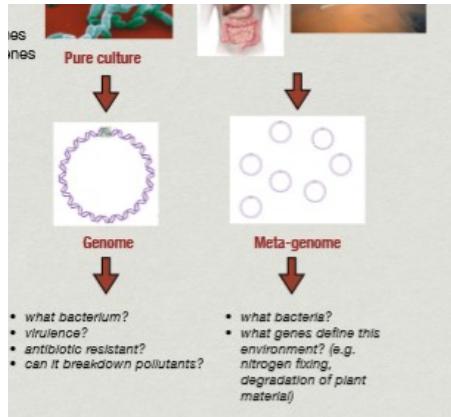
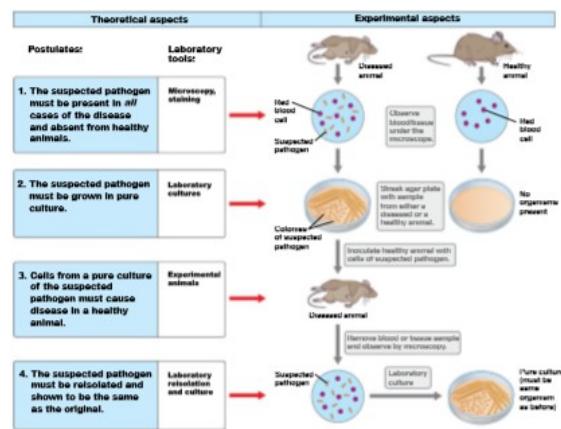
- Infectious disease was thought to be supernatural
- Disproof of spontaneous gen lead to the thought that germs could move and be responsible for spreading

Koch's Postulates

- Confirmed/proved germs were responsible
- His experiment →

Microbiology Tools

- Microscopes: Light micro is most common
- Microbiology Medium (agar plates)
- DNA Sequencing



MICROBIAL DIVERSITY

Diversity Of Microorganisms

Diversity = The range of features or degree of difference between organisms in a particular environment.

3 types

1. Physical/structural (e.g. cell shapes, Gram stain [determines thickness of cell])
2. Biochemical/metabolic (e.g. energy sources, secondary metabolites)
3. Genome (i.e. DNA sequence)

Diversity Of Morphology – Prokaryotes (archaea, bacteria)

cocci (spheres), ~ 1 µm diameter arrangements • diplococci (pairs)

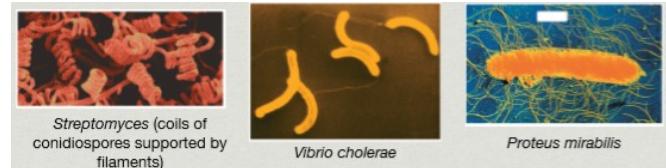
- chains (e.g. Streptococci)
- clusters (e.g. Staphylococci)



- tetrads (4 cocci in a “square”)
- sarcinia (8 cocci in a “cube”)

Bacilli – “rods”, $\sim 1 \mu\text{m} \times 3 \mu\text{m}$

- coccobacilli (very short rods/almost like spheres)
- filamentous (very long rods).
- vibrios (curved rods), commas
- blunt or square ends

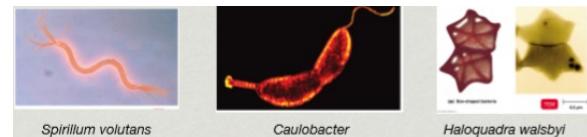


arrangements:

- cells arranged singly, in short chains, palisade, or “Chinese lettering”

Less-common shapes:

- Spirilla - rigid helices
- Spirochetes - flexible helices (usually quite long, internalized flagella)
- Coryneform - variable appearance (usually rodlike)
- Pleomorphic - variable, irregular shapes, occasionally branched
- Flattened rectangles, triangles & trapezoids (rare)



Diversity Of Morphology - Eukaryote

Morphologically more complex than prokaryotes

- Protists (diverse taxa). Protists
- Fungi (one taxon)



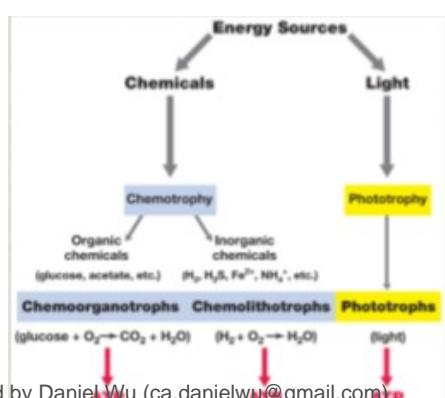
Diversity Of Morphology - Viruses

- Morphologically complex, despite being acellular



Diversity Of Metabolism

- Metabolism refers to all the chemical reactions in a cell or biological system
- Microorganisms show enormous metabolic diversity
- High variable environments have led to microbes evolving diverse ways of getting energy



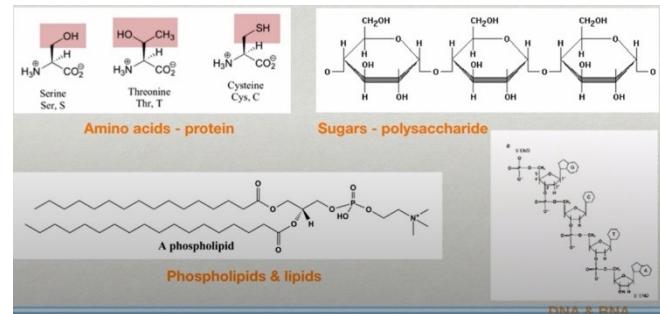
Diversity Of Microbial Genomes

- **Genome/Genotype:** The genetic makeup of a cell
- **Core genome:** the complement of genes that make-up a species
 - Genes (DNA) encode all info for cell structure and metabolism
 - Reflect all aspects of physical and chemical diversity

Week 3 – MICROBIAL NUTRITION

1. Essential Macronutrients

- Composition of microbial cells (composing >95% dry weight = C,H,O): 1. Essential macronutrients - C, H, O, N, S, and P (Carbon, Hydrogen, Oxygen, Nitrogen, Sulfur, Phosphorus)
- Required in fairly large amounts
- Components of:
 - Carbohydrates/sugars
 - Proteins/amino acids – Find nitrogen in amine group, Sulphur in Sistine
 - Lipids/phospholipids – Phosphorous membrane in phospholipids
 - DNA/RNA – Forms nitrogenous base



Nitrogen, Phosphorus And Sulfur

- All organic mater = C,H,O but can also contain N,P,S

Nitrogen

- Needed for synthesis of amino acids, nucleotides, some carbs and lipids
- Supplied organically or inorganically:
- Microorganism use N from amino acids and organic matter
- Some incorporate ammonia directly, some reduce nitrate to ammonia
- Some bacteria “fix” N (atmospheric nitrogen & reduce to NH_4^+)

Phosphorus

- needed for nucleotides (including ATP), phospholipids, cofactors and some proteins and cell components
- Only supplied inorganically
- Most incorporate phosphorus directly
- Low phosphorus can limit growth

Sulfur

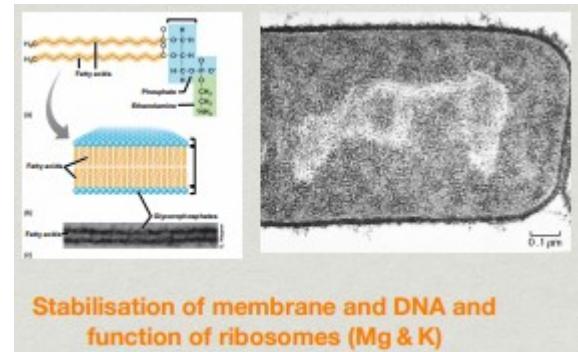
- needed for the synthesis of amino acids cysteine and methionine, some carbohydrates, biotin and thiamine
- usually supplied as sulfate or via organic sulfur compounds

2. Co-Factors - Macronutrients

Difference is these co factors don't make up the macromolecules of the cell but are used for the cell to do things (activity)

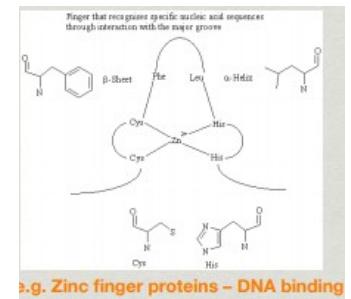
- K and Mg, Ca and Na for some.

e.g. for:



3. Trace Elements – micronutrients Fe, Mn, Zn, Co, Mo, Ni, Cu (iron, manganese, zinc, cobalt, molybdenum, nickel and copper)

- Required in low (trace) amounts for enzymatic functions or protein stabilisation (only some)
- In culture, these are provided in water or media (no need to add)
- In nature, these are rarely growth limiting



4. Growth Factors

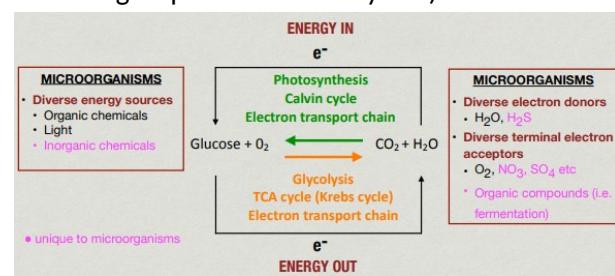
- Some microbes are unable to synthesise their certain molecules and these must be obtained from the environment or provided in growth medium.
- Three categories:
 1. Amino acids (synthesis of proteins) e.g. Lactobacillus spp. and requirement for amino acids
 2. Purines and pyrimidines (synthesis of DNA & RNA)
 3. Vitamins (co-enzymes and functional groups of certain enzymes)

Metabolic Diversity

All life is an oxidation/reduction reaction.

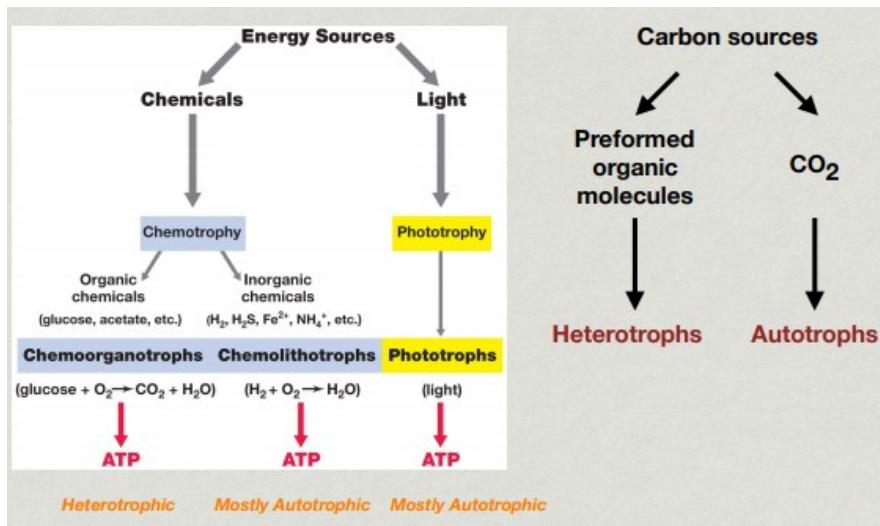
- Microorganisms special as they can use inorganic chemicals

$$\Delta G^{\circ'} = G_f^{\circ} [C + D] - G_f^{\circ} [A + B]$$



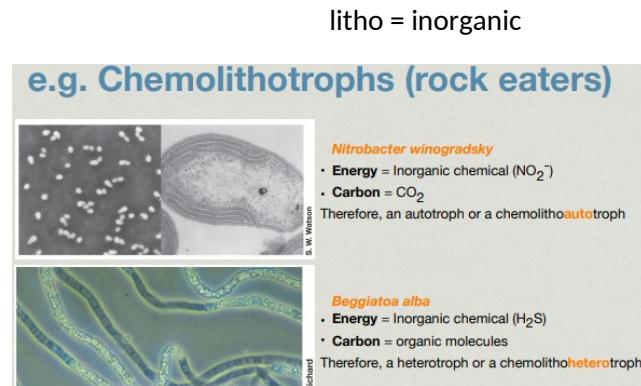
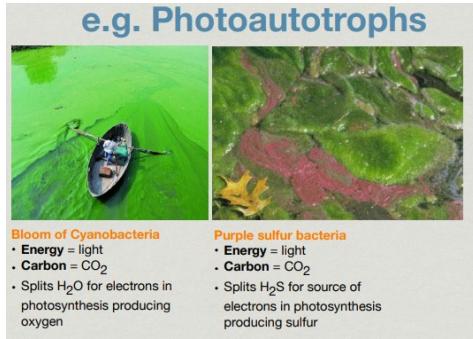
Nutritional Types

All organisms require an energy source and a carbon source.



Phototroph = light as energy Auto = CO_2

Chemo = chemical as energy organo = organic



Week 3 pt 2 - MICROBIAL CULTURE MEDIA

Culture Media

Culture media must contain all the nutrients required by the organism to grow.

Media are classified based on:

1. Chemical constituents (defined or complex)
2. Physical nature (liquid, semi-solid or solid)
3. Function (supportive, enriched, selective or differential).

1. Chemical constituents

- **Defined:** All components and constituents (and concentrations) are known

e.g. All amounts are perfectly defined

- **Complex:** Contain some ingredients that are of unknown composition and/or concentration

Culture Media - Defined

A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as <i>Escherichia coli</i>	
Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride ($NaCl$)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

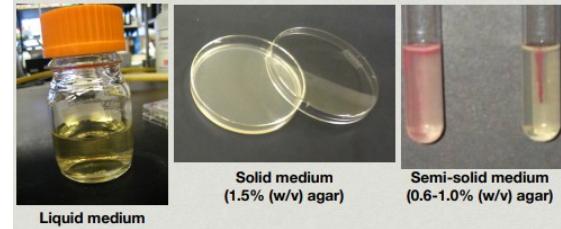
Culture Media - Complex

Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria	
Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

e.g. Peptone and Beef extract are not specific or defined as to what is in it and the quantities.

2. Physical Nature

- Agar (sulfated polysaccharides used to solidify liquid media such as L-broth)
- Extracted from red algae
- Melts at 90°C but once melted doesn't harden till 45°C.
- Plates are used to separate (colonies spread on plate, so you can pick which one you like)



3. Media function

- 1. Supportive - supports many organisms e.g. tryptone soy agar
- 2. Enriched - general purpose media enriched with blood or other special nutrients to support the growth of fastidious bacteria e.g. blood agar (helps 'fussy' microorganisms to grow)
- 3. Selective - allows growth for particular microorganisms while inhibiting the growth of others e.g. MacConkey agar – selects for enterics (i.e. bile salts)
- 4. Differential - distinguished on different groups of microorganisms based on their biological characteristics. e.g. Blood agar - haemolytic versus non-haemolytic bacteria



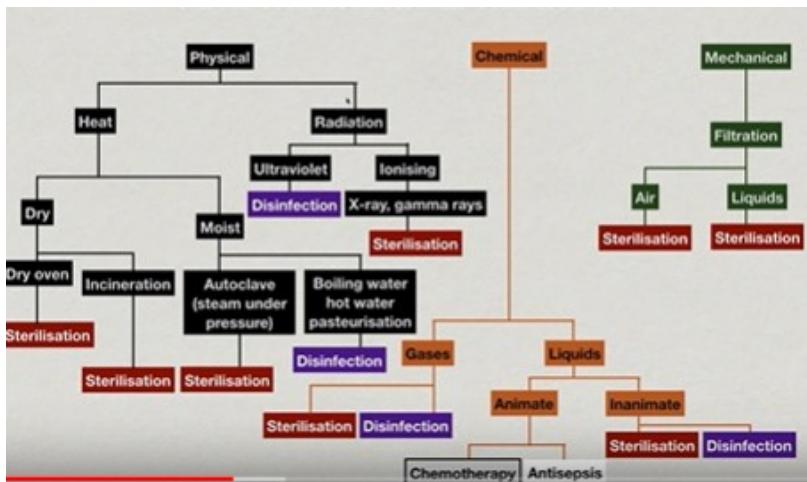
Week 4 - CONTROL OF MICROBIAL GROWTH

Important Definitions

- Sterilization = process by which all living cells, spores and acellular entities (viruses) are destroyed (dead)
- Disinfection = Killing or removal of pathogenic organisms. Aims at reducing microbial population while killing potential pathogens.
- Disinfectants = chemical agents used for disinfection
- Sanitization = reduction of microbial population size to levels considered safe to public health standards.
- Antisepsis = The prevention of infection of living tissue
- Antiseptic = chemical agent applied to tissue to prevent infection often not as harsh as disinfectants as they must not destroy excess living tissue.
- Chemotherapy = use of chemical agents to kill or inhibit the growth of microorganisms within living host tissue. →

...cide - (Latin: cida; to kill) Therefore:.... <ul style="list-style-type: none"> • Germicide - kills pathogens and non-pathogens • Bactericide - kills bacteria • Fungicide - kills fungi • Algaicde - kills algae • Viricide - destroys viruses or simply: Microbicide - kills microorganisms
...static - (Greek: statikos; causing to stand or stop) Therefore:.... <ul style="list-style-type: none"> • Bacteriostatic - inhibits bacteria • Fungistatic - inhibits fungi NB: These agents do not kill, but they prevent growth. If removed, microbial growth will resume. In a drug treatment sense, these drugs are still highly useful as they allow your immune system to clear the infection.

Controls of Microbes: Physical, Chemical and Mechanical Methods



Heat is usually the best choice for sterilization (kill everything).

Mechanical = filter/exclude the microorganism

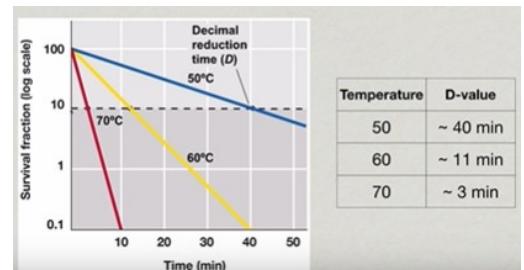
Patterns of Microbial Death

- A microbial population is not killed instantly when exposed to a lethal agent
- Generally exponential/population reduced at constant rate by same fraction
- Thus, exposure time is important
- E.g. →
- Microorganisms can be viable but not culturable (VBNC), meaning they are not dead and can potentially become culturable once again. (hard to know when microorganisms are dead)

The D Value

- D= decimal reduction time = time to kill 90% of the microorganisms in sample
- E.g. 70 degrees = most effective (only takes 3 mins)
- Need to know this to know how long to expose a treatment for it to be effective

TABLE 7.2 Microbial Exponential Death Rate: An Example		Alive	Dead
Time (min)	Deaths per Minute		
0	0	1,000,000	100%
1	900,000	100,000	10%
2	90,000	10,000	1%
3	9000	1000	0.1%
4	900	100	0.01%
5	90	10	0.001%
6	9	1	0.0001% 99.9999%



Factors That Influence Effectiveness of Antimicrobial Agents

- Population size – more cells you have the longer it will take
- Population Composition – Vegetative (growing) cells are more sensitive than spores. So, you can't use a method that kills vegetative cells in this long if there are also spores.
- Duration of exposure – Longer exposed = more organisms die
- Temperature – Increased temp can often enhance antimicrobial activity = lower concentrations of an antimicrobial agent can be just as effective at higher temps.
- Local Environment – Usually population being controlled is not isolated so environmental factors can offer protection or aide in its destruction.
- Concentration of agent - More concentrated = more rapid death. Not always the case e.g. ethanol better at 70% than 90%.

Biofilms (bacteria that form slim layers) are More Resistant to Control

- When they come together, they are protected by a polysaccharide gel
- This resist the penetration of toxic molecules

- Organic matter (EPS) protect microorganisms from heat and chemical disinfectants
- pH gradients effect biofilms
- Biofilm composition
- These must be taken into account when thinking about control

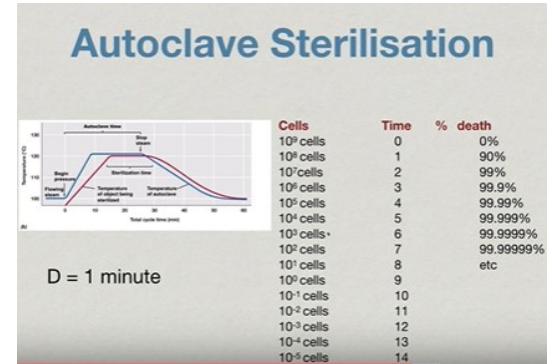
Physical Methods of Control

Moist Heat

- Increasing temperature in a water environment, causes DNA to melt, proteins to denature (that's what kills the cells)
- 100 degrees Celsius for 10 mins = sufficient to destroy most vegetative cells. BUT not for spores (so not sterilisation)

Autoclave Sterilisation

- = saturated steam under pressure – able to penetrate deep into the cells.
- Runs for 12 mins, 121 degrees with 15lb pressure.
- 12-15 to make sure there is no cells.
- **Best way to sterilise** (used in dentists...)



Moist Heat: Pasteurisation

- Controlled heating. Used for milk, beer and other beverages
- This does not kill all microorganisms, but reduces population down (longer shelf life)

Dry Heat (for water sensitive) (2nd best)

- Takes longer/less effective
- Works by denaturing proteins and oxidising cell constituents.
- Doesn't corrode glass or metal instruments
- Can sterilise oils and powders

Low Temperatures

- Stop microbial reproduction due to lack of water and stops/slow growth (unfavourable temps)

Radiation (only if moist and dry heat can't be used)

UV

- Causes DNA to mutate. UV (260nm) lethal to many microorganisms
- Cannot penetrate so only useful on surfaces

Ionizing Radiation (Gamma irradiation)

- Penetrates deep into objects
- Kills everything.
- Good for things like plastics that melt from heat methods
- Expensive so is a last resort

Mechanical Methods

Filtration (good for vitamins and probiotics)

- Exclude organisms
- Reduce microbial populations and even sterilise them by removing the
- Standard filter is $0.22\mu\text{m}$ which gets rid of bacteria and eucaryote but not viruses
- Can be used for liquids and air (gases)

Air Filtration

- E.g. laminar flow.

Chemical Controls

Common Antiseptics

Antiseptics	Mode of action	Notes
Phenolics & Bisphenols	Disrupts cytoplasmic membrane	Effective in organic material, long lasting but can cause skin irritation and smells
Alcohols	Dissolves lipids and denatures proteins	Effective in organic material and most effective at 70%
Iodophors (e.g. betadine)	Iodinates proteins rendering them non-functional	Effective in organic material
Quaternary ammonium compounds	Disrupts cytoplasmic membrane	Effective in organic material
Heavy metals	Inactivates proteins	Effective in organic material but toxic and rarely used anymore
Hydrogen peroxide (3%)	Oxidising agent	Effective in organic material

Alcohols, hydrogen peroxide and iodophors can be antiseptics, disinfectants or sterilants depending on concentration, length of exposure and form of delivery.

Typical Disinfectants

Disinfectant	Mode of action	Notes/use
Phenolics & Bisphenols	Disrupts cytoplasmic membrane	General purpose disinfectant, e.g. floors etc
Alcohols	Dissolves lipids and denatures proteins	General purpose disinfectant for surfaces e.g. lab bench.
Chlorine	In water produces HOCl which is an oxidising agent	Inactivated by too much organic material (e.g. swimming pools). Medical equipment and drinking water.
Quaternary ammonium compounds	Disrupts cytoplasmic membrane	Medical equipment, household use, food/dairy equipment.
Aldehydes (e.g. formaldehyde)	Acylates amines in proteins and cross-links.	Disinfectant or sterilant depending on concentration. Preservative. Toxic.
Ozone	Oxidising agent	Drinking water.

Alcohols, hydrogen peroxide and iodophors can be antiseptics, disinfectants or sterilants depending on concentration, length of exposure and form of delivery.

Often lots of overlap with antiseptics but are used in more concentrated amounts as it is used on inanimate surfaces.

Sterilising Chemical Gas

- Used to sterilise heat sensitive materials
- Uses very toxic ethylene epoxide in chambers
- Combines with and inactivates proteins

Examples of How to sterilise

- Vitamin solution = filtration
- Charcoal powder = Dry heat (doesn't mix well with water so dry heat)
- Eppendorf tubes (heat resistant) = AutoClave
- Petri dishes (heat sensitive) = gamma radiation

- Tips (heat resistant) =Autoclave

- Antibiotic - filtration

How to Disinfect

- Lab bench = 70% ethanol, bleach

- Contaminated shoes = Detergent, UV hanging in sun

- Toilet = Bleach

Week 5 - ANTIMICROBIAL CHEMOTHERAPY

CHEMOTHERAPEUTIC AGENTS

Chemical Agents For Control Of Microbial Growth In Therapy

Antimicrobial chemotherapeutic agents

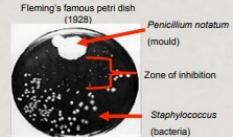
- Chemicals used as medical therapeutic to kill or inhibit the growth of microbes
- Selective toxicity - they target microbe (not host)
- Most = antibiotics: chemicals synthesised by microbes that are effective at controlling the growth of bacteria

The Development of Chemotherapy →

Chemotherapeutic Agents: Antibiotics

- Chemical agent used to treat disease
- Destroy pathogenic microbes or inhibit their growth within host
- Most are antibiotics = microbial products of their derivatives, that kill susceptible microbes or inhibit their growth (textbook definition)

- **1904** - Paul Erlich found that the dye trypan red was active against trypanosomes causing African sleeping sickness.
 - Dye selectively stained microbial cells.
- **1910** - Erlich and Sahachiro developed arsenic-based chemical for treatment of syphilis called Salvarsan.
- **1927** - Gerhard Domagk with support from I. G. Farben-Industrie discovered Prontosil red, a new dye for staining leather protected mice from pathogenic streptococci and staphylococci without toxicity. Led to sulfonamides drugs.
- **1928** - Fleming, accidental discovery of penicillin.
- **1939** - Florey read Flemings work on penicillin.
 - Chain developed the purification protocol of penicillin from the fungus.
- **1945** - Fleming, Florey and Chain – Nobel Prize



General characteristics of anti-microbial drugs

- **Selective toxicity** = ability to kill or inhibit growth of pathogen with little or no damage to host
- **Therapeutic dose** = Drug level required for clinical treatment
- **Toxic dose** = Dose at which drug is too toxic or brings unwanted side effects
- **Therapeutic Index** = ratio of therapeutic dose to toxic dose
- **Side effects** = undesirable effects on the host (all antibiotic have to some extent)
- **Narrow Spectrum drugs** = only attack certain pathogens
- **Broad Spectrum drugs** = effective on different pathogens

Antibiotics produced by microbes

TABLE 20.1 Representative Sources of Antibiotics

Microorganism	Antibiotic
Gram-Positive Rods	
<i>Bacillus subtilis</i>	Bacitracin
<i>Paenibacillus polymyxa</i>	Polymyxin
Actinomycetes	
<i>Streptomyces nodosus</i>	Amphotericin B
<i>Streptomyces venezuelae</i>	Chloramphenicol
<i>Streptomyces aureofaciens</i>	Chlortetracycline and tetracycline
<i>Saccharopolyspora erythraea</i>	Erythromycin
<i>Streptomyces fradiae</i>	Neomycin
<i>Streptomyces griseus</i>	Streptomycin
<i>Micromonospora purpurea</i>	Gentamicin
Fungi	
<i>Cephalosporium</i> spp.	Cephalothin
<i>Penicillium griseofulvum</i>	Griseofulvin
<i>Penicillium chrysogenum</i>	Penicillin

Antibiotics

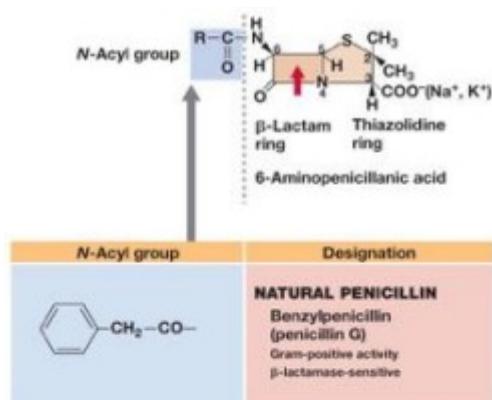
= Microbial products, or their derivatives, that kill susceptible microbes or inhibit their growth (called - Natural Antagonism) e.g. fungi produce chemical to antagonise another organism (like bacteria in the photo)

- Early history of antibiotic = all came from there learnings of antibiotics that were created by microbes (as seen on table)
- Now can be synthetic



Semi-Synthetic Antibiotics

- = naturally produced antibiotics that are chemically modified
- This can make them less susceptible to microbial inactivation
- Allows alteration of antibiotic to increase effectiveness e.g. go from Narrow to broad spectrum...
- E.g. Penicillin got altered, new variations were no longer broken down by β -lactamase



SEMISYNTHETIC PENICILLINS	
Methicillin	acid-stable, β -lactamase-resistant
Oxacillin	acid-stable, β -lactamase-resistant
Ampicillin	broadened spectrum of activity (especially against gram-negative bacteria), acid-stable, β -lactamase-sensitive
Carbenicillin	broadened spectrum of activity (especially against <i>Pseudomonas aeruginosa</i>), acid-stable but ineffective orally, β -lactamase-sensitive

DETERMINING THE LEVEL OF ANTIMICROBIAL ACTIVITY

Minimal Inhibitory Concentration (MIC)

- Concentration of drug that you need to inhibit the growth of a pathogen
- MIC = lowest concentration of the drug that inhibits the growth

Why Is MIC important?

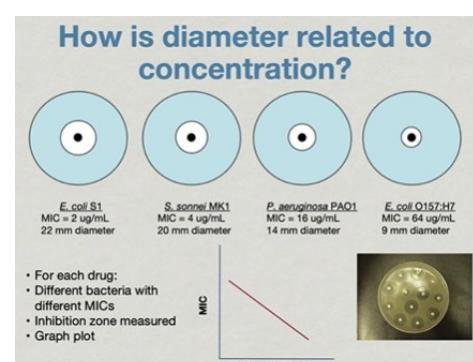
- Determine whether MIC of the pathogen is lower than the concentration of the drug that can reach the site of infection
- Determine if the pathogen is susceptible to the drug e.g. hasn't become resistant

How to Work out MIC

Method 1: Dilution susceptibility test

- Inoculating media containing different concentrations of a drug
- The broth with lowest concentration showing NO growth is MIC

Method 2: Disc diffusion assay



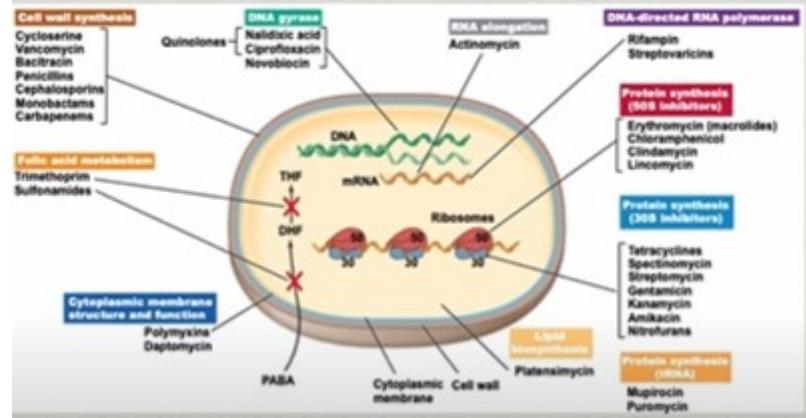
- Disc impregnated with drug placed in agar plates with a test microbe
- Drug diffuses from disc into the agar plate, establishing a concentration gradient
- Clear zones = no growth = MIC (rim before growth)
- Zone size standardised: susceptible, intermediate, resistant (read in mm - diameter)
- Zone diameter Interpretation Standards = preset standards that give you the diameters that are = to the MIC
- NEED to be able to measure diameter then refer to standards to determine if its resistant, sensitive ... (intermediate also)

Drug name (Dose strength)	Zone Diameter (mm)		
	Resistant (mm or less)	Intermediate (mm or less)	Susceptible (mm or more)
Penicillin G (10 µg)	≤28	—	≥29
Oxacillin (1 µg)	≤10	11-12	≥13
Erythromycin (15 µg)	≤13	14-22	≥23
Gentamycin (10 µg)	≤12	13-14	≥15
Tobramycin (10 µg)	≤12	13-14	≥15

MODES OF ACTION OF ANTIBIOTICS

Most act to control microbial growth by the following specific mechanisms:

- Inhibition of cell wall synthesis
- Inhibition of protein synthesis
- Inhibition of DNA or RNA synthesis
- Metabolic antagonist
- Range of drugs that effect these 4 areas →



THESE 4 areas ARE SPECIFIC PATHWAYS

THAT ONLY EXIST IN BACTERIA = kill/inhibit bacteria without effecting a host (e.g. humans)

Inhibitors of Cell Wall Synthesis

Main inhibitors (picture)

β-lactams (e.g. penicillin, amoxycillin, cephalosporin, carbapenem)

- has β-lactam ring structure (attacked by β-lactamases)
- structurally resemble the terminal D-alanyl-D-alanine in the side chain of peptidoglycan subunit of the bacterial cell wall
- This is proposed to block enzymes catalysing peptidoglycan cross-links
- i.e. these agents prevent peptidoglycan synthesis

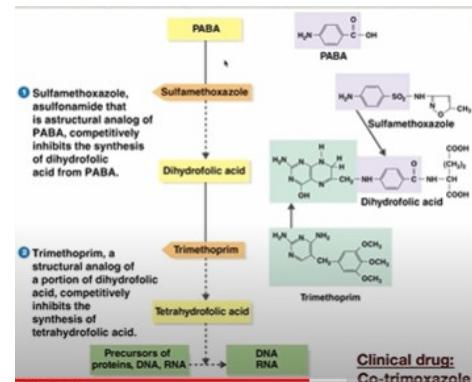
Vancomycin

- blocks cell wall structure through interacting with peptidoglycan
- NB: These agents only act on growing bacteria that are synthesising new cell walls

Diagrams show the β-lactam ring structure, its similarity to D-alanine-D-alanine, and the interaction of Vancomycin with the peptidoglycan layer.

Inhibitors of Protein Synthesis = β-lactams & Vancomycin

- Protein synthesis inhibitors selectively block prokaryotic protein synthesis by binding to prokaryotic ribosome
- These drugs can discriminate between prokaryotic and eukaryotic ribosomes



Metabolic Antagonists →

e.g. sulfamethoxazole and Trimethoprim = drugs block the pathway and stop infection.

Nucleic Acid Inhibitors

- Chemicals that are able to bind to prevent DNA synthesis
- agents inhibit DNA polymerase and DNA helicase, blocking replication or transcription
- Quinolines e.g. ciprofloxacin act by blocking bacterial DNA gyrase (twists) & topoisomerase IV (untwists)

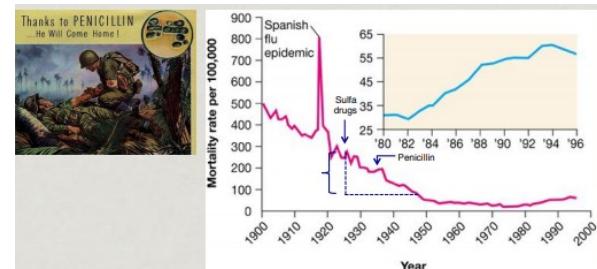
Factors Affecting Effectiveness of Antimicrobial Drugs

1. Ability to get to the site of infection
 - E.g. if you have an eye infection you get eye drops not oral medicine as it is destroyed by stomach acid and is indirect)
2. Susceptibility of pathogen to the drug
3. Ability of the drug to reach concentrations in the body that exceed the MIC of the pathogen
Determined by:
 - amount of drug administered
 - route of administration
 - speed of uptake
 - rate of clearance from the body

ANTIBIOTIC RESISTANCE

The Golden Age of Antibiotics

- Antibiotics caused US deaths to decline by ~230 per 100, 000 in 15 years (Armstrong et al, 1999)
- All other medical technologies reduced deaths by ~20 per 100, 000 over next 45 years



Antibiotic Resistance

- Resistance is causing antibiotics to become ineffective. Crisis is that one day they will no longer be effective
- **Strategies to cope** = reduce use on antibiotics, develop tech, reduce transmission, increase vaccination so we don't need antibiotics...

How Does Resistance Arise?

Mutation

- Bacterial DNA replication: 1 mutation for every 10^{10} bases synthesised.
- The 1 mutated cell survives (others die) and it replicates to create resistance

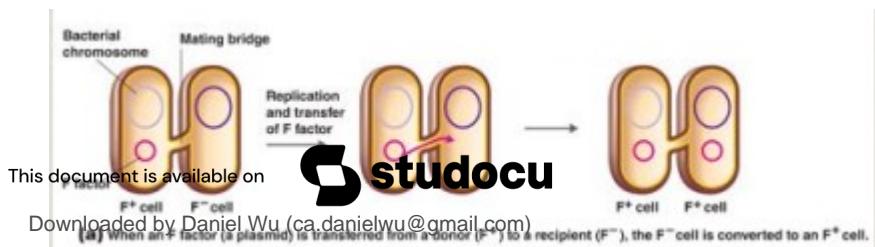
Spread of Resistance Genes

Antibiotic resistance genes can be found on:

- bacterial chromosomes (rare)
- **plasmids** (frequent)... e.g. "resistance" plasmids transposons

Mobile genetic elements (plasmids) are easily exchanged between bacteria!

Lateral gene transfer



- Bacteria mating
- Plasmid transfers

Spread Of Resistance Genes Cont.

Transposons/Insertion Sequences

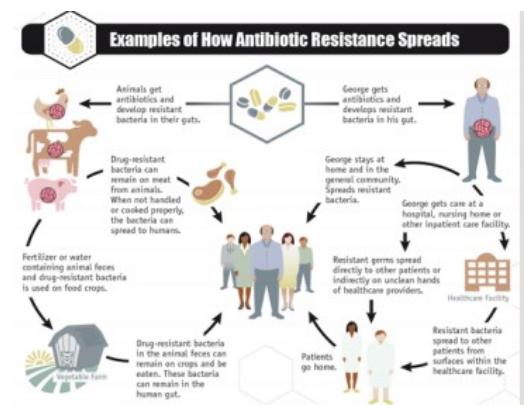
- Sequences of DNA that replicate **can move around** to different positions within the genome or from the genome to an existing plasmid in the single cell.
- Can move other genes with it (e.g. antibiotic resistance genes).

Antibiotic Use Leads To Antibiotic Resistance

- More you use = faster resistance grows

Spread Of Resistance

- Not just from the spread of bacteria but also the spread of the genes



Mechanisms of Resistance

How do resistance genes protect against antibiotics?

1. Reduced permeability (block antibiotic from getting in)
2. Inactivation of antibiotics (B-lactamase breaks down B-lactam ring - penicillin)
3. Alteration of target (e.g. RNA polymerase, Ribosome, DNA gyrase)
4. Development of resistance biochemical pathway
5. Efflux (drug gets in but is immediately pumped out of cell)

Other Chemotherapeutics

Antifungal drugs

- Fewer exist as they are also eukaryotic (hard to find one that hurts them and not us)
- easier to treat superficial mycoses than systemic infections

Antiviral drugs

- Target the attachment phase of viruses (as viruses replicate once attached to an organism's cell)
- Or target a very specific part that differs from our cell replication process.

Antiviral drugs
<ul style="list-style-type: none"> • viruses are intracellular obligate pathogens • viruses use the host cell machinery for viral replication • therefore development of anti-viral drugs has been difficult, as it is difficult to develop drugs that selectively inhibit virus replication and not host cell replication • current antivirals - target virus-specific enzymes <ul style="list-style-type: none"> e.g. acyclovir blocks herpes virus DNA synthesis e.g. amantadine blocks flu virus uncoating e.g. cidofovir blocks viral DNA polymerase e.g. AZT blocks HIV reverse transcriptase, other drugs block HIV protease e.g. rulenza and tamiflu block flu virus entry

Week 6 – Metabolism

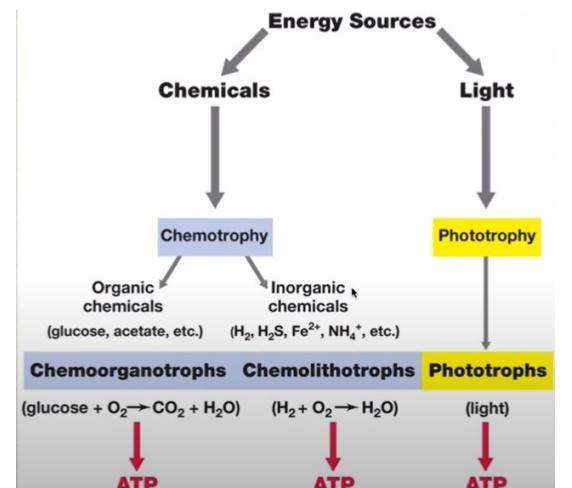
Metabolism = Total of all chemical reactions in the cells

Catabolism = breakdown of larger molecules into smaller, simple molecules with release of energy

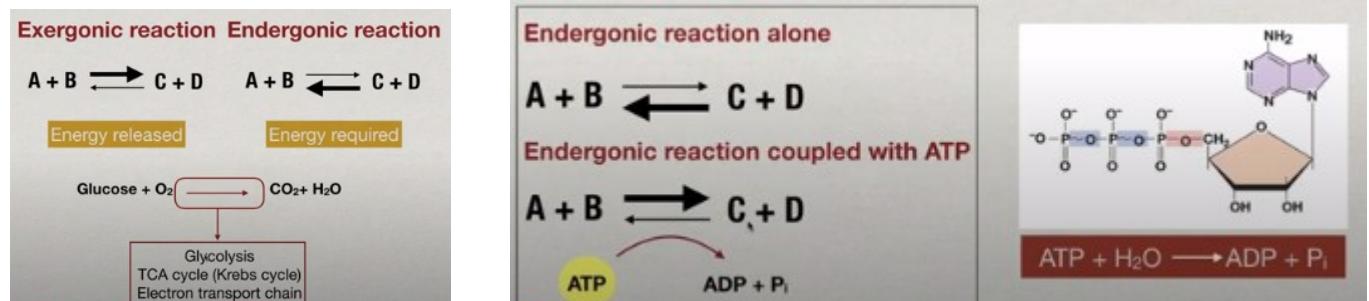
Anabolism = Synthesis of complex molecules from simpler molecules with the input of energy.

Nutritional Groups

- Pathogens are chemoorganotrophs (because they feed off animals/human bodies = composed of organic molecules)
- Microbes and prokaryotes are chemolithotrophs (inorganic). They are important in nutrient cycling.
- Primary producers are phototrophs (create the organic molecules for chemoorganotrophs)

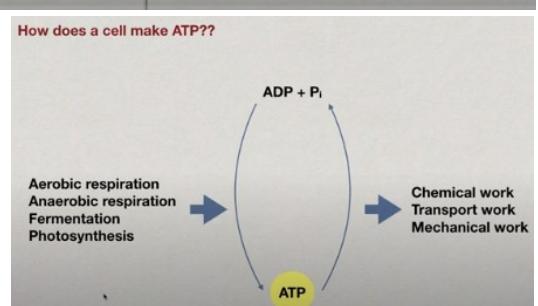


Exergonic and Endergonic Reactions



Energy

- = the capacity to do work
- Chemical work = synthesis of complex biological molecules
- Transport work = energy for the uptake of nutrients
- Mechanical work = required for cell motility and movement of structures within the cells.



How to make energy (ATP)

- Aerobic, anaerobic respiration, fermentation, and photosynthesis.
- This allows for chemical, transport and mechanical work to be done

ATP Synthesis

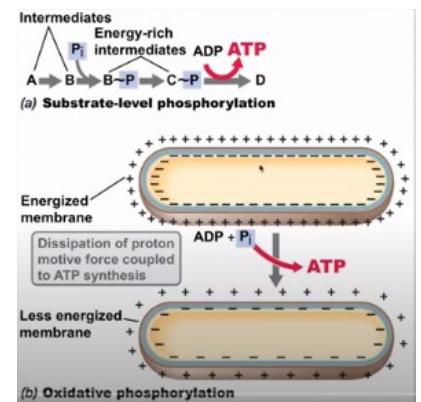
2 ways in which you can derive ATP:

1. Substrate level phosphorylation

- Organic molecule produced by another organism with high phosphate. Take the high phosphate and combine with ADP to make ATP

2. Oxidative phosphorylation

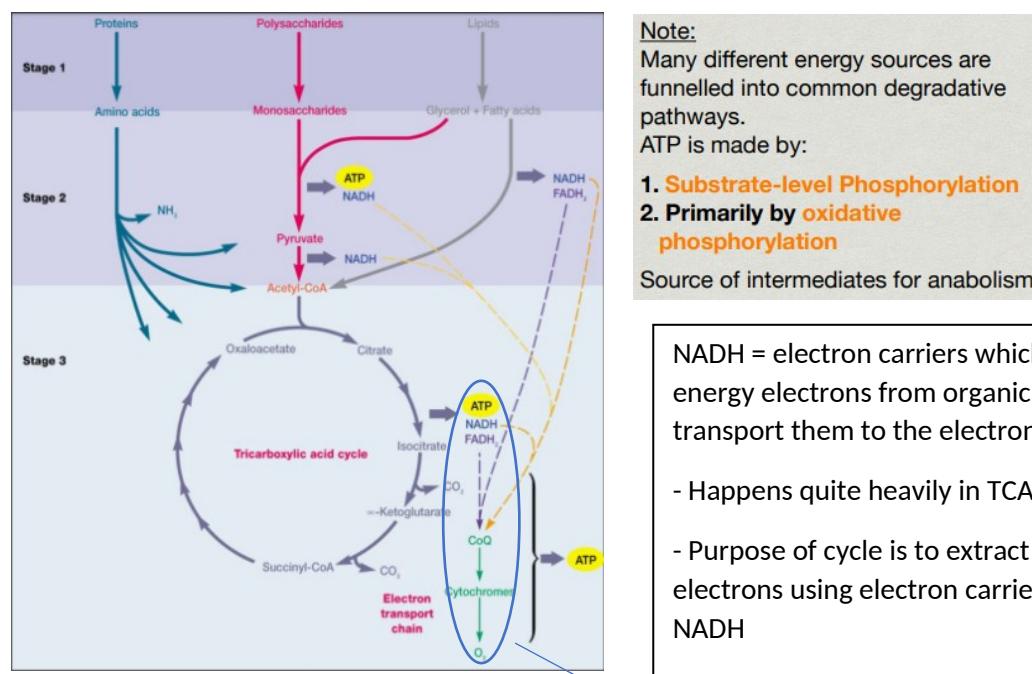
- Uses proton motive force and ATP synthesis to make ATP
- Membrane must be energised like a battery (+ outside - inside) for it to be converted to ATP



Overview of Chemoorganotrophs catabolism of organic energy sources

Overview of **aerobic** catabolism (the three stages in the picture):

- Large molecules into small ones
- Initial oxidation and degradation to pyruvate (this is glycolysis)
- Oxidation and degradation of pyruvate by TCA cycle



Note:

Many different energy sources are funnelled into common degradative pathways.

ATP is made by:

- Substrate-level Phosphorylation**
- Primarily by oxidative phosphorylation**

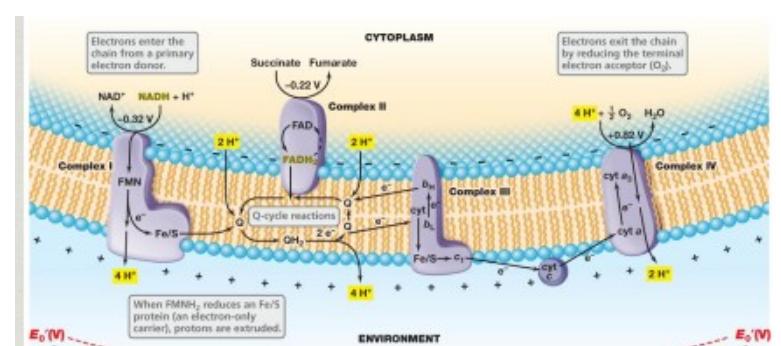
Source of intermediates for anabolism

NADH = electron carriers which pull protons, high energy electrons from organic molecules and transport them to the electron transport chain.

- Happens quite heavily in TCA cycle
- Purpose of cycle is to extract high energy electrons using electron carriers (NAD) to form NADH
- High energy electrons pass through electron transport chain for oxidative phosphorylation

The electron Transport Chain

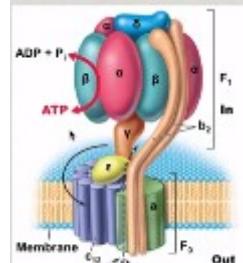
- Composed of electron carriers that transfer electrons from donors (e.g. NADH) to terminal electron acceptors (O_2) for aerobic microbes
- Electrons passed from cytochrome to cytochrome



NADH being converted to NAD^+ when they give the proton to the first protein in the electron transport chain

- protons are being pumped across the membrane = plus minus differential (battery)
- In cytoplasmic membrane (prokaryotes) & mitochondria (eukaryotes)

- Image = ATPase's Protein (motor). It rotates as protons move down
- This process is how ADP is converted to ATP
- This oxidative phosphorylation is the most efficient way to make ATP (compared to substrate phosphorylation)



Energetics Balance Sheet for Aerobic Respiration

(1) Glycolysis: Glucose + 2 NAD ⁺	→	2 Pyruvate + 2 ATP + 2 NADH
(a) Substrate-level phosphorylation 2 ADP + P _i → 2 ATP		
(b) Oxidative phosphorylation 2 NADH → 6 ATP		
	8 ATP	to CAC to Complex I
(2) CAC: Pyruvate + 4 NAD ⁺ + GDP + FAD →	→	3 CO ₂ + 4 NADH + FADH ₂ + GTP (ATP)
(a) Substrate-level phosphorylation GDP + P _i → GTP (ATP)		
(b) Oxidative phosphorylation 4 NADH → 12 ATP 1 FADH ₂ → 2 ATP		
	15 ATP (x 2)	to Complex I to Complex II (See Figure 3.20)
(3) Sum: Glycolysis plus CAC	→	38 ATP per glucose
b) Energy yield from the citric acid cycle		

How is it calculated?

- Based on ATP synthesised per flow of protons through ATP synthase
- 1 NADH = 10 protons = 3 ATP
- 1 FADH₂ = 6 protons = 2 ATP

Anaerobic Respiration and Fermentation

In aerobic respiration, O₂ is the final electron acceptor (excellent)

But without oxygen what happens?

1. Anaerobic respiration
 - Another electron acceptor substitutes oxygen
2. Or... fermentation
 - Derive ATP from only substrate level phosphorylation
- Some prokaryotes can do all three types, some can only do 1, some can flip between aerobic and anaerobic/fermentation depending on oxygen availability

Anaerobic Respiration

- The whole process is the same (proton passed through electron transport chain but the acceptor changes).
- Yields less energy (ATP)

Overview of anaerobic respiration

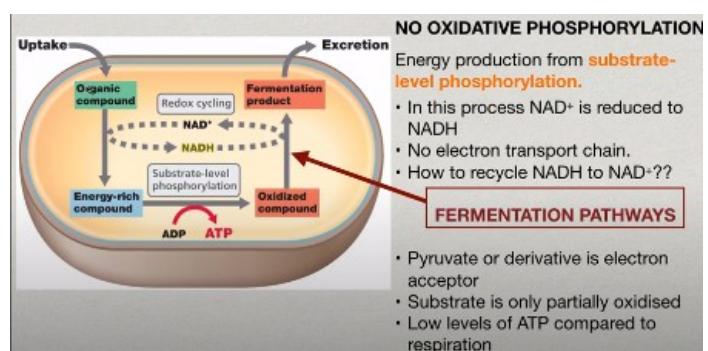
1. Terminal electron acceptor **other than oxygen**
2. Generally yields less energy (because the E₀ of the electron acceptor is less than the E₀ of oxygen)
3. Final electron acceptor may be nitrate (NO₃⁻), sulfate (SO₄²⁻), CO₂, but also iron (Fe³⁺) etc.
4. Electron transport chain is/can be modified

	Electron acceptor	Related products
Aerobic	O ₂	H ₂ O
Anaerobic	NO ₃ ⁻	NO ₂ ⁻
	NO ₂ ⁻	NO ₂ , N ₂ O, N ₂
	CO ₂	CH ₄
	S ⁰	H ₂ S
	Fe ³⁺	Fe ²⁺

e.g. farmers aerate soils as there are organisms that convert nitrate to nitrite making it unavailable to plants. By pumping oxygen, the organisms use oxygen to respire instead.

Fermentation (no respiration in fermentation)

- Takes into consideration the glycolytic pathway (glucose down to pyruvate)
- Uses just that part of the pathway to make ATP
- Fermentation pathway is all about recycling NADH back to NAD⁺
- Least energy produced



Fermentation is used when an organism lacks an electron transport chain or there is not enough oxygen.

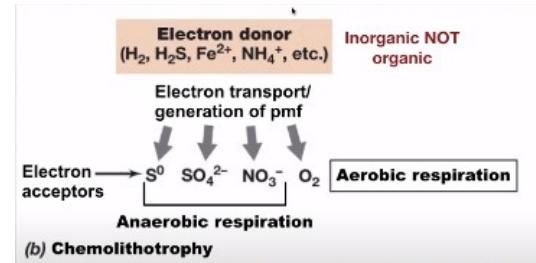
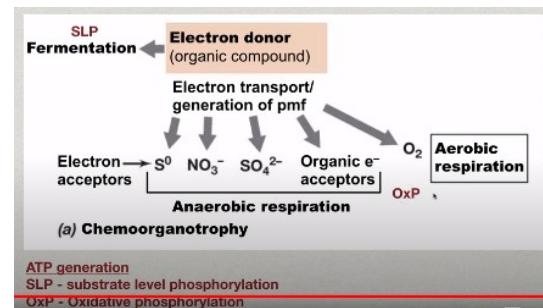
Summary of metabolism in Chemoorganotrophs

- Key is they need organic molecules

Chemolithotrophy

- Key is they are not organic (inorganic)
- Principle is the same but with inorganic molecules...

- Electron donor passes electron to electron acceptor through electron transport chain
- ATP synthesised by terminal acceptor (usually oxygen) or can be anaerobic
- Many chemolithotrophs are autotrophs (have to make organic molecules from CO_2)

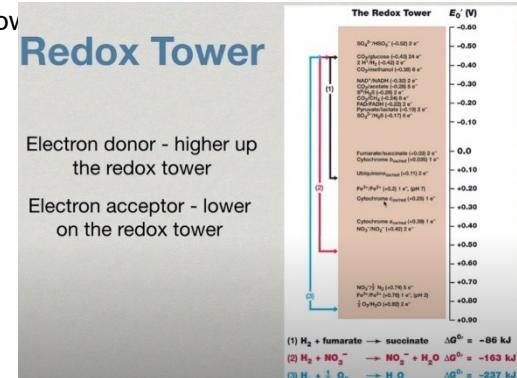


Energy is still much less than in chemoorganotrophs as they are lower down (higher on tower = more energy)

Large difference (distance) between acceptor and donor = better

Central Role of Proton Motive force (=differential charge)

- Phototrophs use light to make photosynthesis to charge an electron (split form water) to make 'battery'. Chemotrophs use electron donor and pass it down the electron transport chain to make proton motive force.
- **Proton motive force** can be used to: 1. Make ATP by powering ATPase. 2. Power flagella motor. 3. Use in Active transport

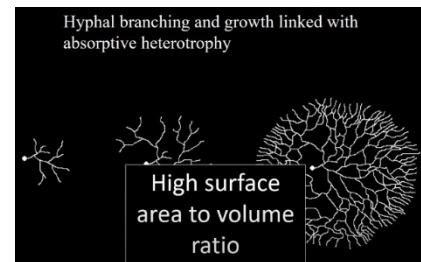


- Cell walls have CHITIN – cell wall = like plant, Chitin = like animals
- Primary storage carbohydrate is GLYCOGEN (like animals)

Fungi are more closely related to animals than they are to plants. Originally put in plants but now have their own place on the tree (next to animals due to similarities)

Fungal Structure

- Single celled fungi = yeasts
- Multicellular = moulds
 - multiple cells form and grow to form **hyphae**: = filaments tangled into a mass (mycelium – the body of the fungus)
- Grow from the original spore like this to increase surface area to volume. Larger surface area makes them lose moisture much faster thus struggle in dry areas.



Asexual Reproduction – **Anamorph** (asexual state)

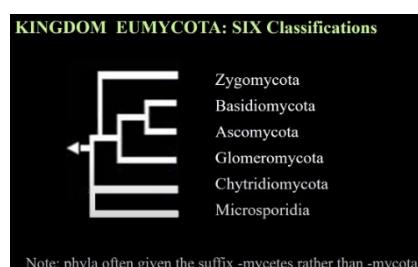
Methods:

- Hyphal fragmentation – any undamaged piece of hyphae can regrow if it has adequate nutrients and cellular parts for reproduction
 - Binary Fission (few fungi) – cell enlarging and dividing in 2
 - Budding (most yeasts) – Fully formed cell pinches off to grow a daughter cell
 - Spores – Contain essential parts for reproduction when it lands on an accepting hyphae
 - Asexual reproduction occurs continuously (no need to find mate)
 - Limited opportunity for genetic change
- Dimorphism (mainly occurs in pathogens) – Where a fungi grows as a mould in a normal environment, but at a certain stimuli it becomes a yeast (e.g. mould at 24 degrees and yeast at 37 degrees. Thought to do so to evade human immune response better)

Sexual Reproduction – **Teleomorph** (sexual state)

- Often seasonal (prefers sexually but can only do so in certain conditions)
- Involves union of compatible nuclei
- Most fungi are sexually indeterminate (no classification of sexual type). Can have up to 8 different mating types (can't tell what these are till they mate)
- Some have more than one mating type

Nomenclature and Classification



- 6 classifications
- Not definitive only current to what we know

Microsporidia – 1500 species

- Simplest form
- Unicellular
- No mitochondria, mitosome instead (reduced form of mitochondria)
- Contain Chitin
- Intracellular parasites (mainly with fish. Have polar tubes that shoot out of the cell and attach to the fish. Mate till the fish explodes)

CHYTRIDIOMYCOTA – 1000 species

- Unicellular or simple aseptate mycelium
- Free living, Saprobes (live on decaying material).
- Asexual and sexual spores both motile (utilise flagella)
- Parasitic forms infect aquatic plants and animals, insects
- Some anaerobes (anaerobic)
- Have wiped out 100 of 600 amphibian species (can wipe out a frog community in a week)

GLOMEROMYCOTA – 250 species

- Plant symbionts (form networks on/in roots) Feed of carbohydrates produced by roots. In exchange, they transfer nutrients (very important for plants)
- Only asexual reproduction has been observed

ZYGOMYCOTA – 1500 species

- Simple aseptate mycelium
- May produce asexual sporangiospores, within sporangia (s. sporangium)
- Sexual reproduction when environmental conditions not favourable
- Some produce sexual zygosporangia
- Diverse ecological roles (because they can reproduce both ways, there are a number of species)
- Used to make amylases, rennets, many organic acids (lactic, citric, etc), part of the cortisone process (some benefits to us)
- Some human pathogens — zygomycosis (bad infection)

ASCOMYCOTA (bathroom moulds) – 65000 species

- Many common fungi eg. *Saccharomyces* spp., *Penicillium* spp., *Aspergillus* spp.
- Asexual reproduction by budding, fragmentation, spores (conidia)
- Conidia are freely borne on hyphae
- Sexual structures: ascospores in ascus, may be surrounded by fruiting body — ascocarp
 - Ascus provides protection, until stimuli leads to spore release
- Many species with diverse morphologies, habitats and life-histories
- Many undescribed taxa
- Many plant, human and other animal pathogens

BASIDIOMYCOTA – 30000 species (macro fungi like mushrooms)

- Morphologically diverse
- Complicated life-cycles
- Some are yeasts, most are moulds
- Dominated by sexual (diploid) state

- Characterised by the presence of sexual basidiospores
- May have a basidiocarp (protection, launching of spores) containing basidia (meiosis to form n spores)

CONSUMPTION OF MUSHROOMS

- some have undesirable effects - from gastro-intestinal distress, hallucination, coma and death
- Most poisonings related to misidentification - Some toxic spp. nearly identical to edible spp.

Week 8 - MYCOLOGY II (applied mycology)

PRIMARY METABOLITES OF INDUSTRIAL IMPORTANCE

Citric Acid

- 1,500,000 Tons/y for food, pharmaceuticals
- *Aspergillus niger* (ferment excess molasses to form citric acid)
- Molasses from sugar refining

Ethanol

- Antiseptics, solvents, car fuelling (e-10)
- 2 fungi used industrially = *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* (kombucha)

Different yeast species are used to make different sorts of beer

- Metabolize sugar extracts from grains (malt) to produce alcohol and CO₂ = beer
- Influences the character and flavour
- 95% beer sold in AUS is made in AUS
- AUS beer industry = \$16.9 B/y = 1.02% AUS GDP
- GM yeast for bitter flavour to remove cost of Hops in beer production



SECONDARY METABOLITES OF INDUSTRIAL IMPORTANCE

- Many of these but here are the 4 most important:
- Penicillin - most effective on gram positive, it inhibits bacterial cell wall synthesis
- Cephalosporins - works same as penicillin (but can affect gram negative in high conc.)
- Griseofulvin - Drug derived from fungi to stop fungi (only oral drug for cutaneous infections). Very toxic so only in rare cases. Prevents mitosis.
- Cyclosporin A - initially an antifungal drug but it gave bacterial infections. Now used for organ transplants.

ENZYMES

Fungi secrete enzymes to the outside of the cell to break down complex OM and transport nutrient back into the cell.

- Extracellular enzymes produced
- Aspergillus spp. are the most important fungi – most diverse secretions and easy to contain
 - Fungal enzymes dominate the market
 - Usually batch fermentations
 - May contain impurities (but cheap)
 - Issues with toxicity (need to detoxify to use for food)

Can use fungi to make SINGLE CELL PROTEIN

- Can use fungi to convert cheap N and C sources into high quality proteins (for foods)
- Only requires a small area (less waste than farming) and there's no seasonality

PROBLEMS

- Microorganisms have a high level of RNA (can cause gout)- Said its been countered but there is still reluctance
- Ultimately unprofitable now.

Fungi can be used as bio-control agents

- Use insect pathogens to control insects (fungi are pathogens)
- Only sparingly (don't know the affects)
- Has potential to control insect to human

Forensic Mycology

- The use of fungi in criminal investigations
- Fungal growth on a corpse (some fungi's can be used as indicators of where a corps is) 2 mushrooms do this at the moment (not used yet)
- Fungi characteristic of disturbed topsoil

CLINICAL MYCOLOGY

FUNGAL PATHOGENICITY

- Fungi and humans have a common ancestor
- This makes mycoses very hard to detect and treat
- When we treat, antifungals are extremely toxic

FUNGI AS HUMAN PATHOGENS

- Most fungi that can infect you are opportunistic not obligate pathogens (except dermatophytes which need to infect to spread)
- For most fungal diseases relate to immunological status (how compromised they are) and environmental exposure

PHYSIOLOGICAL BARRIERS TO FUNGAL GROWTH

- 2 major physiological constraints =
 - Temp - most cannot survive at 37 degrees
 - Redox potential - living tissue is way more reduced than decaying items
- Nonspecific Host defences
 - Mechanical barriers (hair in nose, skin... prevent fungal infection until skin is broken)
 - Surface excretion (tears, saliva... have antifungal properties)

- Endogenous Flora (organisms in our skin, gut... and they outcompete fungi most of the time)
- Cellular defences
 - Non-specific immunity - e.g. neutrophils (attack non human products)
 - Acquired Immunity - Regulated by T-cells (requires exposure for immunity to be made)

PREDISPOSING FACTORS TO FUNGAL DISEASE

- Disturbance of the epithelial barrier (cuts... allows exposure)
- Dysfunction of nonspecific immunity (radiotherapy, chemotherapy)
- Dysfunction of t-lymphocyte cell mediated immunity (HIV, AIDS...)

MYCOTIC INFECTION CLASSIFICATIONS

- Superficial Mycoses
 - Piedras = infections of hair shaft
 - Dermatophytes = infection of the skin (ringworm, tinea...)
 - Most frequent
 - Obligate pathogens = must cause infection to disseminate
 - Contagious
 - Must live on the person
 - Treatment: topical ointments and antifungal agents
- Cutaneous Mycoses
 - Same as superficial
- Subcutaneous Mycoses
 - Infections of the dermis and subcutaneous tissue (under skin)
 - Result from traumatic implantation (e.g. from splinters)
 - Frequent in rural areas and tropics
 - Clinical manifestations - nodules that ulcerate. Organism spreads up lymphatic channels (produces more)
 - Treatment: antifungal agents and surgical excision
- Systemic Mycoses
 - Deep seated fungal infections. Involve more than one organ
 - True pathogens (mostly dimorphic, no recognizable predisposition) or opportunists (less virulent, less adaptive, immunocompromised)

SUMMARY

ROLE OF FUNGI IN DISEASES OTHER THAN MYCOSES

- | | |
|---|--|
| <ul style="list-style-type: none"> - <u>Inhalation</u> - of fungal material: allergic reaction - <u>Ingestion</u> of mushrooms or material altered by fungal growth - <u>Aflatoxin</u> - Aspergillus flavus, associated with peanuts | <ul style="list-style-type: none"> - Most fungal pathogens are opportunistic - Immunocompromised most susceptible - Few antimycotics are available - Illness may result from infection OR toxins |
|---|--|

Week 9 - Parasitology

What is Parasitism

- A relationship in which one species lives on or in another organism
- The parasite obtains essential molecules e.g. carbohydrates, fats or lipids (strong dependency)

- The perfect parasite would live harmoniously without causing disease to the host as they rely on it. They can change host through transmission
- Parasitism is NOT symbiosis, mutualism or commensalism

Types of parasites:

- **Endoparasite** = lives inside host
- **Ectoparasite** = lives outside host on skin
- **Facultative** parasite = can alternate between free living and parasitic stages
- **Obligate** parasite = needs host to complete life cycle

Hosts

Can be described in several ways:

- **Reservoir** host = harbours parasites infective to humans
- **Transport** host = it transports the parasite and the parasite does not need to reproduce
 - E.g. a dog eats meat with a parasite, the parasite passes through and out the dog without developing
 - If the parasite developed and reproduced it is either:
 - **Definitive** = if the parasite undergoes sexual reproduction
 - **Intermediate** = if the parasite only asexually reproduces

Vectors

Many ectoparasites have a relationship with other parasites

- **Vector** = when an ectoparasite is a vehicle of transmission for another parasite
- Many ectoparasites are **arthropods** (not microbes) e.g. mosquito as it needs to feed on blood for development and can transport e.g. malaria
- Can be:
 - **Mechanical** vector = transmitted through contact e.g. fly picks up disease on body and transports through physical contact
 - **Biological** vector = e.g. mosquito, transmitted through biting...

Zoonoses

- = infection that has jumped from animal to human. The parasites are described as Zoonotic
- Some parasites don't normally infect humans but can be accidentally transmitted e.g. from a dog, resulting in disease.

Classification of Parasites (3 we are looking at)

- Protozoa
 - Amoeba
 - Flagellates
 - Ciliates
 - Sporozoans
- Helminths (can often see adults by eye)
 - Worm like metazoan invertebrates
 - Round worms
 - Flat worms
- Arthropods (NOT MICROBES)

- Mostly ectoparasites
- Insects, flies, mosquitos, lice, fleas, ticks, mites

Protozoa

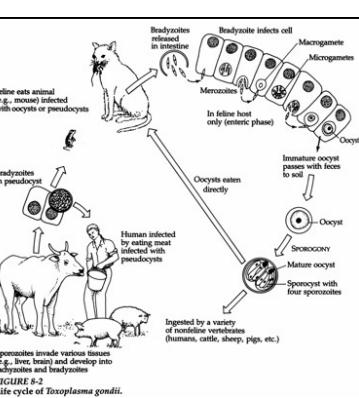
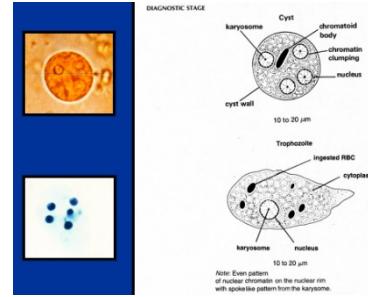
- Are unicellular eukaryotic (single cell containing complex organelles)
- Often asexually reproduce through fission (splitting in half) but some can sexually reproduce
- Limited host distribution
- Transmitted by ingestion or arthropods
- Cystic stages common

Types of Protozoa

- Gut associated
- Blood
- Tissue/viscera

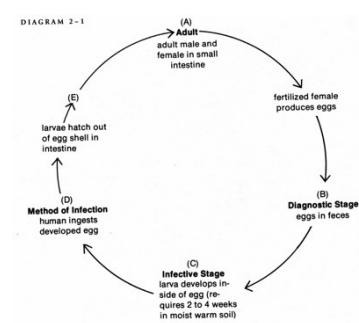
Main groups of protozoa (categorised by how they move, except Apicomplexa)

- Amoeba - pseudopodia
 - Move across solid surface using pseudopodia (only trophozoites using cytoplasmic streaming). Don't have fixed shape.
 - In humans, mainly found in gut (transmitted from faecal oral route, hand to mouth)
 - Two main life cycles: trophozoite and a cyst (often formed after asexual repro of the trophozoite. Cyst = vehicle of transmission through faeces (environmentally resistant to desiccation)
- Flagellates - flagella
 - Move with flagellum and are found in the gut (e.g. Giardia) and blood (e.g. Haemoflagellates, which rely on blood metabolites such as haem)
 - Pathogenic; cause serious deadly diseases (blood mainly)
 - Transmitted by arthropod vectors
 - Giardia have trophozoite (motile with flagella) and a cyst (non-motile).
 - Haemoflagellates possess flagellum in one of their life cycle stages. Contain organelle, Kinetoplast = novel type of mitochondrion responsible for respiration (unique to these types)
- Ciliates - cilia
- Apicomplexa - apical complex (sometimes called sporozoans)
 - Obligatory, contain many important pathogens that affect humans/animals
 - Host restricted
 - Complex life cycle
 - Both sexual and asexual
 - Transmission involves zygote (from sexual reproduction) which develops into an oocyst (cyst)

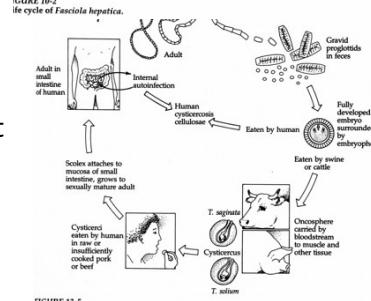
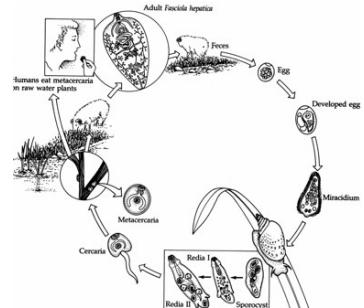


Helminths

- Nematodes (round worms)
 - Free living or parasitic



- Male and female. Well defined morphological features for sexual orientation
- Adult have cuticle (structure of the body wall)
- eggs give rise to larvae, developing larvae through a series of four moults to become adults
- Medical diagnosis of intestinal nematodes is done by investigating the eggs in stool and they are all unique in morphology
- Types of Nematodes
 - Intestinal (majority)
 - Blood and Tissue
- Trematodes (flatworms)
 - Dorsoventrally flattened and have no gut cavity (unlike round worms)
 - No respiratory or blood vascular systems
 - Solid body (internal organs embedded in a parenchyma)
 - Surrounded by tegument (no cuticle)
 - Hermaphroditic (no separate males/females)
 - Types of Flatworms: two main, the flukes (gut and other tissue) and the tapeworms (just gut)
 - Digenea (flukes-non-segmented)
 - Larval stage (miracidium) develops in the egg
 - Involves an intermediate host e.g. snail where the larvae undergo asexual development
 - Final larval stage = cercaria = infective stage
 - Enters definitive host for adult development and reproduction
 - Cestodes (tapeworms-segmented)
 - one segment = a proglottid which contains both male and female reproductive organs.
 - Segments break off and are passed in faeces to infect (as well as eggs).
 - Tapeworm have a scolex, composed of suckers and rostellum that help attach to a gut wall.



Arthropods

- Either an ectoparasite or vector
- Segmented invertebrates
- Exoskeleton, body cavity, highly developed set of body systems in place

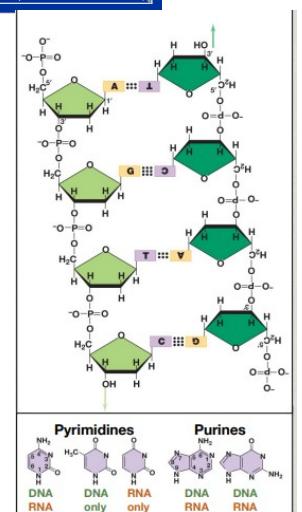
Arthropods and Disease	
Arthropod	Disease
Mosquitoes	Malaria, elephantiasis, dengue, yellow fever
Biting flies	Leishmania, Trypanosoma, Onchocerciasis
Non biting flies	Myiasis
Lice	Typhus
Hard and soft ticks	Tick fever
Bugs	Chagas' disease
Fleas	Plague

Week 10 - MICROBIAL GENETICS

DNA REPLICATION

Revision I: DNA Structure

DNA is usually a double stranded molecule consisting of nucleotides.



- Nucleotide is a phosphate, sugar & nitrogenous base.
- Nucleotides are joined by phosphate esterification. 3' carbon of sugar to 5' carbon of adjacent sugar.
- 4 nitrogenous bases
 - Cytosine (pyrimidine) (C), Guanine (purine) (G), Thymine (pyrimidine) (T), Adenine (purine) (A)

Revision II: DNA Structure

- 2 strands run antiparallel: 5' – 3' & 3' – 5'
- 2 strands held together by hydrogen bonds

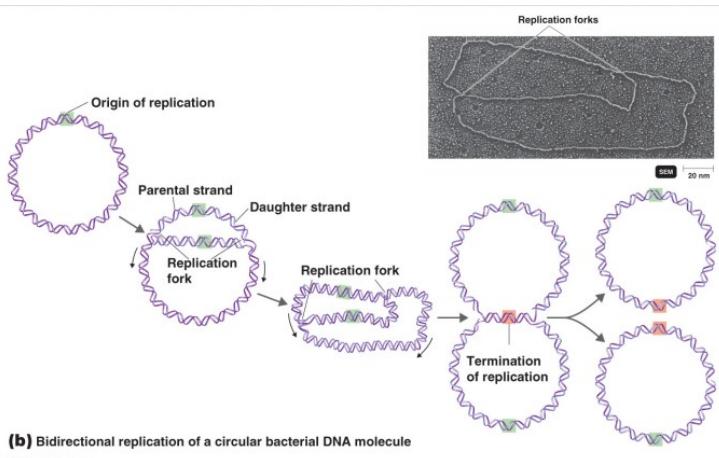
Double-stranded DNA formed by base-pairing:

- 2 hydrogen bonds between A and T
- 3 hydrogen bonds between G and C

DNA sequence is always presented 5' – 3' • e.g. 5'-CCCGTGTATTCC-3'

Bacterial DNA replication

- In most prokaryotes DNA replication occurs bidirectionally from a single origin of replication.



Most prokaryotes have 1 singular circular chromosome (far left). Origin of replication = origin of all replication

Image 2 – DNA strands melt to allow DNA synthesis in both directions (dark purple strand = parental, light purple = synthesised strand going in both direction = daughter strand).

Replication continues all the way around, till it hits the termination of replication where they split, forming two identical chromosomes (1 parental and 1 daughter strand)

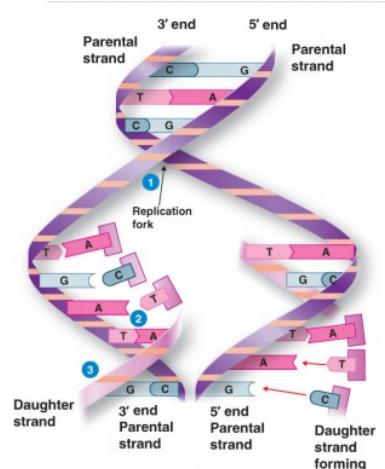
Bacterial DNA replication

DNA replication:

- DNA replication is complex
- It involves numerous proteins which ensure accuracy

Process:

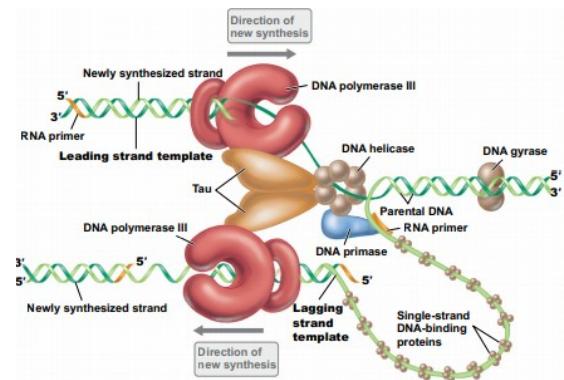
- The two strands separate, each serving as the template for the synthesis of a complementary strand
- DNA synthesis is semi-conservative (i.e. each daughter cell obtains one old & one new strand)
- DNA synthesis is ALWAYS in the 5'-to-3' direction (This results in the formation of a phosphodiester bond)



Steps in DNA replication (at replication fork)

Proteins involved in DNA replication form the replisome

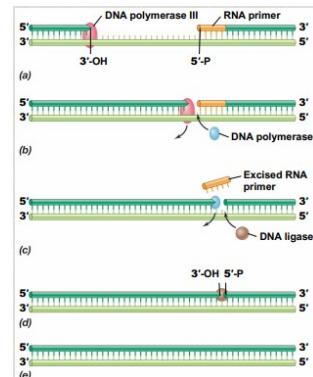
1. Replication starts at **origin of replication**.
2. **DnaA** binds origin of replication and allows for initiation of replication.
3. **DNA Helicase** binds and unwinds DNA – always ahead of replication fork (**breaks H bonds**).
4. **SSBs** (single stranded binding proteins) bind and keep strands separated.
5. To begin synthesis, there is a short RNA "primer" (at 5' side) that is synthesised by **Primase**
6. **DNA polymerase III** synthesises DNA 5'-3' – leading and lagging strands (okazaki fragments). Tau - holds together two core enzymes for the leading and lagging strands.
7. DNA gyrase relieves tension ahead of fork generated by unwinding DNA



Bacterial DNA replication - okazaki fragments

Lagging Strand Synthesis

- **DNA polymerase III** synthesis lagging strand discontinuously (5'-3') through Okazaki fragments (points where the strand join back).
- **DNA polymerase I** removes RNA primer and fills in with DNA fragment
- **DNA ligase** joins Okazaki fragments

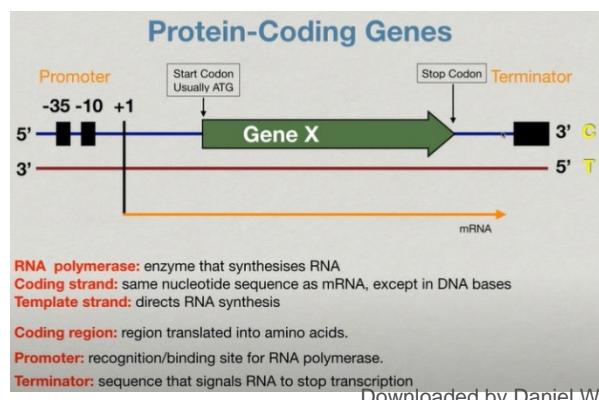


PROTEIN CODING GENES

Genes

What is a gene?

- A nucleotide sequence that codes for a functional product. 3 things can be coded:
 - A protein
 - tRNA (transfer)
 - rRNA (ribosomal)
- To make the DNA turn into something useful, we need **RNA polymerase**
- These can be for:
 - mRNA = messenger RNA (for protein-coding genes). Translated to form protein
 - tRNA (required for translation)
 - rRNA (required for ribosome function)
- Letter = A U G C (the T's from DNA sequencing are replaced by U's in RNA)



Top = coding strand, bottom = template strand.
RNA polymerase codes on the T strand, copying what's on the C strand. (but T is replaced with U)

Two promoters (-35 = 35 base pairs upstream & -10 = 10 base pairs upstream) allow RNA poly to bind

mRNA codes for gene X (replaces T for U) = identical. Terminator makes RNA poly fall off

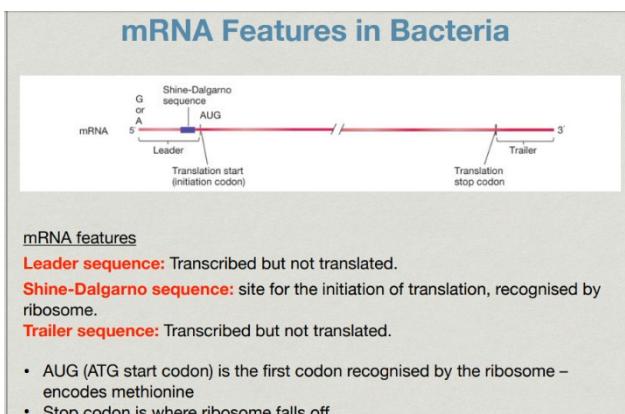
Protein-Coding Genes

Making RNA = transcription,

making protein = translation

Transcription in prokaryotes:

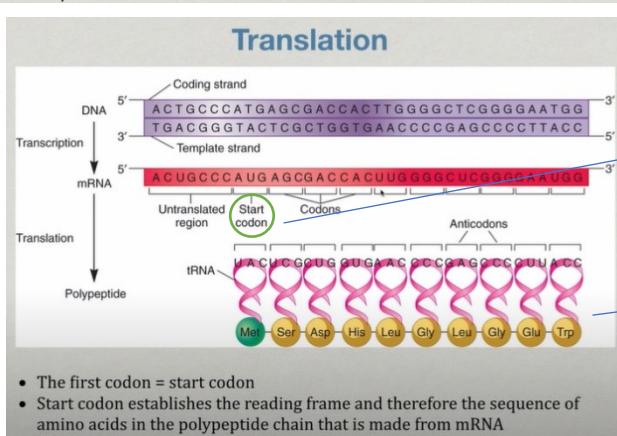
- Most bacterial RNA polymerases contain:
 - RNA poly = **Core enzyme** – 5 proteins
 - The **sigma factor** helps core enzyme recognize the promoter and allow RNA poly to bind
 - Bound RNA poly = **Holoenzyme** = core enzyme + sigma factor
 - Only holoenzyme can begin transcription, but core enzyme completes RNA synthesis once it has been initiated.
 - Different sigma factors recognise different promoters.



AUG = ATG = start codon (first codon that the ribosome attaches to, to begin protein synthesis)

Shine-Dalgarno seq = binds to RNA and finds ATG (AUG)

Ribosome moves across in 3's till it reaches a stop codon. It falls off and synthesis is complete



That process is seen here: mRNA sequence = copy on top (C) coding strand but with U's not T's.

Shine Delgarno finds start codon = AUG

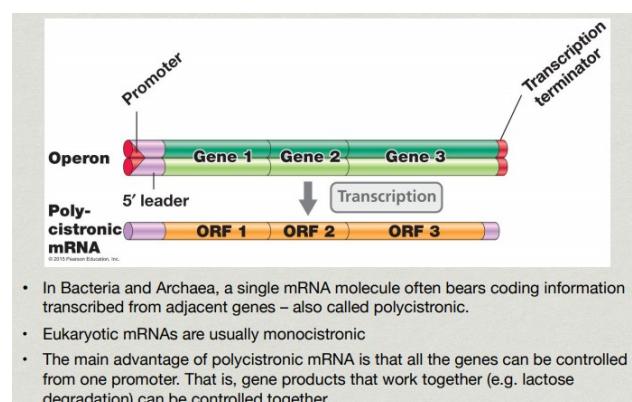
Then moves in three (grouping in codons (anticodons)) until the stop codon

That sequence = the protein

(note: identical, but RNA uses U not T)

Operons: Polygenic mRNA often found in bacteria

- In bacteria you can have **Operons** = when 1 promoter driving the TRANSCRIPTION of multiple genes in succession
- Seen on right (1 promoter coding 3 genes for 3 different proteins)
- When RNA poly binds, it creates one mRNA with 3 open reading frames (ORF) and ribosomes combine to that to make proteins



- DNA portion = operon, RNA portion = poly-cistronic
- Unique to prokaryotes (bacteria and Archaea) not in eukaryotes = beneficial as it can be done all in 1

Prokaryotes couple transcription and translation

- **Coupled transcription translation** = when translation starts before transcription finishes (going at the same time) = Ribosome moves across forming protein before RNA is completed
- Only Prokaryote as well. Because in Eukaryotes mRNA must be transported out of the nucleus first

MUTATION (how do we get changes in DNA to get different proteins = mutation and lateral gene transfer)

DNA Polymerase Error and Evolution

- As DNA poly is coding it can make mistakes = **mutations**
- Mutation = a heritable change in the coding sequence of the organism (passed onto daughter cells)
- Mutation must be BENEFICIAL to be selected. E.g. if its more resistant to e.g. antibiotics, it will grow more and replicate more = selected, if harmful it will die out.
- Every 10 million synthesis of nucleotides, there will be one mutation
- BUT e.g. E.coli has 4 billion nucleotides and doubles every 20 mins. So, @ rate 1 in 10 mil, E.coli will have 30-300 mutants in 24hrs.

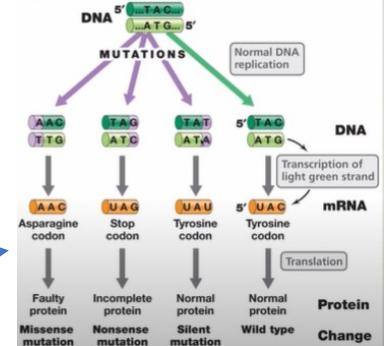
Wild-type strain: Typically refers to strain isolated from nature

Mutant: A strain of any cell or virus differing from parental strain in genome

Mutation: Heritable change in DNA sequence that can lead to a change in phenotype

Point mutation: Mutations that change only 1-basepair. E.g. these:

Frame shift Mutation: When there is an addition or subtraction of a base pair in a codon (insertion or deletion) which throws the sequence out of sync. = More dangerous due to the changes they can make



Mutation

Spontaneous mutations

- Spontaneous mutations result from errors in DNA replication and transposon insertions. (transposons jump)
- All life has a natural spontaneous mutation rate.

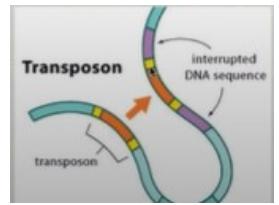
Induced mutations

- Induced mutations are caused by chemical (e.g. nitrosamines – found in tobacco) or physical agents (e.g. radiation) that damages or alters the chemistry of DNA or interferes with DNA repair mechanisms.

- These agents increase the mutation rate.

Mutational insertions by transposable elements

- Transposons: a DNA element that can move from one DNA location to another (transposition).



GENE REGULATION

= Regulation of bacterial Transcription

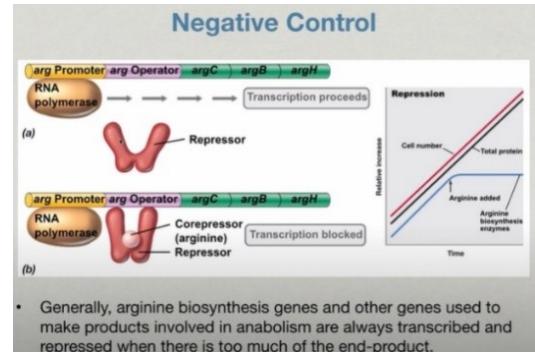
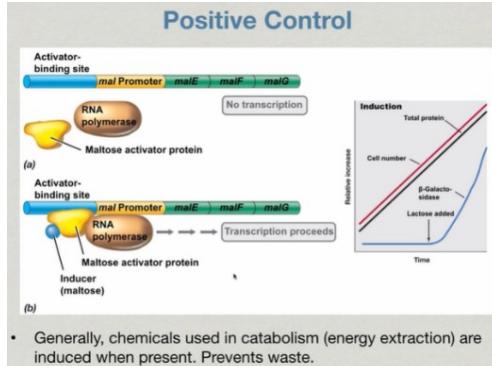
Constitutive gene expression = genes that are required all the time e.g. Housekeeping genes (involved in cellular processes)

Inducible gene expression = Activated only when needed e.g. Genes involved in Catabolism (breaking down of complex molecules e.g. glucose) and only activated when glucose is present

Repressible gene expression = Repressed unless needed e.g. genes involved in biosynthesis. Products are needed most of the time but when in excess the genes are repressed.

How its regulated - Regulation of Bacterial Transcription

- Regulator Proteins
 1. **repression proteins**, which have negative control = prevent the RNA poly from binding or moving down. Represser proteins bind at a site called **operator** = overlapping promotor or downstream = stops the RNA poly
 2. **Activator Proteins**, which have positive control = help RNA poly to bind. Activator proteins bind at a site called **activator binding site** = upstream of promoter
- **Effector molecules** -Effect binding or non-binding. Bind regulatory proteins non-covalently. e.g. sugars, amino acids etc. to affect the binding or non-binding of the proteins.
- **E.g.**



Week 11

- What is a Virus?

Viruses are:

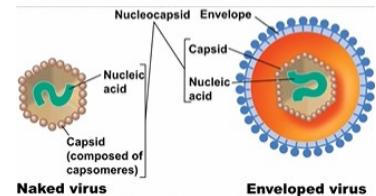
- Acellular infectious agents (not cells)
- Are obligate intracellular parasites (must get inside the organism)
- Consist of one or more molecules of DNA or RNA enclosed in a coat of protein
- Some have additional layers e.g. carbohydrates, lipids, additional proteins...
- ABSOLUTELY DEPENDENT ON A LIVING HOST (cell)

How big is a Virus?

- 10-400nm in diameter
- The smallest = larger than ribosomes and largest = same size as bacterium
- Most only viewable on electron microscope

General Structure of a Virus

- **Virion** = the complete virus particle
 - Consists of nucleic acid and protein coat (capsid) = nucleocapsid
 - Viral nucleic acid encodes viral proteins, some of which are used to form the capsid
 - May have e.g. lipid-based envelope

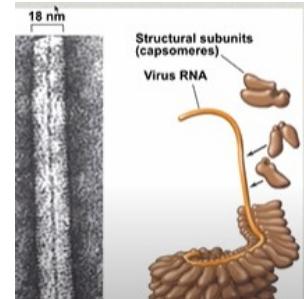


Virion Structure

- Capsid
 - Macromolecular structure of proteins
 - It: protects viral nucleic acid and its transfer between cells
 - Proteins that form capsid = capsomere
 - 3 types of Capsid symmetry
 1. Helical (helix)
 2. Icosahedral (20 triangular faces to form pointed ball like shape)
 3. Complex (everything else. Less common)

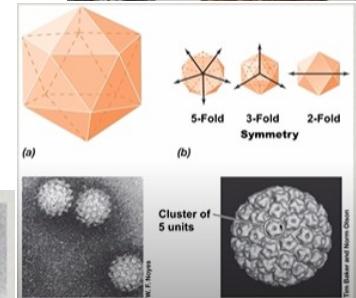
Helical Capsids (most common 2)

- Shaped like hollow tube
- Size is influenced by capsomeres (their size shape and how they interact with one another and nucleic acid within it (appears to determine length of capsid since it doesn't extend far past viral genome or the nucleic acid))



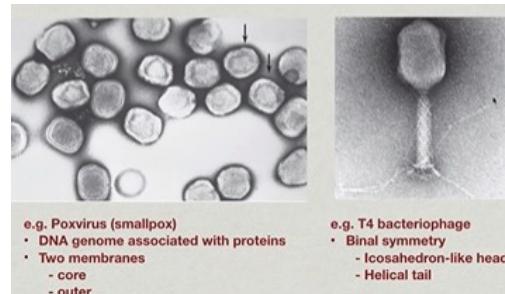
Icosahedral Capsids (most common 2)

- Constructed from ring or knob-shaped clusters of 5-6 capsomeres
- Regular polyhedron of 20 equilateral triangular faces e.g. Adenovirus (common cold)



Complex Capsids

- Some don't fit in the other 2
- Can contain elements of the others



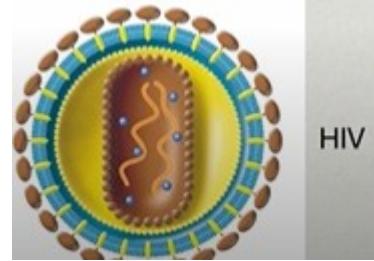
Viral Envelopes

- Many animal, some plant and at least one bacterial virus are bound by an outer membrane = **envelope**

Animal Virus

- Obtain envelope usually by exiting the eukaryotic cell (as they bud out they pick up part of the envelope)
- Therefore, envelope lipids and carbohydrates are acquired from the host

Envelope proteins coded by viral genes and may project as **spikes**, also called **peplomers** (spikes on the outside)



Viral Genomes

Nucleic Acid

- All viruses contain nucleic acid (DNA or RNA). 4 possible types:
 - single stranded DNA 2) dsDNA 3) ssRNA 4) dsRNA
- Animal Virus = all types
- Most plant viruses = ssRNA
- Most bacterial = dsDNA
 - DNA virus = most use dsDNA (linear or circular)
 - RNA viruses = most use ssRNA
 - Many RNA viruses have segmented genomes (pieces of RNA, but not attached)

Viral Genomes

- Viral mRNA – encodes phage proteins
- Plus (+) RNA strand: immediate translational information
- Negative RNA strand: complementation of plus strand
- + DNA strand: same as RNA but with DNA nucleotides
- - DNA strand: same as RNA but with DNA nucleotides

VIRAL REPLICATION AND BACTERIOPHAGES

Generalised Cycle of Viral Multiplication

1) Attachment

- Interactions between specific receptors (e.g. pili, flagella, proteins, LPS) = binds to host
- If receptor common – wide host range (e.g. rabies has common receptor for animals and humans so can jump)
- If receptor rare – narrow host range (e.g. HIV)

2) Entry of viral nucleocapsid or nucleic acid (penetration)

- Injection of nucleic acid
- Nucleocapsid penetration of plasma membrane (e.g. most eukaryotic viruses)

3) Synthesis of viral proteins and nucleic acids

- Viral genome replication strategy depending on nucleic acid
- mRNA synthesis strategy dependent on nucleic acid
- Viral proteins synthesised by host systems
 - Early mRNA: used to code viral copies
 - Late: self-assembly and release proteins

4) Self-assembly of virions

- Proteins and nucleic acids come together to form the capsid
- Complex

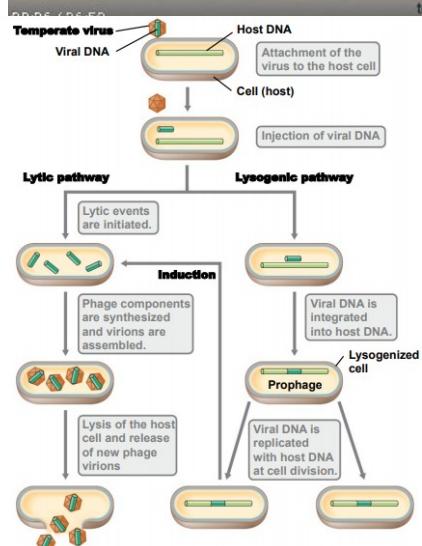
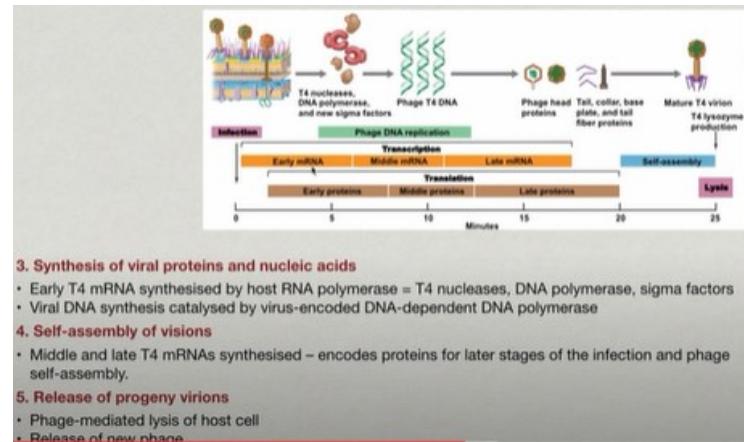
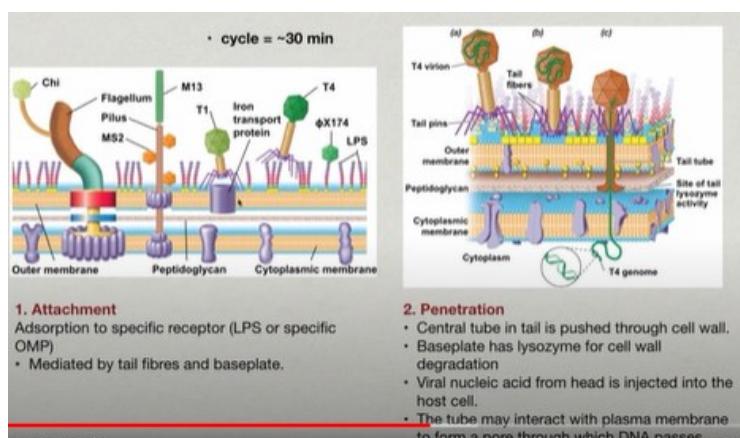
5) Release of progeny virions (virus leaves)

- By cell lysis (host cell dies)
- Budding (host cell survives, weakened)

Viruses that Infect Bacteria and Archaea (These 5 steps above are the same for bacterial and archaea)

- Most are dsDNA

Example – T4 Bacteriophage



Temperate Bacteriophages and Lysogeny

- Many phages are ‘temperate’- the phage genome (DNA) integrates into the host cell genome
- A Temperate phage infect the cell in the **lysogeny** (where the DNA is incorporated into the genome prophage).
- Lysogenic bacteria are normal, except:
 - They cannot be reinfected by the same virus (have immunity)
 - Under appropriate conditions, prophage exercises and enters the lytic phase (**INDUCTION**)

How does a Temperate Phage ‘decide’ which cycle to follow?

- 2 regulator proteins =

- 1) **Lambda repressor** – pushes phage in lysogenic cycle. Blocks transcription of the Cro gene
 - 2) **Cro protein** – pushes phage into lytic cycle. Inhibits transcription of lambda repressor
- If DNA is damaged it induces a **rescue protein** – **RecA** which interacts with the lambda repressor and degrades it which increase the amount of cro protein and pushes it into the lytic cycle. This allows the virus to get out of the damaged host cell

CULTIVATION OF VIRUSES

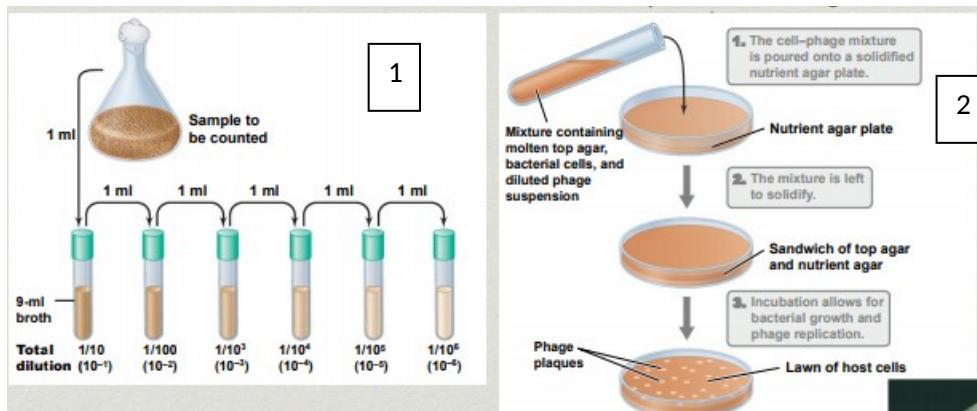
Cultivation of Bacteriophage

How do we culture virus?

- Cannot be cultured without a **host** cell with a **receptor it can bind** too and replicate
- Must use host cells to culture virus

Purification and Enumeration of Bacteriophage

- 1) **Dilution series** of virus or environmental sample (left image)
- 2) Dilute virus or sample with target cells (e.g. e.coli) and warm agar. (right image)
- 3) Count plaques (dilution with 30-300 plaques) and work back to find the original number of viruses were present.



Bacteriophage Therapy

= Use of bacteriophage to treat bacterial infections

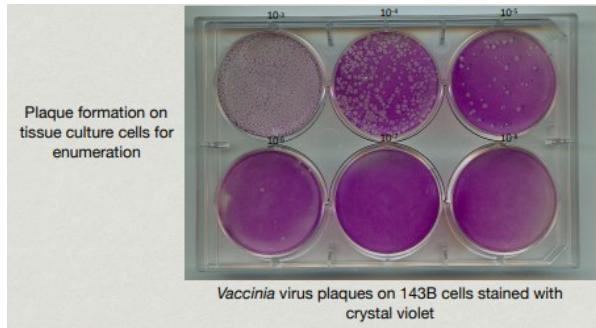
- Cultivate the virus and inject etc
- A possible solution for antibiotic resistance
- Concerns with potential effects but has been used in Eastern countries without any known affects.

Cultivation of Animal and Plant Viruses

- Why cultivate?
- 1. To **study** – like bacteria need to cultivate to see what proteins are produced – how infects etc.
- 2. To produce virus for **vaccine** production

- Influenza seasonal vaccine – egg
- Polio – monkey kidney cells
- Measles – chicken embryo cells

Purification And Enumeration Of Animal Viruses



LEC 12 – What Are Archaea?

Archaea have 2 phyla

- **Phylum Euryarchaeota** (“wide” “ancient”) – very diverse, largest group – Includes methanogens, halophiles, Thermoplasms (lack cell walls), thermophilic sulfate reducers.
- **Phylum Crenarchaeota** (“Spring” “ancient”) – originally isolated from hot springs. Thermophilic or thermoacidophilic.

Archaea – Morphology

- Rods and cocci are most common
- But also have some unusual shapes unique to only archaea

Archaea - Dominant Inhabitants Of “Extreme” Environments

- 1) **Thermophiles** = love the hot (acid)
 - Genome structure, enzymes and membranes help them tolerate high temps
 - Acid is tolerated by active transport of protons out of the cells (e.g. Pyrodictaceae 105°C optimum growth, survives 121°C / 1 h autoclaving).
- 2) **Halophiles** = love high salt conc.
 - Salt concentrations up to 5 M (saturation). Cells maintain high level of K⁺ (potassium) inside, to balance outside concentrations (osmotic stress)
 - Some have unusual shapes
- 3) **Psychrophiles** = cold loving
 - These are abundant: majority of earth's biosphere is cold.
 - Enzymes and membranes adapted to cold
- 4) **Piezophiles** (or barophiles) = tolerate or require high pressure
 - Ocean floors – hundreds of atmospheres of pressure
 - Proteins and membranes specifically adapted to high pressure

Astrobiology

- If we know more about extremophiles, we can better understand if organisms like archaea can live on environments of other planets

Archaea - Mesophiles Are Abundant

- **Mesophiles** = archaea that like the normal environments (our conditions)
- Archaea are as abundant as bacteria here: in oceans, mammals, and soils
- Play an important environmental role (e.g. in nitrogen cycle)

Features Of Archaea: 1. Non-Pathogenic

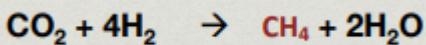
- There are no known archaeal pathogens of animals nor plants
 - Associated with mammals as commensal microorganisms
- There is one example of an Archaeal parasite = *Nanoarchaeum equitans* (parasitic of another archaea)

Features Of Archaea: 2. Different Cell Envelopes

- 1) No peptidoglycan cell walls like bacteria (= no gram positive or negative to identify)
 - Usually contain a protein S-layer instead (a single protein that crystallises to create layer)
- 2) Lipid membranes contain ether linked, branched, chains
 - Contrasts to Eukarya and bacteria which have ester linked, unbranched fatty acid chain

Features Of Archaea: 3. Methanogenesis

- Creation of methane is thought to be unique to some archaea; the "Methanogens". Don't just blame cows



- Carbon dioxide is fixed as a carbon source for biosynthesis. Hydrogen gas is used for energy.
- Methane is partly consumed before entering atmosphere by other microorganisms called methanotrophs.

Archaea and global warming

- E.g. If the permafrost disappears, water will bog the system = anaerobic = archaea can undergo this methane process. This means that there could be a lot of methane released from the melting of the permafrost (which is already melting)

Fracking (drilling to get methane)

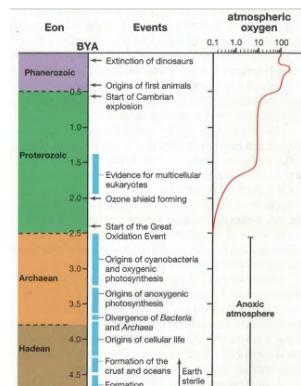
- Most of the natural gas we use is methane from archaea that is in the ground
 - Abiotic – decomposition of sedimentary rock
 - Biotic – methanogens (most)
- Trapped gas from decomposition of plant and dead animals.
- Concerns= Escaping methane – toxic & Groundwater contamination

MICROBIAL EVOLUTION

Diversity In The Microbial World

- How did we get all of this diversity?

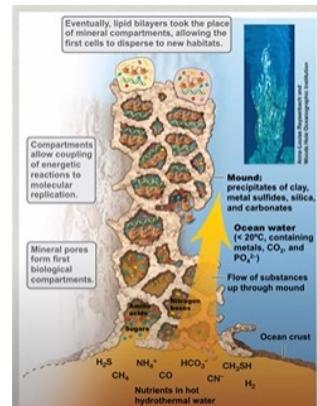
Early Earth



- First forms of cellular (prokaryotic) life = 4bya
- 3.8bya thought that bacteria and archaea diverged
- 3.5bya = origins of anoxygenic photosynthesis (use light as energy, but splitting hydrogen sulphur) = purple and green sulphur bacteria
- Origins of oxygenic photosynthesis = use light as energy, but splitting water molecules = oxidation event
- Allowed ozone layer to form = life on land (eukaryotes)
- Cambrian explosion...

Origin Of Cellular Life

- Origin unknown - submarine mound hypothesis
 - Early earth - hot environment. Energy source = inorganic like H_2S



Microbial Evolution And The Fossil Record

- First direct evidence of cellular life: Swartkoppie chert microbial fossils estimated at 3.4 billion years
- The Archaean Apex chert of Australia are dated to 3.5 billion years ago

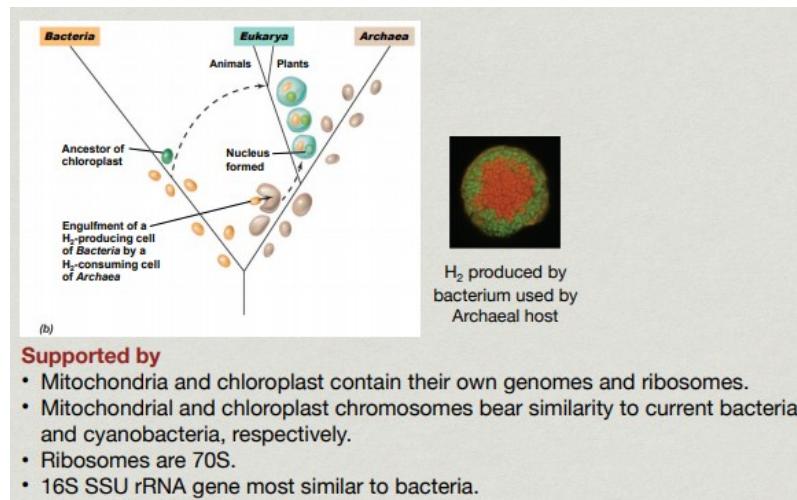
Photosynthesis And The Oxidation Of The Earth

- Cyanobacteria layered in stromatolites
- = Oxygen (splitting H_2O) releasing photosynthesis

The Great Oxidation Event

- Iron oxides precipitated out of the oceans (BIF's) Soluble Fe^{2+} oxidises to Fe^{3+} , which precipitated out
- Collection of O_2 in atmosphere came about after all Fe^{2+} oxidised out
- Formed ozone = protection layer

Endosymbiotic Origin Of Eukaryotes



- because of these similarities in eukaryotes that are found in both bacteria and archaea.

- It is believed that at some point they formed together to create eukaryotic cells

