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L1: The History of Microbiology

Microbial cells evolved 3.5 – 3.8 billion years ago. Cyanobacteria learnt how to release oxygen from water which oxygenated the environment which led to the development of plants and microbes.

Microorganisms are ubiquitous and the dominant life form on earth. They permeate the entire globe, particularly the bacteria. They are able to survive and grow over a wide range of conditions. Most microorganisms live in specific niches, with other microbes, as communities. You don't typically see pure cultures in nature. >99% of microbes are uncultured.

The existence of microorganisms wasn't confirmed until approx. 1625 when **Stelluti used a microscope supplied by Galileo to view them. Antony van Leeuwenhoek later developed a microscope that had a magnification of 50-300x.** 7

People used to believe that living organisms could develop from non-living matter, in a process called spontaneous generation. Louis Pasteur attempted to disprove this theory in 1861. He filtered air through cotton wool and then studied it to find that there were little plant spores that had been trapped. These grew when they were placed in a sterile medium. He hypothesised that air contained microbes capable of growth. He then conducted an experiment to prove this:

> In swan neck flasks, he heated some broth to the point of sterility. Some flasks he left with the neck intact and therefore airborne microbes are trapped at the base and the broth remained sterile. He broke the neck of others which allowed microbes to get in and grow and contaminate the broth. He had proven that the air contains microbes capable of growth.

People also used to think that disease was spread by poisonous vapours called miasmas. Koch disproved this by working with Anthrax, and eventually showed that Bacillus Anthracis caused the disease. Koch established a set of postulates to link a specific microorganism with a particular disease. Pasteur proved that disease was due to microorganisms, not miasmas. Koch showed that specific microorganisms were the cause of particular diseases.

Koch's Original Postulates:

1. The microorganism must be present in every case of the disease but absent from the healthy organisms
2. The suspected microorganisms must be isolated and grown in a pure culture.
3. The same disease must result when the isolated microorganisms is inoculated into a healthy host.
4. The same microorganisms must be isolated again from the diseased host.

These weren't always feasible:

- Some pathogens are part of the normal microbiota
- Some pathogens can't be grown/cultured
- Some pathogens grow/cause disease only in humans
- Sometimes more than one pathogen is involved in a disease eg polymicrobial diseases
- Doesn't consider the immune status of the individual

So, **Molecular Postulates** were formulated:

1. The VG must be found in pathogenic strains of microbes
2. VG must be expressed during infection/disease
3. Mutation (or deletion) of the VG decreases pathogenicity

4. Replacement/restoration of the VG mutation/deletion restores pathogenicity

A virulence gene is a gene that encodes characteristics that allow the microbe to cause disease.

L2: Bacterial Cells I

Taxonomy **is the study of biological classification.** It consists of:

- Classification (the arrangements into groups)
- Nomenclature (the assigning of names)
- Identification (determining if an organism belongs to a certain classification)

Taxonomy used to rely solely on the phenetic system **which was based off similarity of phenotype:** morphological, physiological etc. However, this relied on culture-dependent attributes and >99% of microbes have not been cultured.
Now, we use the phylogenetic system **which compares organisms on the basis of evolutionary relationships – their degree of relatedness.** A lack of good fossil record made this initially problematic.

Ribosomes have 2 components: small subunit (SSU) and large subunits (LSU). The SSU read the mRNA and the large join the AAs. The SSU contains small rRNA molecules. In prokaryotes this is called the 16S, in humans it is the 18S. The SSU rRNA are not subject to mutations and change slowly over millions of years.

Woese and Fox used these SSU rRNA gene sequences to measure relatedness. They found that there were 3 domains:

- Bacteria (16S)
- Archaea (16S)
- Eukarya (18S)

The hierarchy:

- Phylum
- Class
- Order
- Family
- Genus
- Species

A species is a collection of strains that share properties. A strain is the descendants of a single microorganism. A pure culture is cells that are identical because they arise form a single cell.

New methods allow us to enrich taxonomic data with genotypic data to allow us to compare genetic similarity. This is culture independent. Methods include genomic fingerprints and determination of the DNA sequence. They compare the G+C content and whole genome sequences.

Polyphasic taxonomy **is the amalgamation of phenetic, phylogenetic and genotypic information.**

We used to classify into prokaryotes or eukaryotes but biology isn't so black and white and significant overlap presented.

Archaea

- Share some features in common with bacteria:
    - Cell shapes (cocci and rods)
    - Size (1 – 10 um)
- They have different
    - Cell wall composition
        - Does not contain peptidoglycan
        - Made of glycoprotein or polysaccharide
        - L (not D) AAs used in cross links
    - Membrane lipids
        - Ether linked (not ester). Makes them stable to heat and pH extremes (extremophiles)
    - rRNA

Bacteria

- Have a plasma membrane
- No membrane bound organelles
- Cell wall contains peptidoglycan
- Possess 70S ribosomes
- Divide by binary fission
- Produce endospores and capsules (not always)
- 1-5 um
- Two main shapes
    - Cocci and rods
    - Some are pleomorphic
- Exist as single cells or in grouped arrangements as a result of cell division
    - Chains (streptococcus agalactiae)
    - Clusters (staphylococcus aureus)
- Cell envelope consists of PM, cell wall and optional outer layers

Bacteria – Plasma membrane

- 5-10nm thick
- Selectively permeable
- Location of metabolic process (photosynthesis, respiration, lipid synthesis)
- Can sense the environment (with receptor proteins)
- Phospholipid bilayer with amphipathic lipids
    - Saturated – all single C bonds in tail. Grow in high temps
    - Unsaturated – 1 or more double bonds between carbons. Grow in low temps.
- Composition varies between bacteria and eukaryotes
    - Bacterial PM lacks sterols
        - Have **hopanoids** to alter rigidity and fluidity

Bacteria – Cell wall

- Determines call shape and strength due to peptidoglycan
- Is the difference between gram positive and gram negative bacteria
- Protects against osmotic lysis and toxins
- Some components contribute to pathogenicity
    - Lipopolysaccharide (LPS)
        - Present in GN bacteria
- Site of action of several antibiotics

Bacteria – Peptidoglycan

- **Made of alternating strands of N-Acetylglucosamine (NAG) and N-Acetylmuramic acid (NAM).**
- Amino acids link the strands
- Found in helix formation with peptide side chains
- The amino acids alternate between D- and L- on the same chain.
- **DAP is involved in the cross linking between strands**
- Helices are joined by cross links in 2 ways
    - **Direct linkage**
        - There is a link between the terminal Ala and the DAP on different strands
    - **Peptide Interbridge**
        - A glycine bridge forms between the terminal Ala and another AA that isn't a terminal Ala.
- **There is more crosslinking in the GP PG than the GN PG. The GN is more porous than the GP.**

Gram Staining

You use Gram Staining **to find out if the sample you have if GN bacteria or GP bacteria.**

1. Heat fix your sample to your slide so it doesn't wash away
2. Apply crystal violet
3. Add iodine – these 2 then form an insoluble complex in the PG
4. Add decolourisation agent – alcohol or acetone
5. In GN, the CV-I gets washed out but GP are less porous so they remain violet.
6. Add counter stain safranin so you can observe the GN

If you look down your light scope and don't see anything, the following problems could have occurred:

1. Didn't heat fix the bacteria to the slide so they all washed away
2. Didn't do the iodine treatment (decolourisation washes away the crystal violet)
3. No cells in sample

Gram positive cell envelopes have thicker PG, no outer membrane and a smaller periplasmic space. Gram negative cell envelopes have thinner PG, an outer membrane and larger periplasmic space.

Teichoic acids are present in the GP cell envelope. They are polymers of alternating glycerol and phosphate groups. They confer a net negative charge on the cell wall and help stabilise the cell envelope.

LPS endotoxins are in the outer membrane of the GN cell envelope. They confer a net negative charge and help stabilise the OM. They create a permeability barrier to restrict the entry of bile salts and some antibiotics.

Gram positive – *Staphylococcus aureus*
Gram negative – *Escherichia coli*

Capsules are polysaccharide material that isn't easily washed off the cell. They aid in adhesion and biofilm production. They protect against dehydration, phagocytosis, bacteriophage attack and toxins. They can be visualised by negative staining (India ink) and staining the bacteria.

Slime is unorganised polysaccharides that is easily washed off and not easily visualised under a light scope. Slime is produced by gliding bacteria to facilitate motility.

S-Layers are proteins or glycoproteins that self-assemble and are found in GP and GN bacteria and the archaea. They protect the microorganisms and aid in cell shape and rigidity, adhesions and pathogenesis.

The **cytoskeleton** has actin filaments, microtubules and intermediate filaments. It is needed for cell division, cell shape and protein localisation within the cell.

Ribosomes are the site of protein synthesis. Those that are free in the cytoplasm produce proteins that remain in the cytoplasm. Those attached to the PM produce proteins for envelope or export. They are composed of protein and RNA molecules and are smaller than eukaryotic ribosomes.

Inclusions are aggregates of organic or inorganic material. Some are free floating in the cytoplasm and others are surrounded by a single layered membrane. They are mainly used for storage of nutrients and waste.

The **bacterial genome** is comprised of the nucleoid and plasmids. The nucleoid is the region of the cytoplasm that contains the singular, dsDNA chromosome. There may be one or more plasmids and they replicate individually of the chromosome and are dsDNA. They may have between 1 and 40 of the same plasmid.

Endospores are specialised structures resistant to heat, desiccation, radiation and disinfectants. They sporulate when growth ceases due to environmental stresses. The DNA in them are protected by small acid-soluble proteins and they are impermeable to most simple stains so you need to heat them to stain them.