BMS2052- Microbes in health and disease

Lecture 1

Facts:

1. There are 10 times more bacteria in the human digestive system than there are cells in the body
2. Gut bacteria contributes to 1kg of our entire mass (most in large intestine mixed in faeces)
3. Human bite is the worst bite you can contract
4. 1500 species in your belly0button
5. 80 million microbes are transferred through an intimate kiss
6. 1 micron – 7.5 m in length
7. Less than 10-5% of microbes cause disease
8. Candida:
    a. Occurs in vagina and kept in check by Lactobacillus
    b. Failure of Lactobacillus = vaginal candidiasis
9. Hepatitis G virus
    a. Only asymptomatic without an immune compromised system (HIV, organ transplants)
    b. Reduces chance of transmission of HIV from mother to baby by 59%
    c. Reduce mortality of HIV and Ebola patients
10. Pseudomonas aeruginosa: can affect immune compromised individuals or babies
11. TB: top infectious disease killer in the world
12. VRE: Staphylococcus aureus causes major/lethal disease in Australian hospitals
13. Pseudomonas aeruginosa: most humans are colonised but not infected:
    a. It becomes a problem in babies and burns victims
14. Leishmaniasis: spread by sand-flies to and from live individuals causing a flesh eating disease

Virus, bacteria, parasite (NOT ~~bug~~)

Malaria is not caused by Plasmodium virus. It cannot be transmitted through oral sex

Influenza/pneumonia virus

Salmonella bacteria

Genus species OR *H. pylori* OR H. pylori

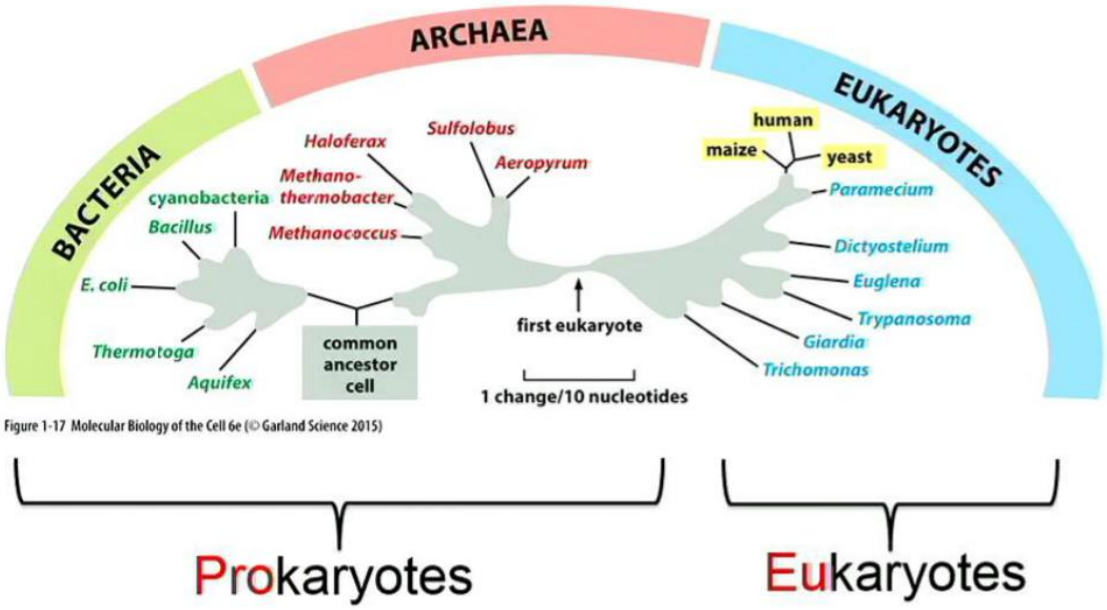
Lecture 2

BAVPE: Bacteria, Archaea, Viruses, Prions, Eukarya (plants/animals/fungi/protists)

Size matters:

- Tools required to identify protists (small) from large bacteria and amoeba are much more expensive, time consuming and difficult to interpret
- Worms do not need a microscope to identify
- Diagnosis in developing countries is difficult when dealing with small micro-organisms due to expensive and complications of the technology required

Complication: left→right
Treatment and diagnosis become more complicated as the organisms become more complicated and more 'human-like'



<u>Bacteria and Archaea</u>

Similarities:

- Anucleate
- Generally, have single chromosome
- Cell envelope
- Vary in shape and size

Differences:

- Difference rRNA sequences
    1. Ribosomal subunit sequences are used to classify archaea as different to bacteria
- Bacteria have plasma membrane containing peptidoglycan
- Unlike bacteria, archaea have no known bone fide pathogens (real) of the human
    1. Possible exception: Methanosphaera oralis: mouth

<u>Archaea and Eukarya</u>

Similarities:

- DNA replication/translation/transcription
- Has histones
- Many enzymes involved in DNA replication

Differences:

- Eukarya contain unique membrane lipids
- Archaea completely lack peptidoglycans (S-layer)

<u>Archaea</u>

- Methanobrevibacter smithii: gut and vagina
- Methanosphaera stadtmanae: gut
- Methanosphaera oralis: mouth

Lecture 3

Bacteria

Cell Wall:

- Complex
- Polymeric
- Shape/support
- Virulence and pathogenicity
- Protection though porous
- Action site form some antibiotics
- Proteoglycan: peptidoglycan
    1. Glycopeptide (sugar/protein)
    2. Unique to bacteria
    3. Structure
    4. Staining

Glycan: two sugars

- Alternating derivatives of glucose covalently linked
- N-acetyl glucosamine (NAG)
- N-acetylmuramic acid (NAM)

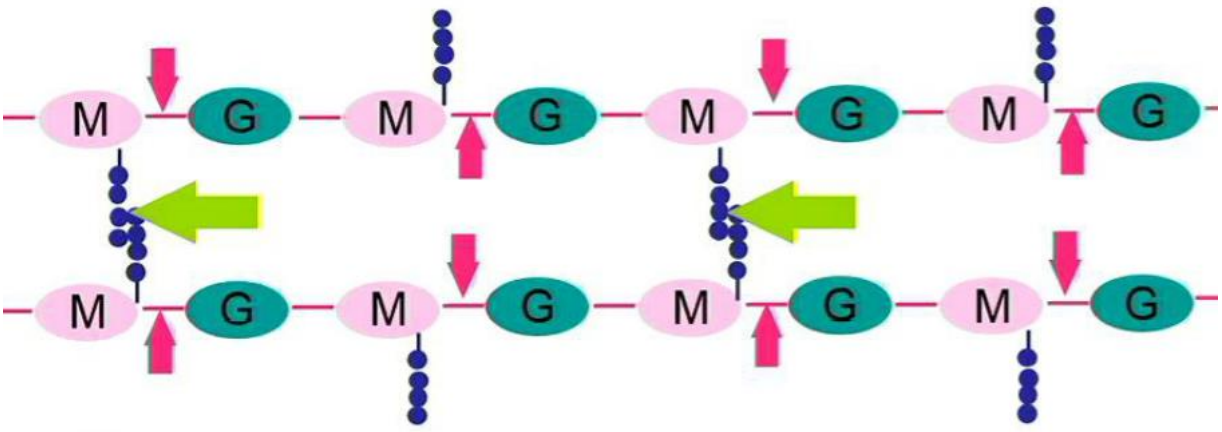
Peptido:

- L-Alanine
- D-Glutamic acid
- Diaminopomelic acid
- D-Alanine
- Alternating
- D forms are rare in AAs (D-glu, D-Ala and DAP are not found in other proteins)
- Gram negative (pink): cross link D-Ala and DAP
    1. E. coli
    2. Thinner and less complex
    3. Complex outer membrane
        - Most common protein: Braun's lipoprotein
        - Binds to peptidoglycan and links outer membrane to cell
        - Lipopolysaccharide (LPS):
        - On the outer membrane edge and very complex
        - Surface for antigen and receptor binding = critical for Gm- bacteria
        - 3 components:
            - Glycan polymer (hangs off)
            - Core polysaccharide
            - Lipid A (anchor)
        - Good:
            - Contributes to structural integrity
            - Forms permeable barrier
            - Increases negative charge = stabilised membrane
            - Essential (therapeutic target: polymyxins are highly toxic antibiotics which are coming back into the market due to increased antibiotic resistance)
            - Highly immunogenic: rapid antigenic variation by glycan O antigen = used to evade host (molecular mimicry) e.g. E. coli 157
        - Bad:
            - Lipid A

        - Endotoxin shock → leads to septic shock
        - Fatal
        - Stimulates pro-inflammatory cytokines, nitric oxide and eicosanoids (active arachidonic acid)
    4. Gram Staining:
        - Crystal violet is first added, followed by Grams iodine
        - Grams iodine molecules are larger than the crystal violet and are insoluble in water
        - Ethanol decolourises and dehydrates the peptidoglycan layer, causing it to shrink
        - The outer membrane is degraded and the crystal violet colour is washed away.
        - Fuchsin is lighter than crystal violet and stains the cell
    5. No teichoic acids
    6. Thinner/complex
    7. Less ridged/more flexible
- Gram positive (purple): cross link D-Ala and L-Lys
    1. Has longer crosslink chains therefore greater amount of space between layers hence denser peptidoglycan layer
    2. S. aureus
    3. Lacking outer membrane
    4. Thicker/simple
    5. Staining:
        - Crystal violet is first added, followed by Grams iodine
        - Grams iodine molecules are larger than the crystal violet and are insoluble in water
        - Ethanol decolourises and dehydrates the peptidoglycan layer, causing it to shrink
        - As the peptidoglycan layer shrinks, it traps the crystal violet molecules, retaining colour
        - The peptidoglycan layer is thicker and also has iodine retaining molecules which increase the affinity for crystal violet binding
        - Because fuchsin is lighter than crystal violet, it does not disrupt the purple colour
    6. Teichoic Acids: phosphate bonded polymers of ribitol or glycerol
        - Teichoic acid: covalently linked to peptidoglycan
        - Lipoteichoic acid: covalently linked to plasma membrane
        - Negatively charged, adding to cell wall negative charge
            - Makes it stable
            - Site for antibiotic attack

Peptidoglycan Layer:

- Site of action of lysosomes and penicillins
- Part of regulation of bacterial control by the immune system
- Transpeptidase emitted by penicillins acts on the transpeptide bonds and break them to destroy the bacteria
- Lysosomes hydrolyse polysaccharide bonds



A-typical Bacteria

- Without peptidoglycan layer

Chlamydia:

- Maintain relatively normal morphology
- Fragile (some rigidity)
- Require the host to live due to fragility
- Global major cause of STIs
- Causes:
    1. Trachoma: C. trachomatis (eyes)
    2. Psittacosis: C. psittaci (lungs)
    3. Intracellular parasites:
        - Rickettsia
        - Mycoplasma

Planctomycetes

Mycoplasmataceae

- Pleomorphic (polymorphic)
- Insensitive to penicillin and β-lactam antibiotics
- Take cholesterol form host to obtain sterols
- Mycoplasma
- Ureaplasma
- Respiratory/UG disease and humans and animals
- Can be sexually transmitted
    1. U. urealycticum
    2. M. genitalium
    3. M. hominis
- M. pneumoniae
- Very small genomes

NOT L-FORMS

- Also without cell wall
- Only ever in vitro
- Most bacteria can produce L-forms
- Cannot survive and infect humans outside this lab environment

Acid fast organisms:

- Mycobacteria:
    1. TB
    2. Leprosy
    3. Mycolic acid, lipoarabinomannan on top of the peptidoglycan layer
        - Thick and fatty
        - Form impermeable membranes
        - Resistant to desiccation: extreme dryness
        - Makes these bacteria very hard to kill
        - Some TB strains are resistant now to all antibiotics
- Nocardia spp:
    1. Pneumonia
- Hydrophobic
- Acid-fast staining:

1. Carbol fuchsin
2. Ziehl-Neelsen
3. Kinyoun's modified Z-N

Lecture 4

1. Understand the differences between bacteria that cause disease and those that don't – (commensal vs pathogenic)
2. Understand how to determine whether a microorganism is able to cause disease
3. Understand the main types of bacterial **virulence factors** and how they contribute to pathogenesis

- How do microbes cause disease?
- How is the disease transmitted?
- How does this inform prevention and treatment?

ONE

Normal: indigenous flora (mostly bacteria)

- Opportunistic pathogens
- Exclusively pathogenic
    1. E.g. M. tuberculosis
- Can have some species that pathogenic and non-pathogenic strains
    1. E.g. E. coli
    2. Shigella spp (1 million deaths per year)- same E. coli strain

Niches for gut colonisation:

- Gut
- Eye
- Outer ear
- Skin
- Respiratory tract
- Female (vagina) and male (urogenital) genital tracts

Presence of microbes out of these area constitutes a disease

Normal flora:

- Help protect us:
    1. Compete with invaders for space and nutrients
    2. Kill other bacteria
        - E.g. lactobacillus lowers pH s other bacteria cannot grow)
    3. Stimulate gut flora which can regulate weight
- As you move deeper into the body, more and more normal flora reside

TWO

Pathogen: organism able to cause disease

Pathogenicity: measure of the ability of an organism to cause disease

- Interaction between pathogen and host
- E.g. Vibrio cholera (causes potentially fatal diarrhoea (only infects humans)

Virulence factor: factors required by microbe to cause disease

- Salmonella typhimurium: mild reaction in humans but severe disease in mice
- Salmonella typhi: severe in humans, asymptomatic in mice

- Virulence factors:
    1. Capsules
    2. Helps colonise niches that they are not usually found in
    3. Help adhesion
    4. Anti-phagocytic and immune evasion response
    5. Tissue damage via toxins (mediate disease)

Koch's postulates

4 criteria to establish a causative relationship between a microbe and disease

1. Suspected organism must be present
2. Microbe must be isolated and grown in a pure culture
3. Disease should be reproduced when introduced to a healthy host
4. Microbe must be recoverable from an infected host
5. *Suggested* effective preventative/therapeutic techniques should eliminate disease

- No longer used now

Limitations:

1. No host factors taken into account
    a. Carrier states
    b. Opportunistic infections
    c. Host has protective measures
2. Not always possible to culture microbe (virus/prions)
3. Can lose virulence/pathogenicity in culture
4. May not require the organism to infect the host (e.g. toxins)- rare
5. Requires susceptible host

Molecular Koch's postulates (revised)

1. Virulence gene is always found in the strain causing disease
2. Gene should be expressed in host
3. Mutation of the virulence gene reduces virulence phenotype
4. Reintroduction of gene causes disease

Causing Disease

Pathogens must:

- Get into the body
    1. Innate immunity:
        - Non-specific
        - First line defence
        - Physicochemical elements:
            - High salt, fatty acids, dry (skin)
            - Acid (stomach, vagina)
            - Mucous & cilia (LRT), peristalsis (GIT), defecation/urination
            - Normal flora
            - Soluble mediators *e.g.* lysozyme (eye, nose, stomach)
        - Biochemical elements:
            - Cytokines: pro/anti-inflammatory
            - Compliment: cascade of proteins that aids clearance
        - Cellular elements:
            - Neutrophils
            - Eosinophils
            - Macrophages

        - Monocytes
        - Dendritic cells (phagocytes)
        - Activates acquired immune response
    2. Acquired immunity
        - T cells:
        - Recognise antigens presented by MHC's and macrophages
        - Activate B cells which produce antibodies and killer T cells
- Colonisation:
    1. Must be able to remain and multiply there
    2. Helicobacter pylori: produces urease which cleaves urea and converts it ammonia (highly basic). Shrouding itself in this cloud of ammonia, it protects itself from the highly acidic stomach.
    3. Involves adhesion via:
        - Pili/fimbriae
            - Rod shaped
            - Helical array
            - Peritrichous (all over cell) or clustered (polar)
            - Tip contains protein pilin and binds to COH residues on glycoproteins/glycoproteins
            - Loosely adhere and later on afimbrial adhesion strengthen bonds
        - Afimbrial adhesions:
            - Between fimbriae
            - E.g. Bordetella pertussis (whooping cough)
        - Capsule
            - Protein carbohydrate mix
            - Creates sticky surface (biofilms)
            - E.g. K. pneumoniae
            - Tooth decay
- Invasion:
    1. Intracellular pathogens with atypical shapes
    2. E.g. Chlamydia
    3. Mycoplasma
    4. Rickettsia
    5. Legionella
    6. Involves:
        - Escape washing out
        - Hide from immune response
        - Proliferate
        - Disseminate (spread) into deeper tissue