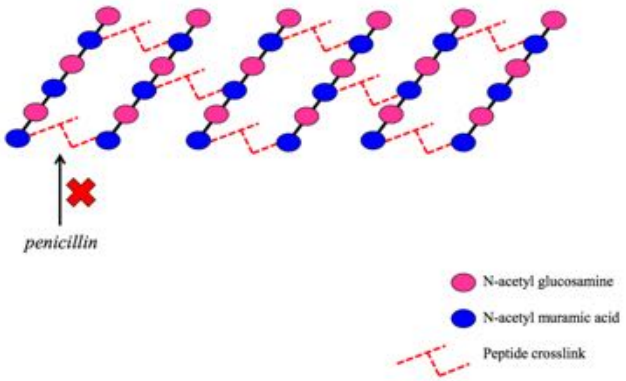
Introduction to Microbes and Host-pathogen Interactions

A microbe is a microscopic organism, which may be single-celled or multicellular. These include prokaryotes – organisms that lack a cell nucleus and other membrane bound organelles, which are almost always unicellular and eukaryotes – organisms which has a cell nucleus and other membrane bound organelles.

There are three major differences between prokaryotic and eukaryotic cells:

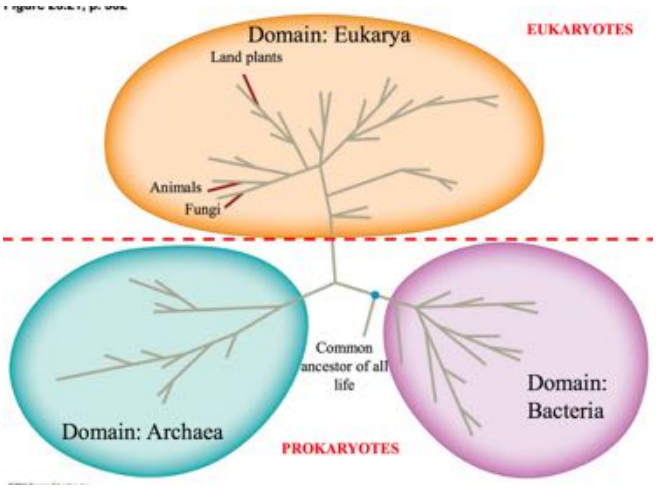
- *Structure of nucleus*: prokaryotic nuclear material is not surrounded by a membrane whilst eukaryotic nuclear materials are
- *Intracellular organelles*: eukaryotes consist of membrane bound cellular organelles whilst prokaryotes do not
- *Cell wall structure*: there are many unique compounds in bacterial cell walls, such as peptidoglycan, lipopolysaccharides and D-amino acids

These structural and biochemical differences between prokaryotic and eukaryotic cells are of critical importance in design of antimicrobial agents of antibiotics as it ensures selective toxicity to these antibiotics. For example, *penicillins* inhibit the synthesis of peptidoglycan, an essential structural component of bacterial cell wall. As it is not found in human cells, it is a good target for selective toxicity.



There are three domains of life: *eukarya, bacteria* and *archaea*.

- *Eukarya* includes all eukaryotic organisms, and thus contains four kingdoms of organisms
    - *Animalia*: multicellular animals
    - *Plantae*: multicellular plants
    - *Fungi*: multicellular fungi and unicellular yeast
    - *Protsita*: unicellular algae and protozoa
- *Bacteria* is a domain which is prokaryotic
- *Archaea* is a diverse prokaryotic group or organism that often live under extreme environmental conditions, such as high salt/temperature, hot springs in New Zealand



Archaea

It is a domain of single-celled microorganisms that are simple in form and only grow in oxygen-free environments. They are thermophiles and live in extreme weather conditions. Archaea and bacteria are generally similar in size and shape. They also have similar hereditary mechanisms, have a cell membrane composed of lipids, a cell wall and has a metabolism based on ATP. However, they do not consist of a peptidoglycan in their cell wall, have unusual lipids in cell membrane and has gene and ribosome structure similar to that of eukaryotes They include organisms that produce methane from CO_2 and H_2, such as methanogens which obtain energy from this process and are also a source of major proportion of our natural gas reserves. Additionally, they also include organisms that can (1) survive and grow in hot, acidic environments *(thermoacidophiles)*, such as hot springs and deep hydrothermal vents, often using H_2S as an energy source and (2) tolerate high salt concentrations *(halophiles)*, such as the Dead Sea.

Similarities with Bacteria and Eukarya

- Common features with bacteria
    - Anucleate – no defined, membrane-bound nucleus
    - Cell envelope
    - Generally possess single circular chromosome
    - Vary shape and size
- Common features with eukarya
    - DNA replication, transcription and translation is more similar to eukarya than to bacteria
    - Archea DNA has histones (modern drug targets) – DNA binding proteins considered to be a defining characteristic of eukarya
    - Many enzymes involved in DNA replication (DNA polymerase, primase etc. are more similar to those found in the eukarya than to bacteria)

Differences with Bacteria and Eukarya

- Distinctive rRNA sequences which are resistant to heat, oxidating agents and are very stable
- Plasma membrane different to bacteria and eukarya and contain unique membrane lipids
- Archaea lack peptidoglycan: key component of the cell wall of bacteria
    - Outer layer of the cell wall – S layer (structured or surface layer) – interacts directly with the plasma membrane, forming a complex lattice

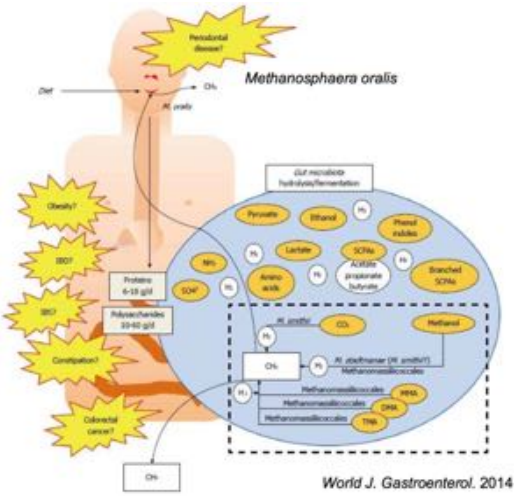
Unlike bacteria, there are virtually no known bonafide pathogens of humans. The diversity of the Archaea in the human body is also substantially lower, including representatives of only one phylum (*Euryarchaeota*)

- *Methanobrevibacter smithii* – gut and vagina
- *Methanosphaera stadtmanae* - gut
- *Methanosphaera oralis* – mouth

Methanosphaera oralis

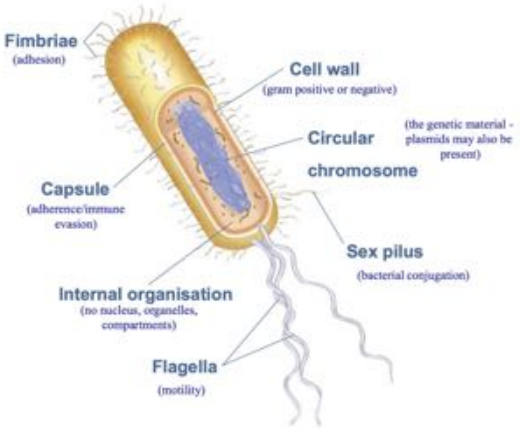
M. oralis is a major archaeal player in the oral cavity. Its proportions increase with the severity of oral diseases/infections, such as periodontal diseases. Apical periodontitis is the result of the infection of a tooth's root canal which can be invaded by oral microflora, such as *M. oralis*.

Methanosphaera stadtmanae and *Methanobravibacter smithii*



M. smitihii is the major archaeal component in the human gut system whilst *M. stadtmanae* is a less frequently detected species. They both are methanogens, microorganisms that produce methane as a metabolic by-product in anoxic conditions, and thus, disorders involved include inflammatory bowel disease, irritable bowel syndrome and colorectal cancer. In contrast, they can also aid in decreasing cardiovascular disease. Trimethylamine (TMA) is oxidised to TMAO to promote atherosclerosis, a disease in which plaque builds up inside the arteries, narrowing and hardening it. Archaea uses TMA as a carbon source, thus reducing the production of TMAO and, consequently, cardiovascular risks. They also aid in preventing Trimethylaminuria (TMAU), fish odour syndrome, a rare metabolic disorder that causes a defect in the normal production of Flavin-containing monooxygenase 3 (FMO3), resulting in fishy sweat, breath and urine. Flavin monooxygenase is responsible for cleaving out TMA in the liver, however, when a defect occurs in this enzyme, TMA is unable to be cleaved out of the liver, resulting in TMAU. Similar, archaea uses TMA, thus reducing the amount of TMA in the liver and preventing TMAU.

Bacteria



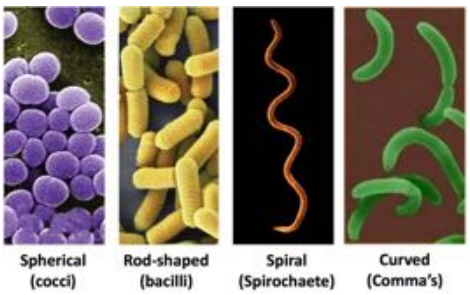
Bacteria is a large group belonging to prokaryotic microorganisms. They are free-living and can vary in size, from 0.1μm to 50μm in diameter. Due to the small size, and the lack of contrast between cells and background, it makes it difficult to observe. Therefore, it is helpful to (1) use high powered 100x oil immersion lens coupled with 10x eyepiece which gives 1000x magnification and (2) stain cells with colored dye for a greater contrast between the cell and the background.

There are important differences between bacteria and eukaryotes and are often compared to what the prokaryotes lack in:

- Membrane bound nucleus
- Internal membranous structures (ER, golgi)
- A cytoskeleton (actin, spectrin, protein)

However, some bacteria do have their DNA enclosed in a membrane (*Planctomycetes*), some do have membrane-bound organelles and extensive intracytoplasmic membrane structures and some do have cytoskeletal elements. Nevertheless, they will continue to be differences between prokaryotes and eukaryotes as a general statement.

Bacterial morphology



- Cocci (spherical)
  - *Staphylococcus aureus* is a common bacterium that lives on the skin or in the nose, thus is considered normal flora. An infection of this, however, is difficult to treat in humans.
- Bacilli (rod-shaped)
  - *Clostridium difficile*
- Spirochaete (spiral)
  - *Treponema pallidum* is the cause of food-borne infections, such as food poisoning
  - *Leptospira spp.*
  - *Camplyobacter jejuni*
- Commas's (curved)
  - *Vibrio cholera*

Some bacteria are a mixture of the two, such as:

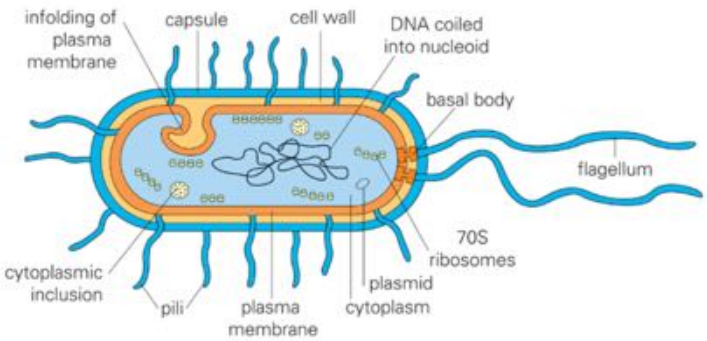
- *Cocco-bacilli* is a type of bacterium with a shape intermediate between cocci and bacilli. It is short, round and rod-shaped.
- *Yersinia pestis* is a rod shaped coccobacillus, and a facultative anaerobic organism that can infect humans, especially indigenous Australians.
- *Acinetobacter baumannii* is a short, round, rod shaped bacterium that is poorly understood. It can be an opportunistic pathogen in humans, affecting people with compromised immune systems.

Pleomorphic bacteria are able to alter their shape or size in response to environmental conditions.

- *Mycoplasma* and *Ureaplasma*
    - They are bacteria commonly found in the reproductive tract of both men and women
    - They are the smallest species of bacterial cells
    - No rigid cell walls around the cell membrane, therefore, they are difficult to get off
- *Rickettsia*
    - A diverse group of bacteria, some of which can be transmitted to humans via the bites of fleas, lice, ticks or mice
    - Present as cocci, rods or thread-like
    - Phylogenetically between bacteria and viruses and are obligate intracellular parasites, therefore, they must live inside the cell
    - Cause important vector borne (ticks/fleas) diseases, such as typhus, scrub typhus and rocky mountain spotted fever

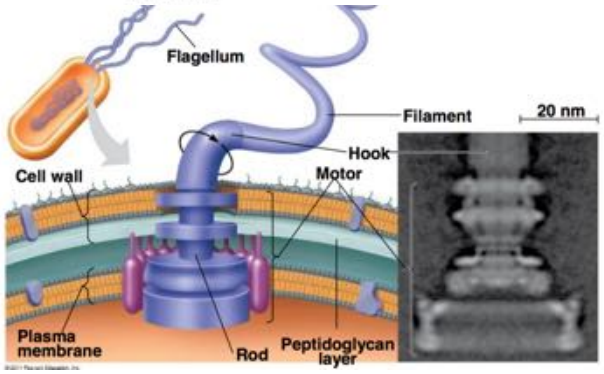
Structural Features

Bacteria have many specialised structures that are important for survival, interaction with host, virulence and pathogenesis.



- *Flagella*
    - Thin, invisible under light microscopy unless special stains used
    - Peritrichoous or polar (orientation)

    - Motility – rotates like a propeller
    - Not all bacteria have flagella, and therefore, not all are motile



The arrangement of flagella can have one, or multiple, depending on the number of flagella and arrangement are terms as:

- Monotrichous – bacteria with a single flagellum
- Amphitrichous – bacteria with a flagellum at both ends
- Peritrichous – flagella that cover the entire surface of the bacterium
- Polar – flagella only at one end or another

- *Pili or fimbriae*
    - Shorter than flagella and more numerous, exposed on the cell surface
    - Composed of protein subunits (pilin)
    - Not involved in motility, but rather important in pathogenesis – mechanism which causes diseases

- Main function is in adhesion – allows bacteria to adhere to mammalian cells
  - Pili allow adherence to epithelial cells of intestine
  - *e.g. Escherichia coli – traveller's diarrhoea*

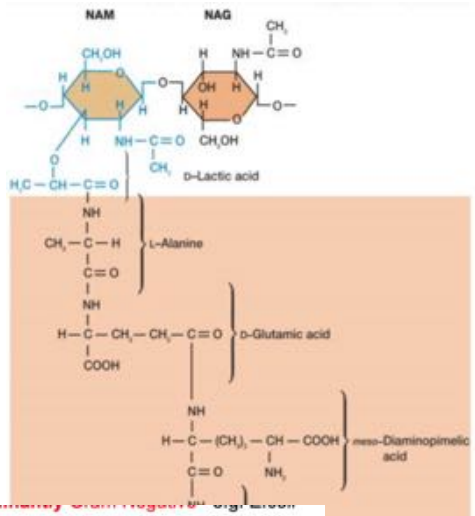
- *Capsules/S-layer (glycocalyx)*
  - Many bacteria secrete a slimy layer onto their cell surface
    - Capsule, slime layer, glycocalyx
    - Usually polysaccharide complexes
    - Different composition in different bacteria
    - Also important in pathogenesis
      - Adhesion of bacteria
        - *e.g. Streptococcus mutans – dental plaque*
      - Prevention of phagocytosis by host cells (a defence mechanism against immune response which makes it difficult for macrophage to undergo phagocytosis)
        - *e.g. Streptococcus pneumonoiae – pneumonia*
    - Protection against desiccation, prevents bacteriophages from attachment and infection, protects against phagocytosis
  - Forms 'biofilms' – complexes of microbial communities embedded in a capsular material mass
  - 'Biofilm' formation is an important virulence factor
    - colonisation of indwelling devices
    - assists oral bacteria to colonise teeth resulting in decay

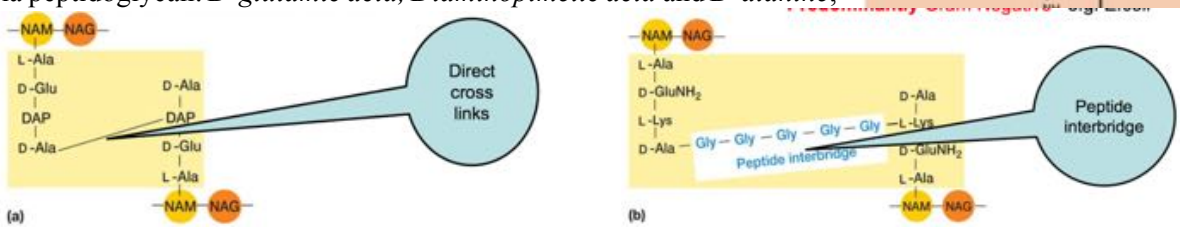
- *Outer membrane (-ve bacteria)*
  - Complex outside later of the cell wall, important in pathogenesis

- *Cell wall*
  - Outside the plasma membrane that encloses the bacterial cytoplasm
  - Rigid, determines shape

- *Peptidoglycan*

Peptidoglycan is a polymer of amino acids and sugars that makes up the cell wall of all bacteria. As humans do not have the enzymes to break down peptidoglycan, this gives bacteria a survival advantage. The 'glycan' is composed of two alternating sugars: *N-acetyl glucosamine* (NAG) and *N-acetylmuramic acid* (NAM), both being derivatives of glucose. NAG and NAM are covalently linked in long chains, alternating with one another. On the other hand, 'peptido' is composed of four amino acids – tetrapeptides – containing both D- and L-amino acids, also in an alternating pattern. It consists of three amino acids that are not found in other proteins and are only found in bacteria peptidoglycan: *D-glutamic acid, Diaminopimelic acid* and *D-alanine*,



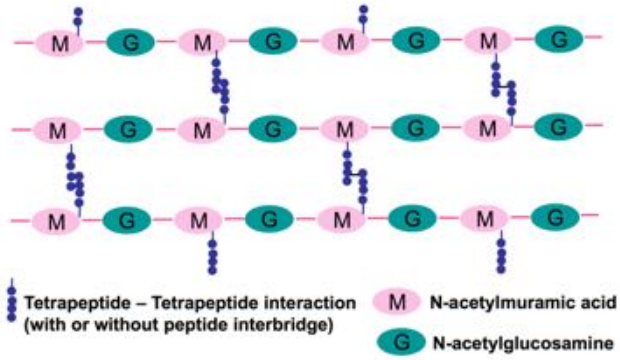


respectively. The terminal D-alanine cross links with another NAM either directly (+ve gram) or indirectly (-ve gram). In regards to linking indirectly, the peptide interbridge spaces the peptidoglycan by 5 peptide residues.

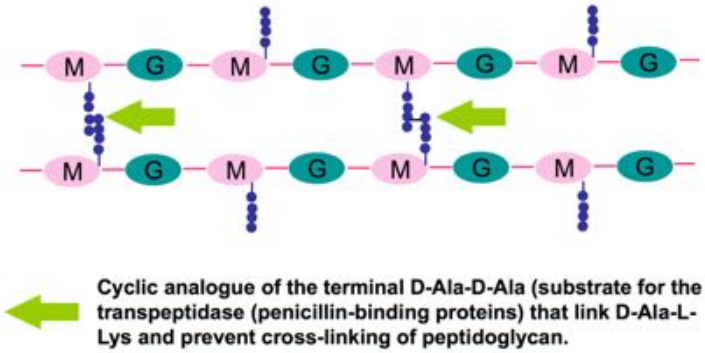
There are also variations to cross-linking polymers.

- Mycobacteria such as *M. tuberculosis* links L-Lys and L-Lys directly with no bridge, even with the lack of terminal D-alanine
- There can be different amino acids or different numbers of amino acids in the bridge, such as for *S. pneumoniae* links
    - D-Ala – D-Ala directly
    - D-Ala-Ser-D-Ala

Tertiary structure of peptidoglycan consists of NAG and NAM linked with a tetrapeptide interaction.



It can be the site of action of penicillin and other beta-lactam antibiotics.



Bacterial Cell Walls

The differences in peptidoglycan form the basis of the Gram strain, differentiating them into two large groups (Gram positive and Gram negative), depending on their ability to retain crystal violet/iodine complex after alcohol wash.