Medical Microbiology - A Brief Introduction

This is intended to introduce you to the nature of microbes and to describe the general properties of different microbial groups amongst the topics covered are:

Introduction
Viruses
Microfungi
Protozoa
Bacteria

Introduction

Medical Microbiology is the study of the causes and management of infectious diseases. These can be caused by viruses, bacteria, microfungi and protozoa. Medical Microbiology may overlap with parasitology, generally considered to be the study of diseases caused by multicellular parasites, where a parasite is defined as an organism that derives its nutrients from another living organism, often but not always to the detriment of its host.

So, why do Medical students need to know about microbiology? We are only 10% human. It has been estimated that there are about 10^{14} cells in the human body. Of these only 10% are of human origin. The remainder are the microbes that comprise our **commensal flora**. These are the microbes that live in and on our various body surfaces. We provide these organisms with food and shelter. In return, the commensal flora can play an important role in preventing infection. The word "commensal" means to share a table. The vast majority of commensal microbes are **bacteria** but we also harbour **fungi** and **protozoa. Viruses** are also found causing latent infections. These are infections where no symptoms are apparent.

Most of the microbes that live on or in humans do no harm. Indeed, they may be positively beneficial. The relationship is, however, finely balanced. Microbes are continually probing our defences and commensals that get into the "wrong" place can do untold damage. **Peritonitis,** for example is the life-threatening infection that results when gut microbes gain access to the peritoneal cavity, for example following a ruptured appendix. **Urinary tract infections** are most frequently caused when gut organisms or the skin flora gain access to the bladder. This is why, considering comparative anatomy, women are much more likely to suffer cystitis than men, for example. Some commensals can cause disease because of our life-styles. **Dental decay** in the Developed world is the result of metabolism of sugars in our diet by our oral flora to produce acid that subsequently etches our teeth. This initiates dental caries.

A very small minority of microbes are **primary pathogens**. These are capable of infecting individuals and causing disease. Because infections are very common and may be life-threatening, it is easy to get the wrong perspective on microbes. Without microbes, life on Earth would not exist. They are responsible, for example for **nutrient cycling**. Certain bacteria, for example, are the only organisms that can **fix atmospheric nitrogen** and make it available for other life forms, all of which depend upon a steady supply of fixed nitrogen to survive. Medical students, like other health professionals are, however, more likely to be interested in the infections that microbes cause. Before we can understand the nature of infection, we must learn about the nature of microbes.

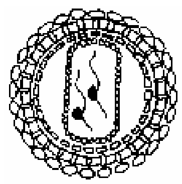
Viruses

It is debatable whether viruses are truly living organisms. They entirely rely upon other cells for their replication. Viruses are obligate intracellular parasites: an obligate intracellular parasite requires to live within a cell in its host.

It is not just humans that are affected by viruses. Every class of organism suffers from virus infection, even bacteria. Viruses are the simplest of microbiological entities. They comprise a nucleic acid core wrapped in a protein coat. Some viruses have an envelope, made of lipid and usually derived from the cell in which they grow. Other viruses are naked and just have their protein coat exposed, protecting the nucleic acid within the centre of the structure.

Specific viruses attack specific types of cells. Respiratory Syncytial Virus infects only the cells of the respiratory tract, for example. There is a special class of virus that attacks bacteria. These are the bacteriophage. Most viruses are simple in shape: round, rod-shaped, icosahedral, brick-shaped or bullet-shaped.

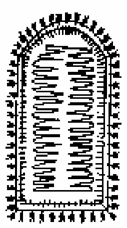
Viruses have a nucleic acid core, either DNA or RNA but not both. Retroviruses are unusual in that the virion carries an RNA copy of the genome but upon infection of a host cell a cDNA copy of the virus RNA genome is made using the enzyme reverse transcriptase. A retrovirus has a genome comprising RNA in the virus particle. From this, the cDNA copy is made following infection of a new cell. The cDNA copy then integrates into its host cell genome. Retroviruses include HIV, the human immunodeficiency virus, associated with AIDS. The term 'virion' is another name for a virus particle.



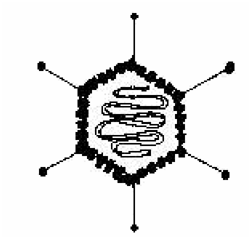
Human immunodeficiency virus - the cause of AIDS

Around the nucleic acid core lies a protein coat, made up of units called capsomeres. Some viruses also have an envelope derived from the host cell membranes. The envelope may be either baggy or tight, depending upon the nature of the virus.

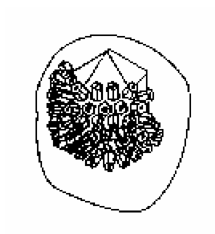
Despite their simple structure, viruses can show an astonishing variety of shapes. Some of the most complex viruses are those that infect bacteria. Some of these bacteriophage (bacteria eaters) look like lunar landing modules. Most viruses have a very narrow host range and can often only affect particular tissues within an infected individual.



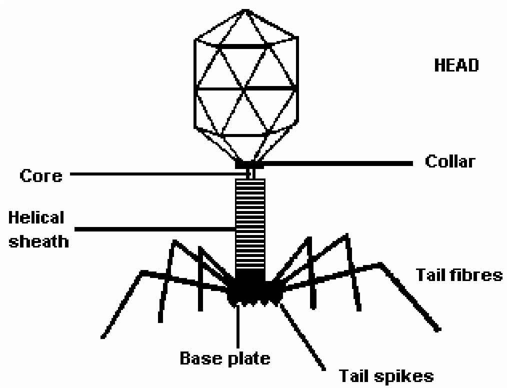
Rabies virus



Adenovirus - associated with respiratory and gastrointestinal disease



Herpes simplex virus (note the lipid envelope)



A T-even bacteriophage that can infect*Escherichia coli*

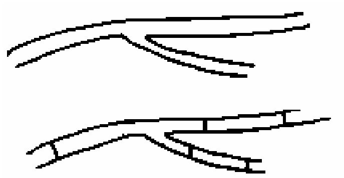
Plants can suffer infection from viroids: naked, infectious RNA molecules that are not associated with any proteins. At one time it was suggested that the causative agent of Creutzfeldt-Jakob disease was a viroid. Now the prevailing opinion is that this disease and other spongiform encephalopathies such as Scrapie in sheep and BSE (mad cow disease) in cattle are caused by infectious proteins known as prions. No animals are known to be affected by viroid infection.

Microfungi

All fungi are eukaryotic, where a eukaryote is defined as an organism in which cells have a membrane-bound nucleus and sub-cellular organelles such as mitochondria, golgi complexes, *etc*. Most possess a cell wall made of chitin: a polymer of N-acetyl glucosamine that is found in the cell walls of the majority of fungi. It is also a major component of the exoskeleton of arthropods such as insects.

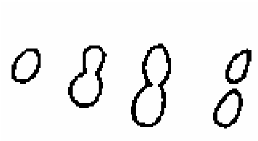
Fungi are heterotrophic. Heterotrophs require complex organic chemicals in order to grow. Autotrophs can grow using simple, inorganic chemicals. Phototrophs use and require light as a source of energy and chemotrophs require a supply of chemical energy, but can grow in the dark. Humans and many microbes are heterotrophs and chemotrophs whilst plants are autotrophs and phototrophs. Saprophytic fungi grow on dead organic matter. Saprophytes are of great importance in recycling organic matter. Parasitic fungi grow on living tissues.

Fungi are probably the most familiar of microbes encountered by non-microbiologists. Which student has not come across the dreaded black mould in the bathroom of the student flat? This is most likely to be a fungus of the genus *Cladosporium*. Who has not seen *Penicillium* spp. growing on stale bread? If you are really lucky, you will have kept pet furry fungi in the forgotten, half-eaten jam-jar at the back of your larder cupboard. These are all moulds: fungi that grow in mats of tiny filaments known as hyphae (singular: hypha, Greek for a thread) or mycelia (singular: mycelium, from the Greek mukes, meaning a mushroom). These may or may not be subdivided into separate compartments by cross walls known as septa (singular: septum).



Aseptate and septate mycelia

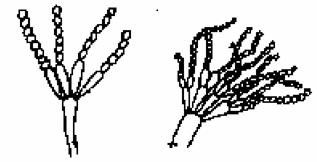
Moulds are multicellular organisms and are in some degree related to the mushrooms and toadstools. There are, however, unicellular fungi: the **yeasts.** Most familiar of the yeasts is *Saccharomyces cerevisiae*. Depending upon your viewpoint, this is baker's yeast, used in the production of leavened bread, or brewer's yeast, used in alcohol production. At one time the yeast used for brewing lager was thought to be of a different species, *Saccharomyces carlsbergensis*, but now it has been re-classified in the species *Saccharomyces cerevisiae*. Yeasts grow and multiply by budding daughter cells off from a mother cell.



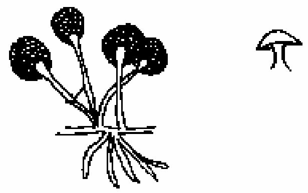
Yeast cells

Fungi also produce a staggering variety of spores - resting cells capable of surviving harsh conditions. These may be produced asexually or sexually and are important in the identification of moulds.

Examples of asexual spore structures produced by fungi



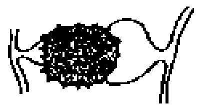
The umbrella structure, shown below, is the spore-bearing structure once it has discharged its load:



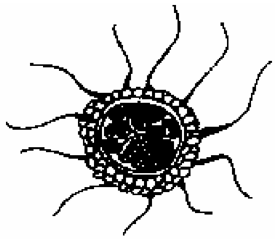
Asexual spores can be enclosed within specialised sacs:

Examples of sexual spore structures produced by fungi

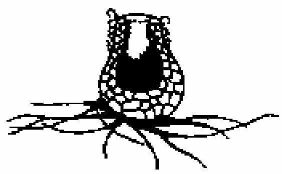
The warty zygospore is suspended between two mycelia of different mating types:



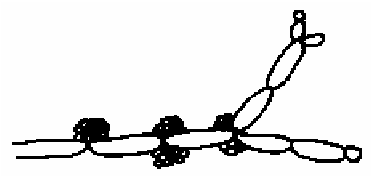
Spores within an enclosed structure - a **cleistothecium**:



Spores contained inside a more open structure - a **perithecium**:



Moulds cause a variety of common, superficial infections such as ringworm and athlete's foot. In compromised individuals they can cause much more severe infections but these are rare. The most common yeast infection is "thrush" caused by *Candida albicans*. Under certain conditions, some yeasts have the ability to develop pseudomycelia. This happens, for example, when the commensal form of *Candida albicans* causes active thrush.



At one time it was thought that fungi were plants that did not need photosynthesis. They lacked chloroplasts but they did have cell walls and they were sedentary organisms. We no longer use this as a basis of our classification. The cell walls of plants are made of **cellulose** whereas the walls of fungal cells are made of **chitin** and other polymers. Chitin is also the material found in insect shells. Plants require only simple inorganic compounds such as carbon dioxide and water to grow. Fungi require a diet of complex organic molecules to thrive. Because of these and other considerations fungi are now classified in their own kingdom, separate from plants and animals. There are two remaining kingdoms in our current classification system: the **protozoa,** comprising unicellular eukaryotes and the **monera,** comprising the bacteria.

Fungi can multiply either sexually or asexually. In classifying fungi, great weight is placed upon the appearance and structure of sexual fruiting bodies. Any fungus that does not exhibit a recognisable sexual structure is difficult to classify. They are grouped in the collection known as "**fungi imperfecti**". One of the most important of fungi imperfecti are members of the genus *Penicillium.* It is from these fungi that we derive penicillins. These were the first true antibiotics and are still among the most used antimicrobial agents world-wide.

Protozoa

Protozoa are unicellular eukaryotes. There are four classes of protozoa: sporozoa, flagellate protozoa, ciliate protozoa and amoebae. Sporozoa include the intracellular parasites of the genus *Plasmodium*. These cause malaria, an illness characterised by recurrent bouts of fever and sweating. It is spread by mosquitoes of the genus *Anopheles*. Flagellate protozoa are motile because they possess flagella (*singular*: flagellum). Ciliate protozoa are motile because the cell surface is covered in tiny hairsc called cilia. There are no ciliate protozoa known to cause human disease. Amoebae move by pushing out pseudopodia (false feet). Human pathogens include *Entamoeba histolytica* and *Naeglaria fowleri*. *Naeglaria fowleri* colonises natural hot water springs, and causes a rare form of meningitis. It is because of the presence of this amoeba that people are no longer allowed to swim in the Roman Baths at Bath. *Entamoeba histolytica* causes amoebic dysentery, a severe diarrhoeal disease.

Many protozoa are free living, but others cause serious infections. Protozoa can infect any human tissue, and are the cause of a variety of diseases. They include intracellular and extracellular parasites. They spread using a variety of strategies. Some produce cysts to survive outside the body, others are spread by insects, and yet others spread during human sexual contact.

Infections caused by protozoa include, for example, toxoplasmosis, amoebic meningitis, malaria, trypanosmiasis, leishmaniasis (Kala-Azar) and amoebic dysentery as well as diarrhoea caused by *Cryptosporidium* spp. or *Giardia intestinalis* (lamblia), the cause of a fatty, foul-smelling diarrhoea. Chronic, persistent diarrhoea caused by cryptosporidia is associated with the onset of AIDS. Vaginal infections may be caused by *Trichomonas vaginalis*. This protozoan causes a foul-smelling vaginal discharge. Men can be asymptomatic carriers, although this protozoan can cause balanitis - inflammation of the penis.

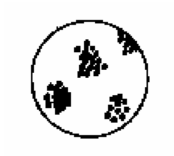
Pneumocystis carinii was for many years considered to be a protozoan because of its microscopic appearance and its behaviour, but molecular biological studies have shown this organism to be a fungus! It is important for patients suffering from AIDS, and causes a type of pneumonia.

Bacteria

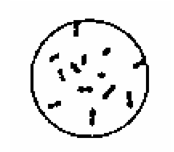
Bacteria are generally simple structures. The bacterial cell lacks a membrane-bound nucleus. Because of this, bacteria are described as prokaryotes. The term prokaryote is derived from two Greek words *pro*- and *καρψον* (karyon), meaning 'before the nucleus'. Bacteria are thus prokaryotes because they lack a clearly defined nucleus and sub-cellular organelles. Despite their simplicity, bacteria have an enormous range of metabolic capacities, and can be found in some of the most extreme environments on earth. Only a small minority of bacteria causes disease.

Bacterial shapes

There are three basic shapes that bacterial cells adopt. They are either round, rod shaped or spiral. Round bacteria are referred to as cocci (singular: coccus, a Latinised Greek word, κοκκοσ [kokkos], meaning 'a berry'), and rod shaped bacteria are known as bacilli (singular: bacillus - the Latin word for a stick). The term 'bacillus' meaning a rod-shaped bacterium should NOT be confused with the genus of bacteria known as 'Bacillus'. The shape of bacterial cells is of fundamental importance in the classification and identification of bacteria. Although bacteria are of three basic shapes, they display an astonishing variety of forms when viewed microscopically.

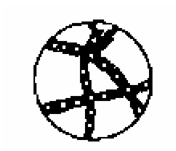


Staphylococci

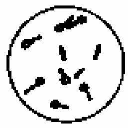


Diplococcal cells of
Streptococcus pnuemoniae

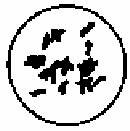
Streptococci



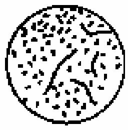
Sporing cells of *Bacillus anthracis*.
Note spores do not stain and do not cause the bacilli to swell



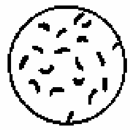
Sporing cells of *Clostridium tetani*.
Note spores do not stain and in this case they DO cause the bacilli to swell



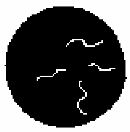
Irregular cells of *Corynebacterium diphtheriae*



Various shaped cells of *Haemophilus influenzae*



Curved rods of *Vibrio cholerae*.

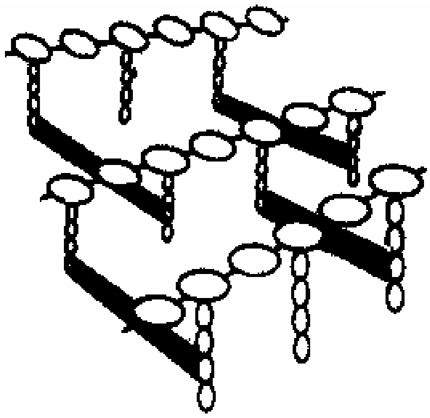


Spiral cells of *Treponema pallidum*.
This bacterium causes syphilis and is so slender that it cannot be seen using conventional light microscopy. It is most easily visualised using dark-ground microscopy.

Bacterial cell walls

The vast majority of bacteria have a cell wall containing a special polymer called peptidoglycan. The cell wall lies outside the cell membrane, and the rigid peptidoglycan is important in defining the shape of the cell, and giving the cell mechanical strength. Peptidoglycan is a unique biological polymer. It comprises a backbone of repeating sugar units, N-acetyl glucosamine and N-acetyl muramic acid. These are joined by short peptides that attach to the N-acetyl muramic acid. The peptides are unique amongst biopolymers because they contain both L- and D-amino acids.

The bacterial cell wall is a unique **biopolymer** in that it contains both D- and L-amino acids. Its basic structure is a carbohydrate backbone of alternating units of N-acetyl glucosamine and N-acetyl muramic acid. The NAM residues are cross-linked with oligopeptides. The terminal peptide is D-alanine although other amino acids are present as D-isomers. This is the only biological molecule that contains D-amino acids and it is the target of numerous antibacterial antibiotics. The cell wall of Gram-positive bacteria lies beyond the cell membrane and is largely made up of pepidoglycan. There may be up to 40 layers of this polymer, conferring enormous mechanical strength on the cell wall. Other polymers including teichoic and teichuronic acids also lie in the cell walls of Gram-positive bacteria. These act as surface antigens.



Properties associated with bacterial cell walls

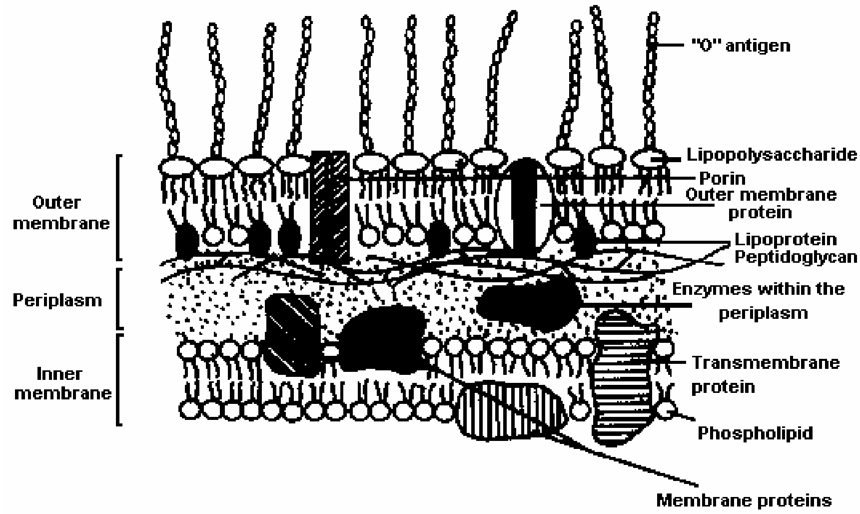
Bacteria may be conveniently divided into two further groups, depending upon their ability to retain a crystal violet-iodine dye complex when cells are treated with acetone or alcohol. This reaction is referred to as the Gram reaction: named after Christian Gram, who developed the staining protocol in 1884. It may seem a very arbitrary basis on which to build one's classification system. This reaction, however, reveals fundamental differences in the structure of bacteria. Electron microscopy shows that Gram-negative and Gram-positive bacteria have fundamentally different structures, related to the composition of the cell wall, amongst other things.

Cells with many layers of peptidoglycan can retain a crystal violet-iodine complex when treated with acetone. These are called Gram-positive bacteria and appear blue-black or purple when stained using Gram's method. The protocol for Gram staining is as follows:

- Cells are heat-fixed onto a microscope slide.
- The slide is treated with crystal violet, then an iodine solution.
- All cells appear blue-black at this stage.
- Acetone is used to remove the crystal violet-iodine complex from Gram-negative cells.
- Only Gram-positive cells can be easily seen at this stage.
- Gram-negative cells are counterstained with the red dye, carbol fuchsin, so that they, too, may be easily seen.

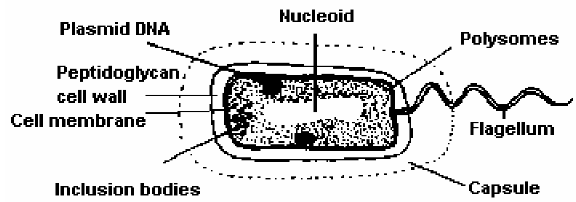
The cell wall of Gram-positive bacteria lies beyond the cell membrane and is largely made up of pepidoglycan. There may be up to 40 layers of this polymer, conferring enormous mechanical strength on the cell wall. Other polymers including teichoic and teichuronic acids also lie in the cell walls of Gram-positive bacteria. These act as surface antigens.

In contrast to Gram-positive cells, the cell envelope of Gram-negative bacteria is complex. Above the cell membrane is a periplasm. This area is full of proteins including enzymes. One or two layers of peptidoglycan lie beyond the periplasm. Gram-negative bacteria are thus mechanically much weaker than Gram-positive cells. Beyond the peptidoglycan of the Gram-negative cell wall lies an outer membrane. This has protein channels - porins - through which some molecules may pass easily. The outer side of the Gram-negative outer membrane contains lipopolysaccharide. This provides the antigenic structure of the surface of Gram-negative bacteria and also acts as **endotoxin**. It is this that is responsible for eliciting the symptoms of Gram-negative shock if it gains access to the bloodstream. Porins and Outer Membrane Proteins (OMPs) act as transporters through the outer membrane.

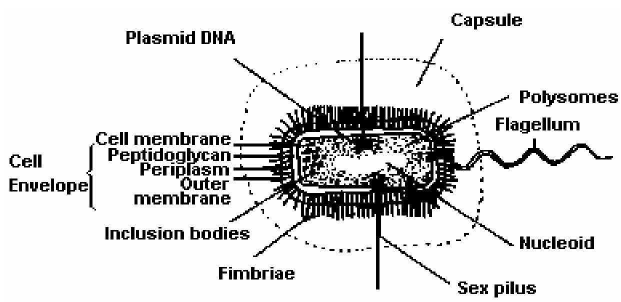


The cell envelope of a Gram-negative bacterium

The nature of the cell wall is also reflected in different cell architectures.



A Gram-positive cell



A Gram-negative cell

A few medically important bacteria do not stain easily using conventional stains, and need to be heated to near boiling point in the chosen dye (carbol fuchsin for light microscopy: rhodamine-auramine for fluorescence microscopy) for at least five minutes. This is to allow the dye to penetrate the waxy cell walls. Having taken the stain, these bacteria resist decolourisation with both acids and alcohol, and are known as acid-alcohol fast bacteria. This is a property of mycobacteria. These include Mycobacterium tuberculosis, the cause of tuberculosis; a chronic infection. Most common is pulmonary tuberculosis, affecting the lung. The kidneys may be infected in renal TB, and there is a rare form of osteomyelitis (bone infection) and meningitis caused by TB. In miliary tuberculosis, the infection is disseminated through the body. Another medically important mycobacterium is Mycobacterium leprae, the cause of leprosy; a chronic infection of the skin and nerves. Nerve damage leads to a loss of sensation, and ultimately to paralysis. This can lead to tissue damage that can lead to the loss of fingers and toes.

The genetic makeup of bacteria

The bacterial chromosomal DNA is located in a region of the cell known as the nucleoid. Bacteria, being prokaryotes, do not have a true, membrane-bound nucleus. Bacteria carry a single chromosome that is circular in structure.

Additional genetic information may be carried on plasmids. These are circles of DNA that lie within the bacterial cytoplasm and replicate independently of the chromosome. . Plasmids carry genes that are typically not essential for survival, but that can confer selective advantages in special circumstances. Not all bacterial cells carry plasmids, but some can carry several plasmids in a single cell. R-factors are plasmids that carry genes that confer antibiotic resistance on the cell. Toxins are sometimes coded for by plasmid genes.

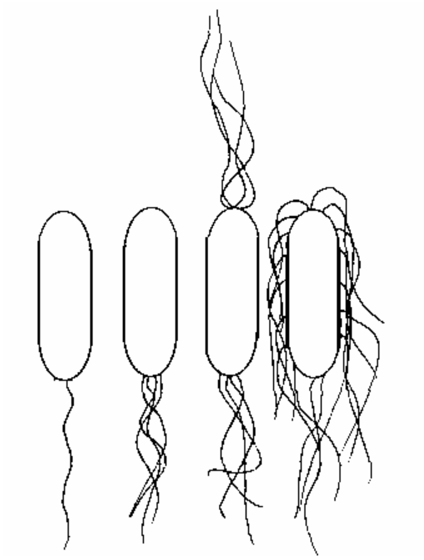
Lysogens are bacteria that have been stably infected with a bacteriophage and that carry the virus as a 'prophage'. The bacteriophage DNA is integrated into the genome of the bacterium. Under special conditions, lysogens can burst to release new bacteriophage particles. Lysogens can be very important. The gene for diphtheria toxin is carried by a prophage, and only the lysogenic strains of *Corynebacterium diphtheriae* can cause diphtheria.

Bacterial cell contents and appendages

The cytoplasm of bacteria contains **polysomes** - a range of **ribosomes** actively translating **messenger RNA** into proteins. Some bacteria also have **inclusion bodies** within the cytoplasm. These are often energy storage resources. Some inclusion bodies are referred to as **metachromatic granules** since they change the colour of dyes used to stain cells. Inclusion bodies found within *Corynebacterium diphtheria*e, the cause of diphtheria, are an important example of metachromatic granules.

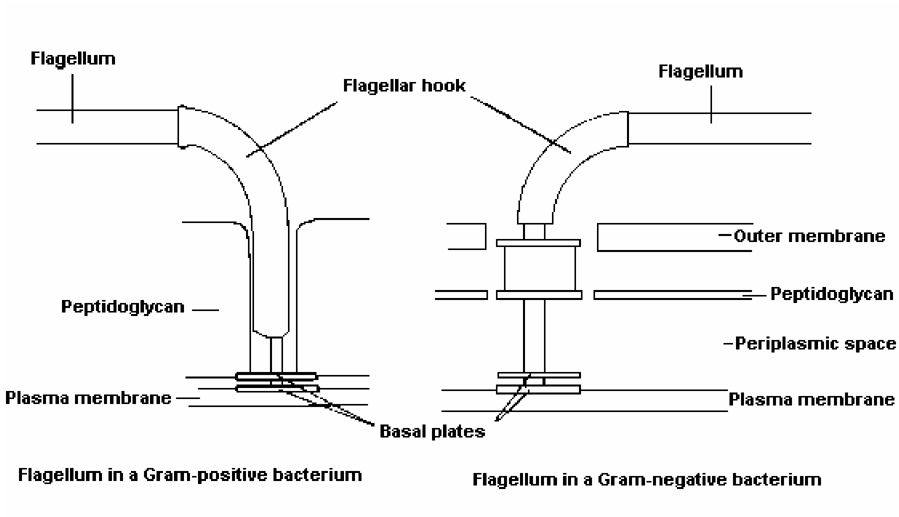
Flagella are responsible for the motility of pathogenic bacteria and can play a role in the production of disease. Gram-negative pathogenic bacteria may be covered in fine hairs called fimbriae (singular: fimbria) these help to stick to body surfaces. Pili can attach two bacterial cells together: sex pili are necessary for the transfer of certain plasmids between bacteria.

Bacterial cells may carry a single flagellum, and are thus described as monotrichous. If the single flagellum is at one end of a rod-shaped cell it is known as a polar flagellum. If the bacterium carries a single tuft of flagella, it is said to be lophotrichous (lophos - Greek for a crest). When the tuft appears at both ends of the cell, the bacterium is amphitrichous (amphi - Greek for 'at each end'). Bacteria that are covered all over in flagella are said to be peritrichous (peri - around).



Arrangements of flagella on bacterial cells

Flagella are inserted through the cell walls of bacteria. At their base can be found wheel-like structures.



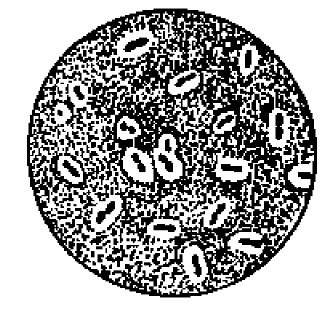
Insertion of flagella into bacterial cells of different types

Gram-negative bacteria have additional structures. They exchange genetic material in a process of conjugation that involves cells being joined by **sex pili**: tube-like structures through which DNA is passed. The surface of Gram-negative cells is also covered in fine, hair-like structures called **fimbriae** (some microbiologists also call these pili, confusing them with sex pili). These are important in adhesion and can play a central role in virulence. If a microbe is to cause an infection, first it must attach to its surface.

Capsules, *etc.*

Some bacteria are enclosed within a capsule. This protects the bacterium, even within phagocytes, helping to prevent the cell from being killed. Encapsulated bacteria grow as 'smooth' colonies, whereas colonies of bacteria that have lost their capsules appear rough. Rough colonies do not generally cause disease. Encapsulated bacteria do not succumb to intracellular killing as easily as bacteria that lack capsules. Strains of *Streptococcus pnuemoniae* that lack capsules do not cause disease. All the bacteria that cause meningitis are encapsulated. Suspending bacteria in India ink is an easy way of demonstrating capsules. Ink particles cannot penetrate the capsular material and encapsulated cells appear to have a halo around them. This is the **Quellung** reaction.

In the 'Quellung' reaction, bacterial cells are resuspended in antiserum that carries antibodies raised against the capsule. This causes the capsule to swell, and this can be easily visualised by suspension in India Ink. The ink particles cannot penetrate the capsule, which this appears as a halo around the bacterial cells.

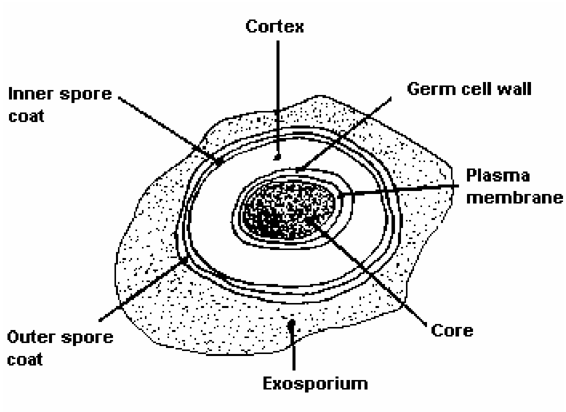


The Quellung reaction

Some bacteria produce slime to help them to stick to surfaces. Slime is produced by several types of pathogenic microbes, and is usually made up from polysaccharides. The slime produced by *Streptococcus mutans* enables it to stick to the surface of teeth, where it helps to form plaque, leading eventually to dental caries. 'Coagulase-negative' staphylococci live on the skin, and some strains produce a slime that enables them to stick to plastics. These bacteria cause infections associated with implanted plastic medical devices.

Bacterial spores

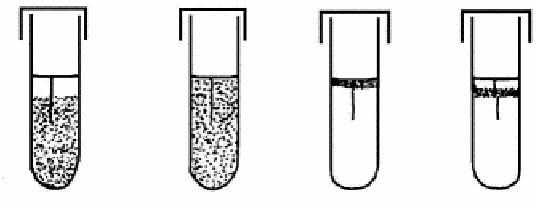
A few species of bacteria have the ability to produce highly resistant structures known as endospores (or simply spores). These resist a range of hazardous environments, and protect against heat, radiation, and desiccation. Endospores form within (hence endo-) special vegetative cells known as sporangia (singular sporangium). Diseases caused by sporing bacteria include botulism (*Clostridium botulinum*), gas gangrene (*Clostridium perfringens*), tetanus (*Clostridium tetani*) and acute food poisoning (*Clostridium perfringens*, again) All these bacteria are 'anaerobic'. The aerobic sporing bacteria can also cause disease. Anthrax is caused by *Bacillus anthracis*. *Bacillus cereus* causes two types of food poisoning.



A bacterial spore

The atmospheric and temperature requirements of bacteria

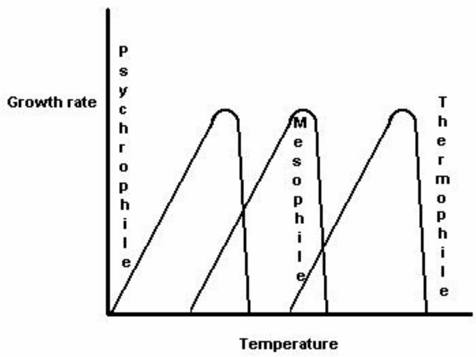
Some bacteria have an absolute requirement for oxygen. These are the obligate aerobes. Others, the facultative anaerobes can survive in the absence as well as the presence of oxygen. The obligate anaerobes are killed by traces of oxygen. A small group of bacteria are killed by normal atmospheric levels of oxygen, but yet require traces of oxygen to grow. These are the referred to as microaerophiles.



The relationship between bacterial growth and oxygen

The culture on the left is an obligate anaerobe, unable to grow in the presence of oxygen, close to the surface of the growth medium. Next is a facultative bacterium, which is indifferent to the presence of oxygen. There then follows an obligate aerobe that can only grow at the surface of the culture medium. The culture on the right is a microaerophile. It can only grow where the oxygen tension is low.

Bacteria that grow at very low temperatures are known as psychrotrophs. Bacteria found to grow at high temperatures are known as thermophiles. Those that grow at moderate temperatures are known as mesophiles.



The relationship between bacterial growth and temperature.

It is not possible to determine the minimum temperature at which some psychrophiles can grow. The laboratory media used to determine these temperatures becomes frozen during the experiments. It would seem probable that the only requirement to support bacterial life is the necessity that surrounding water should be in a liquid state. There are extreme thermophiles that live around underwater volcanoes at temperatures in excess of 200 degrees Centigrade. Human pathogens are mesophiles, and many have an optimum growth temperature close to the normal human body temperature.