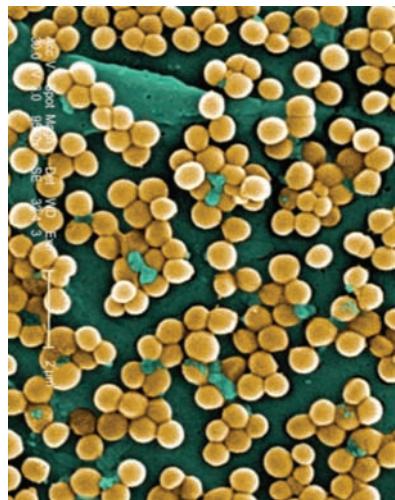


Chapter 3

Bacterial structure and function



Meticillin-resistant *Staphylococcus aureus* - MRSA

KEY FACTS

- Bacteria are prokaryotic, generally single-celled organisms, which exist in a limited number of morphological forms.
- In the majority of cases their cell walls contains peptidoglycan, a unique bacterial component which confers rigidity to the cell.
- Bacteria can be broadly divided into two main groups – Gram-positive and Gram-negative, based upon the structure of their cell walls.
- One of the components of the Gram-negative bacterial cell wall is lipopolysaccharide which is also known as endotoxin; it is responsible for many of the adverse effects of Gram-negative bacterial infections.
- The bacterial chromosome is a single circular molecule of double-stranded DNA. It is not surrounded by a nuclear membrane but exists in a highly compacted state within the cytoplasm.
- A number of small circular extrachromosomal pieces of DNA may also be found within a cell which will have been inherited from other cells. These may confer on the cell additional attributes such as resistance to antibiotics.
- Some bacteria are able to transform themselves into highly dormant, heat-resistant structures called endospores. This is not a reproductive mechanism but acts as a means of surviving adverse conditions. Bacterial spores are a very important consideration in the design of sterilization procedures.

Chapter 1 has emphasized the fundamental differences between prokaryotic cells and human cells. As we shall see later, this is extremely useful as it allows us to target bacteria with antimicrobial agents which have only a limited effect on us. This chapter looks at the basic structure of bacteria and explains the functions of the various internal components of the cell.

3.1 Bacterial morphology

Although there are some hundreds of thousands of individual bacterial species they exhibit rather limited morphology (structure and shape). Broadly speaking, we can consider those cells whose shape is based upon a rod

and those based upon a sphere. Below are some examples with typical dimensions.

3.1.1 Rod-shaped cells

Bacillus

Rod-shaped cells whose dimensions vary depending upon the species. Note the potential confusion in terminology here. The term *Bacillus* is a genus name while the word bacillus also describes a shape. *Escherichia coli* cells are typically 1.0 μm in diameter and 2–3 μm in length, while *Bacillus* species are approximately 2 μm in diameter and up to 7 μm in length.

Vibrio

Rigid curved cells, a typical example of which is *Vibrio cholerae* (the causative agent of cholera). Dimensions are 0.5 μm in diameter by 2 μm in length.

Spirochetes

Thin, flexibly coiled cells e.g. *Treponema pallidum* (the causative agent of syphilis) which has dimensions of 0.5 μm diameter by 5–500 μm in length.

Spirillum

Cells made up of rigid spirals with variable numbers of turns. Examples include *Campylobacter jejuni* (major cause of food poisoning) and *Helicobacter pylori* (implicated in the formation of gastric ulcers). Dimensions are typically 0.5 μm in diameter with variable length up to 60 μm .

Filamentous

Some cells grow as slender, nonseptate, branching filaments, which resemble filamentous fungi rather than typical bacteria. Examples include the streptomycetes, which are soil bacteria responsible for the production of a number of important antibiotics, for example *Streptomyces venezuelae* 0.5–2 μm diameter with variable length.



Fusiform bacilli



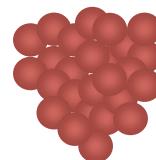
'Cigar-shaped' bacteria. *Fusobacterium* species are normal inhabitants of the mouth, gut and female genital tract. They cause a range of infections including, sinusitis, otitis media and dental infections.

3.1.2 Spherical cells (coccus shaped)

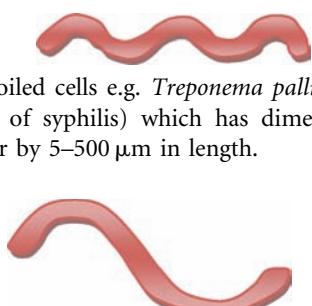
These vary not so much in shape as in degree of aggregation and diameter. The extent and the manner in which they aggregate are determined by the plane of cell division and the strength of adhesion between cells after division.



Staphylococcus



These are found as irregular clusters of spherical cells which are said to resemble a bunch of grapes. Cell division takes place in a number of planes with a high degree of adhesion. *Staphylococcus aureus* is often found as part of the normal microflora of the skin and nostrils but can also give rise to wound and other infections, which can be life threatening. Cells are typically 1 μm in diameter.



Streptococcus



These are spherical or slightly oval cells which occur usually in chains. They divide in one plane only and the degree of adhesion between cells is not very strong, hence chains of cells are easily disrupted. An example is *Streptococcus pyogenes*, which can cause sore throats and also skin and soft tissue infections. The cells are approximately 1–2 μm in diameter.



Diplococcus



These are small cocci or oval cells which occur typically in pairs, usually joined along their longest axis and with adjacent sides flattened. An example is *Neisseria gonorrhoea* (often called the gonococcus- which is the causative agent of gonorrhoea).

Tetrad

This is the name given to bacteria which are typically found in clusters of four cells, for example *Gaffkya* species.

Sarcina

The name given to bacteria found in clusters of eight cells, for example *Sarcina* species.

Pleomorphic

These cells exhibit a variable morphology depending upon how they are grown. For example *Lactobacillus* species can grow either as a slender rod-shaped cell or a coccobacillus depending upon culture conditions.

3.2 The cell wall

The structure of a typical bacterial cell is shown in Figure 3.1. One of the most important structures of the cell and one of the things which sets it apart from mammalian cells is its cell wall. If mammalian cells such as erythrocytes are placed in a hypotonic solution they will swell due to the uptake of water by osmosis and then burst (a process known as haemolysis). If bacterial cells are placed in a hypotonic solution they will not burst because of the presence of their rigid cell wall. This cell wall is also responsible for giving them their characteristic shape illustrated above.

However, not all bacteria have the same type of cell wall, and this was first discovered by Christian Gram in 1884. Bacterial cells subjected to a differential staining procedure known as the Gram stain (see text box) were seen under the microscope either as a blue/violet colour

or a pink colour. The former were named Gram-positive cells and the latter Gram-negative cells.

Gram staining procedure

1. Fix bacterial smear onto a glass slide using heat.
2. Stain with crystal violet solution – the cells appear blue/violet colour.
3. Fix with Gram's iodine (I/KI solution) – the cells same colour as above.
4. Decolourize with alcohol or acetone – some cells appear blue/violet, others colourless.
5. Stain with safranin solution (red colour).
6. Wash with water – Gram-positive cells appear blue/violet colour under microscope, Gram-negative cells appear pink/red colour.

The structures of the two different types of cell wall are shown in Figure 3.2. It can be seen that the Gram-positive cells are much simpler and the predominant component of the cell wall is a polymer called peptidoglycan. The Gram-negative cells are more complex and have a thinner layer of peptidoglycan surrounded by a lipid bilayer comprising lipopolysaccharide and phospholipid. This additional outer layer makes the Gram-negative cells less easily penetrated by drug molecules (including several important antibiotics) and accounts for many of the properties found in these cells.

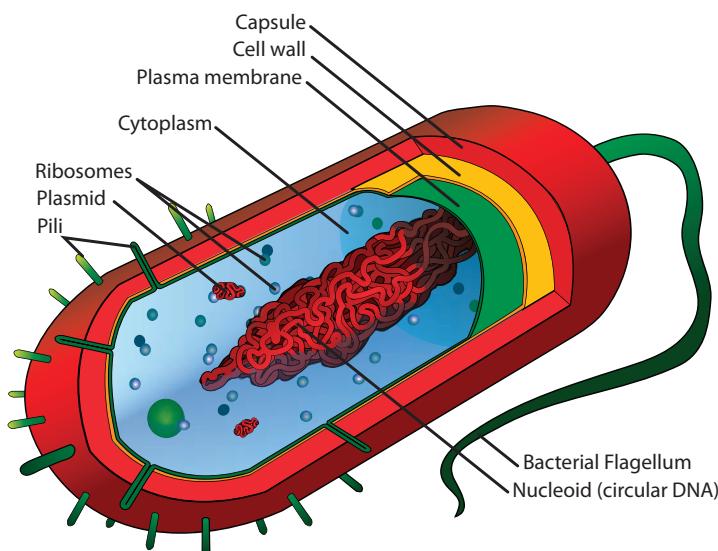


Figure 3.1 Structure of a typical bacterial cell. Source: http://commons.wikimedia.org/w/index.php?title=File:Average_prokaryote_cell_en.svg&page=1.

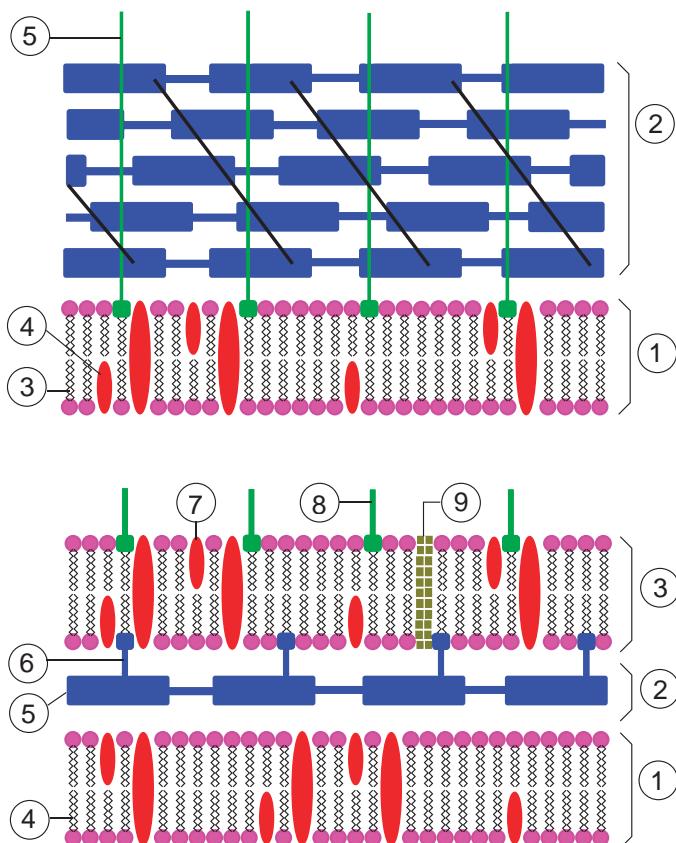


Figure 3.2 Bacterial cell walls. Top: Gram-positive cell wall. 1-cytoplasmic membrane, 2-peptidoglycan, 3-phospholipid, 4-protein, 5-lipoteichoic acid. Bottom: Gram-negative cell wall. 1-inner membrane, 2-periplasmic space, 3-outer membrane, 4-phospholipid, 5-peptidoglycan, 6-lipoprotein, 7-protein, 8-LPS, 9-porins. Source: http://commons.wikimedia.org/w/index.php?title=File:Bacteria_cell_wall2.svg&page=1.

3.2.1 Features of peptidoglycan

- They are made up of linear polysaccharide chains (glycan strands) up to 200 disaccharide units in length, cross-linked by short peptide chains.
- Glycan strands are composed of alternating units of N-acetylglucosamine and N-acetylmuramic acid joined by $\beta 1-4$ glycosidic bonds.
- Peptidoglycan comprises one large continuous molecule surrounding the cell rather like a net.
- The carboxyl group of each N-acetyl muramic acid is attached via a peptide bond to a chain of four amino acids.
- The amino acids which comprise this chain vary but in Gram-negative cells a typical sequence is: L-alanine; D-glutamic acid; meso diaminopimelic acid; D-alanine
- In Gram-positive cells the meso diaminopimelic acid is replaced by L-lysine.
- The tetrapeptide chains on adjacent glycan strands are joined by peptide bonds to give a cross-linked polymer.

The structure of peptidoglycan is shown in Figure 3.3. This is a generic structure and there are variations between different cells particularly with respect to the cross-linking groups.

3.2.2 Antimicrobial agents acting on peptidoglycan (see Figure 3.4)

Enzymes (for example, lysozyme, lysostaphin):

- Many body fluids such as tears and saliva contain the enzyme lysozyme as a protection against invading bacteria.
- Lysozyme breaks linkages between N-acetyl muramic acid and N-acetylglucosamine.
- Their activity is carefully controlled normally to allow for selective hydrolysis when required.
- On death of the bacterial cell these lytic enzymes are activated and the peptidoglycan of the cell wall is degraded.

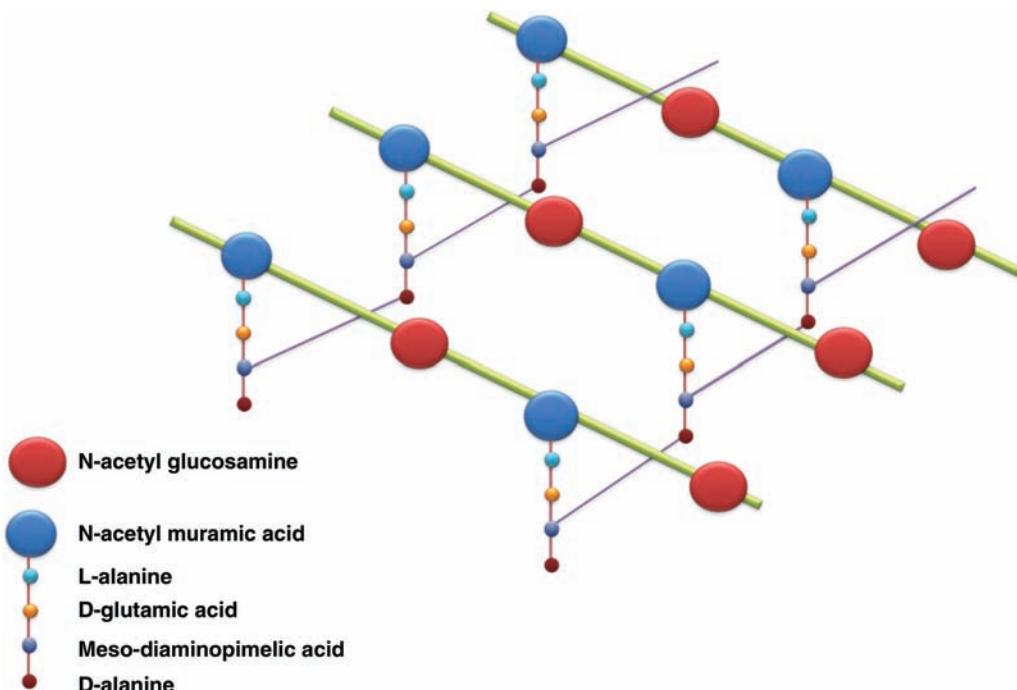


Figure 3.3 General layout of the structure of peptidoglycan.

Antibiotics (for example, penicillins, cephalosporins, bacitracin, vancomycin, teicoplanin):

- β -lactam antibiotics (penicillins and cephalosporins) act by preventing the cross-linking of peptidoglycan during synthesis. There is no effect on intact peptidoglycan, therefore they only act on growing cells.
- Bind to penicillin-binding proteins (PBPs), which are carboxypeptidases and transpeptidases responsible for final stages of cross linking.

- Enzyme inhibition leads to a build up of precursors and release of autolytic enzymes. They form spheroplasts (spherical cells lacking all or part of the cell wall: Figure 3.5) and lysis of the cells results due to fragile outer cell walls.
- MRSA (meticillin resistant *Staphylococcus aureus*) has an additional PBP with lower binding affinity for β -lactams.
- Bacitracin interferes with a lipid carrier responsible for transporting cell-wall precursors across the membrane from the cytoplasm.

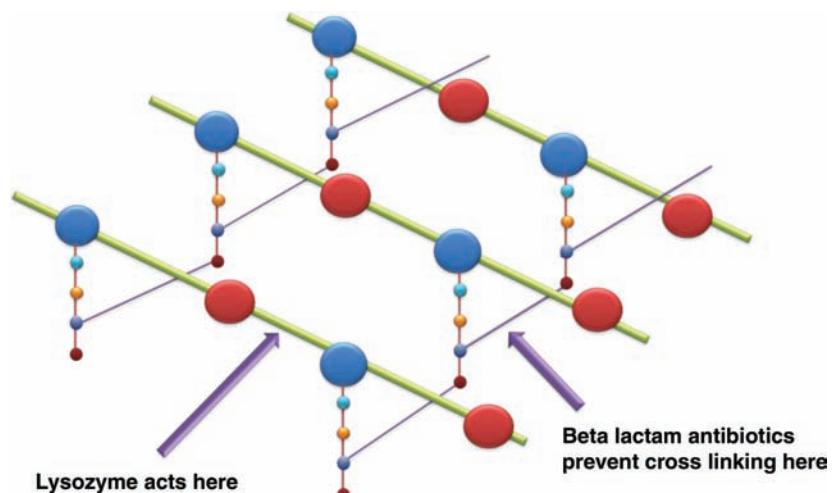


Figure 3.4 Action of lysozyme and beta lactam antibiotics on peptidoglycan.

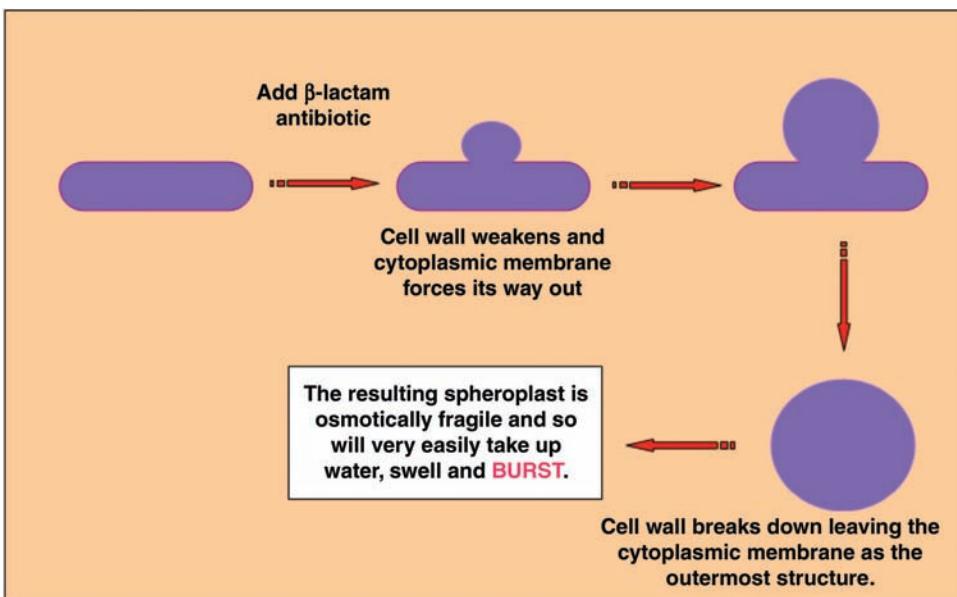


Figure 3.5 The effect of beta lactam antibiotics on a bacterial cell.

- Glycopeptides are large polar molecules unable to penetrate the Gram-negative outer membrane. They are only active against Gram-positive bacteria and act by binding to the end of peptide chains, preventing cell-wall growth.

the lipopolysaccharide found in the Gram-negative cell envelope, commonly called endotoxins or pyrogens. It is therefore important to ensure that injections are not just sterile but also free from endotoxins.

A lipopolysaccharide molecule is made up of three parts:

- An outer region, which is a type-specific O-antigen extending into the external environment.
- A core region which anchors the O-antigen to the membrane.
- The lipid A region, which is similar to phospholipid. This sits inside the membrane and all the significant pharmacological activity of LPS is due to this region (see below).

3.4.1 Toxic effects of endotoxins

Pyrogenicity

- Body temperature is regulated by the thermoregulatory centre in the hypothalamus of the brain and endotoxin interferes with this control.
- It does not act directly but causes release of endogenous pyrogen from macrophages in the body.

Cardiovascular effects

- Initially endotoxin causes hypertension but this is followed by progressive and profound hypotension. Shock and death may follow.

Generalized toxic effects:

- Endotoxin stimulates the release of cytokines (such as interleukins and kinins) from macrophages and other cells.

3.3 Teichoic acids

Teichoic acids are found only in Gram-positive cell walls and constitute up to 45% of the wall of *Staphylococcus aureus*. They do not confer any extra rigidity to the cell wall, but being acidic in nature they may function as sequestering agents extracting essential cations from solution. They are believed to regulate the activities of amidases and glycosidases, which are involved in cell-wall synthesis and also act as adhesins regulating attachment to surfaces. They have also been shown to play a role in inflammation (causing host cells to release inflammatory agents such as cytokines).

3.4 Lipopolysaccharide (endotoxin)

Gram-negative bacteria retain the ability to kill and injure humans even after the cells have died. Suspensions of cells autoclaved for 1 hour at 121°C will be killed and the solution will be sterile. The resulting solution, if injected into a patient, could cause high fever, circulatory collapse and death. Patients with Gram-negative septicaemia (bloodstream infection) are liable to suffer from toxic shock even if given antibiotics. The toxic component is

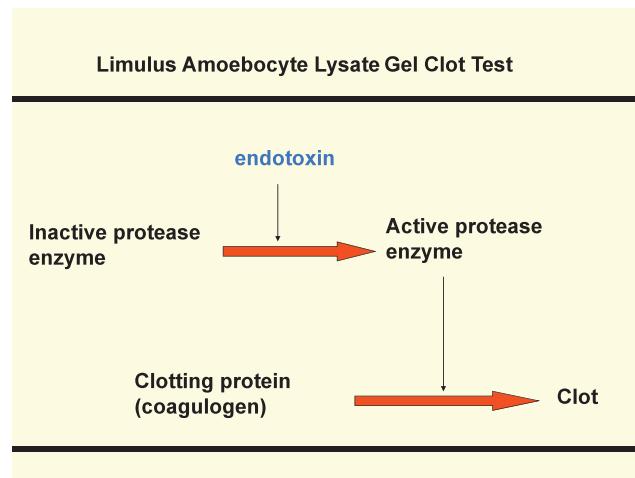


Figure 3.6 Mechanism of LAL clotting test for endotoxin.

- Most of the actions of endotoxin can be attributed to the release of these endogenous mediators.

3.4.2 *Limulus amoebocyte lysate (LAL) test for the detection of endotoxins*

The horseshoe crab (*Limulus polyphemus*) is a living fossil which has remained unchanged since the time of the dinosaurs. It is a large creature 30 cm across with an armour-plated shell and it devours large quantities of shellfish – hence it is not liked by fishermen. The creature is seldom seen and lives in deep waters off the coast of North America, Canada and southeast Asia. Every spring they come ashore in their hundreds of thousands to lay eggs.

It has been known for many years that the blood of *L. polyphemus* forms a solid clot when withdrawn from the

animal. We now know that coagulation is caused by the presence of contaminating bacterial endotoxin reacting with the contents of circulating blood cells (amoebocytes). The crabs are harvested and their blood withdrawn under sterile conditions. The crabs are not harmed by this and are returned to the sea. The amoebocytes are collected and lysed and the contents formulated into LAL reagent. The clotting reaction is very sensitive and can detect nanogram quantities of endotoxin (see Figure 3.6).

3.5 Cytoplasmic membrane (see figure 3.7)

Cytoplasmic (plasma) membranes of Gram-positive and Gram-negative cells are similar. They are made up of protein (60–70%); lipids/phospholipids (20–30%)

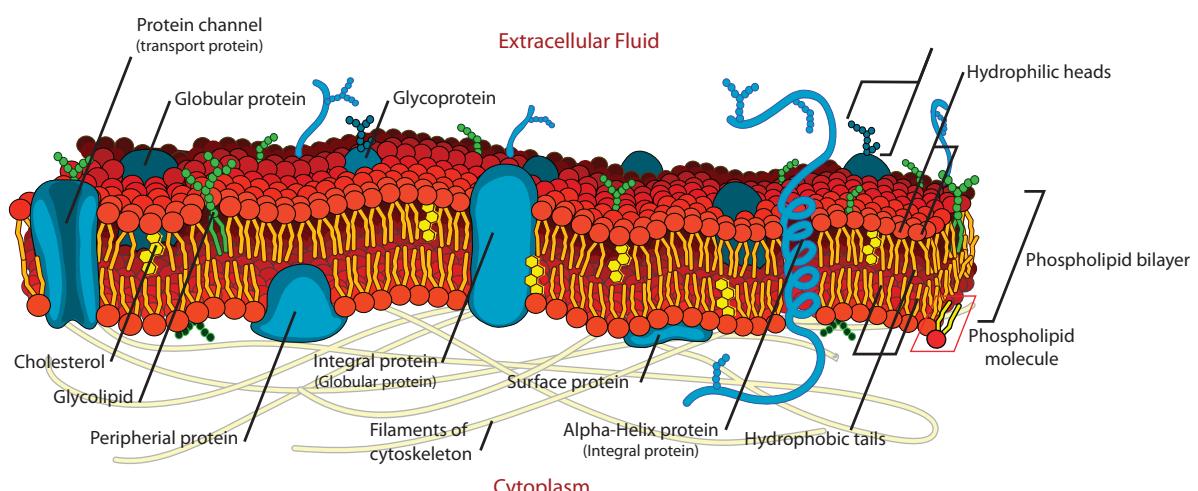


Figure 3.7 Diagram of a bacterial cytoplasmic membrane. Source: http://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_en.svg.

and a small amount of carbohydrate. Sterols such as cholesterol and ergosterol are absent in bacteria but are present in eukaryotic cells where they provide rigidity.

Prokaryotic membranes are very fluid – stabilized by hydrogen bonding and magnesium and calcium ions. The membrane acts as an osmotic barrier allowing only small molecules to enter the cytoplasm. Larger molecules can only enter if their entry is mediated by specific transport proteins and, being hydrophobic, the membrane is impermeable to hydrogen ions.

Oxidative phosphorylation occurs in the membrane. Redox couples (a reducing species and its corresponding oxidized form) pass electrons down the series to oxygen as the final electron acceptor. Energy is used to pump protons out of the cell and this gives a proton and charge gradient across the membrane – known as the proton motive force. Protons are allowed back via a transporter system linked to ATP production.

Bacterial cells establish an internal osmolarity in excess of the environment in order to maintain a constant internal pressure. The cell responds to changes in external osmotic stress by altering the internal concentration of solutes. Solutes include potassium ions, glutamate, glycine betaine and various sugars. Potassium is an important intracellular cation and cells can accumulate high concentrations in excess of the environment (for example staphylococci up to 30x higher).

A large number of general antimicrobial agents (including surfactants) act on the cytoplasmic membrane. They cause loss of membrane integrity, leakage of intracellular contents, disruption of enzyme function and uncoupling of proton motive force.

3.6 Inclusion bodies (storage granules)

Bacteria often accumulate materials within storage granules inside the cell. These can take a variety of forms as shown below:

- Volutin granules
 - are used for storage of phosphorus and energy;
 - consist of polymetaphosphate;
 - accumulate at the end of active growth.
- Glycogen granules
 - are food and energy stores;
 - accumulate under conditions of nitrogen starvation;
 - can account for up to 50% of the dry weight of a cell.

- Lipid granules
 - consist of poly- β -hydroxybutyric acid;
 - are storage products;
 - occupy a large volume in old cells.
- Sulfur/iron granules
 - some cells can accumulate magnetite (Fe_3O_4);
 - impart a permanent magnetic dipole to a cell.

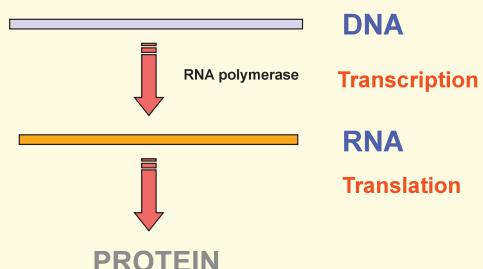
3.7 The bacterial chromosome

The chromosome contains the genetic information necessary for functioning of the cell. It is a single circular molecule of double-stranded DNA approximately 1000 times as long as the cell itself. It exists in a highly folded state and this supercoiling is brought about by topoisomerase enzymes. In prokaryotic cells the chromosome is not surrounded by a nuclear membrane but exists free in the cytoplasm condensed into areas called chromatin bodies. In afeat of extraordinary dexterity the chromosome may duplicate itself every 20 minutes during exponential growth.

The role of the DNA is to act as a source of information for the synthesis of proteins encoded into a sequence of nucleotides. The cell can access this information by firstly transcribing the coded information into RNA by the action of the enzyme RNA polymerase and this is then translated into a peptide sequence by the action of the ribosomes (see box below). The nucleotide sequences on the DNA are collected into discrete areas called genes each with their own control elements.

Prokaryotic genomes are very small with very little space between the genes. *E. coli* has approximately 4000 genes and the length of the DNA molecule is about 1mm. On the other hand a yeast cell has approximately 6000 genes in a genome three times the size of *E. coli*.

Transcription and translation



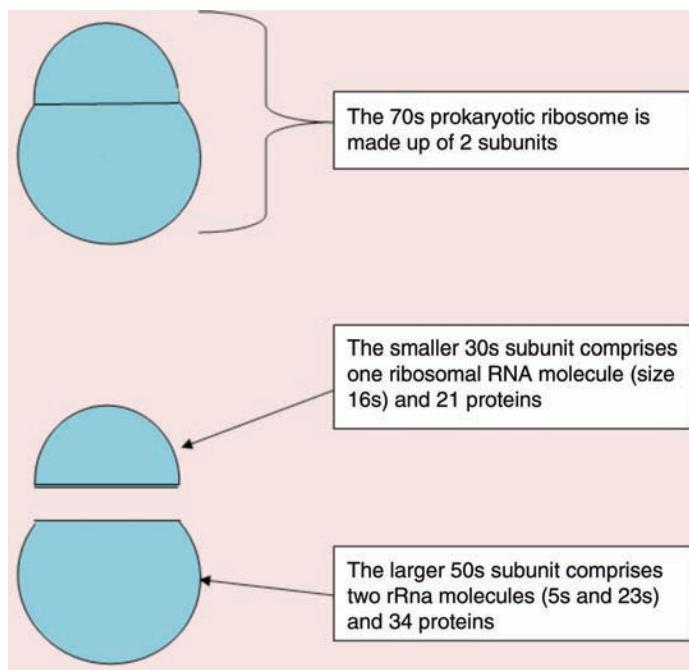


Figure 3.8 Structure of prokaryotic ribosomes.

3.8 Plasmids

Cells can contain additional genetic elements such as plasmids. These are autonomously replicating, extrachromosomal, circular pieces of double-stranded DNA. They vary in size from 1000 to 200 000 base pairs (the *E. coli* chromosome has 4 million base pairs) and encode for accessory functions conferring advantages to the cell, for example the production of toxins, pili, bacteriocins, siderophores and enzymes responsible for antibiotic resistance.

Plasmids replicate faster than the main bacterial genome and so cells usually contain multiple copies. All plasmids contain the information required for replication but some also contain information for cell to cell transfer (called conjugative plasmids). Plasmids may contain multiple genes encoding antibiotic resistance elements and be able to transfer between cells. What is worrying is the ability to transfer plasmids between species, because a relatively harmless bacterium found in our gut, for example, can then donate antibiotic resistance genes to a pathogenic species.

3.9 Ribosomes

Protein synthesis is carried out by the ribosomes which are complex structures approximately 18 nm in diameter.

They have a molecular weight of about 2500 kDa and those from bacteria have a sedimentation coefficient (which is a function of size) of 70s. Those found in eukaryotic cells are slightly larger and have a sedimentation coefficient of 80s. The bacterial ribosomes can dissociate into a large and a small subunit with sedimentation coefficients of 50s and 30s respectively (see Figure 3.8).

Bacterial protein synthesis starts with the interaction between a 30s ribosomal subunit, mRNA and a tRNA (attached to the amino acid formylmethionine). The 50s ribosomal subunit then attaches and the whole ribosome moves along the mRNA chain reading the sequence of nucleotides and constructing the peptide chain dictated by the mRNA.

3.10 Fimbriae (pili)

Fimbriae are common on Gram-negative bacteria but uncommon on Gram-positive. Most of the fimbriae is made up of major structural protein. The minor tip protein gives rise to variability and leads to antigenic variation and adhesion variability. *Neisseria gonorrhoea* possesses fimbriae, which facilitate binding to the urogenital epithelium. The cell can switch production from one type of fimbria to another to evade host responses.

Type I fimbriae (common fimbriae) are chromosomally mediated and found in *E. coli* and other enteric

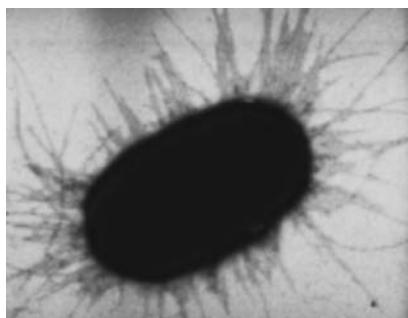


Figure 3.9 Electron micrograph of bacterium with multiple fimbriae. Source: Gross L (2006) Bacterial fimbriae designed to stay with the flow. PLoS Biol 4(9): e314. doi:10.1371/journal.pbio.0040314, © 2006 Public Library of Science.

bacteria including *Salmonella* species. These assist the cell in colonizing the large intestine. In enterotoxigenic *E. coli* (see Figure 3.9) the production of fimbriae is plasmid-mediated and these structures allow colonization of the small intestine. The K88 pilus antigen of some *E. coli* causes diarrhoea in pigs but not in other animals as a result of specific cellular adhesion.

3.11 Capsules

Many Gram-negative bacteria and some Gram-positive bacteria produce extracellular polysaccharides (EPS), which may take the form of a discrete capsule or a more generalized layer of slime (Figure 3.10). The EPS can function in:

- adherence (for example, *Streptococcus mutans*);
- resistance (to biocides, desiccation and macrophages);
- virulence (*S. pneumoniae*).



Figure 3.10 Photomicrograph showing *Bacillus anthracis* cells surrounded by a capsule. Visualized using fluorescent dye tagged to capsular antibody. Source: Oregon State Public Health Laboratory ID #1888; Photo Credit: Larry Stauffer, Centers for Disease Control and Prevention.

Streptococcus pneumoniae depends upon its polysaccharide capsule for pathogenicity. In the absence of a capsule the infecting dose is increased 10,000 times. The biochemical nature of the capsule can affect virulence, for example two types of encapsulated pneumococcus are pathogens: Type 3 gives severe disease; Type 30 gives mild disease.

The human host responds very well immunogenically to protein antigens but less well to polysaccharides and, hence, the capsule shields cells from detection by the immune system. The capsule is referred to as the K antigen.

3.12 Flagella

Flagella are long whiplike appendages that enable bacteria to move (Figure 3.11) and cells lacking flagella are nonmotile. They are made up of repeating units of a simple protein called flagellin and the numbers of flagella per cell are variable. The flagella are antigenic and are given the name H antigen. The structure does not beat or wave but it is a rigid helix, which rotates like a propeller up to 300x per second. Using this mechanism the bacteria can travel 200 times their own length per second.

Motile bacteria have two modes of movement:

- **Swimming**

- Directed by the flagella and cause the bacterium to move in a straight line.

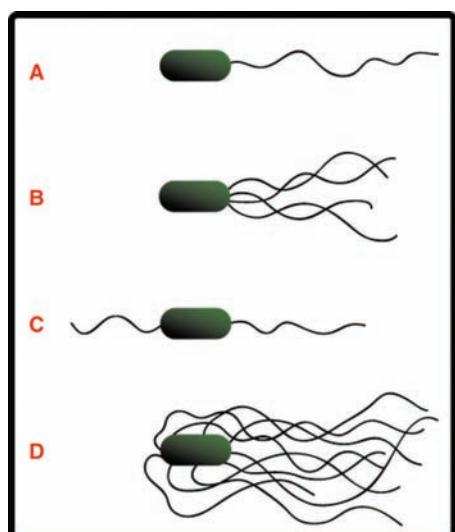


Figure 3.11 Arrangement of flagella on the surface of bacterial cells. (A = monotrichous; B = lophotrichous; C = amphitrichous; D = peritrichous). Image by Mike Jones; <http://commons.wikimedia.org/wiki/File:Flagella.png>.

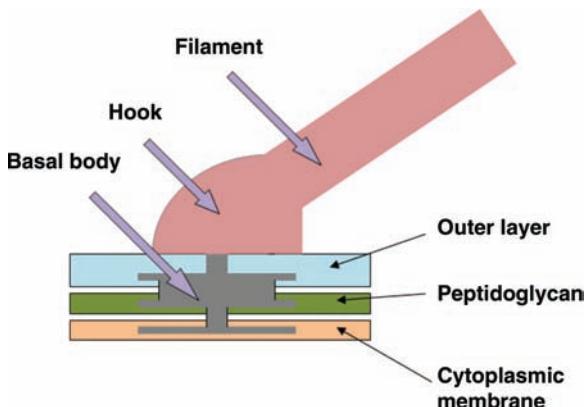


Figure 3.12 Diagram of bacterial flagellum showing basal body, hook and filament.

- **Tumbling**

- Bacterium rotates around its own axis in a random manner but does move from that spot.

Figure 3.11 shows the various arrangements of flagella found on the surface of bacterial cells. The flagellum originates in the cytoplasmic membrane and is firmly anchored in the cell wall (see Figure 3.12). ATP synthesizing systems within the membrane provide energy to drive the action of the flagellum.

3.13 Bacterial endospores

Most vegetative (actively growing) bacteria exhibit the normal growth cycle as shown in Chapter 2. With the advent of nutrient depletion or accumulation of toxic waste products the cell population begins to die. However, a limited number of bacteria can differentiate into highly resistant endospores and so survive extreme environments. The two main endospore-forming genera are:

- *Bacillus* species (aerobic Gram-positive rods);
- *Clostridium* species (anaerobic Gram-positive rods).

The trigger for sporulation to begin is usually nutrient starvation. Sporulation is *not* a reproductive process but a survival mechanism. Spores exhibit extreme resistance to heat, disinfectants, radiation and desiccation and they are highly dormant structures – which can exist in a state of cryptobiosis (where metabolic activity is reduced to an undetectable level). The spores can however, germinate very rapidly when favourable conditions are restored.

The sporulation process takes about seven to eight hours under ideal conditions and each stage is associated with

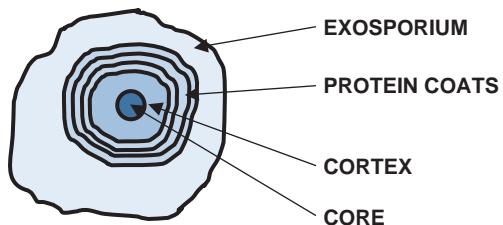


Figure 3.13 Cross sectional diagram of bacterial endospore.

characteristic morphological and biochemical changes. It is important to understand that a single cell gives rise to a single spore due to internal reorganization. The end result is a multilayered dormant spore as shown in Figure 3.13.

3.13.1 Why spores are important

- Extreme resistance makes them difficult to eradicate from pharmaceuticals and food.
 - Sterilization processes are designed for the elimination of spores.
- A number of species are dangerous pathogens.

– <i>Bacillus anthracis</i>	anthrax
– <i>Clostridium tetani</i>	tetanus
– <i>Clostridium perfringens</i>	gangrene
– <i>Clostridium botulinum</i>	botulism
- Issue of dormancy (longevity in the environment) makes them a problem with respect to germ warfare.
- Spore-forming bacteria are commercially important as producers of antibiotics – for example bacitracin, gramicidin and polymyxin – and of insecticides.
- They are a simple example of cellular differentiation.

3.13.2 Longevity of spores

- Spores have been isolated from lake sediments 500–1000 years old and revived within hours.
- Anthrax spores found sealed in jars known to be over 1300 years old were still viable.
- Spores found in the gut of an extinct bee trapped within amber germinated rapidly in the laboratory. The bee was thought to be 25 to 40 million years old.

3.13.3 Heat resistance of bacterial endospores

The extreme heat resistance of bacterial endospores has long been a fascinating phenomenon for microbiologists. Early theories postulated that the whole

Table 3.1 Heat resistance of endospores from different species.

Organism	Time taken to kill at 100°C
Most vegetative bacteria	Seconds
<i>Bacillus subtilis</i>	15 minutes to several hours
<i>Bacillus anthracis</i>	10 to 15 minutes
<i>Geobacillus stearothermophilus</i>	6 hours
<i>Clostridium tetani</i>	60 minutes
<i>Clostridium perfringens</i>	5 to 10 minutes
<i>Clostridium botulinum</i>	6 to 7 hours

spore must be highly dehydrated although this was quickly shown not to be true. A unique spore component, dipicolinic acid, (DPA) complexed with calcium ions was originally also thought to be involved but this was also shown to be incorrect. It is now thought that only the core is highly dehydrated and this is brought about by the cortex squeezing water out.

Spores of different species vary in their ability to withstand heat and Table 3.1 gives examples of the heat resistance of bacterial endospores. The extreme heat resistance of *Geobacillus stearothermophilus* has led to its use as a biological indicator in steam sterilization processes (Chapter 19).

3.13.4 Activation, germination and outgrowth

An endospore will remain in a dormant state until it encounters an environment favourable for growth. The transformation of a dormant, highly resistant endospore into a fully metabolizing vegetative cell takes place via a series of different steps – activation, germination and outgrowth:

- **Activation.** (This can be likened to the alarm clock waking you in the morning. There is no guarantee that it will result in you actually getting out of bed!)
 - It is the process of breaking dormancy of the spore.
 - It is reversible.
 - Processes include heat shocking and exposure to specific chemicals.
- **Germination.** (The alarm clock has woken you and you can't get back to sleep, so you might as well get out of bed.)
 - The process is irreversible and is the change from a dormant spore to a metabolically active cell.
 - Germination is associated with a number of events:
 - loss of heat resistance;
 - release of DPA;
 - decrease in optical density;
 - loss of refractility.
- **Outgrowth** (now you're up and about and fully active).
 - Development of a vegetative cell from a germinated spore.

Acknowledgement

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