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Pauline Lowrie and Susan Wells
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CHAPTER 1

Microbiology

By the end of this chapter you should be able to:

1. describe the distinguishing features of Prokaryotae, Protoctista, Fungi and viruses;
2. describe the general structure of viruses;
3. describe the life cycles of the lysogenic bacteriophage λ, and the Human Immunodeficiency Virus (HIV);
4. describe the organisation of the genetic material inside bacterial cells and viruses;
5. describe the structure and asexual reproduction of *Escherichia coli*;
6. describe the differences in bacterial cell wall structure that are the basis of the Gram staining technique.

The main purpose of this book is to give you an understanding of some of the many ways in which microbiology and biotechnology affect our lives. It is also important that you should understand why microorganisms are particularly suitable for use in industrial processes. However, before you can properly understand all these things, it is necessary that you learn a little about the structure and function of the different kinds of microorganisms.

Microorganisms (microbes) are so small that they can only be seen individually with a good quality light microscope. **Microbiology** is the study of microorganisms and can be sub-divided into several specialist branches such as **bacteriology** (the study of bacteria), **mycology** (the study of fungi) and **virology** (the study of viruses). There are four major groups of microorganisms: Prokaryotae, Protoctista, Fungi and viruses.

All prokaryotic organisms are grouped into the kingdom Prokaryotae. Prokaryotic cells do not have a true, membrane-bound nucleus and organelles, nor flagella with the typical 9+2 arrangement of microtubules such as are found in the eukaryotes (see *Biology 1* in this series). Organisms in the kingdoms Fungi and Protoctista are all eukaryotic. Viruses do not fit into a classification of living organisms because they are dependent on other cells for their reproduction.

Kingdom Prokaryotae

This kingdom contains all the prokaryotic organisms, that is the bacteria and cyanobacteria (the blue-green bacteria, formerly called blue-green algae).

Bacteria

Examples: *Escherichia coli*, *Streptococcus lactis*, *Bacillus subtilis*, *Staphylococcus aureus*

Bacteria are found in a wide range of habitats: in soil, air, water, as well as in or on the surface of animals and plants. They range in size from 0.1–10 μm in length and are usually found in enormous numbers: one gram of soil may contain 100 million bacteria. Some bacteria have an optimum temperature for growth which is greater than 45 °C and are called thermophilic bacteria; some can even thrive in hot volcanic springs at around 70 °C. Psychrophilic bacteria, on the other hand, grow best at temperatures below 20 °C and can withstand long periods of freezing. Bacteria are important because they help to decay and recycle organic waste. Some cause disease, but most are harmless and many are of increasing economic importance in biotechnology.

Structure of bacteria

Figure 1.1 shows a variety of shapes of bacteria and *figure 1.2* shows the structure of a generalised bacterial cell. *Table 1.1* gives more details of their structure. Bacteria have no nucleus. Instead, they have a circular piece of double-stranded DNA which is often referred to as the bacterial chromosome. It is different from eukaryotic DNA because it is naked, i.e. it is not complexed with protein.

SAQ 1.1

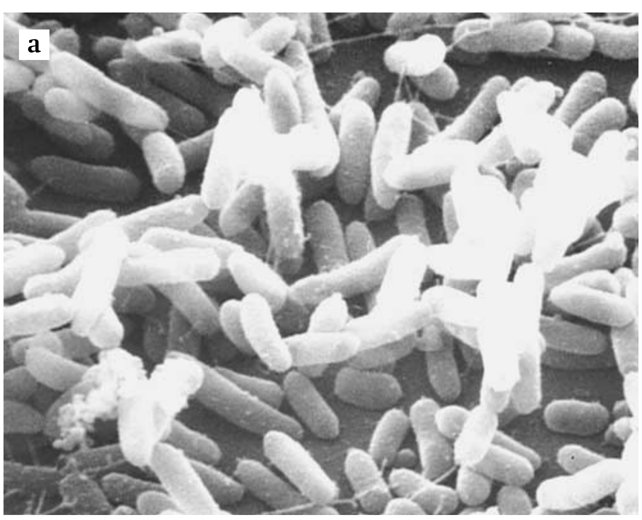
Give two features present in the generalised bacterium which are not present in *E. coli*.

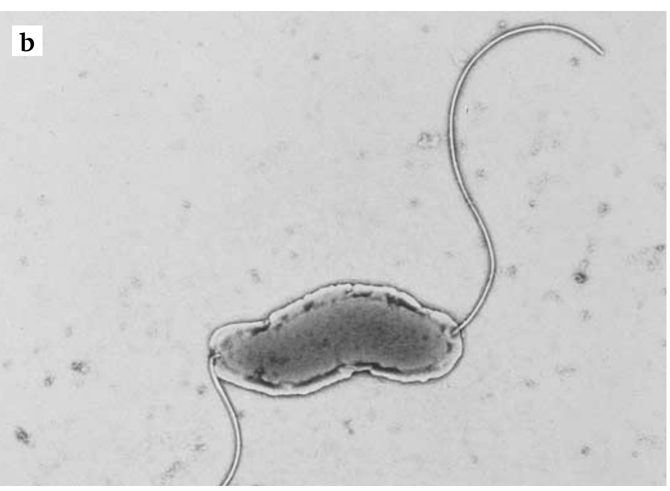
The bacterial cell wall

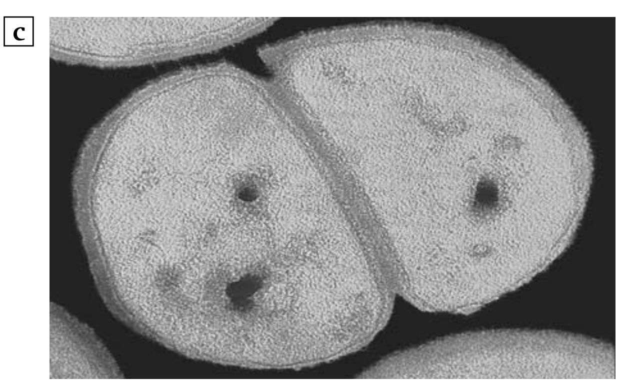
In 1884 Christian Gram developed a way of staining bacteria which divided them into two groups. These were called Gram-positive and Gram-negative. It is now known that bacteria have two different types of cell wall which the staining technique reveals.

The technique developed by Gram is still commonly used today. It involves heat-fixing a smear of bacteria to a clean microscope slide and then flooding it with crystal violet. All bacteria take up this stain. The smear is washed with Gram's iodine to fix the stain and then decolourised with alcohol or propanone. Gram-positive bacteria retain the crystal violet/iodine complex appearing purple, but Gram-negative bacteria do not. Finally the smear is counter-stained with a red stain such as safranin or carbol fuchsin. Gram-negative bacteria take up this stain and become red. Gram-positive bacteria stay purple.

The different reaction to the stain is due to the structure of the two basic types of cell wall (*figure 1.3*). Gram-positive bacteria have a plasma membrane surrounded by a rigid cell wall about 20–80 nm thick. This rigid layer is made of a peptidoglycan, (a polymer of sugars and amino acids) **murein**, which has a complex three-dimensional structure. Gram-negative bacteria also have a rigid layer outside the surface membrane but it is much thinner, only 2–3 nm thick. On the outside of this is an outer membrane which contains lipopolysaccharides instead of phospholipids. This





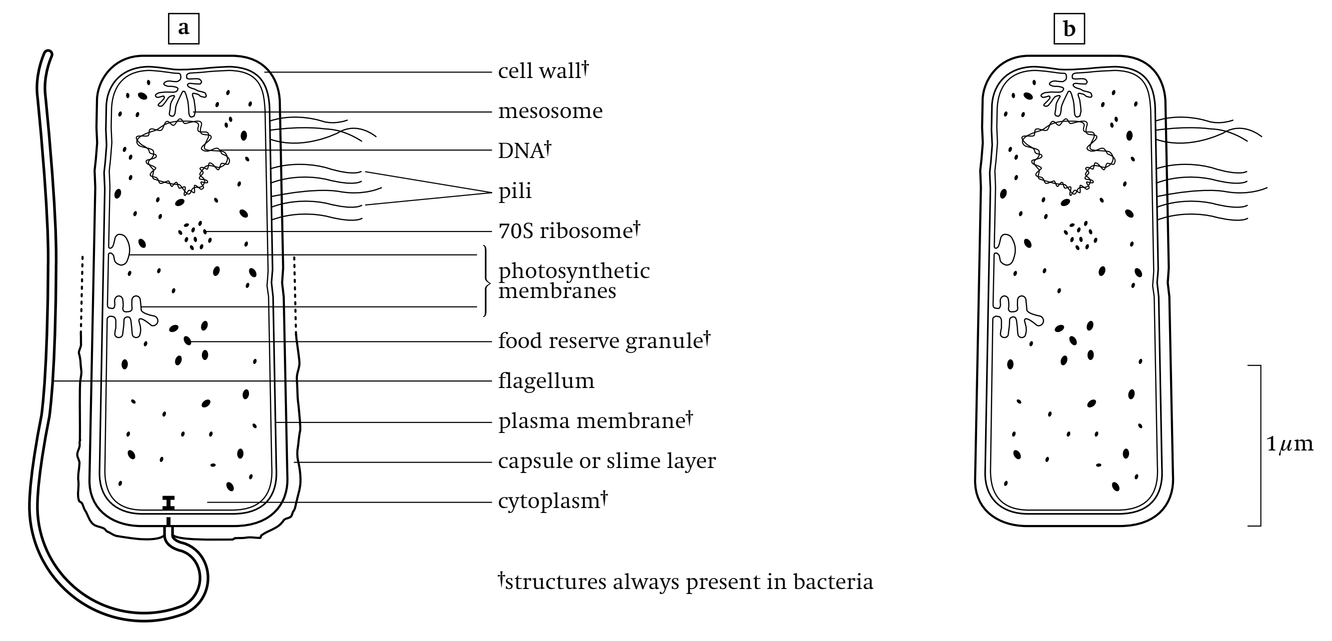


● **Figure 1.1** Some examples of bacteria.
a Scanning electron micrograph (SEM) of the rod-shaped Gram-negative bacterium *Escherichia coli* (× 14 500).
b Transmission EM of *Campylobacter jejunii* which causes food poisoning in humans. The bacterium moves by means of two single terminal flagella (× 9000).
c Transmission EM of *Staphylococcus aureus*. The cell has just divided (× 62 000).

Structure	Features
†cell wall	10–100 nm thick, net-like, multilayered structure made of peptidoglycans (polymers of sugars and amino acids); two distinct types are distinguished by the Gram's stain: **Gram-positive**; thicker, rigid murein network filled with peptides and polysaccharides, e.g. *Lactobacillus* **Gram-negative**: thinner, more complex, made of murein coated with a smooth layer of lipids, e.g. *E. coli*
†plasma membrane	phospholipid bilayer with proteins floating in membrane; proteins include enzymes involved in respiration†, photosynthesis•, nucleic acid synthesis† and electron transport†
†ribosomes	small 70S ribosomes are the site of protein synthesis
†cytoplasm	uniform with few organelles; contains storage granules, ribosomes, plasmids; a nuclear region is usually present
•plasmid	a small circular piece of DNA which is present in some bacteria, containing genes additional to those in the chromosome; some bacteria contain more than one
•flagellum	used in the locomotion of many motile bacteria; a rigid, hollow cylinder of protein, the base of which rotates propelling the cell along, e.g. *Rhizobium*, *Campylobacter* and *Azotobacter*
•capsule (slime layer)	an outer protective layer visible with negative (background) staining; it protects against chemicals and desiccation, stores waste products and protects the bacterium from attack by phagocytic cells; it helps bacteria to form colonies and is important in soil bacteria where it helps bind soil particles into crumbs, e.g. *Azotobacter*
•photosynthetic membranes	sac-like or tubular infoldings of the plasma membrane provide a large surface area for the inclusion of bacteriochlorophyll and other photosynthetic pigments in photoautotrophic bacteria, e.g. *Chromatium*
•mesosomes	tightly folded infoldings of the plasma membrane, that may be the site of respiration and involved in cell division and the uptake of DNA; they might be an artifact of preparation for electron microscopy
•pili (fimbriae)	numerous projections from the plasma membrane through the cell wall found all over some bacterial cells; they are used in conjugation to bind cells together and exchange genetic material; they also have an antigenic effect, e.g. *Salmonella*
•endospores	a hard outer covering forms a resistant endospore which ensures survival in severe conditions of drought, toxic chemicals and extremes of temperature, e.g. *Bacillus anthracis* spores, which cause the disease anthrax, are known to be viable after 50 years in the soil

†always present, •sometimes present

● **Table 1.1** Summary of the structure of bacteria.



● **Figure 1.2** **a** A generalised rod-shaped bacterial cell. **b** *Escherichia coli*.

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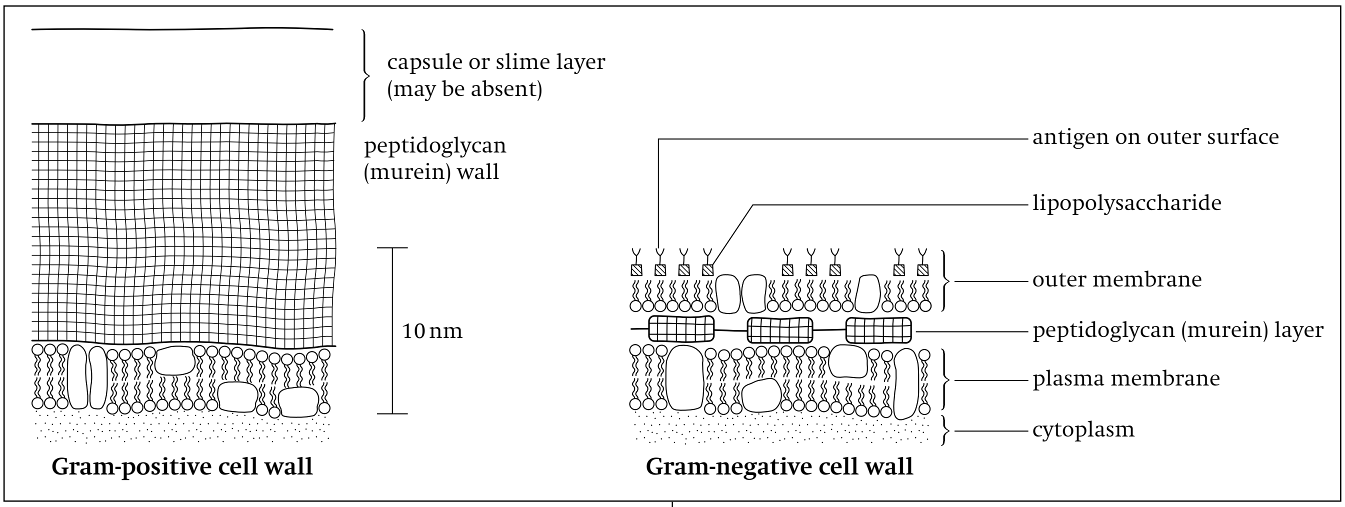


● **Figure 1.3** The structure of bacterial cell walls based on electron micrographs of the cell walls of *Bacillus* (Gram-positive) and *E. coli* (Gram-negative).

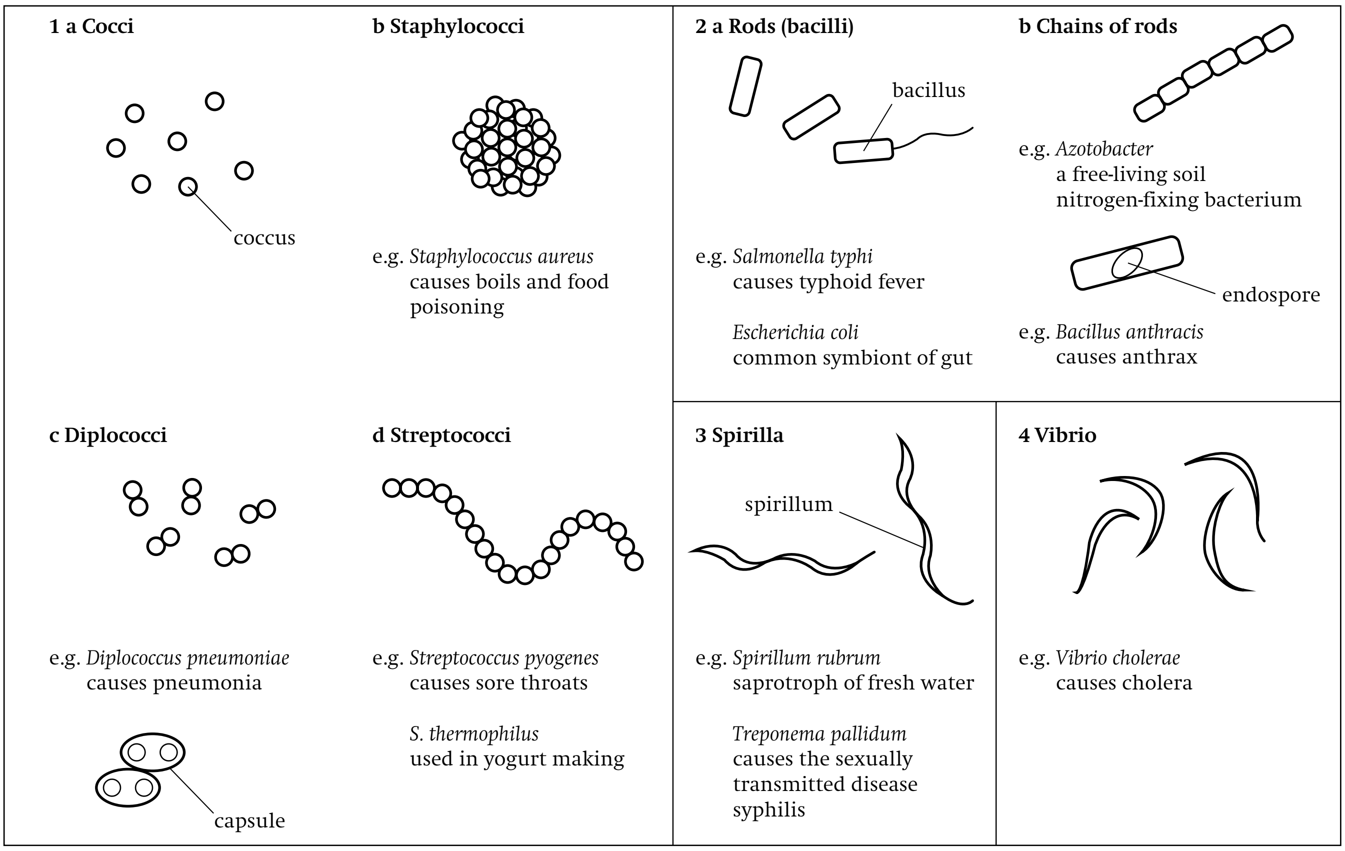
forms an extra physical barrier to substances, such as antibiotics and enzymes like lysozyme, which normally destroy or inhibit bacteria. The crystal violet/iodine complex is a large molecule and it is thought that, during Gram staining, it becomes trapped inside the Gram-positive cell wall, whereas it is more easily washed out of the thinner Gram-negative cell wall.

Shapes of bacteria

When viewed with a microscope, bacteria show several distinct shapes and these may be used to help in identification (*figure 1.4*).

Reproduction

Bacteria grow very quickly in favourable conditions. The generation time may be as little as 20 minutes, though for many species it is 15–20 hours. Division is usually by binary fission (*figure 1.5*).



● **Figure 1.4** The forms (shapes) of bacteria.

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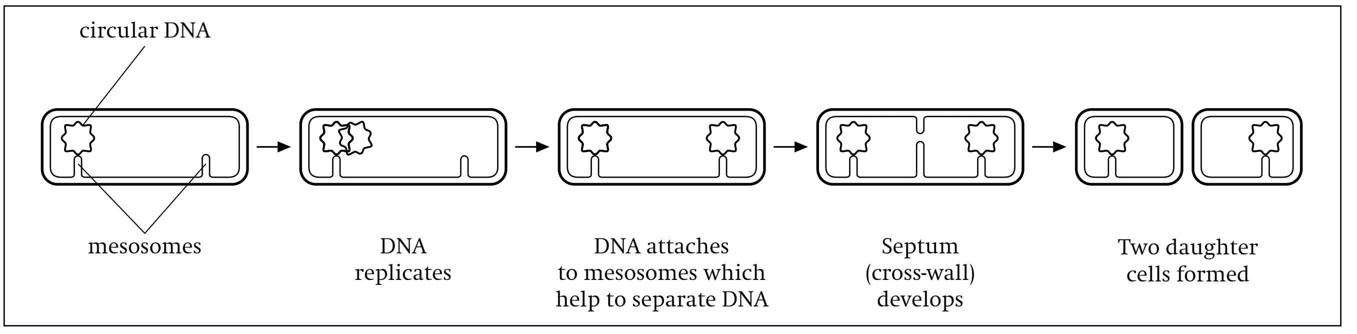


● **Figure 1.5** Binary fission in *Escherichia coli*.

1 The circular bacterial chromosome divides but there is no mitotic spindle. The chromosome attaches itself to the plasma membrane or, in some cases, to the mesosome.
2 A septum starts to be synthesised to divide the cell. This often starts growing where there are mesosomes.
3 The septum grows right across the cell, dividing it into two daughter cells.

Plasmids

The cytoplasm of certain bacteria contains one or more small circles of DNA called plasmids which are able to replicate independently of the main circular chromosome. Plasmids are known to carry genes which may help the bacterium to survive in adverse conditions. For example, plasmids known as R-factors cause resistance to antibiotics, virus infection and ultraviolet radiation. Plasmids can be transferred to another bacterium in conjugation, transformation or transduction.

Conjugation, transformation and transduction

Some bacteria have 'mating' cells which come together and are joined by their pili in a process known as **conjugation** (*figure 1.6*). The donor passes a plasmid called the F-factor, or fertility factor, to the recipient. Genetic information on the F-factor provides the bacterial cell with everything needed to be a donor, including the capacity to synthesise the sex pilus. The F-factor may exist as a free element within the cytoplasm, replicating independently of the bacterial chromosome, or it may become incorporated within the bacterial chromosome and be replicated whenever the chromosome replicates.

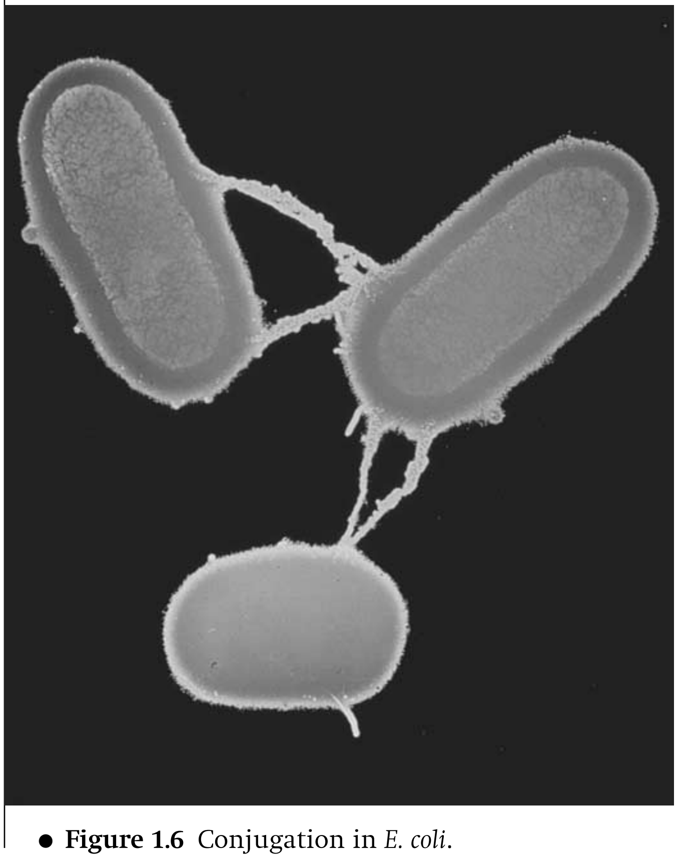
Transformation occurs when one bacterium releases DNA which is absorbed by a second bacterium. The second cell therefore acquires new characteristics.

Transduction is where new genes are inserted into the chromosome of a bacterium by a bacteriophage virus (see page 17).

Conjugation, transformation and transduction are not forms of sexual reproduction since fertilisation does not occur. In each of the above cases, DNA has been transferred from a donor to a recipient.

The economic importance of bacteria

Some genera of bacteria contain species which are commercially useful and some which are harmful to humans. For example, most *Bacillus* spp. live in



● **Figure 1.6** Conjugation in *E. coli*.

the soil, and are either aerobes or facultative anaerobes (see pages 34–35). *Bacillus subtilis* is a strict aerobe which is used in industry as a source of enzymes such as amylases. *Bacillus thuringiensis* has become important in genetic engineering because it causes a paralytic disease in many caterpillars and has been used to produce insect-resistant plants (page 56). However, some species of *Bacillus* are pathogenic, such as *Bacillus anthracis* which causes the disease anthrax.

Table 1.2 lists some bacteria which are commercially useful. Some of these are described in more detail in later chapters. Other bacteria are useful in different ways, for example in recycling nutrients and fixing nitrogen in ecological cycles. As discussed in *Biology 1*, chapter 7, *Rhizobium* is a nitrogen-fixing bacterium which is present in the soil. It invades the root hairs of leguminous plants, causing the cells to divide and form nitrogen-fixing nodules. Many species of *Clostridium* can also fix nitrogen, such as *C. welchii*, *C. pastorianum* and *C. butylicum*, and are important in the nitrogen cycle. (Also see page 7.)

Table 1.3 lists some bacteria which cause disease in humans. Some belong to genera which contain many non-pathogenic species, for example most species of *Staphylococcus* are facultative anaerobes which are found in the normal microflora of the skin. *S. aureus* normally causes boils. However, one strain of this species (called MRSA, methicillin resistant *Staphylococcus aureus*) has developed resistance to most antibiotics, through their overuse, and has become a major problem in hospitals in many countries. MRSA can cause severe blood-poisoning (septicaemia) when it infects wounds, e.g. after sugery. This is difficult to treat and can be fatal.

Name	Gram stain	Form (shape)	Use
Lactobacillus bulgaricus	+ve	rods	yogurt
Streptococcus thermophilus	+ve	filamentous	yogurt
Streptococcus lactus	+ve	cocci	cheese
Streptococcus cremoris	+ve	cocci	cheese
Methylophilus methylotropus	variable	cocci	methane, methanol
Clostridium acetobutylicum	+ve	rods	propanone (acetone)
			and butanol
Leuconostoc mesenteroides	+ve	cocci	dextran
*Bacillus subtilis**	+ve	rods	enzymes
Streptomyces spp.	+ve	filamentous	antibiotics
*Escherichia coli**	−ve	rods	insulin, growth hormone, interferon
Pseudomonas denitrificans	−ve	rods	vitamin B_{12}

* using genetic engineering

● **Table 1.2** Some useful bacteria.

Name	Gram stain	Form (shape)	Disease
Staphylococcus aureus	+ve	cocci	boils
Salmonella typhimurium	−ve	rods	food poisoning
Salmonella typhi	−ve	rods	typhoid fever
Mycobacterium tuberculosis	variable	fine rods	tuberculosis
Bordetella pertussis	−ve	very short rods	whooping cough
Neisseria gonorrhoea	−ve	cocci	gonorrhoea
Treponema pallidum	variable	long spirals	syphilis
Vibrio cholerae	−ve	curved rods	cholera
Clostridium tetani	+ve	rods	tetanus
Clostridium botulinum	+ve	rods	botulism
Corynebacterium diphtheriae	+ve	short rods	diphtheria
Listeria spp.	+ve	round-ended rods	listeriosis
Shigella sonnei	−ve	rods	dysentery
Yersinia pestis	−ve	small rods	plague

● **Table 1.3** Some harmful bacteria.

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SAQ 1.2

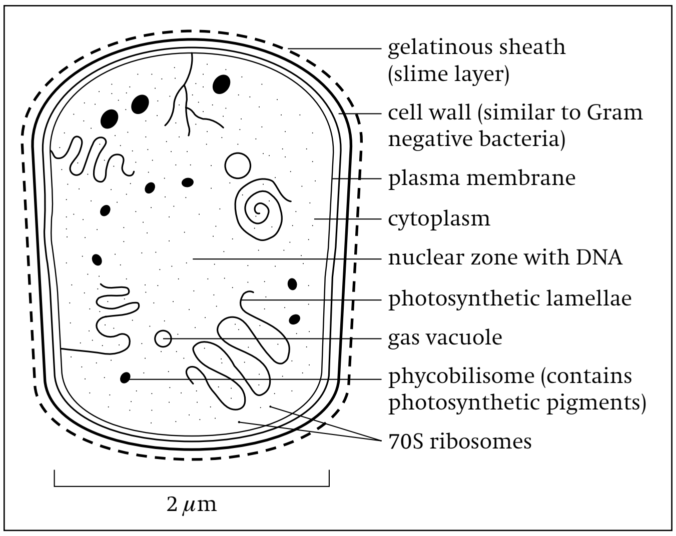
Match the words on the left to the definitions on the right.

plasmid	slimy layer surrounding some bacterial cell walls
mesosome	circular piece of DNA not joined to chromosome
cell wall	made of pilin, used in conjugation in some species
capsule	the main component of this is the peptidoglycan murein
pilus	infolding of membrane, probably used in cell division

Cyanobacteria (blue-green bacteria)

Examples: *Anabaena cylindrica*, *Nostoc muscorum*, *Spirulina platensis*

Cyanobacteria are prokaryotic microorganisms similar to the true bacteria. They are photosynthetic but are not true algae because they do not have membrane-bound nuclei, and are considered to be very ancient life-forms. They have been found in fossil remains from over three billion years ago and may have been some of the first living organisms to evolve on Earth. They are found in the surface layer of fresh and sea water. On land they will grow wherever there is both light and moisture and are found as slime on the surface of mud, rocks, wood and on some living organisms, such as the sloth. Their name comes from the photosynthetic pigments which give them a distinct dark greenish-blue colour.



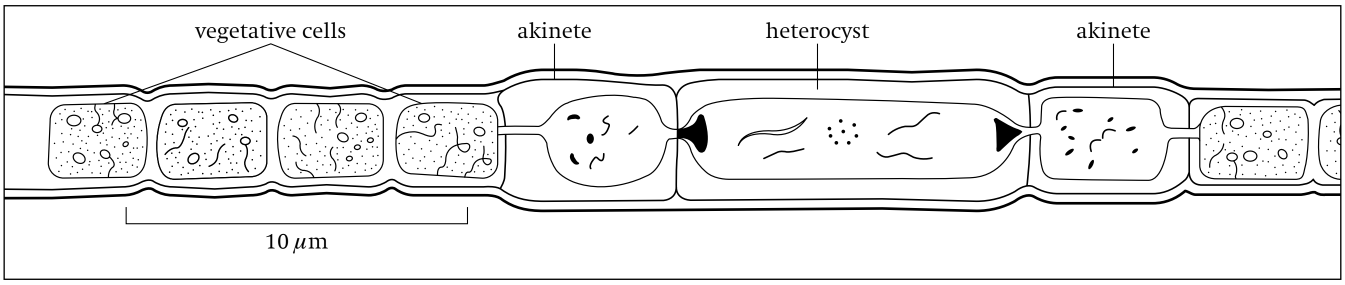
- **Figure 1.7** Diagram of a typical blue-green cell (based on electron micrographs).

Structure of cyanobacteria

Blue-greens have a typical prokaryotic cell structure since they have a naked coil of DNA and no true nucleus (*figure 1.7*). The cell wall is similar in structure and composition to that of Gram-negative bacteria. Protein synthesis takes place on 70S ribosomes in the cytoplasm. Blue-greens are photosynthetic. They have chlorophyll and carotenoid pigments incorporated into infoldings of the plasma membrane, called **lamellae**. They also have photosynthetic pigments, such as phycocyanin and phycoerythrin, which are present in phycobilisomes. These give the cells their distinctive colouration. The cells may occur singly or in colonies, but members of a colony remain independent.

Nitrogen fixation

Only a very few organisms are capable of fixing atmospheric nitrogen by reducing it to ammonia and combining it with organic acids to produce amino acids and proteins. Nitrogen-fixing bacteria can do this and so can some blue-greens. Cells able to fix nitrogen contain the enzyme nitrogenase. This enzyme is inactivated by oxygen and so conditions inside the nitrogen-fixing cell have to be anaerobic. Some blue-greens, such as *Anabaena*, have special thick-walled cells called **heterocysts**.



- **Figure 1.8** A filament of *Anabaena*.

Anabaena has filaments made up of many normal photosynthetic cells that produce sugars and oxygen. Scattered along the filaments are a few distinct heterocyst cells that are able to fix atmospheric nitrogen in this way (*figure 1.8*).

Many filamentous blue-greens are also able to produce **akinetes**, or spores. These are able to survive adverse conditions, such as a period of overpopulation known as an algal 'bloom', and seem to develop from a vegetative cell near to a heterocyst. The cell increases in size and accumulates large food reserves. Photosynthesis within the akinete is reduced and gas vacuoles disappear. This means that the akinete slowly sinks to the bottom of the water. It may survive for several years, and will germinate as soon as conditions become favourable.

The economic importance of blue-greens

Spirulina platensis is a filamentous blue-green found naturally in shallow alkaline lakes in parts of Africa and South America. For thousands of years it has been collected and dried by the local people and used as a food. It is often fried or put in soups and sauces; it is also used as cattle food. *Nostoc* is another blue-green which is used as a food in Peru and in South-East Asia.

In agriculture, nitrogen-fixing blue-greens may be used as organic fertilisers. They are grown on a large scale in China, India, Indonesia and the Philippines, particularly where rice is cultivated in paddy fields. The water may be seeded with a starter culture of blue-greens at the beginning of the growing season. This method has been shown to increase the yield of rice by 15–20%.

Research is taking place into the use of blue-greens in a solar energy conversion system. As you have just learnt, *Anabaena cylindrica* has heterocysts to fix nitrogen and is also able to give off oxygen by photosynthesis in the vegetative cells. In the absence of atmospheric nitrogen it gives off hydrogen by nitrogenase-catalysed electron transfer to H^+ ions in the heterocysts. Both oxygen and hydrogen are in demand industrially.

SAQ 1.3

Why are blue-greens classified as prokaryotes?

SAQ 1.4

It is thought that blue-greens may have been the first photosynthetic organisms on Earth and that they represent a very early stage in the evolution of life. Give as many reasons as you can why this might be so.

Kingdom Protoctista

This kingdom has been created to contain all groups of eukaryotic organisms which are neither animals, plants, fungi nor prokaryotes. These groups are not really related though they do have some similarities. They include all protozoa, all nucleated algae and the slime moulds (*figure 1.9* and *table 1.4*).

The **protozoa** is a collective term for the phyla Rhizopoda, Zoomastigina, Apicomplexa and Ciliophora. They are found wherever moisture is present, in sea water, fresh water and soil. There are commensal, symbiotic and parasitic species in addition to many free-living types. Protozoa are eukaryotic. The nucleus has a **nuclear envelope**, and movement is by means of a variety of locomotory structures such as flagella, cilia or **pseudopodia**. Since the cytoplasm of freshwater protozoans is usually hypertonic to (more concentrated than) the aqueous environment, they take in water by osmosis. To counteract this, they have **contractile vacuoles** that act as pumps to remove excess water from the cytoplasm. However, contractile vacuoles may also be found in some marine protozoans. All types of nutrition are found in protozoans: some are autotrophic, others are saprotrophic and many are heterotrophic. Digestion of food takes place in **food vacuoles** in the cytoplasm. Gas exchange is by diffusion across the plasma membrane. Waste products from cell metabolism diffuse out of the cell. The main nitrogenous waste is ammonia.

The economic importance of protozoa

Many ciliates are saprotrophs and are vital in the recycling of organic wastes, particularly in sewage treatment. Parasitic forms, such as *Entamoeba*, which causes amoebic dysentery, and *Plasmodium*, which causes malaria, may cause loss of life.

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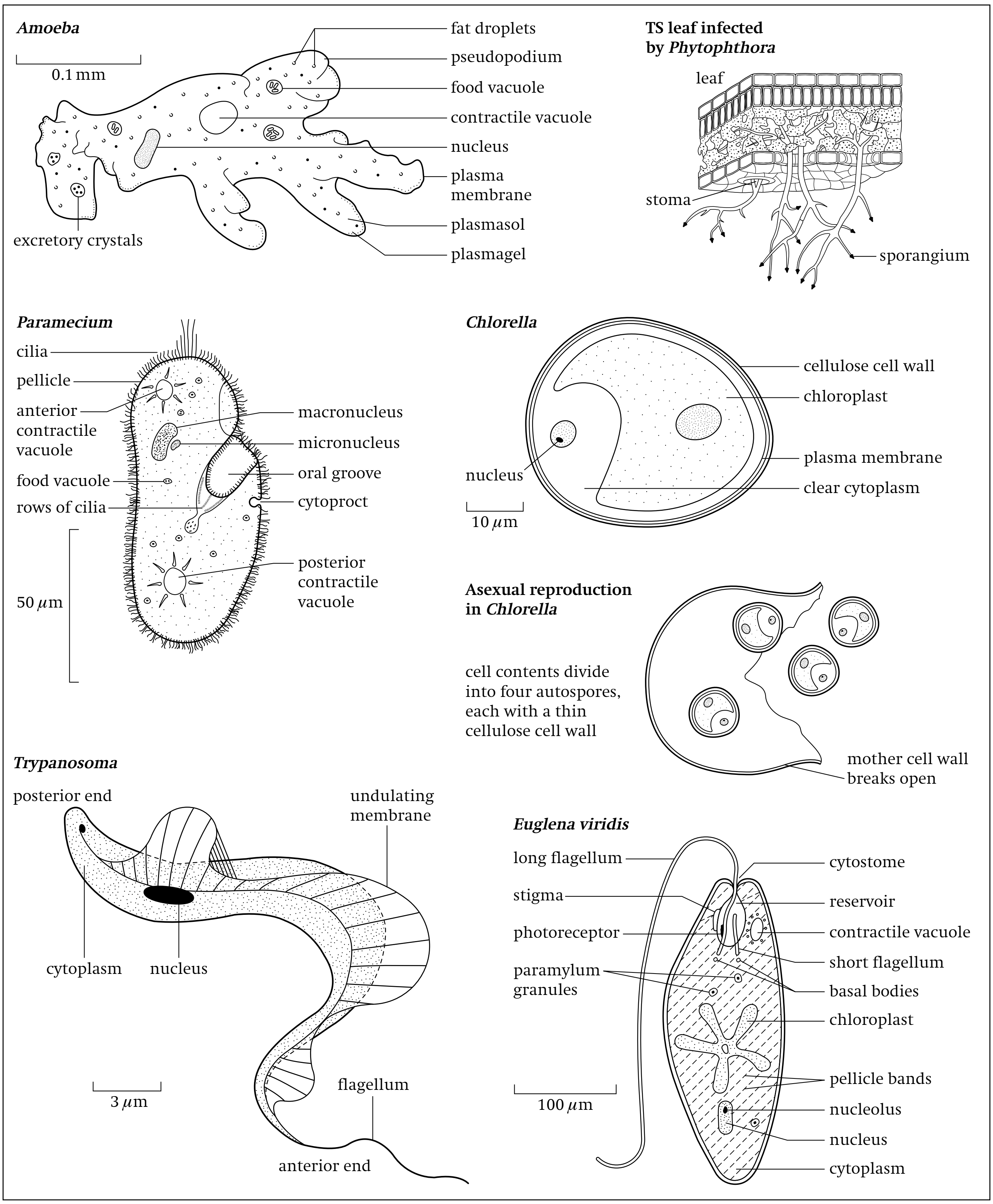
Malaria, for example, can be devastating to the economy of developing countries, incapacitating millions of workers every year (*Biology 1*, chapter 15).

SAQ 1.5

List three features shared by the four phyla classed as protozoa, and two features which distinguish them from each other.



● **Figure 1.9** The structure of some representative protoctists.

Phylum	Example	Structure	Locomotion	Nutrition	Reproduction	Importance
Rhizopoda	*Amoeba proteus*	0.5 mm long, single cell, cytoplasm exists in two states: plasmasol and plasmagel	pseudopodia	phagocytosis and food vacuoles formed	asexual by binary fission	*Entamoeba histolytica* causes amoebic dysentery
Apicomplexa (sporozoans)	*Plasmodium vivax*	10 µm long, different forms during life cycle: sporozoites long and thin, infective particles; merozoites smaller, pear-shaped	microtubules which slide over each other to penetrate host tissue	phagocytosis	asexual by multiple fission and sexual by gametocytes	*P. vivax* causes parasitic disease malaria, mosquito is the vector
Ciliophora (ciliates)	*Paramecium caudatum*	100 µm long, enclosed by a pellicle with cilia, oral groove leads to 'mouth' with cytostome	cilia beat together in coordinated way	cilia beat and sweep food towards cytostome, where phagocytosis takes place	asexual by binary fission, sexual by conjugation	live in fresh water, important in sewage treatment and decomposition
Zoomastigina (flagellates)	*Trypanosoma gambiense*	unicellular, elongated spindle-shaped.	single flagellum bound to the trypanosome along the length of the cell by the undulating membrane, free beyond that; wave-like movements propel the cell through the viscous blood plasma	heterotrophic, parasitic; lives in human blood plasma; soluble nutrients diffuse into cell	longitudinal binary fission in the blood of the host starting at anterior end	causes trypanosomiasis or 'sleeping sickness'; spread by the tsetse fly as it feeds on human blood; symptoms include headache, anaemia, inflammation of brain and spinal cord
Euglenophyta (euglenoid flagellates)	*Euglena viridis*	300 µm long slender cell, pellicle covers outside, blunt anterior end with reservoir	two flagella arise from base of reservoir, light-sensitive stigma used to orientate movement	chloroplasts present, therefore photoauto-trophic; stores food granules of paramylum, needs vitamins B_1 and B_{12} for growth	longitudinal binary fission (asexual)	found in fresh and salt water, and damp soil

● **Table 1.4** A summary of the Kingdom Protoctista (continued opposite).