1 The Background

1.1 Meet the cast

The main point about microbes is that they are very small. Their one unifying feature is that they are too small to be seen without the aid of a microscope – although even that definition, as we will see later on, is blurred at the edges, as some of the 'microbes' I will consider are actually quite big. Within the basic definition, there is a substantial range of diversity. You will probably have heard of some of these microbes, such as the influenza virus or the bacterium known as MRSA, as they regularly make the news because they inflict themselves on us. Others may also be familiar because of their everyday role in fermentation – think of the yeasts that are needed for the production of bread, wine and beer, and the 'friendly' bacteria that are used for yogurt making. As we will see later on, there are very many more examples of a wide range of microbes that are of direct importance to us, both in disease (and health) and economically.

But this is only the tip of an enormous iceberg. Microbes are all around us, in vast numbers and diversity, especially in soil and water. It has been estimated that the world has 10^{31} bacteria – that's 1 with 31 zeros after it – with a total biomass greater than all the plants and animals combined (and that is just the bacteria, before we add in the other microbes – viruses, fungi, algae and protozoa). They play a massive role in shaping our environment, including fixing carbon and nitrogen from the air – and, by degrading organic matter, in releasing these elements again into the air. Our knowledge of the diversity of microbes in the environment has increased enormously in recent years. Molecular techniques that we will encounter in a later chapter have shown that most (perhaps 99 per cent) of these organisms were previously totally unknown and have never been grown in the laboratory.

We can begin the story in the 17th century, in Holland. Antonie van Leeuwenhoek was born in Delft, in 1632. After an apprenticeship with a cloth merchant, he set up his own drapery business and became a prosperous and influential citizen of Delft.

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Having seen the magnifying glasses used by textile merchants for examining the cloth, he developed an interest in the use of lenses and started to make his own as a hobby. Although his 'microscopes' were simple by modern standards – consisting of just a single lens – the superb quality of his lenses, and his skill and patience in using them, enabled him to make many important observations. These included the first descriptions of microscopic single-celled organisms (which he called 'animalcules'), which he reported to the Royal Society in London in 1676.

These observations met with a considerable amount of scepticism but, eventually, after much further investigation, his achievements were recognized and he became a Fellow of the Royal Society in 1680. He continued to make many further detailed observations, such as the description of bacteria in plaque from teeth, until shortly before his death in 1723. Unfortunately, he kept secret some of the crucial details as to how he made his lenses, so, with his death, that part of the story came to an end.

However, others had also developed and used microscopes at around that time. Robert Hooke (1635–1703) is perhaps best known today for his study of the elasticity of materials, described by the mathematical relationship we still know as Hooke's Law. But that was far from the total of his interests or achievements, which ranged from experimental science to architecture. The part of his work we are concerned with here was his role in the development of the compound microscope (which, like a modern microscope, contained two lenses rather than the single lens used by van Leeuwenhoek).

He used this microscope to make a large series of observations of diverse biological materials, which was published in 1665 as a book, *Micrographia*. Notably, his description of the microscopic structure of slices of cork was the first identification of the cellular structure of, in this case, plant material (he coined the word 'cell' for them because of their resemblance to cells in a monastery). Although the microscopes used by Hooke (and other similar ones of that period) were more like a microscope of today than van Leeuwenhoek's single lens instruments, the technical difficulty of making them, and the superb craftsmanship of van Leeuwenhoek, meant that they were actually inferior to van Leeuwenhoek's.

It's now time we met the cast so, for the first members, let's consider viruses. These are so different from other microbes that it is only a matter of convenience that we do include them as 'microbes'. Indeed, it is questionable as to whether we should consider them as 'living' at all (I'll come back to that question in Chapter 10).

Viruses are not able to replicate, or to do anything at all, outside a host cell. The simplest viruses consist just of a nucleic acid molecule (which can be RNA or DNA, but not both), surrounded by a protein coat. These contain a limited number of genes. For example, one of the most basic viruses, called MS2, which infects *E. coli*, has just three genes: one codes for the coat protein, one is needed for copying the viral genome (RNA in this case) and the third is used to organize the assembly of the virus particle.

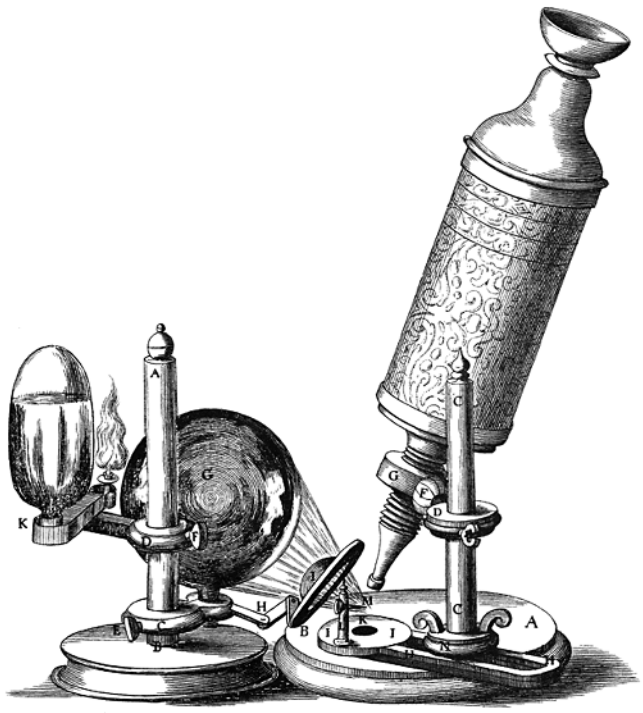

Figure 1.1 Robert Hooke's microscope. Drawing from 'Micrographia'.

However virus structures, and sizes, are quite diverse. Many, including some important human pathogens, are much larger than MS2 and have complex structures including, in some cases, a lipid coat. But virtually all viruses are so small that the human eye cannot see them, even with the aid of a light microscope –an electron microscope is needed to 'see' them. There are, however, some that are larger. The largest known viruses, such as the mimivirus which infects protozoa, are similar in size to some of the smallest bacteria, and they have a genome size to match. But even the largest and most complex viruses are completely unable to replicate without infecting a suitable host cell.

At several points within this book we will consider viruses that infect bacterial cells. These are called *bacteriophages*, or just *phages* for short (the word 'phage' being derived from the Greek '*phagein*', meaning 'to eat'). These are very widespread in nature and they can be of real practical significance – a phage infecting a bacterium that is used, for example, in the production of yoghurt can cause serious economic losses. But they have also played a major role in scientific research. Much of our knowledge of how genes work comes originally from studies of phages, where the simplicity of their structure, and the ease with which they can be grown and manipulated, made them invaluable models.

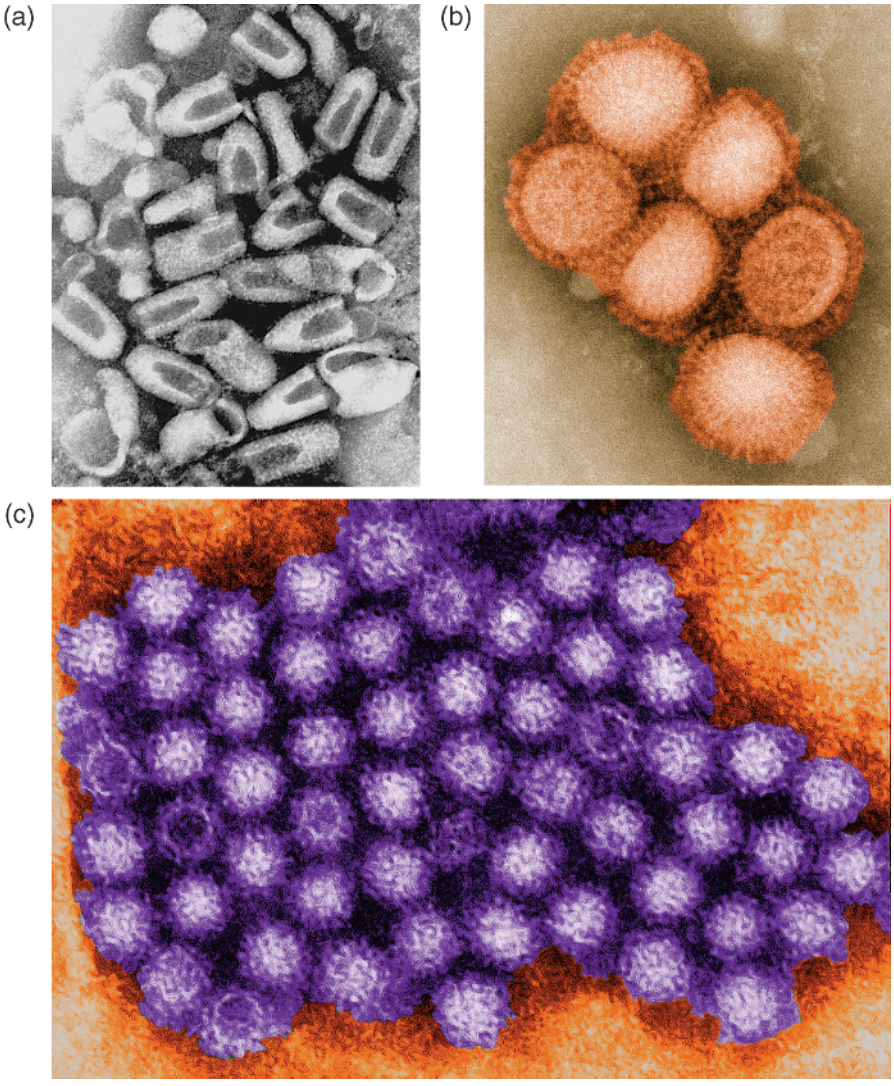

Figure 1.2 Electron micrographs of virus particles. (a) Rabies virus (courtesy of Frederick A Murphy http://www.utmb.edu/virusimages/); (b) influenza virus (colourized) (content provider(s): CDC/C. S. Goldsmith and A. Balish); (c) norovirus (colourized) (content provider(s): CDC/Charles D. Humphrey).

When a phage infects a bacterial cell, its nucleic acid (DNA or RNA, depending on the phage) is injected into the cell. Some of its genes are then recognized by the cell's machinery, which obligingly makes the relevant proteins that those genes code for. These proteins then divert the cell's activity away from its own genes and towards the production of many copies of the phage nucleic acid. At some point in this process, the DNA of the host cell is usually broken down and the bits are used for making the nucleic acid of the virus. The proteins that make up the external

structure of the phage (the coat) are then produced, and the nucleic acid is packed into that structure. The consequence of this is the lysis of the bacterial cell and the liberation of hundreds or thousands of copies of the virus. The whole process, from infection to lysis may take perhaps 20–50 minutes (depending on the phage). The details of this process vary considerably from one phage to another, but the general principles are similar. An equivalent, but more complex, process occurs when viruses infect higher (eukaryotic) cells, including human cells.

Although we usually think of viruses as causing diseases, this does not always happen. Some viruses have the ability to remain latent within an infected cell. We may only realize that they are there when the latency breaks down, perhaps due to a drop in our immune defences. This happens, for example, with the herpes virus that causes cold sores, typically around the lips, and the varicella-zoster virus, which initially causes chickenpox but can subsequently remain dormant until causing an outbreak of shingles many years later.

The best understood example of latency is a virus (bacteriophage), known as lambda, that infects *E. coli*. This has a very elegant mechanism for ensuring that, in a proportion of newly infected cells, the expression of all the virus genes is turned off, apart from one gene that codes for a protein that is responsible for maintaining this repression. In this state, known as lysogeny, the DNA of the virus is integrated into the chromosome of the host cell and is therefore copied, along with the rest of the DNA, as the bacterial chromosome is copied during growth. Each daughter cell therefore receives a copy of the virus DNA. Studies of genome sequences have revealed that most bacteria (and, indeed, animal cells, including our own) contain a number of copies of a variety of viruses, stably integrated into the chromosome and never showing any signs of their presence.

Our second class of microbes is the bacteria and, by way of introduction, I will look at one bacterium in particular: *Escherichia coli*, or *E. coli* for short (unfortunately, most bacteria – and many other microbes – do not have simple common names, so we have to get used to using Latin ones). This is described as a rod-shaped organism, but it is better to visualize it as a short cylinder with rounded ends. Later on we will encounter bacteria with other shapes, especially ones such as *Staphylococcus*, which are spherical, as well as bacteria which grow as filaments or in spiral shapes.

E. coli, which is a common inhabitant of the human gut (as well as being able to cause some nasty diseases), is 2–3 μm long (a μm, or micrometre, is a millionth of a metre, or a thousandth of a millimetre) and 0.5–1 μm wide. It is a favourite model organism for bacteriologists because it will grow readily in a simple medium – all it needs is a sugar such as glucose and a nitrogen source such as an ammonium salt. It will grow even better if it is given a richer medium containing, for example, yeast extract. In a rich medium, it will divide every 20 minutes or so (bacteria typically grow in an apparently simple way – a cell gets bigger until it reaches a certain size, then it divides into two cells, which in turn grow and then divide again – so they multiply by dividing!).

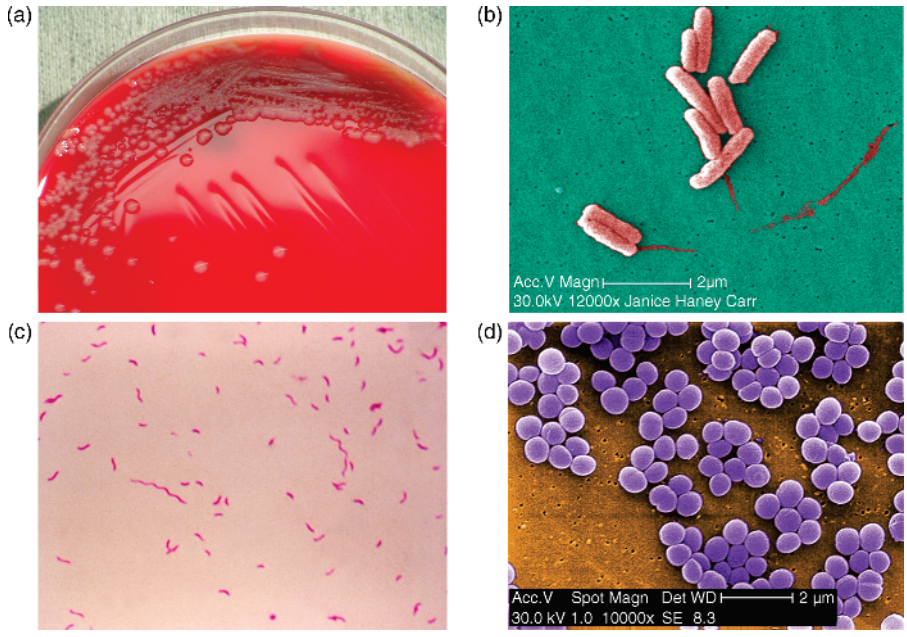

Figure 1.3 Bacteria. (a) Bacterial colonies on an agar plate (content provider(s): CDC/Amanda Moore, MT; Todd Parker, PhD; Audra Marsh); (b) Colourized electron micrograph of *Legionella pneumophila* (content provider(s): CDC/Margaret Williams, PhD; Claressa Lucas, PhD; Tatiana Travis, BS; Photo Credit: Janice Haney Carr); (c) Light microscopy of stained *Campylobacter* (content provider(s): CDC); (d) Colourized electron micrograph of *Staphylococcus aureus* (content provider(s): CDC/Matthew J. Arduino, DRPH; Photo Credit: Janice Haney Carr).

If we start with one cell, after 20 minutes there will be two, after 40 minutes four cells and, by one hour, eight cells. This is known as exponential, or logarithmic growth – it starts off slowly, but very soon reaches astronomical numbers. After ten divisions, there will be about one thousand cells; after another ten divisions, the number will reach a million; after a further ten divisions, it will be up to a thousand million cells.

When we get to such large numbers, they become very difficult to handle in the usual way, so we use what is known as scientific notation (see Appendix 2 for further explanation). A thousand, for example, is $(10 \times 10 \times 10)$ so we call it 10^3 rather than 1,000. A million (1,000,000) is 10^6, and a thousand million (1,000,000,000) is 10^9. So, after 30 cell divisions (about ten hours), we would have some 10^9 bacteria in our flask. This process does not continue indefinitely of course. After a while, the bacteria start to run out of nutrients (diffusion of oxygen into the medium is usually the first limiting factor for *E. coli*) and they will stop growing. For *E. coli*, this will usually happen when the concentration of bacteria reaches about 10^9 cells per millilitre. In other words, a 5 ml teaspoon would contain five billion, or five thousand million, bacteria.

One practical consequence of these massive numbers is worth a slight digression here. Disinfectant manufacturers will commonly make claims such as ‘kills

99 per cent of household germs'. This sounds impressive – until we consider the numbers involved. If we start with say 10^6 bacteria (which is not really very high), then killing 90 per cent (or leaving ten per cent remaining) will reduce the numbers to 10^5; even killing 90 per cent of those (which leaves one per cent of the original), there will still be 10^4 bacteria. So killing 99 per cent (or leaving one per cent untouched) merely reduces the numbers from 10^6 to 10^4 (which we refer to as a 2-log reduction). Even if we kill 99.9 per cent, we still have 10^3 bacteria. It is a useful effect, but not as dramatic as the original claim sounds.

When we grow a bacterium in a liquid medium (a liquid culture), it goes through several recognizable stages. When the culture is inoculated (that is, a relatively small number of bacteria are put into the broth), nothing much appears to happen for a while. This is the so-called *lag phase*. Essentially, the bacteria are getting used to the change from the resting state in which they have been stored, and they are responding to the availability of food by making all the various components needed for growth. Some genes (those needed for the resting stage) are switched off, while the genes needed for active growth are switched on. We'll look further at what is involved in these switches in Chapters 7 and 8.

When the cell is ready, it will start exponential growth. At the end of the log phase, when it runs out of food, the process is, in effect, reversed. The genes needed for active growth are switched off, and the cell enters *stationary* phase. This is not merely the absence of growth. A number of functions are necessary if the cell is to stay alive in stationary phase, so these genes have to be switched on.

Many bacteria, such as *E. coli*, survive quite well in stationary phase, but not all do. Some will start to die, presumably because they do not have the genes needed to keep the cell alive in the absence of growth. Later on, we will also encounter bacteria that are able to form specialized cells known as *spores*, some of which can survive almost indefinitely without any detectable metabolic activity. These dormant structures are extremely important in a practical sense, as they may be extremely resistant to heat and disinfection. Examples of spore-forming bacteria include the organisms responsible for tetanus and botulism (see Chapter 5).

The conventional way of identifying bacteria in a mixture – such as might be obtained from a clinical specimen such as a wound swab, or from an environmental sample – is to look at its biochemical properties. In other words, what chemicals it can grow on, what products it makes, and so on (just looking at them down a microscope usually doesn't tell us very much, although it can help). This means there is a need to purify individual bacteria from the mixture. This is easier than it sounds, provided the bacteria will grow in the lab.

Instead of putting them in a liquid culture (a 'broth'), we would use plastic dishes which are known as Petri dishes, after Julius Richard Petri (1852–1921), who invented them while working as an assistant to the more famous bacteriologist Robert Koch (1843–1910). Into these dishes, we put a medium which is made solid by adding agar (a jelly-like substance made from seaweeds). The bacteria do not move around on this; they just stay where they land.

If a dilute sample is spread on an agar plate, this will create a random pattern of isolated bacteria. They cannot be seen at this stage but, if the plate is incubated at an appropriate temperature, for 1–2 days for many bacteria, the bacteria will multiply. Since they cannot move, this will produce a small blob of bacteria, known as a colony, at the site where they started. If the bacteria were sufficiently well spread out initially, then each colony will have come from a single bacterial cell. An individual colony can thus be picked off and used to make as many cultures as are required. The result is a pure culture of a specific bacterium from the initial mixture, which may originally have contained a lot of different bacteria. This is actually 'cloning' in the original sense of the word – producing a population of identical individuals, all derived from a single bacterium by asexual reproduction.

Bacteria are often referred to as 'prokaryotes', which means that they do not have a nucleus or other compartments such as mitochondria within the cell (but see the section on Archaea later on). All of the reactions within the cell, including replication of the DNA, expression of the genes, and generation of the energy they need, take place within the cytoplasm of the cell. This should not be taken to mean that the cytoplasm is an amorphous soup – it does have structure, but it is quite subtle.

Other microbes have a cell structure that is much more like those of plants and animals; these are the eukaryotes. They have a nucleus, which contains the chromosomes carrying the genetic material, and mitochondria, which are the powerhouse of the cell in that they are largely responsible for energy generation. Some (especially plant cells) also have chloroplasts, which are responsible for photosynthesis. Mitochondria and chloroplasts are interesting, as they also contain DNA, as well as ribosomes (see below), which are responsible for protein synthesis. Thus, mitochondria and chloroplasts resemble organisms in their own right which have become adapted to an existence within the eukaryotic cell. Indeed, it is believed that this is how they originated (see Chapter 10).

The main groups of eukaryotic microbes include fungi, protozoa, and algae. Including fungi may seem surprising, as fungi are familiar to all of us as mushrooms and toadstools. One fungus, a specimen of *Armillaria* that occupies about 1,000 hectares (10 sq km) in Oregon, USA, is often referred to as the largest known living organism; its weight is estimated at over 600 tons (compared to the 200 tons of a blue whale). Similarly, algae include seaweeds.

Surely these are not 'microbes'? To answer this, it is necessary to look at the cellular structure of these organisms. Many fungi can exist either as single cells or as collections of many cells. Mushrooms normally grow in the soil as a network of filaments, called a mycelium, which is composed of many cells joined end to end. Whether we should think of this as a single organism or as a collection of individuals is debatable. In a true multicellular organism (such as ourselves), there is communication and interaction between individual cells, and also differentiation – each cell (or groups of cells) has a specific function and forms a specific structure. So we have a liver, heart, kidneys, and so on. In the mycelium, there is virtually no differentiation, and only a limited amount of communication. However, when

Figure 1.4 Fungi. (a) The mould *Penicillium multicolor*, growing on an agar plate (content provider(s): CDC/Dr. Lucille K. Georg); (b–d) miscellaneous fungi.

conditions are appropriate, differentiation (and communication) does occur, and the fungus produces fruiting bodies, which are the familiar mushrooms. Some cells produce the different parts of the stalk and the cap, and some produce the spores which enable the mushroom to propagate and spread.

It is worth noting that some bacteria – especially the *Streptomyces*, which are common in soil – also grow as a sort of mycelium and can produce spore-bearing structures, although on a much smaller scale. We will come across *Streptomyces* again, as they are the principal source of naturally occurring antibiotics. In a later chapter, we will also come across some other bacteria that show elements of communication and differentiation, and behave in a way that resembles that of a multicellular organism.

The real reason for including fungi as an example of a microbe is their ability to grow as dispersed single cells. Some, such as the yeasts used for baking and brewing, always grow like that, while many others can be grown as single cells in the laboratory. Similar considerations apply to the algae. However, if we apply this too literally, we have a further problem. Many plants can be grown in the laboratory as cultures of single cells (and subsequently induced to form intact plants). Some animal cells can also be grown in this way (although it is not usually possible to regenerate an intact

animal from them). Should we therefore also consider plants and animals as 'microbes'? Conventionally we do not (although for a while the microbiology degree course at my university did include a module on plant and animal cells).

In my list of organisms I included algae and protozoa, but these should really be considered together as a larger group known as protists, because there is a considerable degree of overlap. At one extreme, we have what can be regarded as a typical protozoan, which resembles an animal cell in that it does not have a rigid cell wall, so it is a very flexible organism which moves around by extending its surface in one direction; it typically feeds by simply engulfing part of the liquid around it and digesting whatever it contains. In some cases, protozoa can feed on simple nutrients in the medium, but more interesting is their ability to ingest bacteria. The ability of protozoa to feed on bacteria is an important factor in soil and water ecosystems. These organisms are referred to as amoebae. Many protozoa have more complex structures, including in some cases a 'mouth' and cilia which waft particles into the 'mouth'.

At the other extreme, we have typical algae, which are photosynthetic (they have chloroplasts containing chlorophyll), with a rigid cell wall (and so are similar to

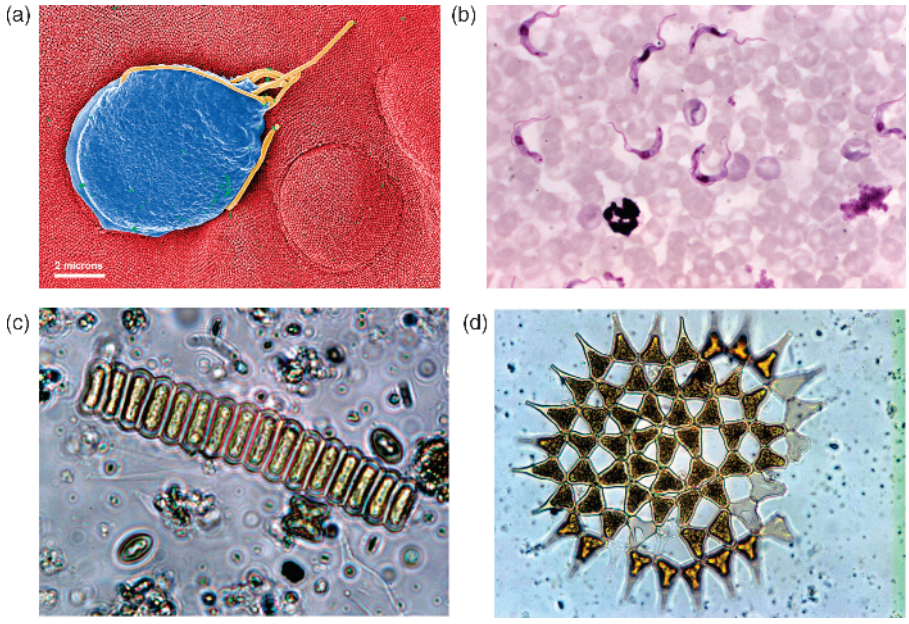

Figure 1.5 Protozoa and algae. (a) Colourized scanning electron micrograph of the protozoon *Giardia muris* adhering to an intestinal epithelial cell (content provider(s): CDC/Dr. Stan Erlandsen); (b) Stained light photomicrograph of the protozoon *Trypanosoma brucei* in a blood smear (content provider(s): CDC/Dr. Mae Melvin); (c) and (d) Green microalgae: (c) *Scenedesmus bijuga* (*Source:* United States Environmental Protection Agency Great Lakes National Program office); (d) *Pediastrum simplex* (*Source:* United States Environmental Protection Agency Great Lakes National Program office).

plant cells). Many of them can move around using flagella. However, the protist group also includes a number of important organisms which do not fit properly into either camp. Especially worth a mention are the diatoms, which have a beautiful glass-like wall made of silica, and the dinoflagellates, which are commonly responsible for the so-called 'algal blooms' that sometimes occur in seawater; some of these produce powerful toxins which have caused severe food poisoning from contaminated shellfish. Other protists have some similarity to fungi. These include the oomycetes, many of which are serious plant pathogens – an oomycete was responsible for the Irish potato famine in the 1840s.

Here it is worth mentioning the organisms that are sometimes, erroneously, referred to as 'blue-green algae'. These are not algae at all but are photosynthetic bacteria, and they are more properly referred to as cyanobacteria. We will come across cyanobacteria in several contexts in this book, as they are very important components of our ecosystem. Indeed, it was the ability of cyanobacteria to carry out photosynthesis, leading to the incorporation of carbon dioxide into living matter and the corresponding release of oxygen, that led to the rise in oxygen levels in our atmosphere which made possible the subsequent evolution of many forms of life as we know it today.

Even more remarkable are the slime moulds. These live in the soil, as single cells, feeding on decaying matter or bacteria, until the food starts to run out. Then, if there are enough of them in a particular niche, they come together as an aggregate and form a tiny slug-like structure, a few millimetres long. Subsequently, the various cells within the 'slug' start to differentiate. Some form a base plate, others contribute to a stalk, and yet others turn into spores within a fruiting body. This cycle between unicellular and multicellular organization makes them a valuable subject for scientists who are interested in the mechanisms underlying development and differentiation.

Clearly, the slime moulds can only do this if there are enough of them present to form all the various bits of the final structure. How do they know when there are enough? This is an example of a widespread phenomenon known as 'quorum sensing'. Each cell secretes a chemical which acts as a signal and is recognized by other cells. If there are enough cells in the immediate neighbourhood, the concentration of this chemical will reach a critical value that permits the subsequent development. We will encounter more examples of quorum sensing later on, as it plays an important role in the way in which some bacteria cause disease, as well as in other phenomena.

The next characters to be introduced are less likely to be familiar. Studies of microbes from various environments, including extreme conditions, such as hot springs and salt lakes, discovered some unusual organisms that, in some ways, resembled bacteria, especially in lacking a nucleus; since they were thought to be primitive organisms, they were dubbed Archaebacteria. However, it was subsequently realized, from comparisons of genome sequences, that they were fundamentally different from bacteria, and that they are actually more closely related to

the eukaryotes. So, they are now called Archaea. As well as living in extreme environments, other members of the Archaea occur in diverse situations ranging from waterlogged soils to the gut of animals (including humans) – and some have the significant property of producing methane.

Since the Archaea do not have a nucleus, they also fall within the Prokaryotes which I referred to earlier. Many microbiologists now regard this as misleading, as the Archaea are quite distinct from bacteria, and therefore the term 'prokaryotic' is now out of favour in some quarters.

I want now to turn back to the other end of the scale of sizes. I started with viruses, and the question of whether these are really 'living'. However, viruses do resemble living organisms in having nucleic acid (DNA or RNA) that codes for proteins. The final members of the cast are even more different, in that they have neither DNA nor RNA. These are the prions, which rose to notoriety in spectacular fashion with the advent of BSE ('mad cow disease', or bovine spongiform encephalopathy) and its human variant Creutzfeldt-Jakob disease. The infectious particle consists solely of a misfolded form of a normal protein. The 'replication' of the prion resembles growth of a crystal, in that copies of the misfolded form of the protein come together as aggregates, which act as seeds to induce further misfolding of other copies of the normal protein. The aggregates increase in size and then split to seed more misfolded aggregates. This process is quite different from replication of living organisms, so we should not really consider them as a form of life. However, they can be transmissible, so this, to some extent, justifies an inclusion here.

However, I should emphasize that they are not always transmissible. Transmission of BSE to humans only occurred through eating meat from a cow with BSE, so human to human transmission was not a problem (although a disease called 'kuru' did occur among people in New Guinea with the cultural habit of eating the brains of dead relatives – often, but misleadingly, referred to as 'cannibalism', although it was more of a mark of respect for the dead rather than the image that the word 'cannibalism' conjures up). I will look further at BSE in Chapter 3.

Prions may have an importance beyond their role in BSE and similar diseases. In particular, there are suggestions that the mechanisms behind degenerative diseases such as Parkinson's and Alzheimer's have some similarity to those involving prions – although they do not seem to be infectious.

In subsequent chapters, we will look at various aspects of microbial behaviour – how they cause disease, their activity in the environment, and how we can, and do, use microbes in a variety of ways. But first, I need to cover some basic concepts that will recur throughout the book. Some aspects are also included in Appendix 1, which covers some basic material as well as going rather further into selected topics than is included here.

1.2 Food for microbes

If we think about those bacteria that are able to grow on simple media, the main components needed for growth are sources of carbon and nitrogen, plus an energy source. Many bacteria, such as *E. coli*, can combine a carbon and energy source, using simple organic compounds such as glucose (or other sugars) to serve both purposes. The glucose is broken down through a series of reactions (ultimately to carbon dioxide and water). At several stages in this process, energy is released, in a controlled manner, so that it can be coupled to the synthesis of the wide variety of materials needed for growth – proteins, nucleic acids (DNA and RNA), lipids, and all sorts of other things. For a nitrogen source, *E. coli* is quite happy with an inorganic substrate such as an ammonium salt.

One of the main ways in which the energy is released in a controlled manner occurs by the passage of electrons (see Appendix 1) from one intermediate to another, until it is eventually passed on to a final electron acceptor. The energy released at each step is harvested by coupling the reaction to the production of a chemical known as adenosine triphosphate (ATP). This is the major energy resource within the cell and it is used in many reactions where energy is needed. For many of the bacteria we will be looking at, which are able to grow aerobically (in the presence of air), this passage of electrons along a chain of cytochromes, known as aerobic respiration, involves oxygen as the final electron acceptor, and the chain of intermediates is therefore called the *respiratory chain*. Some bacteria use a similar process even in the absence of air (*anaerobic respiration*), in which case some other substance (such as nitrate) is used as the final acceptor of electrons.

I need to introduce two further chemicals that are central to these, and many other processes. These are the related compounds known as NAD and NADP. Both can act as electron acceptors, which converts them to the reduced forms NADH and NADPH. Conversely, NADH and NADPH act in other reactions as electron donors, which changes them back to the oxidized forms NAD and NADP (oxidation of a substance is equivalent to the removal of electrons from it, and the converse process, reduction, is the addition of electrons – see Appendix 1 for further explanation). So NAD and NADP act as catalysts in many of the oxidation and reduction systems within the cell.

Not all bacteria have a respiratory chain. Instead, they obtain their energy through the process of *fermentation*. Several of the steps involved in the breakdown of a sugar such as glucose can be directly coupled to the production of ATP, without using a respiratory chain. However, this process is much less efficient, that is far fewer molecules of ATP are produced from the fermentative degradation of glucose than can be achieved using a respiratory mechanism. Many bacteria, and other microbes such as yeasts, can also use fermentation pathways under appropriate circumstances, even though they have a respiratory chain.

Other bacteria, such as the photosynthetic cyanobacteria, are even more versatile. They do not need an organic source of carbon because they are able to fix carbon dioxide – that is, they can use carbon dioxide and convert it into organic compounds

within the cell. The carbon dioxide comes ultimately from the air, although the form in which they use it is in solution in the water in which they live. This does not provide them with energy; indeed, it requires energy for it to happen. This energy is provided by sunlight, and this is captured, in cyanobacteria, as in green plants, by the pigment chlorophyll. Further information is provided in Appendix 1, but the overall message is that light provides the energy needed for splitting water molecules, liberating oxygen, and for the production of ATP and NADPH, both of which are needed for fixation of carbon dioxide. Surplus ATP and NADPH are used for the production of other material within the cell.

That deals with carbon fixation. The other side of the coin is the release of carbon back into the air. This is done first and foremost by all organisms that carry out aerobic respiration – microbes, animals (including us), even plants and other photosynthetic organisms in the dark. We breathe out carbon dioxide as the waste product from our metabolism. Even microbes that are fermenting their carbon sources rather than carrying out aerobic respiration still emit carbon dioxide – think of the yeasts that are used for making beer. In anaerobic environments, such as the sediment at the bottom of a lake, microbes produce methane, rather than carbon dioxide, as the end product of their breakdown processes (as they also do in the gut of ruminants and, to a lesser extent, of other animals, including us).

The combination of these processes – the fixation of carbon dioxide by photosynthetic organisms and the breakdown of organic compounds into either carbon dioxide or methane – is referred to as the *carbon cycle*. The balance of this cycle is an important factor in maintaining the composition of the Earth's atmosphere and, of course, it has important consequences for the 'greenhouse effect' and climate change (which I will deal with in later chapters).

There is an analogous cycle operating for nitrogen. The fixation of nitrogen from the air occurs by an energy-requiring process, using the enzyme *nitrogenase*, to produce ammonia (NH_3), which dissolves in water to produce ammonium ions (NH_4^+). This can be oxidized by other bacteria to nitrite (NO_2^-) and nitrate (NO_3^-) – a process known as *nitrification*. These soluble forms of nitrogen can be used by many microbes, and by plants, and are incorporated into amino acids for the formation of proteins and other nitrogen-containing organic compounds, of which the nucleotides that form the nucleic acids are the most important.

The other side of the nitrogen cycle involves the release of this fixed nitrogen back into the air. The decomposition of dead organic matter includes breakdown of proteins to amino acids (using enzymes known as *proteases*). The amino acids are further degraded to release ammonia. We can also add in the nitrogen that is excreted by animals as ammonia, urea or uric acid. The nitrogen cycle is completed by *denitrification*, by yet further microbes in the soil and water, which involves the conversion of ammonia to nitrogen gas through a series of intermediates including nitrate and nitrite. Excess nitrate in the soil, such as may occur through the use of fertilizers, can also be subject to denitrification in this way. I will come back to carbon and nitrogen cycles in Chapter 6.

There are two other elements that are present at significant (although lower) levels in organic matter. These are phosphorus (P) and sulphur (S). Phosphorus is important in nucleic acids, as the link between adjacent nucleotides in the chain, as well as in numerous other compounds such as ATP. It is widely present in the environment as phosphate (PO_4^{3-}) – indeed, it is almost exclusively in this form – which can be used directly by microbes (and other organisms). So we do not need to consider it further in this context, except to say that in the environment, especially in water, phosphate levels may be low, and microbial growth can be stimulated very substantially by addition of phosphate (as occurs in the run-off from agricultural land).

Sulphur is needed in small quantities (it forms just one per cent of the cell's mass), and it is usually taken up as sulphate (SO_4^{2-}). However, reduced forms of sulphur also exist in the environment – hydrogen sulphide (H_2S), elemental sulphur, and in combined forms in rocks and metal ores. Some bacteria are able to use these forms of sulphur by oxidizing them to sulphate. Conversely, other bacteria are able to reduce sulphate to hydrogen sulphide (by anaerobic respiration – i.e. they are using sulphate as the final electron acceptor instead of oxygen). This anaerobic reaction commonly occurs in situations such as the mud at the bottom of a lake, and it is responsible for the foul smell that results if the sediment is stirred up.

Microbes also need a range of trace elements, and they have evolved efficient mechanisms for obtaining these from their environment, so we do not usually need to add them to our culture medium in the laboratory. However, in the environment, especially in the oceans, metals such as iron may be present at levels that are too low for optimum growth of microbes, so growth can be stimulated by addition of minerals (as is the case with phosphate, as mentioned above). Some microbes, especially pathogenic ones, also need some vitamins or other organic compounds to be supplied. In a diagnostic medical microbiology laboratory blood or blood products, or sometimes more specific supplements, are often added to the medium to enable the isolation of these more fastidious bacteria.

It should be realized that this is a rather superficial and generalized account of bacterial nutrition. A full treatment would take a whole book.

1.3 Basic molecular biology

Although this is not a molecular biology book, the techniques and concepts of molecular biology have become so important to many aspects of our understanding of microbial behaviour – not just in biotechnology – that a basic coverage is inescapable. I will try to keep it simple. Some of it is also covered in Appendix 1.

The basic genetic information in the cell is encoded in DNA, in a series of 'bases' or nucleotides, referred to as A, G, C and T. Most bacteria have between 4–10 million bases in their DNA (although some genomes are smaller). Eukaryotes tend to have more – human DNA has a thousand times as much. Eukaryotes also tend to have a number of chromosomes, while bacteria usually have just one DNA

molecule (although we will see in Chapter 7 that it is not always so). This DNA is double-stranded, with one strand being a sort of mirror image of the other – for each A in one strand there is a T in the other, and each G is matched by a C. We say that the two strands are complementary. So, if we know the sequence of one strand, we can deduce the sequence of the other. This double-stranded nature is important in copying the DNA. The two strands gradually peel apart, and each strand is used as a template to make a new complementary strand.

The genes in the DNA code for proteins. Each gene is not physically separate; they are just part of a continuous sequence. When a gene is expressed, an enzyme called RNA polymerase recognizes a DNA sequence as a position to start copying one DNA strand into a slightly different nucleic acid called RNA, and it makes an RNA molecule corresponding to a single gene (or, in many cases in bacteria, a group of related genes). It is this *messenger RNA (mRNA)* that is used as the information for production of a specific protein.

Protein synthesis involves structures known as *ribosomes*. These recognize a specific site on the mRNA and start protein synthesis from there. The mRNA is read in groups of three bases (*triplets*, or *codons*), each codon corresponding to a specific amino acid. For example, where there is a CCG triplet, the amino acid arginine will be incorporated in the protein; if the codon is ACG, the amino acid incorporated will be serine. There are 64 possible triplet codons and 20 amino acids, so some of the amino acids are coded for by more than codon. There are also three *stop codons*, which are signals for the synthesis of that protein to stop.

Each gene codes for a specific protein (although that is a simplification). A typical bacterium might have 4,000 such genes. Proteins do a variety of things within the cell. For example, many of them are *enzymes*. These are biological catalysts, and are responsible for all the biochemical systems that occur within the cell, ranging from simple reactions such as an individual step in the breakdown of a sugar molecule to the synthesis of complex structures such as DNA. Other proteins are located in the membrane and are responsible for taking up chemicals from the surrounding medium, such as sugars or other nutrients. They can do this in a highly specific and controlled manner. In some cases, they form a pore through the membrane which will permit the passage of the relevant substance. Since this is a diffusion process, it only works if there is a higher concentration outside the cell than inside. Other proteins can take up substances against a concentration gradient – they pump it into the cell, so this is an energy-requiring process. Conversely, membrane proteins can pump unwanted chemicals out of the cell. Both processes can influence the concentration of an antibiotic within the cell, and can therefore affect the cell's sensitivity to that antibiotic (see Chapter 4).

Another important function that is controlled by specific proteins is the regulation of the cell's metabolic activity. For example, there is no point in *E. coli* making the enzyme β(beta)-galactosidase (which breaks down lactose into glucose and galactose, which is the first step needed for using lactose as a carbon source) if there is no lactose present. Therefore, it makes a protein (a repressor) that binds to a specific

DNA sequence, at the start of the β-galactosidase gene, and prevents it being expressed. If lactose is present, it binds to the repressor protein, and this causes a change in its shape so that it can no longer bind to this DNA site. Thus, the gene is expressed, and the bacterium can start breaking down the lactose. There are a large number of such regulatory proteins, and they play a key role in the adaptability of the cell to different environments.

This is just the basics. Bacterial genetics and molecular biology is much more exciting than this, and we will see in Chapter 7 how our knowledge of molecular biology can explain, and elucidate, the fundamental behaviour of bacteria in important respects, as well as how we can manipulate genes and determine the complete genome sequence of bacteria and other organisms.