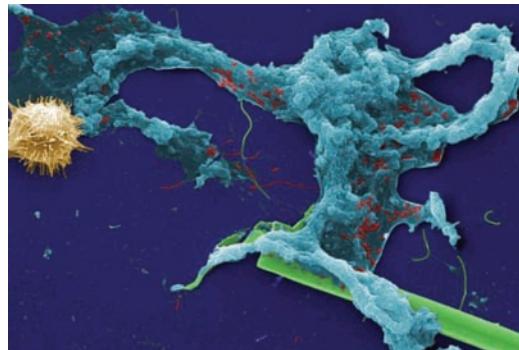


Chapter 1

The microbial world



A mixture of bacteria, protozoa and algae in a water sample from a stream

KEY FACTS

- Microorganisms are all around us in enormous numbers and are present both on and within our bodies. Some, termed pathogens, cause disease; others are beneficial and are of commercial importance but the vast majority are harmless.
- Infectious diseases can be caused by agents which are not living microorganisms: prions are simply 'rogue' protein molecules, and viruses usually consist of nucleic acid and protein but have no cellular structure.
- Bacteria represent the simplest living cells. Most of those of pharmaceutical interest can be grown easily in the lab.
- Fungi and protozoa are more complex than bacteria and most of them can exhibit sexual reproduction.
- Relatively few fungi are pathogenic; most are important as contaminants and spoilage organisms in manufactured medicines.
- Protozoa are only of pharmaceutical interest as pathogens; they are not spoilage organisms.

1.1 Microorganisms around us

Microorganisms are present in almost every location and environment on earth. They are in the air, soil and water, on all plants and animals and in such extreme environments as Antarctic ice and rocks 3 km below the earth's surface where the temperature is 60° C or more. Besides growing at extremes of temperature and pH, many bacteria survive and grow in the absence of oxygen; for these bacteria, described as anaerobes, oxygen is toxic. Micro-organisms are present, too, in huge numbers and variety. The bacteria in the average human gut are estimated to comprise about 500 different species, and their total

number, approximately 10^{14} (one hundred trillion), is about 10 times the number of human cells in the body and more than 10 000 times the human population of the earth. It is impossible to obtain precise data on the relative numbers of harmless and disease-causing (pathogenic) organisms for two main reasons: because new species are being identified all the time, and because of the difficulty of deciding what is harmless and what is not. Organisms that present no threat to a healthy individual might be pathogenic for a person with impaired immunity. Nevertheless, despite the extensive media attention on bioterrorism organisms and the so-called hospital 'superbugs', the harmless bacteria, together with those that are actually beneficial, grossly outnumber the pathogens; one estimate is by a ratio of more than 200 000 to 1.

Table 1.1 Examples of benefits, uses and problems associated with microorganisms.

General benefits and uses	Pharmaceutical applications	Problems and disadvantages
<ul style="list-style-type: none"> Essential role in carbon and nitrogen cycles In brewing, dairy and food industries In the manufacture of several industrial solvents and other chemicals As an insecticide Chemical detoxification In oil extraction 'Biological' detergents 	<ul style="list-style-type: none"> In the manufacture of: antibiotics, steroids, vaccines and many biotechnology products Used in assays to measure antibiotic concentrations As biological indicators of sterilization (Chapter 19) Used in tests to detect metabolic disorders and mutagenicity 	<ul style="list-style-type: none"> Cause infections Even harmless species may transmit antibiotic resistance Even dead bacteria may cause fever (endotoxins) Contaminate and spoil nonsterile medicines Cause noninfectious diseases, e.g. gastric ulcers and some cancers

1.2 The benefits of, and problems with, microorganisms

Microorganisms can be essential, passively beneficial or positively useful (Table 1.1). They are essential for the maintenance of life on earth as part of the carbon and nitrogen cycles, for example; without them, dead animals and plants would not decompose and the fertility of soils would fall. Their passive benefits include the protection afforded by probiotic ('friendly') bacteria, which compete with disease-causing species for nutrients and attachment sites on body tissue; they also limit opportunities for harmful bacteria to establish infections in the body by producing antimicrobial chemicals. The practical uses of microorganisms include their long-established rôles in the brewing, dairy and food sectors, and their applications in the pharmaceutical industry, which have multiplied enormously in recent years. Bacteria and fungi have been used since the 1940s to make antibiotics, and since the 1950s in the production of contraceptive- and corticosteroids, but it was the 1980s, a decade which brought major advances in genetic engineering, which saw bacteria used for the manufacture of insulin, human growth hormone, vaccines and many other biotechnology products (see Chapter 20).

Despite their applications in industry and the increasing recognition of their benefits, it is still the case that the main pharmaceutical interest in microorganisms is in killing them or, at least, restricting their contamination and spoilage of medicines. The reasons for this interest are listed in Table 1.1, and although the

first of these – that microorganisms cause infection – is quite obvious, the other problems that microorganisms pose are less well recognized. It is tempting to suppose both that harmless bacteria are irrelevant and that dead bacteria do no harm. Unfortunately, neither supposition is correct: harmless bacteria can carry genes responsible for antibiotic resistance, which they may transfer to disease-causing species, and components of the cell walls of dead bacteria (termed endotoxins) cause fever if they enter the blood stream. Consequently, in order to avoid the risk of fever from residual endotoxins when an injection is administered, it is necessary to ensure that the injection, which must be sterile (free of *living* organisms) anyway, has not been contaminated with high levels of bacteria during its manufacture. However, it is not only sterile medicines where microorganisms can present a problem: the great majority of medicines are not sterile, and the risk here is that the living organisms they do contain may damage the product, either by altering its physical stability or by breaking down the active ingredient.

1.3 The different types of microorganisms

Living organisms are made up of cells of two types: prokaryotic and eukaryotic. Bacteria used to be considered as the only category of prokaryotic cells, but in 1990 a second group, the archaea, were recognized as having equal status to bacteria. Archaea tend to live in inhospitable conditions (high temperatures, extremes of pH or salinity for example) and often possess unusual modes of metabolism, but because no pathogenic archaea have yet

been discovered this group will not be considered further. All other organisms are eukaryotic, so the major groups of microorganisms (fungi, protozoa and algae), as well as parasitic worms and mites, and all plants and animals up to and including humans, are eukaryotes. Viruses do not have a cellular structure and so some scientists do not even regard them as living but merely mixtures of complex chemicals; nevertheless, they are indisputably agents of infection and for that reason are usually considered as part of the microbial world.

Recap: the major differences between prokaryotic and eukaryotic cells. (This is not intended to be a full list; several further differences are described in biology textbooks.)

Characteristic	Prokaryote	Eukaryote
Cell nucleus	Do not possess a true nucleus	Have a nucleus surrounded by a nuclear membrane
Nuclear division and reproduction	Mitosis and meiosis are absent so reproduction is asexual	Exhibit both mitosis and meiosis, so reproduction may be sexual or asexual or both depending on species
Genetic variation	Resulting largely from mutations	Resulting both from mutations and the creation of new gene combinations during sexual reproduction
Mitochondria, chloroplasts and ribosomes	Mitochondria and chloroplasts absent; ribosome size is 70s	Mitochondria and chloroplasts may be present; ribosomes larger: 80s
Chemical composition	Do not possess sterols in the cell membrane but do usually have peptidoglycan in the cell walls	Do possess sterols in the cell membrane but no peptidoglycan in the walls

1.3.1 Viruses and prions

Viruses are parasites that infect all kinds of organisms: animals, plants, protozoa and bacteria too. They vary a lot in size and structure, but all contain both nucleic acid and protein; the protein surrounds and protects the nucleic acid core, which may be single-stranded or double-stranded DNA or RNA (Figure 1.1). The largest common viruses are about 300 nm in diameter (e.g. chicken pox virus) and the smallest about 20 nm (e.g. common cold virus), although some which are elongated (e.g. Ebola) may be up to 1400 nm long but very narrow, so none of them can be seen with an ordinary laboratory microscope but only with an electron microscope.

All viruses can only grow inside a host cell and usually the range of hosts is very narrow – often just a single species; rabies is a notable exception. Because they cannot be grown on Petri dishes in the same way as bacteria, they are difficult, time consuming and expensive to cultivate in the laboratory using fertile chickens' eggs or artificially cultured mammalian cells as hosts. Many viruses only survive for a few hours outside their normal host cell, but a few survive much longer and so may, in theory, be present as contaminants of pharmaceutical raw materials of animal origin. However, viruses are relatively susceptible to heat and organic solvents so they are unlikely to arise in materials like gelatin, for example, because of the processing conditions used in their manufacture. Some of the larger viruses have been studied as vectors (carriers) to deliver genes to cells, as in gene therapy for cystic fibrosis for example, but, in general, viruses are, like protozoa, important primarily as pathogens. Although viruses possess genes coding for enzymes to be made by the host cell, the majority contain few, if any, enzymes as part of their

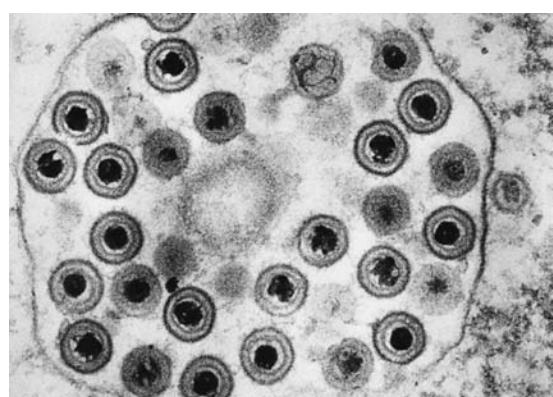


Figure 1.1 Herpes simplex virus viewed under the electron microscope. *Source:* PHIL ID #10231; *Photo Credit:* Dr. Fred Murphy and Sylvia Whitfield, Centers for Disease Control and Prevention.

structure. One consequence of this is that they are unaffected by the antibiotics used to treat bacterial and fungal infections. This does not mean, though, that it is not possible to create antiviral drugs. One consequence of the HIV/AIDS pandemic is that the number of synthetic antiviral drugs on the UK market increased from fewer than 10 in the mid-1980s to more than 40 by 2010.

Prions represent the simplest infectious agents, which, despite the fact that they are definitely nonliving, are nevertheless usually considered with microorganisms because of their capacity to transmit disease from one person to another. They are similar to viruses in that they have no cellular structure, but differ in that they do not even possess nucleic acids. They are merely atypical mammalian proteins that have the capacity to interact with normal proteins and induce structural changes so that the normal molecules are, in turn, changed into prions that are incapable of fulfilling their normal function. Prions are responsible for fatal, nerve-degenerative diseases termed transmissible spongiform encephalopathies, such as bovine spongiform encephalopathy (BSE; 'mad cow disease') in cattle, and Creutzfeldt–Jakob disease (CJD) in humans. They are particularly stable and difficult to inactivate by disinfectants, gamma-radiation and even by steam-sterilization conditions that far exceed those required to kill the most heat resistant spore-forming bacteria.

1.3.2 Bacteria

Bacteria are responsible for a wider range of diseases than protozoa or fungi, and they were discovered at least 200 years before viruses. For those reasons, and because most of them can easily be grown in the laboratory, bacteria were the most widely studied group of microorganisms throughout much of the nineteenth and twentieth centuries. Typically, they are spherical or rod-shaped cells about 1–10 μm in their longest dimension, so when suitably stained they can easily be seen with an ordinary light microscope (Figure 1.2).

Compared to human cells, bacteria are quite robust: they have a cell wall which protects them against rapid changes in osmotic pressure. Many bacteria will easily survive transfer into water from the relatively high osmotic pressure at an infection site in the body, whereas human cells, without a wall to protect them, would rapidly take in water by osmosis, burst and die. Bacteria are also more tolerant than human cells of wide variations in temperature and pH, and will withstand exposure to higher intensities of ultraviolet light, ionizing radiation and toxic chemicals.

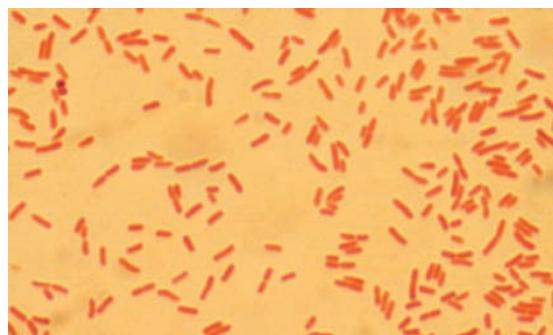


Figure 1.2 The common bacterium *Escherichia coli* (*E. coli*).
Source: http://commons.wikimedia.org/wiki/File:E_choli_Gram.JPG.

The recap box above highlights the major differences between bacteria (prokaryotes) and eukaryotes, but from a pharmaceutical perspective it is particularly relevant to contrast bacteria with mammals and consider the implications of their differences for the avoidance of microbial contamination and the treatment of infectious diseases. Two of the most fundamental distinctions are that bacteria reproduce asexually whereas mammals exhibit sexual reproduction, and that bacteria may reproduce in as little as 20 minutes but mammalian cells take many hours or days to divide. The cell-division process in bacteria is termed binary fission; this simply involves the chromosome being copied, and the cell enlarging. One copy of the chromosome, together with half the cell contents, becomes separated from the other by the formation of a cross-wall in the cell; a constriction may form which eventually causes the two so-called 'daughter cells' to separate. One bacterial cell doubling every 20 minutes can become over 16 million within 8 hours, and such a large number of cells located together on a Petri dish may become visible to the naked eye as a bacterial colony (Figure 1.3).

The consequence of bacteria reproducing asexually is that they are much more reliant on mutations as a means of producing genetic variation, and the fact that they grow so rapidly means that a mutant can quickly be selected and become the dominant cell type in the population. However, bacteria grow more slowly at an infection site in the human body than on a Petri dish (because they are attacked by the immune system and have to compete for food and oxygen with the body cells) but nevertheless, it is quite possible for antibiotic-resistant mutants to be selected during a course of antibiotic treatment.

The cell structures that are unique to bacteria may be both a benefit and a disadvantage. The cell wall, for



Figure 1.3 Colonies of *E. coli* growing on a Petri dish.

example, protects not only against osmotic pressure changes but against drying; consequently, many bacteria survive for long periods in dust. However, the bacterial enzymes that make the cell wall polymers are the targets for a number of important antibiotics, such as penicillins, which achieve their selective toxicity (killing bacteria without harming the patient) simply because human cells do not make cell walls and do not have the enzymes. The same situation applies with respect to ribosomes possessed by bacteria; these are structurally different from those of eukaryotic cells, so antibiotics like tetracyclines and erythromycin interfere with protein synthesis in bacteria but not in humans.

Despite the fact that all bacteria conform to the general description of prokaryotes, they still differ significantly in terms of shape, size and complexity, and these variations have in the past caused problems with classification. Chlamydia and rickettsia are both groups of small, pathogenic bacteria that are obligate, intracellular parasites (meaning that they can only grow within a host cell in a similar way to viruses), whilst mycoplasmas differ from most bacteria in that they do not have a cell wall so they are unaffected by penicillins and other antibiotics that interfere with cell wall synthesis.

1.3.3 Fungi: yeasts and moulds

Fungi are normally thought of as being the toadstools and mushrooms seen on damp, rotting vegetation, but these visible parts of the fungus represent only one stage in their life cycle and other stages involve cells of microscopic dimensions; it is for this reason, and the fact that many fungi never produce structures large enough to be seen with the naked eye, that they are regarded as micro-organisms. The word 'fungus' covers both yeasts, many of



Figure 1.4 Individual cells and filaments of the yeast *Candida albicans* viewed under the light microscope.

which are only slightly larger than bacteria (Figure 1.4), and moulds of the type seen on old food in the fridge.

Yeasts can exhibit sexual reproduction, but more commonly they divide in the same way as bacteria by binary fission or by budding. When growing on a Petri dish their colonies are often similar in appearance to those of bacteria, though usually larger and more frequently coloured (Figure 1.5).

Very few yeasts are capable of causing infection and their pharmaceutical significance is mainly as contaminants of medicines and as spoilage organisms. The term 'mould' is used to describe those fungi that do not produce large fruiting bodies like mushrooms and toadstools. Moulds consist of a tangled mass of multicellular filaments, which, collectively as a colony on a Petri dish, are referred to as a mycelium (Figure 1.6).



Figure 1.5 Colonies of *Candida albicans* growing on a Petri dish.



Figure 1.6 Three colonies of *Aspergillus niger* starting to form pigmented asexual spores in the centre of the colonies.

The mycelium is a branched network of tubes called hyphae, which vary in width from 1 to 50 μm and may contain multiple identical nuclei. Like yeasts, moulds are eukaryotes, but this does not mean that sexual reproduction is common. More frequently, moulds reproduce asexually and it is the formation of asexual spores (Figure 1.7) that is often responsible for the characteristic colours seen in many fungal colonies. The periphery of the colony, which is the actively growing region, is often colourless (Figure 1.6). Again, moulds are more significant as contaminants of manufactured medicines than as pathogens, although some are capable of causing severe illness in immunocompromised patients.

Some fungi exhibit different appearances under the microscope depending on their growth conditions. The organism responsible for the infection known as thrush

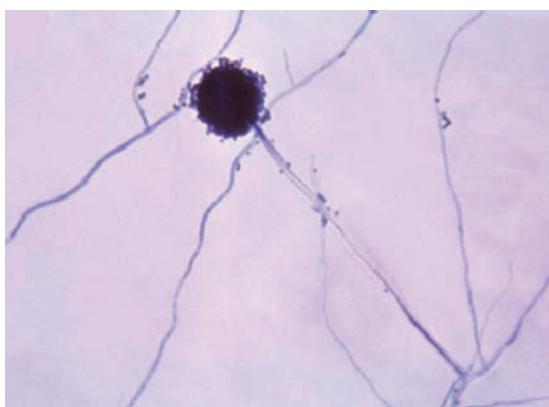


Figure 1.7 Asexual spore-bearing structure of *Aspergillus niger*; the black spherical conidiophore contains many tiny spores that are released when it bursts. *Source:* PHIL ID #3964; *Photo Credit:* Dr. Lucille K. Georg, Centers for Disease Control and Prevention.

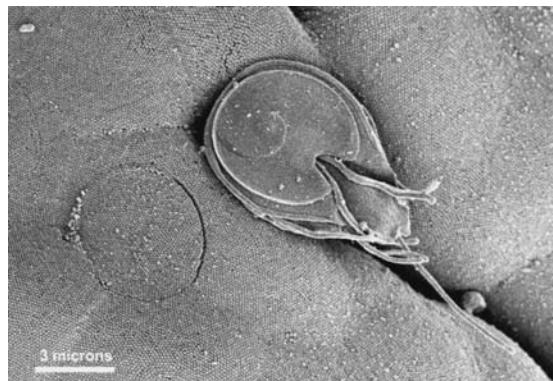


Figure 1.8 A protozoan (*Giardia* species) attached to intestinal epithelium viewed under the electron microscope. *Source:* PHIL ID #11647; *Photo Credit:* Dr. Stan Erlandsen, Centers for Disease Control and Prevention.

(*Candida albicans*) often looks like a yeast when grown in the laboratory, but exhibits a pseudomycelium (Figure 1.4) and under the microscope looks more like a mould when isolated from an infection site or from body fluids.

1.3.4 Protozoa

Protozoa are single-celled animals that are found in water and soil. The cells are typically 10–50 μm but can be much larger, and are usually motile (Figure 1.8). Some of them feed on bacteria and can be grown in bacterial cultures in the laboratory, but most are difficult to cultivate artificially and they do not, therefore, arise as contaminants of raw materials or manufactured medicines. The great majority are harmless, but a few, such as the organisms responsible for malaria and amoebic dysentery, are capable of causing severe infection, and it is for this reason that they are of pharmaceutical interest.

Table 1.2 summarizes some of the more important distinguishing features of the various groups of infectious agents ranging from the simplest, prions, to the most complex, protozoa.

1.4 Naming of organisms

All microorganisms except viruses are given two names: that of the genus (written with a capital initial letter) followed by the species (with a small initial letter) e.g. *Candida albicans*; it is normally written in italics to indicate that it is a proper name of an individual organism rather than, say, a collection of organisms having similar characteristics e.g. *pseudomonads* (which

Table 1.2 Distinguishing characteristics of the major groups of infectious agents.

	Cellular structure?	Prokaryote or eukaryote	Genetic material	Laboratory cultivation	Pathogenic potential
Prions	No	Not applicable	No nucleic acids	Only within living organisms	All mammalian prion diseases are untreatable and fatal
Viruses	No	Not applicable	DNA or RNA	Only within living organisms	Most cause active disease ^a
Chlamydia and rickettsia	Yes	Prokaryotes – parasitic bacteria	DNA in a single chromosome but not in a nucleus	Only within living organisms	Many are human pathogens
Bacteria	Yes	Prokaryotes	DNA in a single chromosome but not in a nucleus	Most of those causing human infection can be grown easily	Despite many being pathogens, most are harmless
Fungi	Yes	Eukaryote	DNA in multiple chromosomes in a nucleus	Most can be grown easily	A few are pathogens but the majority are harmless
Protozoa	Yes	Eukaryote	DNA in multiple chromosomes in a nucleus	The majority are difficult to grow in the lab	A few are pathogens but the majority are harmless

^aBut some may exist in a latent form within the host, causing no obvious disease.

describes bacteria similar to *Pseudomonas aeruginosa*), which would not be in italics; coliforms, staphylococci, streptococci and clostridia would similarly be in roman type without an initial upper case letter.

The name of the genus may be abbreviated to a single letter if that abbreviation is unambiguous, so *Escherichia coli* is more frequently written simply as *E. coli*. Names of organisms written in roman type and underlined are still occasionally encountered; this was an old convention by which a typesetter was instructed to set a word in italics,

and predates modern word processing, which permits italics to be used directly.

Acknowledgement

Chapter title image: PHIL ID #11715; Photo Credit: Janice Haney Carr, Centers for Disease Control and Prevention.

