Chapter 1
Micro-organisms and the Microbiome

The term ‘Micro-organism’ refers to any living thing that is too small to be seen with the naked eye. It covers an astonishing array of life forms that began with the earliest living occupants of our planet. Of the three recognized domains of life, two (Bacteria and Archaea) consist exclusively of micro-organisms. The third (Eukaryota) contains all of the macroscopic, multi-celled organisms that we recognize as plants and animals, but it also includes many micro-organisms. ‘Microbiome’ is a collective term[1] for all of the micro-organisms belonging to these three domains, while the ‘Biome’ comprises all life on earth. For most of earth’s history until the evolution of multicellular Eukaryota around 600 million years ago, however, the earth’s Biome consisted only of micro-organisms (Fig. 1.1). Even now the Microbiome is estimated to represent more than half of the total living matter (*biomass*) on the planet [1]. Their invisibility makes it easy for us to overlook the vast impact that micro-organisms have on the sustainability of the planet and of life on earth. While this book will focus on the gut-associated Microbiome, it is important that we start with a look at the wider microbial world.

Bacteria and Archaea

Members of the two exclusively microbial domains of life, Bacteria and Archaea, appear superficially similar under the microscope, but they are recognised as distinct because of fundamental differences in the way that their cell structures and genetic material are organised [2]. Evolutionary divergence between the ancestors of the

[1]The term ‘Microbiome’ is used here to refer to the microorganisms themselves and is synonymous with ‘*microbiota*’. Somewhat confusingly, ‘Microbiome’ has also been used by some to refer to the collective genetic material of these microorganisms (synonymous with the term ‘*metagenome*’, discussed later (Chap. 3)).

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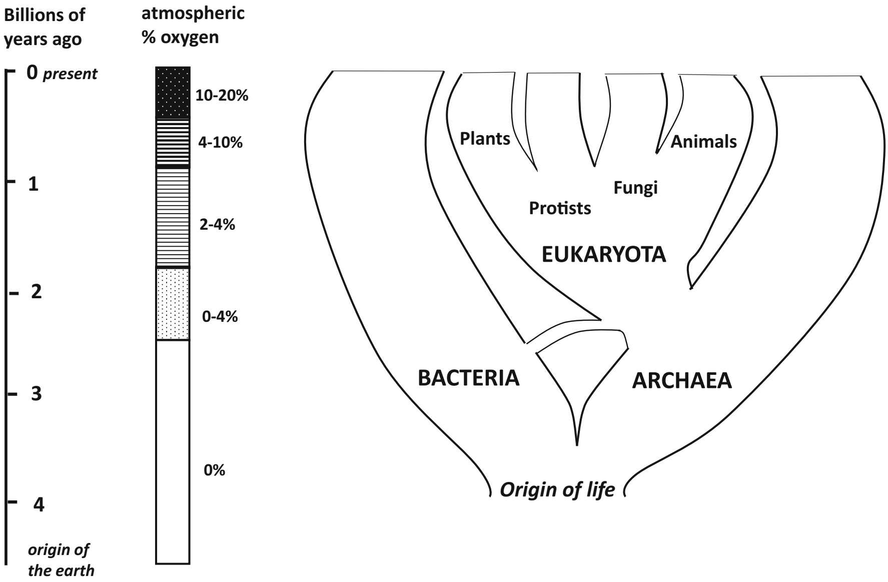

Fig. 1.1 Schematic of the three domains of life. Timescale and change in atmospheric oxygen are shown on the left

Archaea and Bacteria is thought to have occurred very early in the evolution of life on earth, at least 3.5 billion years ago.

All living organisms require a supply of the chemical elements of which they are made (notably carbon) together with a source of energy. This gives them the ability to make, or to obtain, the complex molecules that they require to grow and to reproduce themselves. Clearly the first cellular life forms on earth must have been able to gain their energy and nutrition without relying on any other living organism. Such organisms are called *autotrophs* and are still well represented within the present-day Microbiome. Some autotrophs are able to gain their energy from chemical reactions and their carbon from carbon dioxide or methane (by processes known as *chemosynthesis*). For example, bacteria found in hydrothermal vents deep in the ocean gain energy from the conversion of hydrogen sulfide to sulfur, and this process helps to support a complex food web that includes huge tube worms with which the micro-organisms are closely associated [3]. Indeed, recent evidence suggests that deep ocean vents are a possible site for the origin of life, with chemosynthesis as the driving force [4]. Other extremely important autotrophs, such as the Cyanobacteria, are able to gain energy from sunlight through the process of *photosynthesis*, while absorbing carbon dioxide and producing oxygen. Early evidence of life on earth is provided by 3.5 billion year-old fossilised mats of Cyanobacteria known as ‘stromatolites’ and it was the photosynthetic activity of these organisms over a period of two billion or more years that first introduced oxygen into the earth’s atmosphere. Even now, the marine cyanobacterium *Prochlorococcus* and its close relatives are estimated to account for 50% of global consumption of atmospheric carbon dioxide by the oceans [5]. By no means all photosynthetic bacteria produce oxygen. Autotrophic green sulfur and purple sulfur bacteria (*Chlorobium, Chromatium*) are photosynthetic organisms that are able to use hydrogen or reduced sulfur compounds to make their cell components from carbon dioxide, meaning that photosynthesis can occur in environments (both now and in the distant past) that lack oxygen. As shown in Fig. 1.1, atmospheric oxygen levels were minimal or very low during the first 3 billion years of microbial evolution.

The Archaea include some truly remarkable organisms, many of them autotrophs. Among them are the only living things that are able to gain energy by forming methane gas (*methanogens*). An extraordinary square-celled archaeon (*Haloquadratum*) that is able to use sunlight for energy exists in salt pans in salt concentrations 10 times that of sea water. Other archaeal '*extremophiles*' (organisms that prefer extreme environments) are able to exist at the temperature of boiling water in hot springs (*Pyrococcus furiosus*), or at high pressures found in the deep ocean, or at extremes of acidity and alkalinity. Extremophiles are also found among the Bacteria, for example *Deinococcus radiodurans* which has the ability to withstand extremely high levels of radiation. It is a reasonable assumption that extremophiles evolved initially under the extreme environments that prevailed during the early life of the planet, but they continue to survive today in extreme habitats such as saline lakes and hot springs. Other groups of Archaea play very important roles in the environment by oxidising methane and ammonia.

The great majority of present-day micro-organisms are not autotrophs that feed themselves, but *heterotrophs* that gain their energy and carbon by making use of compounds derived from other living organisms (i.e. *organic* compounds). Leaf litter, soils and sediments contain huge quantities of organic matter that provide abundant nutrients for microbial growth. The highest concentrations of micro-organisms in nature are found in the intestinal tracts of animals, particularly herbivores, where they benefit from the availability of plant material that is not digestible by the host animal [1]. As we will see later, such associations are often mutually beneficial (*symbiotic*), with the host able to benefit from the products of microbial activity and the micro-organisms benefitting from a stable environment and guaranteed food source. Important symbiotic interactions also occur between plants and microorganisms, for example in the nitrogen-fixing root nodules of clover. On the other hand, microbial interactions with multi-cellular plants and animals can also be very one-sided, and we are all too familiar with the fact that particular bacteria (termed *pathogens*) can cause serious diseases in their hosts. Archaea have not so far been implicated directly in disease causation.

While most bacteria and archaea exist as single cells, others grow as long branching strings (filaments). In some cases, more complex organisation occurs within filaments through branching or cell specialisation. Many bacterial species have been shown to form spores that enable them to survive high temperatures and adverse chemical environments (e.g. alcohol) that kill the normal (vegetative) cells. Such resistant spores can lay dormant for very long time periods. Spore-formers that are pathogens (e.g. anthrax, several *Clostridium* species) are of particular concern as we require extreme chemical treatments or the high temperatures and pressures created through *autoclaving* to destroy them and so ensure sterility. A salutary example is provided by Gruinard Island in northwest Scotland, which was used for the experimental release anthrax spores in 1942. 48 years later the island was finally declared safe, but only after treatment of the entire 196 hectare surface with formaldehyde and removal of much of the topsoil. Unlike many Bacteria, Archaea are not known to form spores.

Eukaryota

Diverse single-celled organisms related to algae, fungi and protists are also referred to as micro-organisms, but because their cells show a complex internal organisation that is absent from bacterial and archaeal cells they clearly belong to the third domain of ‘Eukaryota’ (often called ‘eukaryotes’). In particular, their genetic material is contained within a membrane-enclosed *nucleus* and they possess other specialist *organelles* such as *mitochondria* within the cell. The first eukaryotic cells are believed to have evolved around 2 billion years ago. One of the most astonishing conclusions of evolutionary biology is that these first eukaryotic cells apparently originated from an intimate co-operation between an archaeal precursor cell and a bacterial cell. Thus, the energy-generating organelle (the mitochondrion) that is found within the cells of almost all eukaryotes was originally derived from a free-living bacterial cell, while other cell components apparently owe more to the distant archaeal predecessor. Mitochondria play a vital role in the ability of eukaryotic cells to gain energy via respiration. It is also considered that the *chloroplast* structure that is responsible for photosynthesis within the eukaryotic cells of algae and green plants was originally a symbiotic cyanobacterium. Thus the basic machinery required for photosynthesis and respiration in plants and animals had evolved within the Microbiome long before the evolution of macroscopic life forms.

Our view of the relatedness of different single-celled eukaryotes has changed drastically over the past 50 years. Most fungi are now considered as close to animals as to plants in their evolutionary origins. Single-celled organisms once referred to collectively as ‘protozoa’ (such as flagellates, amoebae and ciliates) are now considered to belong to multiple, well separated branches on the evolutionary tree, and the same is true for different groups of photosynthetic algae (diatoms, red, green and brown algae). It follows that multicellular life forms must have evolved not once, but many times over in different evolutionary lineages. There is an incredibly wide range of growth habits among eukaryotes intermediate between the single-celled state and a truly multicellular state that involve varying degrees of organisation, aggregation, communication and specialisation, as illustrated by slime moulds, algal chains and colonies. Many fungi grow as microscopic filamentous *hyphae* that form an extensive network, or *mycelium*. On the other hand many of them also develop complex structures that make it hard to describe them as micro-organisms, suddenly appearing as large, highly visible fruiting bodies (mushrooms). Likewise, the red, green and brown algae that we call seaweeds can clearly exist as very large and complex structures. Nevertheless, many single-celled eukaryotes (such as yeasts and amoebae) are truly microscopic and are quite properly counted as members of the Microbiome. Eukaryotic micro-organisms include many important plant and animal pathogens, both among the fungi and protists. Most of them are non-pathogens, however, that play extremely important roles in the environment through photosynthesis and in the recycling of organic material.

The Virome

Viruses are entities that reproduce and propagate themselves by subverting the reproductive machinery provided by archaeal, bacterial or eukaryotic cells. Although they must therefore have evolved after the first living cells, the nucleic acid relationships between viruses and other life forms indicate an early origin. In fact, viruses are strictly non-living structures since they consist only of genetic material (which can be either DNA or RNA depending on the virus) in a protective coat. They are referred to collectively as 'the Virome'. Their impact on the living organisms of the Biome is, however, profound. We are most familiar with viruses as agents of human diseases ranging from chickenpox and influenza to ebola and coronavirus. Viruses are also responsible for many diseases in crop plants, and indeed tobacco mosaic virus (TMV) was the first virus of any kind to be studied in detail. But there are also huge numbers of viruses that infect bacteria and archaea, and these play a major, if still little understood, role in influencing the cell populations and evolution of their target micro-organisms.

Symbiosis

The huge significance of symbiosis between living organisms is a recurring theme in biology. We will use the term symbiosis here to mean 'living together for mutual benefit' (also known as 'mutualism') as distinct from a situation in which one partner benefits and the other comes to no harm from the relationship (*commensalism*). Many important and fascinating symbioses occur between multi-cellular plants or animals and microorganisms. A particularly close interaction occurs between leguminous plants such as clover and specific bacteria, with the plant's roots forming special nodules that house the bacteria (often *Rhizobium* species) responsible for converting ('fixing') atmospheric nitrogen to form ammonia [6]. As a result, the plant gains a supply of usable nitrogen that does not rely on nitrogenous compounds being supplied from the soil. This symbiotic relationship has been, and continues to be, of enormous significance for agricultural production as it reduces our reliance on chemical fertilisers.

Another vital symbiotic relationship occurs between land plants and fungi. It is estimated that 70–90% of land plant species are involved in associations between their root systems and fungi called *mycorrhizae* [7]. Indeed, just a cubic millimetre of soil can contain hundreds of metres of mycorrhizal fungal hyphae. These extensive fungal networks improve uptake of minerals and of phosphate by the plant, while giving the microbial partner a protected environment and access to photosynthetically produced energy in the form of sugars.

We will look at symbioses between animal hosts and gut micro-organisms in the next chapter. One particularly intriguing association is worth mentioning here, however, that illustrates a symbiotic association between an animal and a non-gut micro-organism. Many marine organisms are bioluminescent—that is to say, they emit light. In the Hawaiian bobtail squid, the ability to emit light is entirely due to a bacterial symbiont, *Vibrio fischeri*, which possesses the light producing-enzyme luciferase. The squid manages to select this symbiont from the environment very early in its development and allows it to proliferate within a special light organ, thus giving it the ability to glow in the dark [8]. *Bioluminescence* is thought to have a variety of benefits for ocean dwellers, but in this case, it is suggested that the squid is camouflaging itself from predators in shallow water by replacing the natural light blocked by its body with bioluminescence!

Many other symbioses are known to occur between microorganisms themselves. A spectacular example is provided by lichens, which are associations between two or more fungal partners and one or more green algae or cyanobacteria. As the photosynthetic partners, the algae or cyanobacteria produce carbohydrates by using light energy, and these in turn become available as energy sources to the fungal partner. Meanwhile the fungal network of filaments (hyphae) helps to anchor the photosynthetic cells and protect them from desiccation. This association is extremely successful, since lichens are well known as the first pioneering colonisers of barren surfaces from rocks to tree bark. Although composed of micro-organisms, lichens are visible as macroscopic structures that are often highly coloured and structurally quite complex. The relationship is clearly symbiotic, although the precise balance of benefit between the partners is likely to vary widely among the 20,000 lichen species that have been described!

Nutrient Cycling

Almost any organic (carbon-based) molecule that is made by one organism can be made use of by another organism within the Microbiome under some environmental conditions. As a result, there is very efficient carbon recycling and this tends to limit the accumulation of carbon-based molecules on the earth. Massive accumulations do occur, however, both in the present and over geological time—peat bogs represent the accumulation of organic matter when soil conditions are too waterlogged and acidic to allow the normal breakdown by micro-organisms. Most obviously, fossil fuels such as oil and coal are the product of un-degraded carbon that was deposited and then subjected to enormous temperatures and pressures resulting from geological upheaval hundreds of millions of years ago.

Microbial activity is not only crucial to the carbon economy of the planet, but also to that of most of the major elements found in living things. Atmospheric nitrogen is converted into soluble forms of nitrogen (nitrate, ammonia) by nitrogen-fixing bacteria which can be found in root nodules of many plants, and also by other nitrogen-fixing micro-organisms that are free-living. Since animals and plants cannot use gaseous nitrogen, this activity provides the usable nitrogen needed to make their proteins and nucleic acids. Conversely, ammonia oxidation by bacteria and archaea leads the formation of gaseous nitrogen, leading to a complex cycle of nitrification and de-nitrifcation.

How Many Microbiomes?

The remainder of this book will focus increasingly on the microbiota of the animal and human gut, but we have started with consideration of the global Microbiome for good reasons. Micro-organisms are incredibly numerous and occur in almost all environments found on the surface of the planet. They are capable of dissemination pretty much anywhere via the atmosphere, water and wind and via biological vectors, excreta and fluids. As a result they do not respect boundaries and the term ‘Microbiome’ is best applied to the whole planet. The soil that we walk on, the air that surrounds us and that we breathe, the food that we eat and the surfaces that we touch are teaming with all kinds of micro-organisms and their spores. While reference is often made (including in this book) to the ‘Gut Microbiome’ or the ‘Skin Microbiome’, we should always remember that this is just part of the global Microbiome. Certain human gut micro-organisms, including some pathogens, are capable of surviving and propagating themselves in other host species, or indeed outside the gut. Others appear more host-specific, but still have to be transmitted between individuals, especially between parents and offspring. More fundamentally, we should not forget that the evolutionary origins of gut micro-organisms lie with the ancient microbial world that existed long before the emergence of multicellular life forms and the development of digestive tracts.

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