INTRODUCTION TO MICROBIOLOGY

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Summary

Microbiology studies organisms, which are too small to be seen by the naked eye. Microorganisms are more abundant and diverse than the organisms we can see. The subject and science emerged as a consequence of the first microscope, revealing this invisible world in the late 17th century. Later Pasteur linked microorganisms to the decay of organic matter. Then Koch introduced the principle approach to demonstrating that a certain microorganism is the causative agent of a particular infectious disease. Microbiology as a highly interdisciplinary and applied subject, advances by using newly available methodologies, and in turn contributes to knowledge and technology.

Microbial morphological and physiological diversity reflects adaptation to even extreme habitats. Microorganisms shaped our world as we know it. Our planet and human life could not exist without microorganisms. Cyanobacteria started to produce oxygen about 3 billion years ago, microorganisms feature in the endosymbiont theory of eukaryotic evolution, and the evolution of mammals has been virus-driven. Microbial species interaction and communities are essential to keep all basic matter cycles going. Unique features of microorganisms are relevant to their industrial use, also made accessible by means of genetic engineering. Microorganisms provide, preserve or contribute to our nutrition, and even produced some of our fossil fuels. On the other hand they can damage our livelihood and our health. Socio-economics of infectious diseases has impacted on us in the past, present and future. How will the future of microbiology develop?

This chapter introduces microbiology (its nature, history and advancement) with a holistic perspective in context of pushing the boundaries of microbiological knowledge and application. Basic knowledge and concepts are addressed by means of integrative examples of everyday life (e.g. food, disease control) and current hot topics (e.g. antibiotic resistance, global change, artificial cell) covering the range of Prokaryotes, Eukaryotes and non-living entities (viruses, prions). In light of an educational approach, some material is explained in detail when it is most appropriate within the natural progression of the chapter, and not necessarily when mentioned first. There are questions provided for the reader to monitor understanding.

1. General Introduction and Historical Contexts

Microorganisms are everywhere. They are all around us, in us and on us. It is estimated $2.5 \times 10^{30}$ microbial cells live on the planet. Yet, we rarely see microorganisms directly, because the individual cells are too small for the naked eye. They are more abundant and diverse than what we actually can see. Their fascinating diversity will be explored and explained in detail in the following parts of this chapter. Non-cellular forms, that are merely proteins (prions such as the causative agent of Bovine Spongiform Encephalitis) of the size of few nanometers, or viruses (measuring 20-400 nm, where the Pox virus is rather large, Influenza virus is medium-sized [100 nm], and the Polio virus is small) being a combination of proteins and nucleic acids. Bacteria (prokaryotic cells) are bigger: the diameter of *Streptococcus* is about 1 µm, *Bacillus* measures about 1 µm by 2 µm. Eukaryotic cells are even bigger. We can find protozoa and algae of 300 µm. Yeasts (belonging to Fungi) are about 5 µm small.
Microorganisms can exist and grow in all environments: in plentiful or adverse conditions, from the extreme cold to extreme heat, with or without oxygen. We mainly notice the effects of microbial presence and activities. Some spoil our food or give it its characteristic taste. Some cause disease, but also allow cows to digest their food. Some cause the beautiful stripy petals we see in tulips (The Tulip breaking virus causes these color breaks due to alterations in the presence of anthocyanins.), but others cause foliage to wilt. Some bring us the smell of fresh soil, and some that of decay. Or we see microorganisms when they have grown in large numbers and colonized certain places. Our planet could not be without them. And in fact we humans would not be without them.

For several decades, the divergence of placental mammals some 200 million years ago has been linked to viruses. Retrovirus infections of the germline occurred millions of years ago. We find such integrations in the genomes of all vertebrates. They are often considered ‘junk DNA’ without any obvious function. The genetic distinctions between host and viral genomes disappeared, because the host needed the virus for survival. Here those endogenous retroviruses are involved in placental development and therefore considered fundamental in the evolution of mammals and viviparity. It is necessary for growth and survival of the embryo. Another example of virus-driven evolution is that of the yeast mating type locus before speciation of *Saccharomyces* and *Schizosaccharomyces*.

Even the evolution of life in general is attributed to microorganisms. For about 80% of planet Earth's history, microorganisms have been the only inhabitants, with cellular life appearing around 3.8 billion years ago. Fossilized bacteria-like structures (stromatolites) exist that are at least 3.5 billion years old. Initially, these were methane-producing microorganism using the energy of the sun. About 3 billion years ago cyanobacteria started to produce oxygen by photosynthesis even in extreme environments. Eukaryotes date back 2 billion years, following the last universal common ancestor LUCA's divergence into Bacteria and another branch about 3.7 billion years ago, then splitting into Archaea and Eukaryotes 2.8 billion years ago. Even the organelles of eukaryotic cells descend from microorganisms according to the endosymbiont theory. Mitochondria originate from an endosymbiotic common ancestor with alpha-Proteobacteria *Rickettsiales, Rhodospirillum* or *Bradyrhizobium*, and chloroplasts from cyanobacteria-like organisms. There is a single primary origin for each organelle. In secondary endosymbiotic events plastids evolved from a eukaryotic algal symbiont. Organellar genes disappeared or integrated in the nuclear genome over time, a state of syntrophy or metabolic symbiosis was established, where a metabolic product is used by the other organism as a substrate.

Irrespective of where life might have originated, microorganisms are highly relevant to all theories. After biomolecules spontaneously formed from components of the primitive atmosphere and hydrosphere, self-organization into precursors of life (proteobionts) is discussed, which then developed into progenotes, evolving further into contemporary organisms. But did all this take place on Earth? The panspermia theory (life exists everywhere in the universe, and is spread via bodies such as meteoroids) was introduced in 1865 by Richter. Spores of the bacterium *Bacillus subtilis* could possibly survive an interplanetary journey. Most likely progenotes would have been much more
vulnerable to ultraviolet radiation. The earth-centered Haldene–Oparin theory was supported by Miller and Urey, when they demonstrated in 1959 that a reducing atmosphere, acted on by solar UV and electrical discharges, produces organic molecules from a prebiotic chemistry. And what about the ‘RNA world’? It could have been established on earth or elsewhere given that we find organic particles in space and freeze-dried bacteria and bacterial spores in clouds in the interstellar space. Some can remain viable, because bacteria can survive high amounts of radiation at low temperature and without water or oxygen in a vacuum.

So microorganisms shaped our world as we know it! And now they are essential to keep all basic matter cycles going, provide (e.g. mushrooms)/ preserve (e.g. cheese) food or contribute otherwise (e.g. rice plantation) to our nutrition and medication. They even produced some of our fossil fuels: methane and crude oil originate from microorganisms. Marine photosynthetic bacteria, together with a very low amount of plant and animal material, sedimented on the sea floor. Anaerobic bacteria deoxygenated the organic matter and produced saturated hydrocarbons, which accumulated within pores of rock. Upon extreme pressure and heat, the natural gas methane is formed within the rock.

Yet, microorganisms can also be extremely dangerous to us, can cause starvation by decimating our crop and cattle, can cause epidemics and corrode man-made structures.

Microbiology studies all these microorganisms too small to be seen by the naked eye. Given this nature of the subject, in order to visualize and characterize what is too small to be seen, microbiologists always apply newly available technologies to their subject. The then gained knowledge allows for general progress, development and immediate application in the context of other sciences (e.g. cell biology, biochemistry, genetics, molecular biology, ecology), of technology and medicine. Microbiology therefore needs other disciplines, and its subjects and methods (e.g. laboratory culture, sterilization, microscopy) are applied to and used as tools for other disciplines such as genetics, biotechnology, ecology. For example in the 1960s bacteriophages were the model systems leading to the discovery of mRNA, understanding the genetic code and how gene expression is controlled. Thus microbiology is a highly interdisciplinary and applied subject, and allows us to constantly push the boundaries of knowledge and understanding.

Naturally the history of microbiology dates back to the introduction of the first microscope and its subsequent use to reveal the hidden world. In the late 17th century Anthony van Leeuwenhoek made drawings from the human mouth flora and described 'Little animalcules' as observed using his microscope. It took until the middle of the 18th century until it was shown that such life was not spontaneously generated. Louis Pasteur infused animal or vegetable material in water and let it stand until it was decayed following a proliferation of microorganisms, which were introduced from aerial contamination. By adding gelatin to such infusions, a solid culture medium was made available and further improved when Fannie Hesse suggested using agar instead of gelatin, which some bacteria can degrade. Such solid media are used in the dishes (Figure 1) invented by Richard Petri. Isolating and growing pure colonies of bacteria

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(Figure 2) from a mixed population is only possible on solid media and the essential prerequisite to study and characterize the organisms.

Figure 1. Petri dish with solid medium (nutrient agar used for cultivating bacteria)

Figure 2. Pure culture of *Micrococcus luteus* on nutrient agar. A dilution streak was performed for inoculation to obtain single colonies.

We can easily see how such seemingly simple technological developments were groundbreaking in increasing microbiological knowledge. Without pure cultures Robert Koch in the late 19th century could have never developed a scientific approach (now called Koch’s Postulates) to demonstrate that a certain microorganism is the causative
agent for a particular infectious disease. Until then supernatural forces were considered to cause the diseases. Koch’s and Pasteur’s work paved the way for the 'Golden Age of Bacteriology' in the early 20th century, when causes of the most important infectious diseases of the days were established. In the present and the future a more systemic approach to microbiological research is taken.

2. Microbial Diversity

All the amazing diversity of microorganisms is the result of the competition for nutrients. This competition is still a crucial driver of further evolution and diversification. Often in nutrient-poor environments diversity developed in the context of adapting to surviving in extreme conditions, and of optimizing nutrient up-take. In nutrient-rich environments diversity reflects an adaptation to fast growth, competing against other microorganisms and evading the defenses of the host. So the gradual adaptation to their habitat (e.g. soil, air, water, extreme sites such as volcanic vents, other organisms [in all arrangements from symbiosis to parasitism]) resulted in morphologically, ecologically and physiologically diverse microorganisms.

2.1. The Big Divide: Prokaryotes and Eukaryotes

We have already mentioned, that we distinguish prokaryotic and eukaryotic cells, based—as the name implies- on the presence or absence of a nucleus to contain the chromosomes. A phospholipid bilayer (plasma membrane) surrounds all cells. Additionally there can be cell wall structures.

The linear chromosomes of Eukaryotes are situated in the nucleus. The cells have also other organelles such as mitochondria. The cytoskeleton is based on microtubules also found in flagella in a typical nine-plus-two arrangement (9 doublets around the edge, 2 singles in the center). Cells contain the Golgi complex and the endoplasmic reticulum. Transcription takes place in the nucleus. Splicing is necessary to remove non-coding stretches from the mRNA. The 5' cap and the 3'poly A tail are added, then the mRNA leaves the nucleus for translation at the 80S ribosomes. Eukaryotic cells undergo mitosis and asexual cell division, and can undergo meiosis and sexual cell division.

Prokaryotes have no cell compartmentalization and the circular chromosome plus extrachromosomal DNA is free in the cytoplasm. Ribosomes (70S) are generally smaller. Archaea are prokaryotic cells, but have some features in common with eukaryotic cells.

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Biographical Sketch

**Beatrix Fahnert** has a background in industrial, applied and medical microbiology, and teaches related subjects at undergraduate and postgraduate level. She studied biology with a focus on microbiology at the Friedrich Schiller University of Jena (Germany), where she graduated in 1997. Then she worked on *Chlamydia* (virulence). At the now Leibniz Institute for Natural Product Research and Infection Biology-Hans-Knoell-Institute in Jena (Germany) she worked on recombinant production of cystine-rich proteins (e.g. hBMP-2) in *Escherichia coli* and on transcriptomics. In 2001 she received a PhD in microbiology from the Friedrich Schiller University of Jena (Germany) and subsequently the "Thesis of the Year"-Award. Having received a Marie-Curie-Individual Fellowship, she went to Finland (University of Oulu) to work on recombinant Wnt proteins. In 2005 she joined Cardiff University (Cardiff School of Biosciences), where she is currently in the position of a Senior Lecturer. Her scientific interests concern the application of postgenomic approaches to studying virulence, pathogenesis and pathogen-host-interactions, and utilizing related findings for prevention and therapy of infectious diseases. She is committed to sharing her passion for science and to making the necessary knowledge, understanding and skills accessible. Therefore, she is interested in certain aspects of learning (e.g. meta-learning; standards; employability) and teaching (e.g. academic culture; continuous professional development; community of practice) in the Biosciences.
within the framework of the changing landscape in Higher Education and related processes. Scientific engagement of the public (particularly public awareness of infectious diseases prevention and appropriate treatment) is another area of her interest. Beatrix Fahnert is a member of the Society for General Microbiology, where she at present serves as Chair-Elect of the Education Division. She is also a Fellow of the UK Higher Education Academy.