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Introduction to Microbiology

1.1 Historical

Only a few years after the first descriptions and isolations of microorganisms by Louis Pasteur, Robert Koch, Gerhard Hansen, and others, the public was already aware of an essential property of these mostly unicellular microorganisms: They are ubiquitously distributed, i.e. everywhere! Also, that they can cause fever in the bodies of humans and animals or cause diseases, partly with death consequences, was already known, likewise already disinfection measures like the use of the agents carbolic acid and iodine. Still in 1906, in the 8th edition of the “Lehrbuch der Botanik für Hochschulen” by Eduard Strasburger, among other names of fission fungi (Schizomycetes), the cyanobacteria are called fission algae [1].

Humans have been taking advantage of the services of microorganisms for thousands of years, but without knowing of their existence for very long. The Sumerians brewed a beer-like beverage as early as 5000 years ago, and the Assyrians fermented grape juice into wine about 3500 years ago.

The first person to see microorganisms with his own eyes was probably the Dutch draper Antony van Leeuwenhoek (1632–1723). He experimented with homemade single-lens microscopes, with which he achieved magnifications up to 270× and resolutions down to 1.5 μm. In 1675, he examined an infusion of peppercorns and discovered tiny creatures. He discovered more of these creatures, then called “*animalcula*,” in dental plaque. Van Leeuwenhoek made drawings of these creatures, which he sent by letter to the Royal Society in London in 1683 [2].

The French chemist Louis Pasteur (1822–1895) made several groundbreaking discoveries in the field of microbiology. He experimentally disproved the primordial hypothesis, explained the nature of fermentation using the examples of alcoholic fermentation and lactic acid fermentation, developed methods for disinfection and sterilization, and introduced procedures for combating infectious diseases by vaccination (e.g. rabies vaccination in 1885).

In 1873, the Norwegian physician Gerhard Hansen (1841–1912) microscopically discovered the causative agent of leprosy, *Mycobacterium leprae*, as one of the first bacteria to be recognized as a pathogen [3]. To this day, this bacterium cannot be cultured in culture media. Diagnosis is made with the microscope on biopsy material or scrapings of the nasal mucosa. The propagation of these mycobacteria is only

successful in the paws of mice and the *Armadillo*. Pathogen-specific DNA can be detected using the polymerase chain reaction (PCR).

In 1876, the German physician Robert Koch (1843–1910) proved that microorganisms are the causative agents of infectious diseases, using the anthrax pathogen *Bacillus anthracis* as an example. He established the following four postulates:

- 1) Bacteria must be detectable in the infected organism.
- 2) These bacteria must be isolated and brought into pure culture.
- 3) Infection with these isolated bacteria will cause the disease again in the healthy organism.
- 4) The same infectious agent can be isolated from the host again.

Koch developed culture media, e.g. meat extract broth, which he initially solidified with gelatin and later with agar–agar. Koch’s plate-casting method, still used today in all bacteriological laboratories, goes back to him.

Microorganisms are grouped into two taxonomic domains of their own (Bacteria and Archaea) and thus distinguished from the domain Eukarya (fungi, animals, and plants). Based on the cell structure of microorganisms, they are divided into prokaryotes (Bacteria and Archaea; Greek: *bakteria* = rod; Greek: *archaios* = ancient, original) and eukaryotes (fungi, yeasts, algae, and protozoa).

1.2 Importance

Medical microbiology is concerned with the study of pathogens of significance to humans and animals, their habits, and their effects on the human or animal organism; it is thus primarily concerned with obligate pathogens (pathogenic in any case) and facultative pathogens (pathogenic under certain circumstances), i.e. with germs that are to be regarded as dangerous or as “pests” due to cell destruction or the release of toxic metabolic products. However, microorganisms are generally much more likely to be considered “beneficial organisms.” Biological equilibrium without microorganisms is not possible at all. By mineralizing organic matter (e.g. plant material), they ensure the recovery of carbon, nitrogen, sulfur, phosphorus, etc., which are then available again to the plants (material cycles). In the gastrointestinal tract of humans and animals, microorganisms play an important role in the digestion of food. The skin and mucous membranes of humans are also colonized. To illustrate the orders of magnitude: A human being consists of about 10^{13} cells. The gastrointestinal tract is home to about 10^{14} and the skin to about 10^{12} microorganisms, which together weigh about 1.25 kg [4]. The human body thus harbors more microorganisms than it has cells of its own.

Microorganisms find application in the food industry. Examples are:

- Yeasts in the manufacture of bread, beer, sake, and wine;
- Lactic acid bacteria in the production of yogurt, kefir, sauerkraut, and salami;
- Acetic acid bacteria for the preparation of vinegar;
- Molds in cheese production (Gorgonzola, Roquefort, etc.) and for the preparation of soybeans (in East Asia).

Microorganisms are used for the recovery of:

- Vitamins;
- Amino acids;
- Hormones;
- Steroids;
- Enzymes, e.g. amylases (starch cleavage), proteases (digestion, leather tanning), lipases (fat cleavage), and pectinases (fruit juice clarification);
- Antibiotics;
- Alcohols (ethanol, butanol, butanediol, glycerol, etc.);
- Active ingredients, some of which are also produced by genetically modified microorganisms (e.g. insulin).

Microorganisms are essential in the treatment of wastewater and waste composting.

1.3 World of Microorganisms

An overview of the various groups of microorganisms and other causative agents of infectious diseases is given in Table 1.1. Microorganisms are not visible to the naked eye; for their observation, a light microscope is required, and in the case of viruses – with very few exceptions – an even higher magnification electron microscope is required.

The average size of bacteria is between 0.3 and 10 μm . The diameter of cocci, which belong to the human skin flora, is approx. 1 μm . If you think of 500 cocci of this size strung together, the diameter of the dot at the end of the sentence would be reached. Another size comparison: a hair on the head is approx. 40–120 μm , on average 80 μm , thick (see Table 1.2). The human eye can recognize objects up to approx. 25 μm (resolving power).

Table 1.1 Groups of microorganisms and biological agents.

Subcellular biological objects	Mostly unicellular organisms (microorganisms)
Prions	Prokaryotes
Viroid	Eubacteria
Bacteriophages	Chlamydia
Viruses	Rickettsia Mycoplasma Archaea
	Eukaryotes
	Fungi, yeasts, algae, and protozoa

Table 1.2 Sizes of particles and cells.

Cell or particle	Size
Egg (bird)	In the centimeter range (ostrich egg: $d = 15$ cm)
Ovum (human)	200 μm
Human hair	$d = 40\text{--}120$ μm , average 80 μm
Human and animal cells	20–30 μm
Human erythrocyte	7.5 μm
Human sperm cell	6.5 μm long
Pollen	7–100 μm
Dust	0.1–100 μm
Aerosols when sneezing	10–300 μm
Protozoa	5–150 μm
Mushrooms	5–10 μm
Bacteria	0.3–10 μm
<i>Nanobacterium equitum</i> (Archaeon)	0.4 μm
Mycoplasma	0.3–0.8 μm
Chlamydia	0.3–1.0 μm
Rickettsia	0.5–1.0 μm
Viruses	0.016–2.0 μm
Viroid	2 \times 40 nm
Macromolecules	1–10 nm
Prions	<5 nm
Atoms	0.1 nm

d , diameter.

The world of microorganisms consists of the following groupings (although the following first three groupings are not living organisms in the strict sense, but biological agents).

1.3.1 Prions

Infectious prions PrP^{Sc} are misfolded forms of a small (molar mass about 30 000 Da) cellular glycoprotein. The misfolding occurs in cattle between amino acids 121 and 230 and is inaccessible to protease digestion [5]. Stanley Prusiner derived the name from “proteinaceous infectious particle” [6]. PrP^{Sc} causes diseases in sheep and goats (scrapie), cattle and cats (bovine spongiform encephalopathy (BSE) and feline spongiform encephalopathy (FSE)), mink, deer, and ungulates. Humans can also be infected (Kuru, Creutzfeldt–Jakob disease, and Gerstmann–Sträussler–Scheinker syndrome). Incubation periods can last for many years. In the course of these diseases, the brain tissue decays in a spongiform manner. BSE first appeared on a larger

scale in the United Kingdom toward the end of the 1980s, while scrapie has been known for more than 260 years [7]. Presumably, the prions were transmitted via insufficiently heated meat-and-bone meal containing PrP^{Sc} from scrapie-infected sheep, which was fed to cattle.

1.3.2 Viroid

Viroids are circular, single-stranded RNA molecules of low molar mass (approx. 12×10^4 Da, approx. 360 nucleotides). The RNA is “naked,” i.e. not coated by protein. Viroids cause plant diseases, e.g. potato spindle tuber viroid.

1.3.3 Viruses

Viruses (lat. virus = poison, mucus) are predominantly ultramicroscopic, obligate cell parasites that contain only either DNA (e.g. poxvirus and herpes simplex) or RNA (e.g. influenza, rhinitis, and rabies viruses), have no enzyme systems for energy production and no systems for protein synthesis, and cause infected host cells to synthesize the virus building blocks. Viruses consist of at least a nucleic acid-containing inner body and a protein coat called a capsid. They may be enveloped, i.e. surrounded by a lipid bilayer (such as pathogens of smallpox, herpes, measles, influenza, rabies, acquired immunodeficiency syndrome [AIDS], and severe acute respiratory syndrome [SARS]) or be unenveloped (such as pathogens of polio, hepatitis A, rhinitis, and foot-and-mouth disease). Poliovirus can be characterized by the chemical molecular formula $C_{332652}H_{492388}N_{98245}O_{131196}P_{7501}S_{2340}$ [8]. On December 9, 1979, the WHO declared the world free of smallpox.

The size of viruses varies from 20 nm (picornaviruses and arboviruses) to 2000 nm (plant viruses such as the *Citrus tristeza* virus). Viruses that infect bacteria are called bacteriophages. T phages (coli phages) are well studied in molecular biology; their size is 70 nm \times 200 nm. The bacteriophage T4 contains linear double-stranded DNA (dsDNA), and its genome size is 168 903 (see Figure 1.1).

In 2003, large viruses were found in amoebae. They were called mimiviruses. With sizes up to 800 nm, they are visible under the light microscope [8]. Viruses are detected using tissue culture, animal experiments, egg culture methods, PCR, and immunological methods. Approximately 1500 viruses are currently known, of which slightly more than 200 are human pathogens [9].

1.3.4 Archaea

Archaea (Greek: *archaios* for old, original) live in extreme locations, for example, in salt lakes (e.g. Dead Sea with approx. 30% of different salts, corresponding to an a_w value of 0.75), hot sulfur springs, and the deep sea. Archaea include methanogenic (produce methane, CH₄), thermophilic (live at high ambient temperatures), and halophilic representatives. Their cell wall structure is different from that of bacteria. So far, more than 250 species of archaea have been described, although pathogenic representatives are not yet known [9].

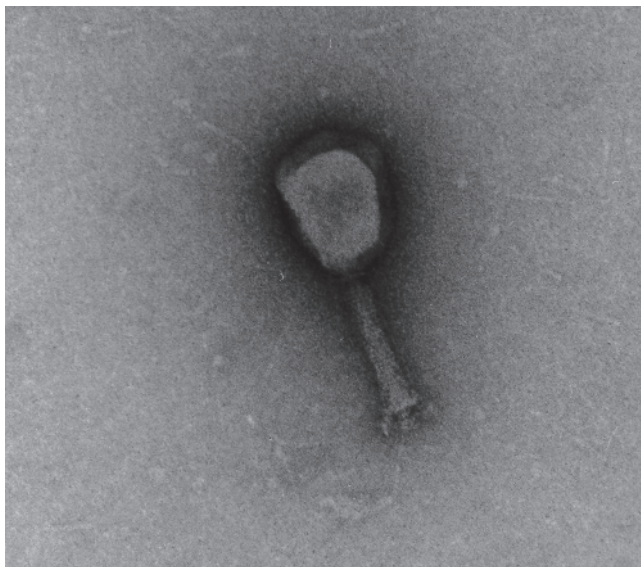


Figure 1.1 Bacteriophage T4, acceleration voltage 80 kV, uranyl acetate as contrast medium, magnification 184 800 \times . Electron Microscope EM 301, Philips, Eindhoven, The Netherlands.

The smallest representative of the archaea is *Nanoarchaeum equitans*. Although this organism has its own ribosomes, it uses part of the metabolic functions of the host cell. Archaea were defined as a separate bacterial kingdom (domain) by the American microbiologist C.R. Woese in 1977 [10].

1.3.5 Bacteria

Bacteria reproduce asexually by transverse division. They have a rigid cell wall of varying thickness that ensures shape and stability. The nuclear structure (which is not a true nucleus) is called a nucleoid. Meanwhile, more than 1000 bacterial genomes have been sequenced (the first sequence analysis was achieved in 1995 on the genome of *Haemophilus influenzae*). To date, more than 10 000 bacterial species have been described [11], and several hundred are added each year. Approximately 340 of the species known to date are human pathogens, and among the causes of death, infectious diseases occupy second place, with the consequences of tobacco consumption in the first place [12].

1.3.6 Chlamydia

They are obligate cell parasites that possess all the typical structural elements of bacteria. Chlamydiae undergo a developmental cycle (from the 0.3 μm elementary bodies to the 1 μm initial bodies). An example is the causative agent of the parrot disease psittacosis, *Chlamydia psittaci*, which can also infect humans, developing flu-like symptoms. Infection happens through inhalation of dust containing chlamydia from bird excrement. Many pigeons in cities are infected with *Chlamydia* species.

1.3.7 Rickettsia

They are also obligate cell parasites of 0.5–1 μm size. They reproduce by transverse division with the aid of host cell cofactors. An example is the causative agent of spotted fever, *Rickettsia prowazekii*. The bacteria are transmitted by ticks, mites, lice, and fleas. Another pathogen is *Coxiella burnetii*. Domestic and wild animals are infected by tick bites. Humans become infected by dust containing *Coxiella* from animal feces. The disease is called Q fever, and its diagnosis is done serologically.

1.3.8 Mycoplasma

This group includes bacteria without a rigid cell wall; as a result, they appear polymorphic and show high plasticity. Their size is 0.3–0.8 μm . Examples are the pathogens of pneumonia (*Mycoplasma pneumoniae*) and urinary tract infections (*Ureaplasma urealyticum*). Normal flora include *Mycoplasma buccale* (on the oral mucosa) and *Mycoplasma hominis* (on the mucosa of the intestine). From *Mycoplasma genitalium*, the genome was sequenced. It is 580 kb in size and contains only about 500 genes. In the Gram stain, the mycoplasmas react variably. They are resistant to penicillins and sulfonamides but not to tetracyclines and streptomycin.

1.3.9 Fungi

Fungi (mycobionta, molds, and yeasts) are a very heterogeneous group of ubiquitous eukaryotic organisms in many forms and colors, with more than 110 000 species. They can be divided into the following four groups: Basidiomycota with about 30 000 species, Ascomycota with about 46 000 species (including about 1000 species of yeasts or Endomycetes), Zygomycota with about 650 species, and *Fungi imperfecti* (or Deuteromycota) with about 30 000 species. Almost all human and animal pathogenic fungi, as well as most molds, belong to this last group [13]. Fungi are estimated to make up 25% of the biomass of our planet. Fungi can even colonize optical lenses in objective lenses. About 300 species of fungi are pathogenic to humans [9], and most diseases of crops are due to fungi. Fungi can produce toxins (more than 500 mycotoxins are known to date), some of which are lethal to humans and animals (in Germany, about 50 people die annually as a result of fungal poisoning). In addition, toxic and carcinogenic metabolites can be produced, especially by molds (e.g. Aflatoxins, Ochratoxins, Patulin, and Fusarium toxins). The Food and Agricultural Organization estimates that up to a quarter of the world's food production is contaminated with mycotoxins. The allergenic potential of the fungi, on the other hand, has so far been classified as low.

Common to all fungi is a rigid cell wall containing chitin (a polysaccharide), cellulose, glucans, etc., and the true nucleus. Fungi cannot photosynthesize, so they feed on finished organic matter: they are C-heterotrophs. Fungi either feed on dead organic matter (see Figure 1.2) or live as parasites on or in other living organisms. While most of the fungi reproduce asexually, some fungi also reproduce sexually. *Fungi imperfecti* are only known to reproduce asexually, for example,