Discover the World of Microbes

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Environment

Intections

Biofuels

Bacteria, Archaea, Viruses

Biotechnology

Gerhard Gottschalk



Microbes & Men

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Wilson, M. Bacteriology of Humans An Ecological Perspective 2008 ISBN: 978-1-4051-6165-7 Gerhard Gottschalk

Discover the World of Microbes

Bacteria, Archaea, and Viruses

WILEY-BLACKWELL

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Preface

Numerous discussions have repeatedly made it clear to me that microbes are a mystery to most people. After all, they are invisible. Microbes cause disease; they are involved in all kinds of novel production processes; they can easily be manipulated genetically - so it's best to keep your distance! Besides, it requires some effort to fathom the secrets of these organisms. However, it is well worth the time. Microbes are fascinating in their diversity, activities, and achievements. Considering their abundance and their involvement in the global cycles of carbon, nitrogen, and sulfur, it is no exaggeration to say that microbes rule our planet. The object of this book is to spark interest in these multifaceted organisms. It consists of two parts, a reading section and a study guide which has been added to the thirty-two essays to allow this book to serve as an introductory text. Formulas and equations have been kept to a minimum whenever possible.

This book was written to include a fictitious conversation with someone who has an interest in the subject without being an expert and who asks questions every now and then. This form has been chosen to keep the text down-toearth. Statements of highly respected colleagues have lent authenticity and brilliance to many of the chapters, for which I am truly grateful. I am also honored that they have read the respective chapters and complemented them in such a persuasive and competent manner. They are: for Chapter 1: Frank Mayer, Stade (DE); Chapter 3: Ralph Wolfe, Urbana, IL (US); Chapter 4: Manfred Eigen, Goettingen (DE), Gerald Joyce, La Jolla, CA (US); Chapter 5: Joachim Reitner, Goettingen (DE); Chapter 6: Karl Stetter, Regensburg (DE), Gregory Zeikus, East Lansing, MI (US); Chapter 7: Aharon Oren, Jerusalem (IL), Colleen Cavanaugh, Cambridge, MA (US), Antje Boetius, Bremen (DE); Chapter 8: William Whitman, Athens, GA (US), Karl-Heinz Schleifer and Ludwig, (DE), Wolfgang Munich Dieter Oesterhelt. Martinsried (DE), Volker Mueller, Frankfurt/Main (DE). Andrew Benson, Santa Barbara, CA (US); Chapter 9: Joerg Overmann, Munich (DE), Jack C. Meeks, Davis, CA (US); Chapter 10: Holger Brueggemann, Berlin (DE), Michael Blaut, Potsdam-Rehbruecke (DE); Chapter 11: Oliver Einsle, Freiburg (DE), Alfred Puehler, Bielefeld (DE); Chapter 12: Gijs Kuenen, Delft (NL); Chapter 13: Douglas Eveleigh, New Brunswick, NJ (US), Rolf Thauer, Marburg (DE); Chapter 15: Peter Duerre, Ulm and Hubert Bahl, Rostock (DE), Michael Young, Aberystwyth (UK); Chapter 16: Douglas Clark, Berkeley, CA (US); Chapter 18: Hermann Sahm, Juelich (DE), Karl Sanford, Palo Alto, CA (US); Chapter 19: Jan Andreesen, Goettingen (DE), Hans Guenter Schlegel, Goettingen (DE); Chapter 20: Timothy Palzkill, Houston, TX (US), Beate Averhoff, Frankfurt/Main (DE); Chapter 22: David Hopwood, Norwich (UK); Chapter 23: Julian Davies, Vancouver (CA); Chapter 25: Werner Arber, Basel (CH), Chapter 26: Peter Greenberg, Seattle, WA (US), Anne Kemmling, Goettingen (DE); Chapter 27: Eugene Rosenberg, Tel Aviv (IL); Chapter 28: Michael Rey, Davis, CA (US), Gregory Whited, Palo Alto, CA (US), Alexander Steinbuechel, Muenster (DE), Garabed Antranikian Hamburg (DE); Chapter 29: Stefan Kaufmann, Berlin (DE), Joerg Hacker, Berlin (DE), Werner Goebel, Munich (DE), Michael Gilmore, Cambridge, MA (US), Julia Vorholt, Zuerich (CH), Ulla Bonas, Halle (DE); Chapter 30: Eckard Wimmer, Stony Brook, NY (US), Karin Moelling, Zurich (CH), Stephen Gottschalk, Houston, TX (US), Patrick Forterre, Paris (France); Chapter 31: Claire Fraser-Liggett, Baltimore, MD (US), Michael Hecker, Greifswald (DE), DeLong, Cambridge, MA (US), Edward Rolf Daniel, Goettingen (DE); Chapter 32: Kenneth Nealson. San Francisco, CA (US), Friedrich Widdel, Bremen (DE), Douglas Nelson, Davis, CA (US), Michael McInerney, Norman, OK (US), and Koki Horikoshi, Tokyo (JP).

This book tells the story of microbes and the discoveries revolving around these organisms. Some of the scientists involved in these discoveries have been mentioned, whereby the list of names makes no claim to be complete. If in connection with such discoveries the work of some colleagues has not been acknowledged, I would explicitly like to request their understanding.

The compilation of this manuscript was supported by Daniela Dreykluft, for which I am truly grateful. I would especially like to point out the contribution of Dr. Anne Kemmling, who designed a great number of the drawings and illustrations. Dr. Petra Ehrenreich also deserves credit for some of the figures. I am indebted to Theodor Wolpers, Emeritus Professor of English Literature, for his contribution of Shakespeare quotes, Colleen Cavanaugh for discussions on the title of this book, Martin Keller for his advice on Chapter 14, and Eckard Wimmer for his advice on Chapter 30.

Special thanks go to Wiley-VCH, notably Anne du Guerny, Dr. Gregor Cicchetti, and Dr. Andreas Sendtko, for the constructive cooperation in a pleasant atmosphere.

This book would not be what it is without Dr. Lynne Rogers-Blaut. She grew up in Ohio (US), came to Germany, and obtained her PhD in my department, so she is qualified to convert my English into readable English and to discover any inaccuracies and inadequate explanations. Thank you, Lynne – it was a wonderful cooperation.

Last but not least, I thank my wife Ellen for her patience and her support.

> *Gerhard Gottschalk* Goettingen, 2011

Prolog

Bacteria: they are a real threat to mankind. They cause plague and cholera, and in previous centuries more people were killed by them than in wars. Still today we may suffer from tuberculosis, intestinal infections, bronchitis, and many other diseases.

Bacteria contaminate water and food. No, I don't want to know more about these creatures. There is already a lot of information in the press and in package inserts of drugs.

This is not fair towards the bacteria. I admit, they do cause diseases, but only a very small percentage of bacteria is responsible for this. Most microbial species on our planet are peaceful and extremely useful. Without them, life on earth, would not be possible. They also affect our climate and are irreplaceable in the manufacture of biotechnological products.

Aren't you exaggerating? I have read that bacteria help clean beaches and waters after tanker accidents. They are also used in waste water treatment. But I can't believe that life on earth, depends on them, and I am sure viruses are not useful at all. By the way, what are archaea?

The discovery of archaea is an exciting story. It will be reported in Chapter 3. These microorganisms look more or less like bacteria, but they are fundamentally different. They represent a separate domain of life, and were already different from the bacterial domain three billion years ago.

Let's start with bacteria, with their smallness and their enormous activity.

Part One

Reading Section

Chapter 1

Extremely Small But Incredibly Active

It is the greatest dream of a bacterial cell to become two bacterial cells

François Jacob

A visit to our Department of Microbiology was on the agenda of a high-ranking politician. How to impress him? We started with the smallness of bacteria but not in the usual way by stating that bacteria are approximately 1 μ m long, so 1000 bacterial cells lined up end-to-end would measure just 1 mm. We tried a different way:

"Sir, this test tube contains nearly 6.5 billion bacterial cells in a spoonful of water. Thus, the number of bacteria nearly equals the number of human beings on our planet." He took the test tube, looked at it, and could hardly recognize the slight turbidity. One billion bacterial cells in one ml or 1000 billion cells in a liter are barely visible. Then we pulled out a photograph the size of a letter pad and said, "Here are two of these 6.5 billion cells (<u>Figure 1</u>)." The Minister was impressed with the smallness of bacteria, which makes them barely visible even in large numbers, and with the enormous power of the methods used to examine them, for example, electron microscopy.

Figure 1 Test tube with a suspension of 6.5 billion bacteria, of which two are shown in an electron micrograph. The cell on the right has nearly completed cell division. The flagellae (long thread-like structures) provide motility to the cells.

(Source: Frank Mayer and Anne Kemmling, Goettingen, Germany.)



Electron Microscopy? I Used a Light Microscope When I Was at School, But What Is the Principle Behind the Electron Microscope?

Let's have the expert Frank Mayer (Goettingen, Germany) tell us about this:

"Well, the "light" required for electron microscopy is a beam of electrons. This one is invisible to our eyes, but the pictures produced can be made visible. Because of the shorter wavelength of electron beams, much smaller details of biological objects can be seen than by light microscopy. Even enzyme molecules can be made visible, for example, on photographic paper. The disadvantage of using electrons is that a vacuum is required. Therefore, water has to be removed from samples before they can be examined, and this may cause damage to the objects. But recent improvements in electron microscopy make it possible to avoid damage to the objects by removing water from the objects in the frozen state."

Isn't it fascinating that electron microscopy makes it possible to magnify objects 100 000 times? Even light microscopy is capable of enlarging objects 1000-fold. This already impressed the plant physiologist Ferdinand Cohn (1828–1898), who wrote,

"If one could inspect a man under a similar lens system, he would appear as big as Mont Blanc [in the Alps] or even Mount Chimborazo [in Ecuador]. But even under these colossal magnifications, the smallest bacteria look no larger than the periods and commas of a good print; little or nothing can be distinguished of the inner parts and of most of them their very existence would have remained unsuspected if it had not been for their countless numbers."

Ferdinand Cohn obviously exaggerated somewhat: a man two meters tall magnified 1000 times would be two thousand meters (6600 feet) tall, nearly half the elevation of Mont Blanc and one third that of Mount Chimborazo.

It is difficult to imagine that clear water can actually be highly contaminated, or that one cubic meter of air can contain one thousand microbial cells. Air, of course, is only slightly inhabited by microorganisms, but it is different when we look at our skin, which is densely populated by bacterial cells (see Chapter 10) with amazing biological activities. There are many sites in nature where they are able to multiply rapidly. *Escherichia coli* (*E. coli*, for short) resides in our intestine and is able to divide every 20 minutes! To put it casually, if one trillion bacterial cells in my intestine go with me to the movies, and if they manage to grow and divide optimally, then 16 trillion cells will leave the cinema with me 80 minutes later.

Good Example, But Why Do Bacteria Multiply So Astonishingly Fast?

It's because bacteria have a high metabolic activity due to their high surface-to-volume ratio. Let me give you an example: If we put a cube of sugar into a glass of tea and, at the same time, the same amount of table sugar into a second glass, the table sugar will dissolve faster than the cube of sugar. Its surface-to-volume ratio is larger. A cube with an edge length of 1 cm has a surface-to-volume ratio of 6: 1, between the total surface area of the sides, 6 cm^2 , and the total volume, 1 cm^3 . If we cut the cube into "bacteria-size" cubes with an edge length of 1 µm, we would end up with 100 million cubes with an overall surface area of 60 000 cm^2 . The total volume would be the same but the surface-to-volume ratio would increase by a factor of 10 000.

That has its consequences. Compared to cells of higher organisms, bacteria have a much larger surface area at their disposal, allowing the faster import of nutrients and export of waste products. Therefore, cell constituents can be synthesized more rapidly, a prerequisite for the rapid multiplication of cells. That's why bacteria have the highest multiplication rates: some species have a record of around 12 minutes, so every 12 minutes two cells emerge from one. This, of course, cannot be generalized. There are also slowgrowing bacteria that divide every 6 hours or even once every few days. Bacteria living in the "land of milk and honey" grow and divide rapidly, whereas the organisms in nutrient-deficient habitats such as oceans are much slower when it comes to cell division.

The Ability of Bacterial Cells to Divide Every 20 Minutes, or Even Every 12 Minutes, Is Quite Impressive. What Does that Mean for a Bacterial Population?

Let's look at a single bacterial cell multiplying every 20 minutes under optimal conditions. How many cells and how much cell mass would be produced after 48 hours? We have to do some simple calculations. One cell (2^0) would give rise to two cells (2^1) after 20 minutes; four cells (2^2) after 40 minutes; and eight cells (2^3) after 60 minutes. Three divisions per hour would make a total of 144 divisions in 48 hours, resulting in a total of 2^{144} cells. This number probably doesn't impress you. Let's do a few more calculations: Conversion into common logarithm а (144 \times 0.3010), with 10 as a base, yields 10⁴³ cells. The weight of one bacterial cell is around 10^{-12} g, so 10^{43} cells weigh 10^{31} g or 10^{25} tons. Our planet weighs 6 × 10^{21} tons, so after 48 hours the total bacterial mass would be nearly 1000 times that of our planet.

Very Impressive, But Certainly Not Realistic.

Of course not, but the calculation is correct. However, the assumption that cells would divide every 20 minutes for a period of 48 hours is incorrect. Nutrients would have become limited after a few hours, so growth would have slowed down and stopped eventually. Perhaps the situation can be compared to that of a large pumpkin, which after reaching a critical size will also stop growing because of shortage of nutrients and accumulation of metabolic byproducts.

I Have Learned Something New. I Would Like to Know How Bacteria Compare with Higher Organisms.

Chapter 2

Bacteria Are Organisms Like You and Me

Nature would not invest herself in such shadowing passion without some instruction *William Shakespeare, Othello*

But What About Archaea and Viruses?

Archaea, to be introduced in Chapter 3, are living organisms like bacteria, but viruses aren't like them at all because several characteristic features are missing. Viruses look and often act like little golf balls, just lying around or flying through the air. They aren't able to do much by themselves. But as soon as they have entered a host cell, they start their devilish work. Viruses are able to cause epidemics, so they must somehow have life in them (see Chapter 30).

Bacterial and archaeal cells actually have much in common with plant and animal cells. Of course, you can't compare a single-celled organism such as our intestinal bacterium *Escherichia coli* with an oak tree or an elephant. Comparisons have to be made at eye level, for example, comparing an *E. coli* cell with a cell from an oak leaf or with a muscle cell from an elephant. Then, the features common to all cells will become apparent. Let's first look at the cell constituents. All cells contain DNA (DeoxyriboNucleic Acid), but there is one qualitative difference. The DNA in plant and animal cells is localized in the nucleus, a compartment surrounded by a membrane. Plants and animals are therefore called eukaryotic organisms. A simple eukaryotic cell, the yeast cell, is depicted schematically in <u>Figure 2</u>a. Bacteria, on the other hand, are prokaryotic organisms whose DNA more or less floats in the cytoplasm (<u>Figure 2</u>b), which is a sort of gel. This intracellular space contains many proteins, nucleic acids, amino acids, vitamins, and salts.

Figure 2 The eukaryotic and the prokaryotic cell. (a) The eukaryotic cell contains a nucleus (center) surrounded by a membrane (with pores), a vacuole (light blue), the endoplasmatic reticulum (green), the Golgi-apparatus (purple), mitochondria (yellow/orange), ribosomes (black dots) and cytoplasm. The cell is surrounded by a cytoplasmic membrane and a cell wall. Diameter of the yeast cell depicted: 10 µm. (b) The prokaryotic cell contains a circular, coiled-up chromosome; ribosomes; and cytoplasm. The cell is also surrounded by a cytoplasmic membrane and a cell wall. A flagellum is depicted on the left (not present in all bacteria). Bacterial cells have an average length of 1 µm. (c) The cytoplasmic membrane consists of a phospholipid bilayer. In living organisms the membrane is charged, negative inside and positive outside. Proteins (red) are inserted into the membrane.

(Watercolor and gouache: Anne Kemmling, Goettingen, Germany.)



All cells contain three types of RNA (RiboNucleic Acid). The ribosomal RNA, together with the ribosomal proteins, makes up the ribosomes, the protein synthesis factories of the cells. The second type is messenger RNA which transmits DNA-imprinted messages to the protein synthesis factory. Messenger RNA passes the instructions from the DNA to the ribosomes, where proteins are synthesized on the basis of these instructions. There are mechanisms to ensure that only those proteins are synthesized that are required under certain physiological conditions. Not all proteins encoded on the DNA are continuously needed. The third type of RNA, transfer RNA, is required for the alignment of amino acids to form proteins. Each cell contains at least 20 of these transfer RNAs, which are specific for the 20 amino acids present in proteins. According to the synthesis protocol of the messenger RNA, the transfer RNAs, each linked to a respective amino acid, are lined up in the prescribed order then the amino acids are connected.

In all cells the entire machinery discussed above is surrounded by the cytoplasmic membrane, which is negatively charged on the inside and positively charged on the outside (Figure 2c). The membrane contains checkpoints for the transport of materials into the cells. These transport processes are highly specific; for example, there are checkpoints that allow potassium ions to pass but not sodium ions. The interior of most bacteria is high in potassium ions but low in sodium ions. If we were somehow able to taste the interior of a bacterium from the ocean (intracellular volume around 1 μ m³, 1 cubic micrometer), it wouldn't taste salty. Without its charge, the cytoplasmic membrane would be unable to fulfill its functions to ensure that the composition of the cell's interior differs dramatically from the surrounding fluid. Inside the cell there are favorable conditions for cell division, irregardless of the conditions outside. The cytoplasmic membrane and its functions is one of the greatest miracles of evolution. How the membrane is charged will be described in Chapter 8.

Those Are the Cell Constituents, But How Does One Cell Become Two Cells?

To answer this question we have to look at the processes of life at a cellular level. Which processes are involved when two cells are formed from one? As already discussed, DNA is the carrier of genetic information needed to generate two *E. coli*-cells from one *E. coli*-cell. First of all, energy is required for the generation of a new cell. Here, the magic word is ATP, the abbreviation for adenosine-5'-triphosphate. ATP is the energy currency of all organisms on our planet. It powers processes such as thinking or muscle work, also growth, motility, and reproduction in bacteria. When ATP fulfills its role as an energy source, it is at the same time devaluated; it loses one phosphate residue, and adenosine-5'-diphosphate (ADP) is formed. This conversion is coupled with a release of chemical energy that can be invested in the energy-requiring reactions mentioned above.

Before a cell can divide into two cells, the chemical constituents of the cell have to be synthesized. It is as if a completely furnished house is to be converted into a completely furnished duplex. The "furniture" has to be assembled and set up or installed, so that two viable cells will have been formed from one. If we disregard the membrane, the cell wall, and any reserve material such as starch, the cell essentially has to deal with the synthesis of DNA, RNAs, and proteins, the three types of constituents already introduced. Before we go into protein synthesis, let's look at the role of proteins.

Most of the proteins of a cell are enzymes, except for proteins such as collagen in higher organisms (part of the supporting tissues) or capsular proteins in certain bacteria. Enzymes are also called biocatalysts, and their names usually end in "-ase," as in lipase or protease. The enzymes consist of 20 different building blocks, called the 20 natural amino acids (Study Guide). These amino acids are found in proteins, not only once but in multiple copies. The chain length of proteins is variable; proteins may consist of 100-

300 building blocks. The amino acids have different chemical properties, SO their chains fold to vield complicated structures to which metal ions. such as magnesium or ferrous (iron) ions are often bound. Every enzyme contains a catalytic center. This is the place of action, where the enzyme-catalyzed reactions take place. The diversity of enzymes is fantastic. Even our commensal E. coli is able to synthesize approximately four thousand different enzymes. They all have a specific function at defined sites of metabolism. For example, enzymes make the synthesis of DNA and RNA possible. Enzymes exhibit specificity, which means that the catalytic center of a particular enzyme has been designed to fit certain reaction partners. A DNA polymerase is capable of elongating DNA strands but it cannot cleave fat - that's the job of the lipases. It is important to note that enzymes dramatically increase reaction rates because they bring the reaction partners into optimal spatial positions. Without enzymes, enzvmes themselves would not exist. The even interdependence of DNA, RNA, and protein (enzyme) synthesis will become clear when we look at these processes (Figure 3).