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Rolf D. Schmid and Claudia Schmidt-Dannert

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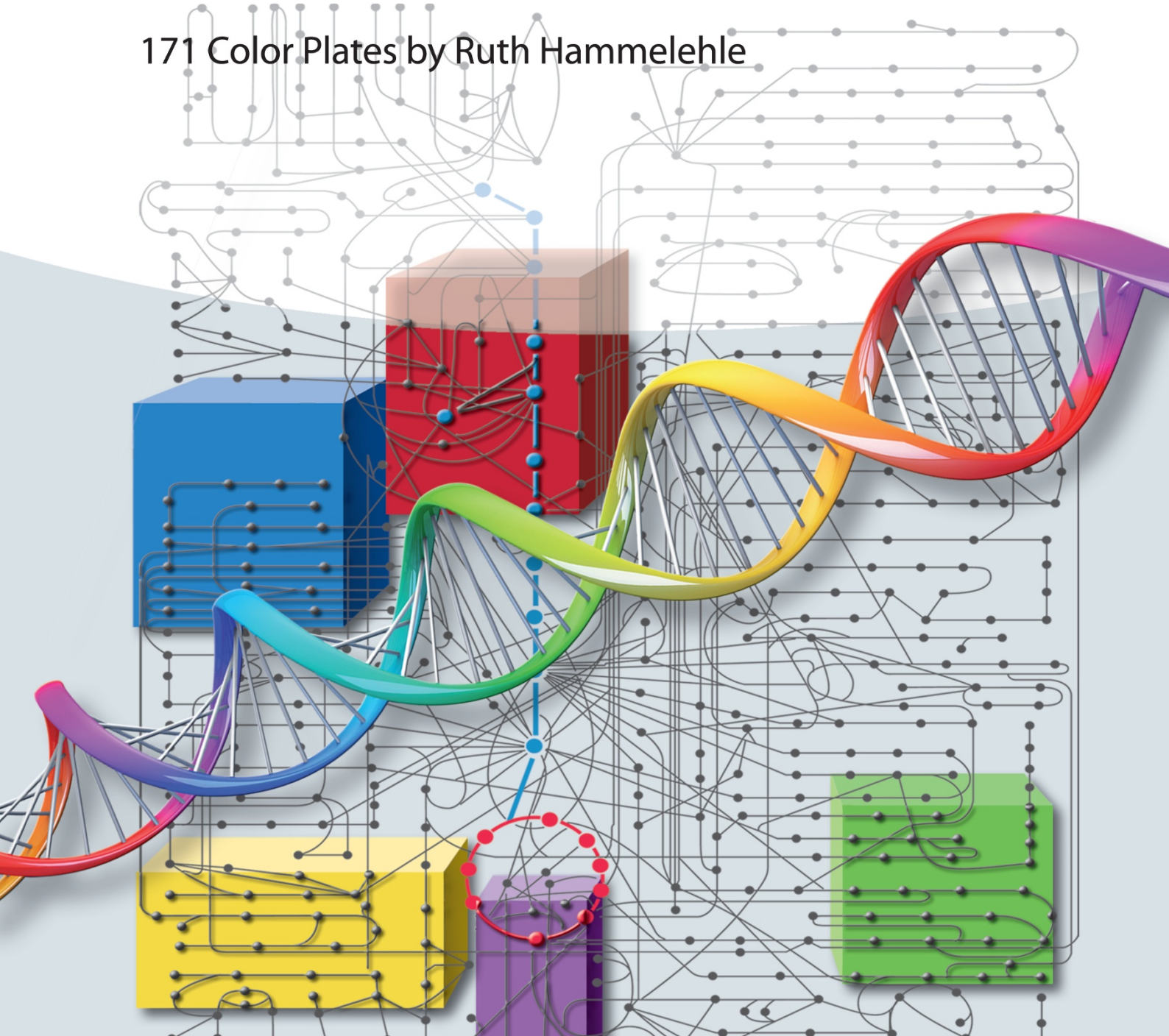
An Illustrated Primer

171 Color Plates by Ruth Hammelehle



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Preface to the 1st edition

Biotechnology, a key technology of the 21st century, is more than other fields an interdisciplinary endeavor. Depending on the particular objective, it requires knowledge in general biology, molecular genetics, and cell biology; in human genetics and molecular medicine; in virology, microbiology, and biochemistry; in the agricultural and food sciences; in enzyme technology, bioprocess engineering, and systems science. And in addition, biocomputing and bioinformatics play an ever-increasing role. Against this background, it is of little surprise that few concise textbooks try to cover the whole field, and important applied aspects such as animal and plant breeding or analytical biotechnology are often missing even from multivolume monographs.

On the other hand, I have experienced during my own life-long studies, and also when teaching my students, how energizing it is to emerge occasionally from the thousands of details which must be learned, to look at a unifying view.

The Pocket Guide to Biotechnology and Genetic Engineering is an attempt to provide this kind of birds-eye perspective. Admittedly, it is daring to discuss each of this book's topics, ranging from "Beer" to "Tissue Engineering" and "Systems Biology", on a single text page, followed by one page of graphs and tables. After all, monographs, book chapters, reviews, and hundreds of scientific publications are devoted to each single entry covered in this book (many of them are provided in the literature citations). On the other hand, the challenge of surveying each entry in barely more than 4000 characters forces one to concentrate on the essentials and to put them into a wider perspective.

I hope that I have succeeded at least to some extent in this endeavor, and that you will find the clues to return safely from the highly specialized world of science, and its sophisticated terms, to your own evaluation of the opportunities and challenges that modern biotechnology offers to all of us.

This English version is not a simple translation of the original version, which was published in German in December, 2001, but an improved and enlarged second edition: apart from a general update of all data, it contains three new topics (Tissue Engineering, RNA, and Systems Biology).

At this point, my thanks are due to some people who have essentially contributed to this book. Above all, I wish to acknowledge the graphic talent of Ruth Hammelehle, Kirchheim, Germany, who has done a great job in translating scientific language into very clear and beautiful graphs. Marjorie Tiefert, San Ramon, California, has been more than an editor: she has caught and expressed the original spirit of this book. My thanks also to the publisher, in particular to Romy Kirsten. Special thanks are due to the many colleagues in academia and industry who have contributed their time and energy to read through the entries in their areas of expertise and provide me with most useful suggestions and corrections. These were: Max Roehr, University of Vienna; Waander Riethorst, Biochemie GmbH, Kundl; Frank Emde, Heinrich Frings GmbH, Bonn; Peter Duerre, University of Ulm; Edeltraut Mast-Gerlach, Ulf Stahl and Dietrich Knorr, Technical University Berlin; Udo Graefe, Hans-Knoell Institute, Jena; Jochen Berlin, GBF, Braunschweig; Allan Svenson, Novozymes A/S, Copenhagen; Helmut Uhlig, Breisach; Frieder Scheller, University of Potsdam; Bertold Hock, University of Munich-Weihenstephan; Rolf Blaich, Rolf Claus, Helmut Geldermann and Gerd Weber, University of Hohenheim; Hans-Joachim Knackmuss, Dieter Jendrossek, Karl-Heinrich Engesser, Joerg Metzger, Peter Scheurich, Ulrich Eisel, Matthias Reuss, Klaus Mauch, Christoph Syldatk, Michael Thumm, Joseph Altenbuchner, Paul Keller and Ulrich Kull, University of Stuttgart; Thomas von Schell, Stuttgart; Joachim Siedel, Roche AG, Penzberg; Rolf Werner and Kerstin Maier, Boehringer-Ingelheim, Biberach; Frank-Andreas Gunkel, Bayer AG, Wuppertal; Michael Broeker, Chiron Bering GmbH, Marburg; Bernhard Hauer and Uwe Pressler, BASF AG, Ludwigshafen; Frank Zocher, Aventis Pharma, Hoechst; Tilmann Spellig, Schering AG, Bergkamen; Akira Kuninaka, Yamasa Corporation, Chosi; Ian Sutherland, University of Edinburgh; Julia Schueler, Ernst & Young, Frankfurt. Among the many members of my institute in Stuttgart who have patiently helped me with the manuscript I wish

to especially acknowledge Jutta Schmitt, Till Bachmann, Jürgen Pleiss and Daniel Appel.
In spite of all efforts and patient cross-checking, it would be a miracle if no unclearness or errors exist. These are entirely the author's fault. I would be most grateful to all readers who will let me know, via the web address www.itb.uni-stuttgart.de/pocketguide, where this book can be further improved.

Rolf D. Schmid
Stuttgart, New Year 2002/2003

Preface to the 2nd edition

In the 10 years since the first edition of this booklet in English, the developments in biotechnology have further accelerated. This is true for the science, which has generated new methods such as synthetic biology, genome editing or high-throughput sequencing of genomes, generating big data which provide us with ever more detailed perceptions of the living world. New applications in industry have followed suit – in the medical sciences, eminent examples are the therapeutic antibodies, iPS-derived stem-cell technologies or a personalized medicine based on SNP analysis and companion diagnostics; in industrial biotechnology, the emerging concepts of a "bioeconomy" based on renewable resources such as biomass, waste or carbon dioxide provide certainly a megatrend. It goes without saying that a little booklet can only provide short sketches for each of these fields. An updated literature suvey attempts to compensate for this shortcoming.
It is my great joy that Professor Claudia Schmidt-Dannert, University of Minnesota, has accepted to join this and future editions as a co-author. This will help to keep the wide information provided in this book as updated as possible in a global setting.
Our sincere thanks go, beyond the individuals mentioned in the first edition, to numerous friends and colleagues who have helped again with their professional knowledge. Our particular appreciation goes to Wolfgang Wohlleben, Tuebingen University; Karin Benz, NMI Reutlingen; Ulrike Konrad, Protagen; Karl Maurer, ABEnzymes, Darmstadt; Bernhard Hauer, Georg Sprenger and Juergen Pleiss, Stuttgart University; Ulrich Behrendt, Munich; Dirk Weuster-Botz, Munich Technical University; Joern Kalinowsky, Bielefeld University; Vlada Urlacher, Düsseldorf University, and Frieder Scheller, Potsdam University.
The high quality of the artwork is due to Ruth Hammelehle, Kirchheim, of the final editing to Bernhard Walter, both of epline Co., Kirchheim u. T. Our deep thanks to both of them, to the editorial team, Dr Gregor Cicchetti, Dr Andreas Sendtko and Dr Claudia Ley at Wiley-VCH in Weinheim, Germany , and to Dr Sarah Perdue and Dr Bradford Condon at the University of Minnesota, St. Paul. The contribution of Dr Alexandra Prowald, who provided an excellent index to this book, is also highly appreciated.

Rolf D Schmid, Claudia Schmidt-Dannert
Stuttgart, Germany and St. Paul, Minnesota, Summer 2015

Introduction

This pocket guide is written for students of biology, biochemistry and bioprocess engineering who are looking for a short introduction to the many different areas where modern biotechnologies are making an impact. It is also intended as a handy reference for teachers, patent attorneys, managers and investors seeking a quick, yet professional answer surrounding an upcoming topic of industrial biotechnology. To this end, specialized knowledge from a wide range of scientific disciplines has been condensed over a total of 171 color plates and further described on the accompanying text page, as well as complemented by a comprehensive survey of the literature. Cross-references provide additional help in jumping from technical applications of biotechnology, for example, to the fundamental science behind the application.

Completely updated and supplemented by many new topics, this second edition retains the modular format, but the structure of the book has been changed. It now begins, after a brief historical survey, with short introductions to the basic fields of modern biotechnology: **microbiology**, **biochemistry**, **molecular genetics**, **cell biology** and **bioprocess engineering**. It is only in the second part that the focus is on applications, such as **food and food additives**, **industrial products**, **enzyme technology** and, most comprehensively, the many contributions of biotechnology to the medical field, including the manufacture of **antibiotics**, biologicals such as **antibodies**, but also in **medical technology**. This section is rounded off with a description of the applications in **agriculture,** such as animal or plant breeding, and in **environmental protection**. The third section of the book deals with the current megatrends in the applied life sciences. These include **genomics** and such post-genomic trends as **personalized medicine,** with **bioinformatics** seen as an answer to current needs in big data processing, but also **cell technology** and **gene therapy,** as well as technologies devoted to building a new so-called **bioeconomy, i. e.** sustainable in energy and material use. The text ends with five chapters devoted to various aspects of **safety and ethics,** including patent and registration-related topics.

The objective of this book is to provide readers with a compact reference on the wide and expanding field of modern biotechnology. We hope that we succeeded not only in offering an attractive and stimulating read, but also in instigating in the reader the desire to dig deeper into this fascinating area of human endeavor.

Early developments

History. The origins of what we call biotechnology today probably originated with agriculture and can be traced back to early history. Presumably, since the beginning people have gained experience on the loss of food by microbial spoilage; on food conservation by drying, salting, and sugaring; and on the effects of fermented alcoholic beverages. As the first city cultures developed, we find documents and drawings on the preparation of bread, beer, wine, and cheese and on the tanning of hides using principles of biotechnology. In Asia, fermented products such as Sauerkraut (China), Kimchi (Korea) or Gari (Indonesia) have been produced for thousands of years. In Europe, starting in the 6th century, the monasteries with their well organized infrastructure developed protocols for the arts of brewing, wine-making, and baking. We owe our strong, alcohol-rich stout beers to the pious understanding of the monks that "Liquida non fragunt ieiunum" (Liquors do not interfere with the chamfering time). Modern biotechnology, however, is a child of microbiology, which developed significantly in the late 19th century. The First and Second World Wars in the first half of the 20th century next probably provided the strongest challenge to microbiologists, chemists, and engineers to establish modern industrial biotechnology, based on products such as organic solvents and antibiotics. During and after this period, many ground-breaking discoveries and developments were made by biochemists, geneticists, and cell biologists and gave rise to molecular biology. At this point, the stage was set for modern biotechnology, based on genetic and cell engineering, to come into being during the 1970s and '80s. With the advent of information technology, finally, modern biotechnologies gave rise to genomics, proteomics and cellomics, which promise to develop into the key technologies of the 21st century, with a host of applications in medicine, food and agriculture, chemistry and environmental protection.

Early pioneers and products. Biotechnology is an applied science – many of its developments are driven by economic motives. In 1864 Louis Pasteur, a French chemist, used a microscope for the first time to monitor the fermentation of wine vs. lactic acid. Using sterilized media ("pasteurization"), he obtained pure cultures of microorganisms, thus laying the foundation for applied microbiology and expanding this field into the control of pathogenic microorganisms. At the start of the 20th century, it occurred to the German chemist Otto Roehm and to the Japanese scientist Jokichi Takamine that enzymes isolated from animal wastes or from cultures of molds might be useful catalysts in industrial processes. Otto Roehm's idea revolutionized the tanning industry, since tanning up to this time was done using dog excrements. In the field of public health, the introduction of biological sewage treatment around 1900 was a milestone for the prevention of epidemics. During World War I, Carl Neuberg in Germany and Chaim Weizmann, a Russian emigrant to Britain and of Jewish origin, developed large-scale fermentation processes for the preparation of ammunition components (glycerol for nitroglycerol and acetone for Cordite). The Balfour declaration and the ensuing foundation of the state of Israel, whose first president Weizmann became, is thus directly linked to an early success in biotechnology. In the postwar period, 1-Butanol, the second product from Weizmann's Clostridium-based fermentation process, became highly important in the USA as a solvent for car paints. The serendipitous discovery of penicillin by Alexander Fleming (1922), much later turned into a drug by Howard Florey, initiated the large-scale production of penicillin and other antibiotics during World War II. As early as 1950, > 1000 different antibiotics had been isolated and were increasingly used in medicine, in animal feeds, and in plant protection. This was accompanied by a rising tide of antibiotic resistance, triggering research on the mechanisms of microbial defense mechanisms. Since 1950, the analytical use of enzymes, later of antibodies, began another important field of modern biotechnology. The first glucose biosensor was introduced by Leland C. Clark in 1954, initiating a concept for blood glucose monitoring which now commands a market of several billion US-$. In the shadow of the 1960s' oil crises and the emerging awareness of overpopulation, the conversion of biomass to energy such as bio-ethanol and of single-cell protein from petroleum or methanol was developed. Now, in 2014, "biorefineries" are under active development.

Biotechnology in early Egyptian drawings

1 beer brewing
2 bread baking
3 hide tanning

early history	sugar-containing juices are fermented to various alcoholic beverages
	sour milk and sourdough products are prepared by lactic acid and yeast fermentation
	hides are bated to leather using reagents such as animal feces
1650	France: Orléans procedure for the preparation of vinegar from ethanol
~1680	The Netherlands: Anthony van Leuwenhoek observes bacteria through a microscope
1856	France: Louis Pasteur separates brewers yeasts from lactic acid bacteria
~1890	France, Germany: Louis Pasteur, Robert Koch develop the first vaccines
1900	Japan: Jokichi Takamine uses α-amylase for starch degradation
1908	Germany: Otto Roehm uses pancreatic trypsin in detergents and for leather bating
1916	UK: Chaim Weizmann develops a fermentation process for acetone, n-butanol
1920	citric acid is industrially produced by surface fermentation using *Aspergillus niger*
1928/29	UK: Alexander Fleming discovers penicillin
1943	USA: Selman Waksman discovers streptomycin
1949	USA: microbial transformation of steroids on industrial scale
1957	Japan: glutamic acid is industrially produced by tank fermentation of *Corynebacterium glutamicum*
1960	Denmark: *Bacillus* proteases are used in detergents
1965	Denmark: microbial rennet for cheese production
1970	USA: high-fructose syrups produced by enzyme technology replace saccharose in softdrinks
1972/73	USA: Stanley Cohen and Francis Boyer develop a procedure for in-vitro recombination of DNA, using plasmid vectors
1975	UK/Switzerland: César Milstein and Georges Koehler prepare monoclonal antibodies using hybridoma cells
1977	recombinant proteins can be manufactured by fermentation using bacteria
1982	first transgenic plants (herbicide resistance) and animals (knockout)
1985	USA: Kary Mullis discovers the polymerase chain reaction (PCR)
from 1990	USA: the human genome project (HUGO) is initiated
1995	transgenic tomatoes (Flavr Savr) are registered as food in the USA and the UK
1995	gene therapy experiments on humans
1996	the yeast genome is completely sequenced
1998	Dolly the sheep is the first cloned animal, a replicate of its mother
1998	over 2 billion basepairs are stored in DNA sequence databases
1999	the Drosophila genome with 1.6 billion bp is completely sequenced in ~ 4 months
1999	human stem cells can be maintained in culture
1999	the sales of recombinant therapeutic proteins exceed 10 billion US$/yr
2001	Craig Venter's Celera and the international Human Genome Consortium (HGP) present a sketch of the human genome
2008	the USA produces over 30 billion L of bio-ethanol from corn
2012	Shinya Yamanaka, Japan, receives the nobel prize for transforming differentiated cells into autologous stem cells (iPS technology)
2014	transgenic plants are grown on >180 million ha land in 28 nations

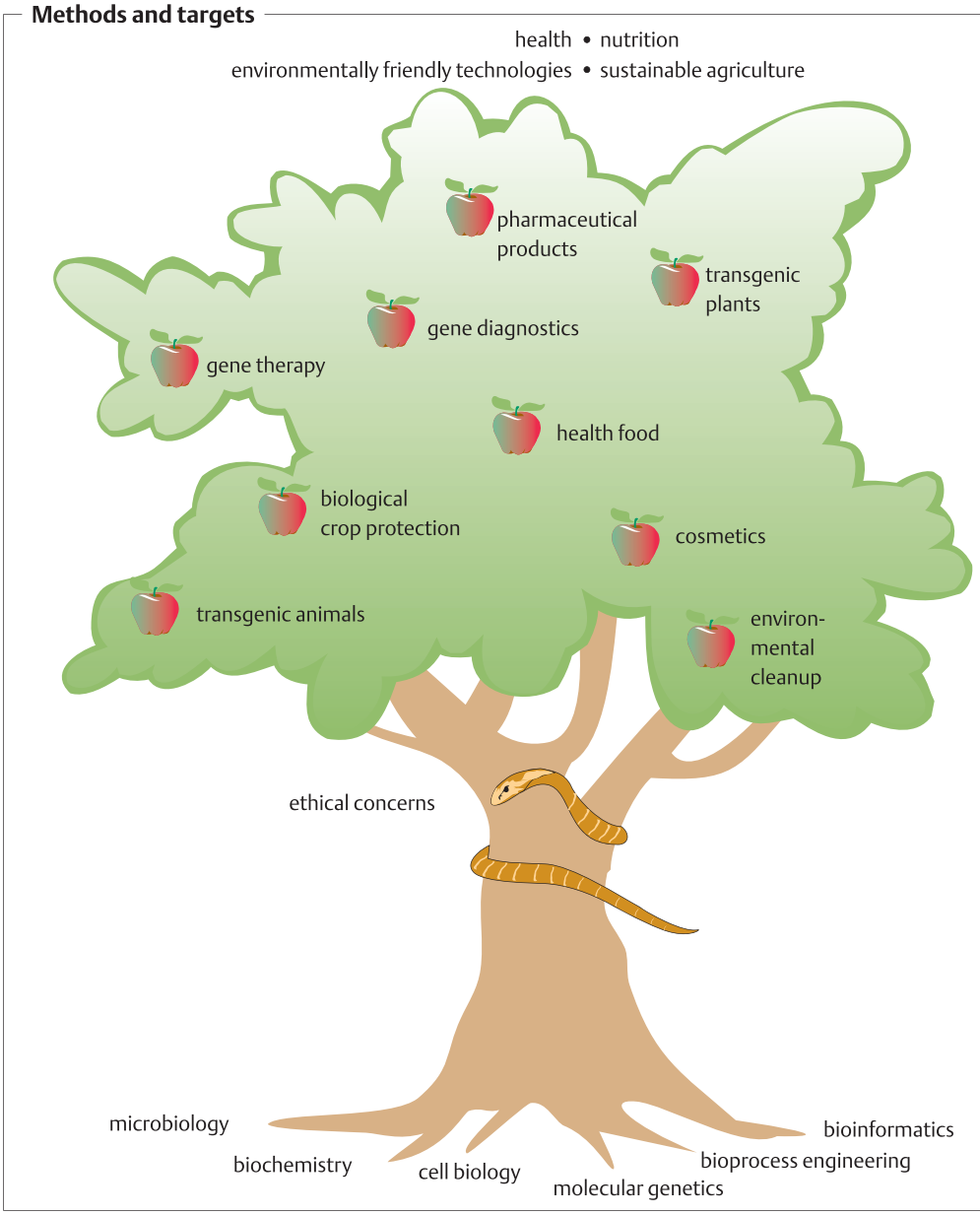
Biotechnology today

Genetic Engineering and Cell Technology. In 1973, Stanley Cohen and Frederick Boyer in San Francisco were the first to express a designed foreign gene in a host organism. After about 10 years the first recombinant drug, human somatotropin, was registered. Since then, more than 50 genetically engineered proteins have been registered as therapeutic agents, including insulin (for diabetics), erythropoietin (for anemic patients), factor VIII (for hemophiliacs), interferon-β (for multiple sclerosis patients), recombinant antibodies and vaccines. Many hundred more are under development. Although the new technologies were first applied to medicine, their innovation potential in agriculture and food production soon began to emerge. Thus, transgenic crops were bred that were resistant to herbicides, insects, or viruses. Today, they are predominantly grown in North America. Flowers have been genetically modified to exhibit new colors, vegetables or fruits to show enhanced nutritive properties, and woods to contain less lignin for improved paper production. In the chemical industry, biopolymers, prepared from biomass-derived chemicals such as starch or cellulose, have begun to replace petrochemical products, and "biorefineries" have appeared which generate biofuels and chemicals from biomass. These technologies are changing the face of agriculture. High-throughput gene sequencers and supercomputers are making the sequencing of human genomes relatively cheap and routine, and genome-based information is now widely used to understand the molecular basis of diseases and to develop novel drugs by a target-oriented screening approach. Novel approaches, such as proteomics and structural biology, are contributing to our fundamental understanding of the chemistry of life and disease. Using gene therapy, we attempt to replace malfunctioning with correctly functioning genes. These developments are in step with great advances in cell biology, which focus on the complex interactions of cells in a multicellular organism. Human differentiated cells such as cardiomyocytes or neurons can now be obtained from embryonal stem cells or even from adult human cells by genetic reprogramming via induced pluripotent stem cells (iPS). Tissue engineering has become a surgical approach to repairing wounded tissue such as skin, bone or cartilage.

Public acceptance. The sheep Dolly, born in 1998, was the first animal ever cloned from a somatic cell of and thus identical to her mother. The thrust and possible consequences of such developments, e. g., for embryonic manipulations or individual (prenatal) genetic fingerprinting, have led to emotional public discussion. Typical subjects are: at what stage does human life begin and when does it need to be protected? Do we accept the cloning of humans? To which extent can we accept a deterministic view of individual health risks, e. g., by an employer or an insurance company? How will molecular genetics and gene therapy affect the age distribution in our societies? Is it ethical to genetically modify plants and animals at will? To what extent are such manipulations in harmony with the ecosystem and its natural diversity? How will the new biotechnologies influence the relationship of industrialized and developing economies? None of these questions has been completely resolved yet. As we begin to understand and interfere with the functions of the human brain, answering these questions on a global scale will become even more urgent.

Foundations. The body of this pocket guide is devoted to the many and growing applications of biotechnology, including discussion of today's "megatrends" (2014), which include bioinformatics. In the introduction to this book, however, the multidisciplinary foundations of the field are briefly outlined. We start with *microbiology*, which is the oldest discipline and has led the way to many contemporary technologies. This is followed by *biochemistry*, the science of life's building blocks, their metabolism and its regulation. A key property of life is to propagate. As a consequence, the basics of *molecular genetics* and *genetic engineering* will be presented. *Cell biology* and *immunology* continue to have a great impact on biotechnology, and some basics are introduced. Finally, without *bioprocess engineering*, a discipline mastered by engineers, the manufacturing of bioproducts could not be done in an economical way. It is obvious that the space available does not allow a thorough discussion of all these fields, but current literature will be provided to the reader interested in further reading.

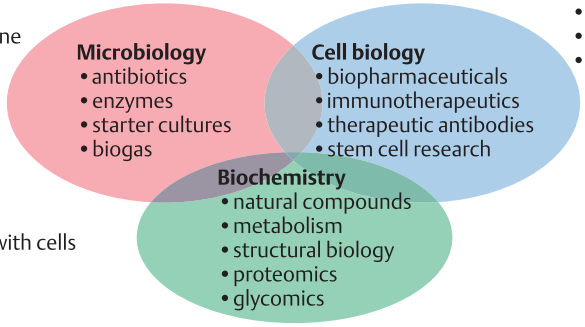
Methods and targets



Scientific foundations

Gene technology/genome research
- recombinant products
- personalized medicine
- gene diagnostics and gene therapy
- animal and plant breeding
- synthetic biology

Bioprocess engineering
- production technology with cells or enzymes
- waste water treatment
- bioenergy



Bioinformatics
- databases
- analysis of "big data"
- metabolic engineering
- systems biology

Viruses

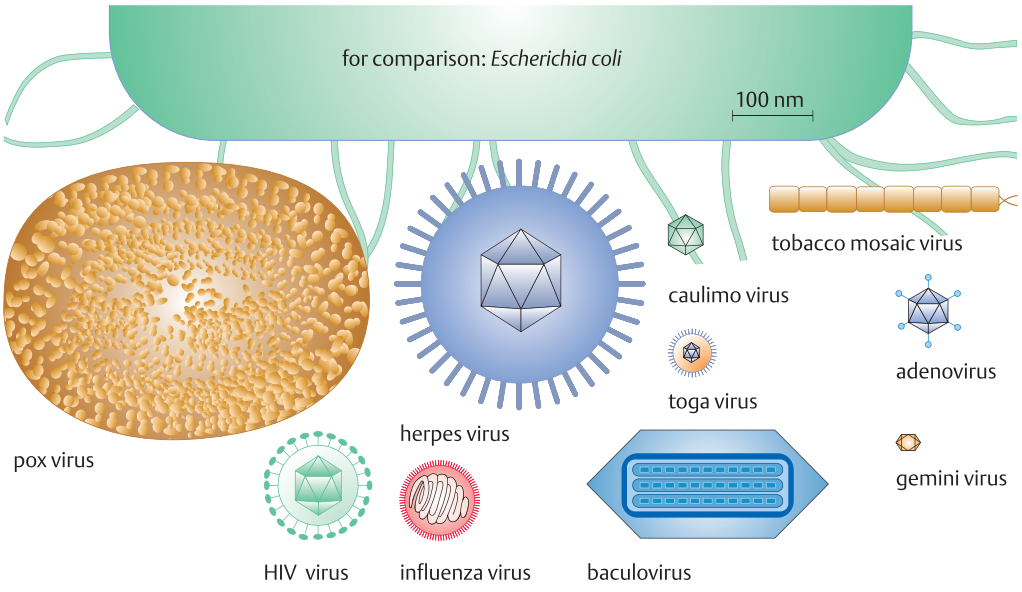
General. A virus is an infectious particle without indigenous metabolism. Its genetic program is written in either DNA or RNA, whose replication depends on the assistance of a living host cell. A virus propagates by causing its host to form a protein coat (capsid), which assembles with the viral nucleic acid (virus particle, nucleocapsid). Viruses can infect most living organisms; they are mostly host-specific or even tissue- or cell-specific. Viruses are classified by their host range, their morphology, their nucleic acid (DNA/RNA), and their capsids. In medicine and veterinary medicine, the early diagnosis, prophylaxis and therapy of viral human and veterinary diseases plays a crucial role. AIDS (HI virus), viral hemorrhagic fever (Ebola virus), avian flu (H5N1-, H7N9-virus) (→250) or hepatitis (several virus families) are important examples of viruses involved in human diseases, as are Rinderpest (Morbillivirus) or infectious salmon anemia (ISA virus) in epizootic veterinary diseases. In biotechnology, viruses are used for the development of coat-specific or component vaccines and for obtaining genetic vector and promoter elements which are, e. g., used in animal cell culture and studied for use in gene therapy.

Viruses for animal experiments. The first cloning experiments with animal cells were done in 1979, using a vector derived from simian virus 40 (SV40) (→98). This virus can infect various mammals, propagating in lytic or lysogenic cycles (lysis vs. retarded lysis of host cells). Its genome of ca. 5.2 kb contains early genes for DNA replication and late genes for capsid synthesis. Expression vectors based on SV40 contain its origin of replication (ori), usually also a promoter, and a transcription termination sequence (polyA) derived from the viral DNA. For the transfection of mouse cells, DNA constructs based on bovine papilloma virus (BPV) are preferred. In infected cells, they change into multicopy plasmids which, during cell division, are passed on to the daughter cells. Attenuated viruses derived from retro, adeno, and herpes viruses are being investigated as gene shuttles for gene therapy (→304). Retroviruses, e. g., the HI virus, contain an RNA genome. They infect only dividing cells and code for a reverse transcriptase which, in the host cell, transcribes the RNA into cDNA. HIV-cDNA is then integrated into the host genome where it directs, via strong promoters, the formation of viral nucleic acid and capsid proteins. Some hundred experiments with retroviral vectors having replication defects have already been carried out for gene therapy. A disadvantage of using retroviral vectors lies in their small capacity to package foreign DNA (inserts), whereas vectors derived from adenoviruses can accommodate up to 28 kb of inserted DNA. In contrast to retroviruses, adenoviruses can infect non-dividing cells, but their DNA does not integrate into the host chromosomal DNA. For gene therapy targeted to neuronal cells, e. g., in experiments related to Alzheimer's or Parkinson's disease, *Herpes simplex*-derived vectors are often used. Their large genome of 152 kb allows them to accommodate inserts > 20 kb of foreign DNA. A similar insert size is reached with Vaccina viruses, which may infect a wide range of cell types.

Viruses for plant experiments. Most plant viruses have an RNA genome (→280). Only two groups of DNA viruses are known that infect higher plants, caulimo virus and gemini virus. Caulimo viruses have a quite narrow host range: they infect only crucifers such as beets and some cabbage varieties. Their small genome reduces their potential for accommodating foreign inserts. Gemini viruses infect important agricultural plants such as maize and wheat and thus bear significant risks for application. Moreover, their genomes undergo various rearrangements and deletions during the infection cycle, rendering the correct expression of foreign DNA inserts difficult.

Baculoviruses infect insects but not mammals. After infection, host cells form a crystalline protein (polyhedrin), which may constitute > 50 % of the insect cell. The polyhedrin promoter is therefore useful for the heterologous expression of proteins, using cell cultures of *Spodoptera* (a butterfly). An advantage of this system is that posttranslational glycosylations in this system resemble those of mammalian cells (→262). Scale-up of this system is, however, limited, rendering it most useful for laboratory experiments. In Japan, silk worms (*Bombyx mori*) are considered an interesting system for expressing foreign proteins. The nuclear polyhedral virus BmNPV is being used for their transfection.

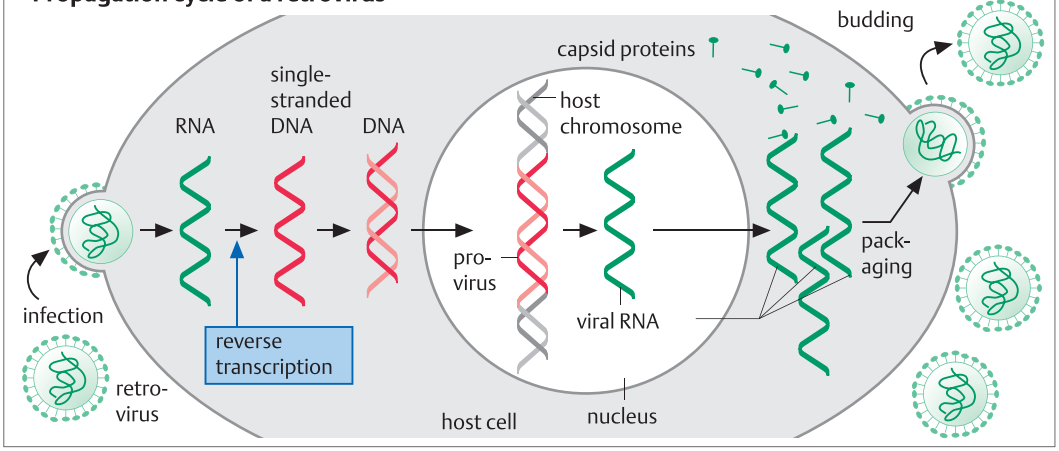
Forms



virus	host	disease	capsid	nucleic acid
smallpox	man, cattle	smallpox	complex coat	linear DNA, d
hepatitis B	man	hepatitis B	polyhedric capsid	circular DNA, d
toga	man	measles	polyhedric capsid	(+)-RNA, s
herpes	man, birds	belt rose, herpes	polyhedric capsid	linear DNA, d
HIV	man, primates	AIDS	round capsid	2 × (+)-RNA, s
influenza	man, mammals	influenza	helical coat	(–)-RNA, segmented
adenovirus	man	common cold	polyhedric capsid	linear DNA, d
papilloma	cattle	warts	polyhedric capsid	circular DNA, d
tobacco mosaic	tobacco plant		polyhedric capsid	RNA, s
caulimo	cabbage		polyhedric capsid	circular DNA, s
gemini	dicots		double polyhedron	circular DNA, s
baculo	insects		polyhedric capsid	circular DNA, d

s = single strand, d = double strand, + = sense direction, – = antisense direction

Propagation cycle of a retrovirus



Bacteriophages

General. Viruses that attack bacteria are termed bacteriophages or simply phages. Their taxonomy is determined by the International Committee on Taxonomy of Viruses, ICTV. Phages occur everywhere in nature, and are abundant in metagenomic analyses of water samples (→74). As there are historic reports of healing by "holy waters," they have been widely studied for the treatment of bacterial infections but results are equivocal. Fermentation processes, e.g., starter culture production (→114), are always endangered by phage infections. As a preventive measure, attempts are usually made to select phage-resistant strains. Phages are useful in genetic engineering, e.g., for the development of cloning vectors or promoters, for DNA sequencing, and for the preparation of gene and protein libraries (→62, 64, 68). Since most cloning experiments use E. coli, phages specific for this bacterium (λ-, M13-, Qβ-, T-phages) play a key role.

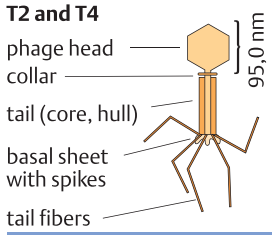
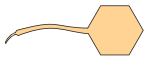
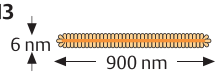
λ Phage. When infecting E. coli, λ phage can follow two routes: either its linear double-stranded DNA (ca. 48.5 kbp, ca. 1% of the E. coli genome) is propagated independent of the E. coli genome, resulting in lysis (lytic cycle), or it is integrated into the E. coli genome, resulting in lysogenic cells containing latent prophages, which replicate with the bacterium over several generations. Upon stress, such as a rise in temperature or UV irradiation, the prophage is excised from the E. coli genome and lyses the host cell. λ is able to form cohesive or sticky ends of 12 unpaired nucleotides each (cos sites), which are necessary for circular λ DNA formation and for its integration into the E. coli genome. The sticky ends also form the recognition signal for the formation of the viral gene product A, an exonuclease. After replication of the λ DNA into a concatemer of linear λ genomes, endonuclease A cuts at this position, initiating the packaging of progeny into its capsids. Cosmids, an important tool for the construction of large gene libraries, are derived from the λ phage, as is a family of λ plasmids such as λEMBL4, which can be induced by a rise in temperature.

The M13 phage infects E. coli according to a different mechanism. It contains single-stranded DNA of ca. 6.4 kb, which after infection directs the synthesis of its complementary strand. The resulting double-stranded phage DNA is not integrated into the E. coli genome but is continuously replicated in the cytoplasm, giving rise to up to 1,000 phage particles/cell. During host cell division, the phage infection is passed on to the daughter cells (ca. 100/cell). Genes that have been cloned into a vector derived from M13 can be obtained as single-stranded DNA – a technique used for classical DNA sequencing (→56). Prior to the invention of PCR, M13 vectors were used for site-directed mutagenesis of proteins.

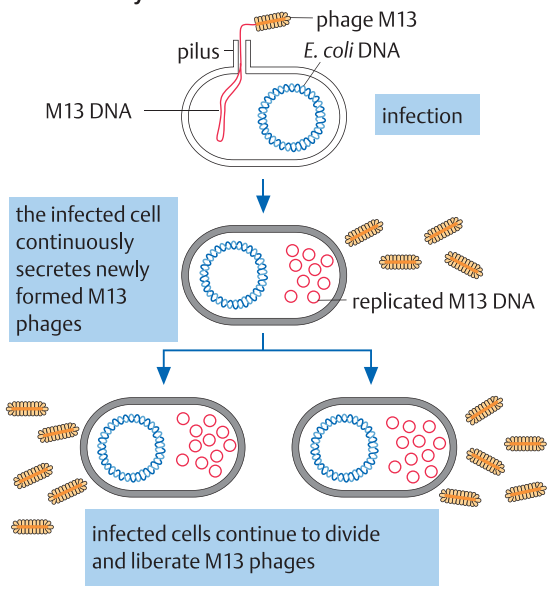
T Phages occur in 7 different types. For genetic engineering, two enzymes coded by T phage genomes are useful: the DNA ligase of T4, which links DNA fragments regardless of the quality of their ends (sticky or blunt), and the DNA polymerase of T7, which polymerizes DNA on a single strand DNA matrix; it is used in gene sequencing (Sanger–Coulson method). The promoter of the T7-RNA polymerase is used in several E. coli expression vectors. T7-RNA polymerase transcribes DNA into RNA, which in turn serves as mRNA in cell-free protein synthesis, based on mRNA, tRNAs, ribosomes, amino acids and ATP.

Phages of other bacteria. Among the > 1,000 classified phages (some 2800 in total), > 300 are specific for enterobacteria, > 230 for bacteriococci, and > 150 each for Bacilli and Actinomycetes. Another group (at present 13 phages), described only recently, is the Ligamenvirales which attack archaeabacteria. Their structure and function are closely related to those of other viruses, including those specific for E. coli. Some of them can be either virulent or lysogenic, similar to the λ phage. Lactobacilli-specific phages are a major problem in the manufacture of milk products. Resistant bacteria prevent adsorption or replication of these phages. Among the 5 groups of Bacillus phages, ø105 and SPO2 are often used for transformation, and PBS1 has been used in construction of the B. subtilis genome sequence map. Phage D3112 is the preferred vector for the transformation of Pseudomonads, and SH3, SH5, SH10, or øC31 are preferred for the genetic engineering of Streptomyces.

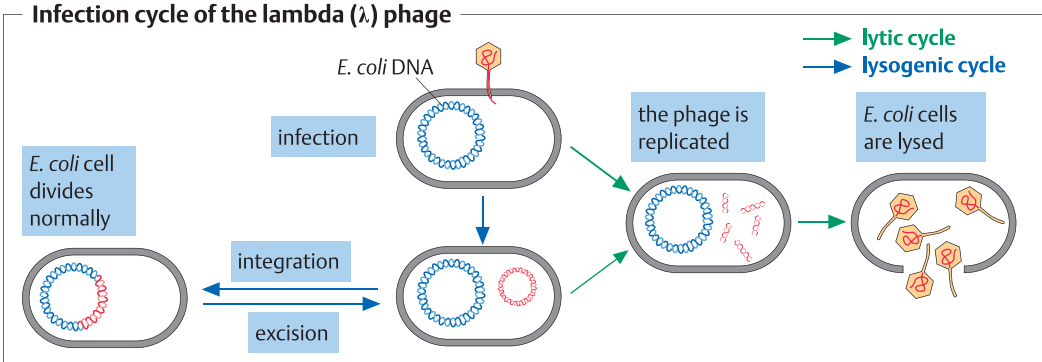
E. coli phages (select)

name	form	genetic material
T2 and T4		DNA (double-stranded)
T7		DNA (double-stranded)
lambda (λ)		DNA (double-stranded)
M13		DNA (single-stranded)

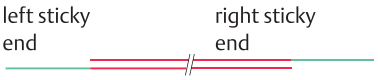
Infection cycle of M13



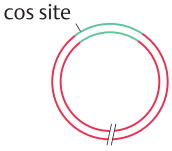
Infection cycle of the lambda (λ) phage

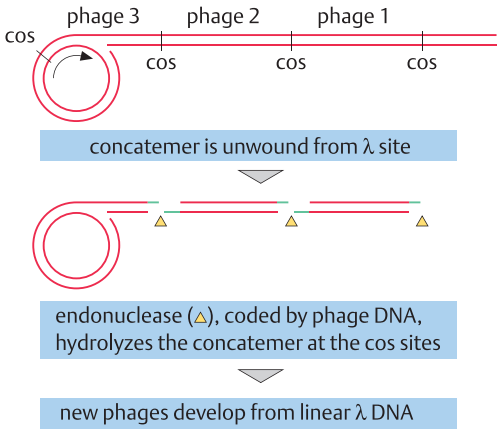


λ DNA in linear form

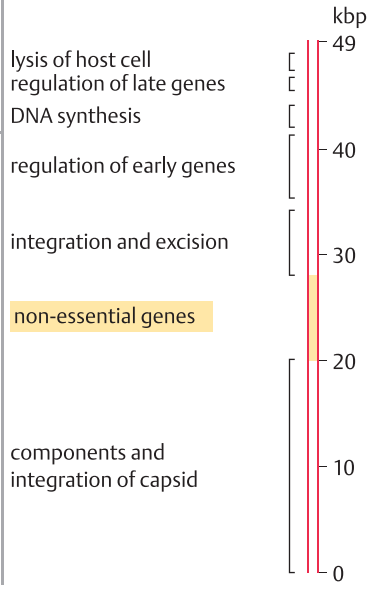


λ DNA in circular form





Genomic map of the λ phage



Microorganisms

General. Microorganisms play a key role in the chemical cycles on earth. They are involved in the biodegradation of many compounds; these processes occur not only in the environment, but also in symbiosis with other organisms (e. g., lichens, intestinal and rumen bacteria). Some microorganisms are parasites or pathogens, impairing the health or life of other organisms. In biotechnology, nonpathogenic microorganisms are used to produce various products such as citric and glutamic acid, antibiotics, xanthan, and enzymes; for the aerobic and anaerobic treatment of wastewater, sludges, soils, and air; and as host organisms for the manufacture of recombinant proteins. Due to their unicellular structure, well established methods for creating and selecting mutants, and their short generation time, they serve as model organisms for understanding the biochemical, genetic, and physiological mechanisms of life, and as a preferred host for the manufacture of recombinant proteins. Based on some fundamental differences, prokaryotic and eukaryotic microorganisms can be distinguished; the former are further subdivided into eubacteria and archaebacteria (> 10,000 different fully characterized strains).

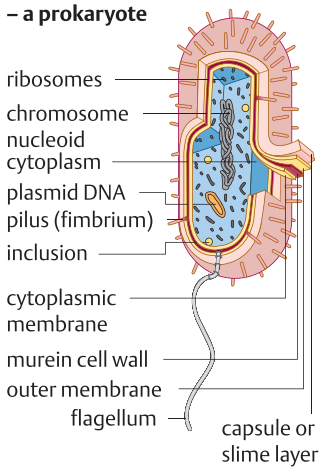
Eubacteria are unicellular organisms that propagate by cell division. Their cell diameter is usually on the order of 1 μm. They have no cell nucleus, and their chromosomal DNA is formed into a tangle, the nucleoid. Frequently, part of their genetic makeup occurs on nonchromosomal genetic elements, the plasmids (→44). Plasmids are often horizontally transferred to other bacteria – a useful mechanism, from the human perspective, for evolving biodegradation pathways for xenobiotic compounds in the environment and sewage plants, but a very dangerous capacity with respect to the evolution of antibiotic resistances. The cell wall, made of peptidoglycan, is more complex in Gram-negative microorganisms and often covered with a slimy layer from which flagella may protrude, which ensure mobility. In the cytoplasm, storage chemicals such as polyhydroxybutyric acid polyphosphate, cyanophycin, or others may be deposited. Eubacteria have a wide potential for variations in metabolism and thus can grow in a much wider range of habitats than higher organisms. Such highly specialized species often surprise us by their unique proteins and cofactors. Thus, the purple membrane of the halobacteria is a unique functional unit of this genus, exhibiting some analogies to photosynthesis and the chemistry of vision in higher organisms.

Archaebacteria (archaea) are believed to resemble the oldest forms of life on earth. Their footprints have been detected in geological formations many hundreds of millions of years old. They often live anaerobically and are usually specialized for growth in unique biotopes. As just one example, the methanobacteria form the most important group of sludge consortia, reducing acetic acid to methane (→288). They differ from the eubacteria in structural and genetic properties, e. g., in the construction of their cell membrane from ether lipids instead of phospholipids. The function of their enzymes is adapted to their often extreme habitats and have been used in biotechnology. For example, a DNA polymerase from a deep-sea bacterium, Pyrococcus furiosus, is often used for PCR reactions with particular high fidelity (→50, 196).

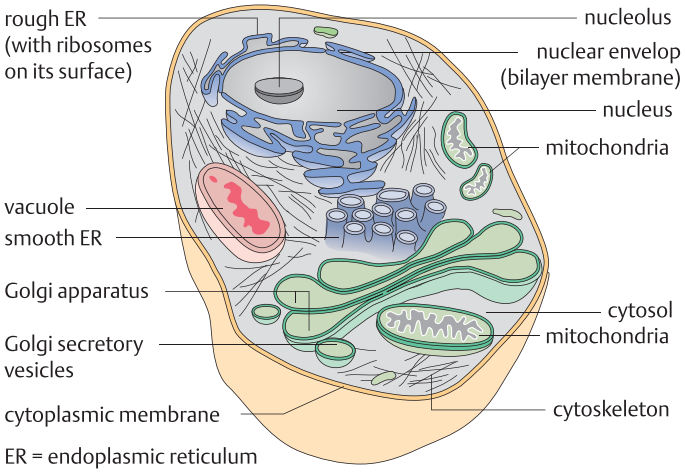
Yeasts and fungi are eukaryotic organisms and so far constitute the largest group of cultivatable microorganisms: about 70,000 different strains have been taxonomically classified. In contrast to prokaryotes, they contain a cell nucleus and other subcellular functional units, and their cell wall is made of chitin, sometimes also from cellulose. Most yeasts and fungi live aerobically. Their wide differences in reproduction and life cycles provide the most useful basis for their taxonomic classification. The vegetative body of fungi is composed of a hairy network, the mycelium, which can propagate sexually or asexually. Asexual reproduction usually proceeds by spore formation, or occasionally by budding. Sexual reproduction of the lower fungi (Phycomycetes) proceeds via gametes, of the higher fungi via fruiting bodies (asci) which have the form of a sac (Ascomycetes) or a club (Basidiomycetes). Yeasts (e. g., Saccharomyces cerevisiae, Schizosaccharomyces pombe) and fungi (e. g., Aspergillus oryzae, Trichoderma viride) are frequently used hosts for the manufacture of recombinant enzymes and other proteins. Unlike prokaryotic hosts, they perform post-translational modifications such as glycosylation (→262), an often important feature for the production of pharmaceutical proteins (glycobiology).

Microorganisms

***Escherichia coli* – a prokaryote**

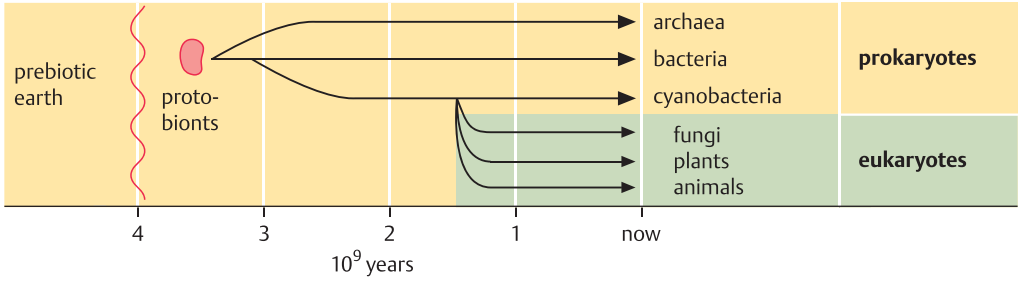


***Saccharomyces cerevisiae* – a eukaryote**



	E. coli	*S. cerevisiae*	for comparison: plant and animal cells
cell nucleus, organelles	no	yes	yes
diameter [µm]	~ 1	~ 10	~ 100
volume [μm^3]	~ 1	~ 1000	>10 000
respiration [µL O_2/mg TS • h]	1000	100	10
generation time [h]	0.3	1.5	> 20
genes	~ 4 300	~ 6 000	> 30 000

Position of the microorganisms in evolution



Archaea, eubacteria, and lower eukaryotes

	archaea	eubacteria	fungi, yeasts
cell type	prokaryote	prokaryote	eukaryote
cell wall	heteropolysaccharide or glycoprotein	peptidoglycan	glucan, chitin
membrane lipids	ether lipids from isoprenoid building blocks	phospholipids	phospholipids
initiator tRNA	methionine	formyl methionine	methionine
genetic material	small circular chromosome, plasmids, histone-type proteins	small circular chromosome, plasmids	complex nucleus with > 1 chromosome and linear DNA, histones
RNA polymerase	complex	simple	complex
size of ribosomes	70S	70S	80S

Bacteria

General. Bacteria can be classified by a variety of morphological, biochemical, and genetic methods, as well as by their nutrient requirements. The *International Code of Nomenclature of Bacteria* (ICNB) governs the scientific naming of bacteria and presently includes about 2,200 genera and 11500 species. The analysis of taxonomically relevant DNA isolated from soil seems to indicate, however, that the number of bacterial species that have not yet been cultured is much larger (→74).

Eubacteria. The oldest method of classifying eubacteria is based on their morphology. Under a simple light microscope, rods, cocci, and spirilli can be seen, some of them forming multicellular aggregates (filaments, colonies) and exhibiting structural details such as spores or flagella. Staining provided further differentiation. Thus, staining according to H. C. Gram's method allows for a classification according to cell wall structure: Gram-positive bacteria have only one cell membrane, covered by a thick murein cell wall, whereas Gram-negative bacteria have two cell membranes, enclosing a periplasmic space. The outer membrane is covered by a thin murein cell wall from which lipopolysaccharides may protrude. Physiological and biochemical criteria have led to additional methods of differentiation. Some important features are:

Response to oxygen: microorganisms can be subdivided according to their ability to grow under aerobic, anaerobic, or both conditions,

Form of energy generation: energy can be generated by photosynthesis (phototrophs), respiration, or fermentation (chemotrophs),

Preferred electron donors: organotrophic microorganisms use organic compounds, and lithotrophic microorganisms use inorganic compounds such a H_2, NH_3, H_2, S, CO, or Fe^{2+}.

Carbon source: autotrophic microorganisms can fix CO_2; heterotrophic microorganisms obtain carbon from organic compounds,

Relation to other organisms: saprophytic microorganisms are autonomous; parasitic microorganisms depend on a host organism.

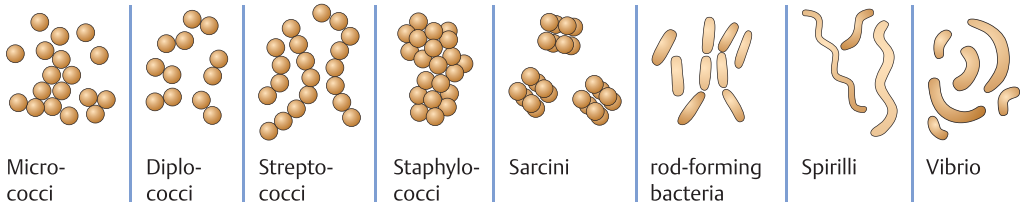
Phage typing: the susceptibility to phages can also be used for taxonomic identification,

Adaptation to environment: mesophilic microorganisms grow under ordinary conditions, whereas extremophiles are adapted to extreme conditions of temperature, pressure, pH, or electrolyte concentration. Cell inclusions, pigments, chemical components of the cell wall and cell membrane (fatty acid composition), immunological differentiation of the cell surface (serology), and susceptibility to antibiotics provide further possibilities for phenotype differentiation. Recently, genotyping of bacteria has become more and more important. For example, the GC content of bacterial DNA enables a rough classification. Complete sequencing of microbial genomes enables the most precise differentiation. A particularly useful method for taxonomy, discovered in 1972, is sequencing the DNA coding for the 16S, 18S and 23S rRNA (S: Svedberg units characterizing sedimentation behavior). This DNA contains sequences that were highly conserved throughout evolution, and analyses of the sequences suggest three families of living organisms: archaebacteria, eubacteria (the prokaryotes), and the eukaryotes. If DNA is isolated from environmental samples, and sequences coding for 16S, 18S or 23S rRNA are compared to those of microorganisms deposited in culture collections, there is less than 5 % identity, suggesting that > 95 % of all microorganisms contained in these samples have not yet been cultivated (s. metagenome) (→74).

Characterization and taxonomy. Rapid taxonomic identification of bacteria is important in hospitals, veterinary medicine, food production, environmental hygiene, and also in microbial and genetics laboratories. Most of the above methods are used, e. g., microscopy, staining procedures, determining the "analytical profile index API" (based on growth on various substrates), fatty acid composition of the membrane, or DNA analysis of taxon-specific sequences coding for the 16S, 18S or 23S rRNA. Precise classification of microorganisms is often far from trivial and requires the consideration of a wide range of experimental data; it is usually done by laboratories that archive culture collections.

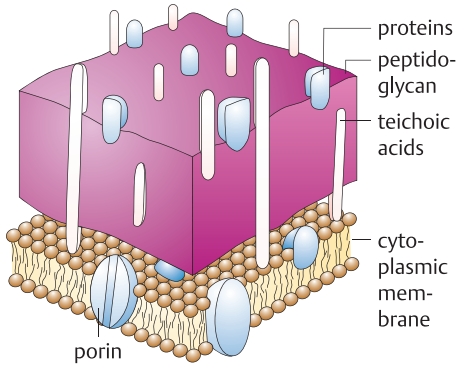
Genome sequencing. As of 2013, genome sequences for some 2,100 bacteria and over 140 archaea are completed. This includes many genomes of human pathogens such as *Mycobacterium tuberculosis*. The analysis of microbial genomes has shown that many variations of metabolic pathways exist, which can be exploited by metabolic engineering.

Forms of unicellular bacteria

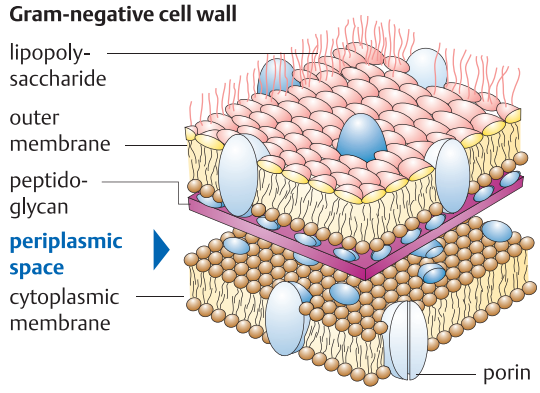


Cell wall composition and Gram-staining

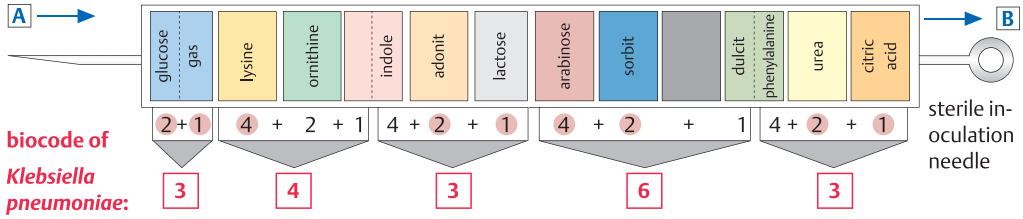
Gram-positive cell wall



Gram-negative cell wall



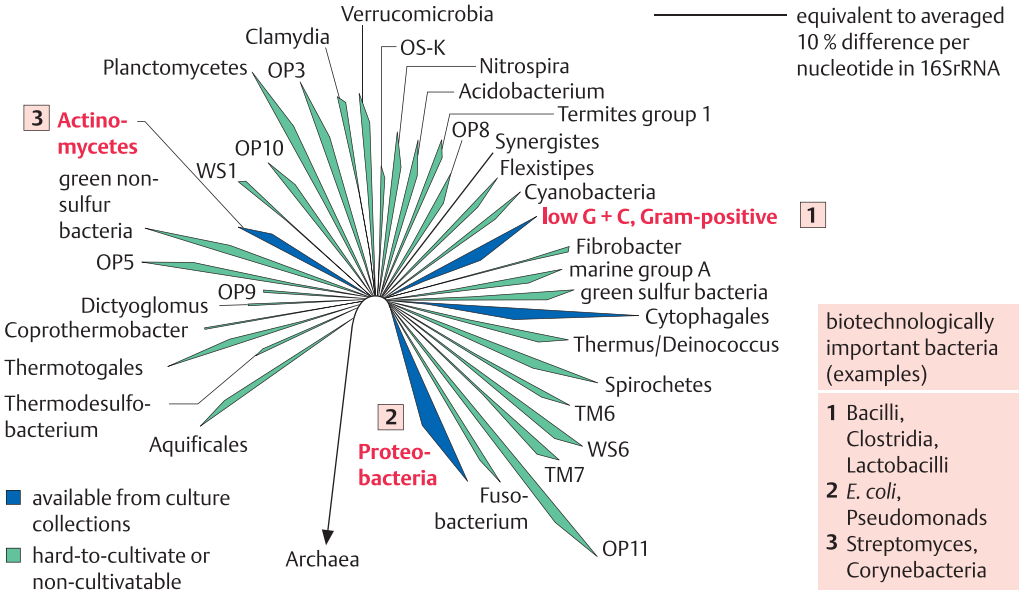
Biochemical characterization



biocode of *Klebsiella pneumoniae*:

color reactions after inserting the inoculation needle into a clinical probe A, draw through all compartments B, incubate

Phylogenetics and cultivation



biotechnologically important bacteria (examples)

1 Bacilli, Clostridia, Lactobacilli
2 *E. coli*, Pseudomonads
3 Streptomyces, Corynebacteria

Yeasts

General. Yeasts are a subgroup of the Ascomycetes. Because they propagate by budding, they are also termed budding fungi. They grow heterotrophically, preferring acidic media (pH 3.5–5.0) and usually do not form mycelia. Their cell wall is made of chitin. *Candida albicans* is an important human pathogen and model for studying pathogenesis. Yeasts of importance for biotechnology are *Saccharomyces cerevisiae*, *Candida utilis* and other *Candida* strains, *Schizosaccharomyces pombe*, *Hansenula polymorpha*, and *Pichia pastoris*.

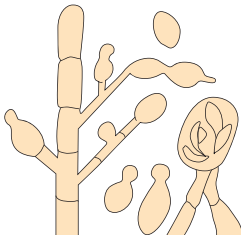
Saccharomyces cerevisiae (synonyms: baker's yeast, brewer's yeast, yeast) (→120) can propagate in either a haploid or diploid manner, thus providing an excellent organism for genetic investigations. Haploid laboratory strains belong to one of two mating types (*MATa* or *MATα*), which can only mate reciprocally. Asexual reproduction proceeds by forming conidia, followed by immigration of either a diploid or a haploid nucleus. Sexual propagation occurs by the fusion of two haploid gametes, followed by meiosis and formation of 4 haploid ascospores, whose phenotype can be separately observed, allowing for simple genetic analysis of the observed traits (tetrad analysis). Due to the simple cultivation of both haploid and diploid cells, the completed genome sequence (12 Mbp, on 16 chromosomes), the general absence of introns, and the short doubling time (90 min), *S. cerevisiae* has become a preferred model organism for the molecular genetics of a simple eukaryote. Another advantage is that yeast occurs with a natural plasmid, termed 2μm (60–100 copies in the cell nucleus), and that a second extrachromosomal element, the killer virion, is also available for recombination experiments. Many cloning vectors have been developed for yeast transformation, which either allow the replication of foreign genes outside the yeast chromosome (YRP = yeast replicating plasmids or YEP = yeast episomal plasmids) or integration of the foreign gene into the chromosome (YIP = yeast integrating plasmids). Artificial yeast chromosomes (YAC = yeast artificial chromosomes) allow for the cloning of large DNA fragments of 600–1,400 kbp; they have been widely used for preparing genome libraries, but have a tendency to recombine and thus have been mostly replaced by bacterial artificial chromosomes (BACs) (→72). The ca. 6,000 genes of yeast, located on 16 linear chromosomes, often show high homology to human genes. Thus yeasts have widely served as a simple model system for metabolic and regulation studies. In biotechnology, yeasts are used in the preparation of food products such as beer (→112), wine (→110), and bread (→120). It is also used in the manufacture of industrial ethanol (→138). Recombinant yeasts have become important host organisms for the manufacture of products such as insulin (→222), interferons (→234), and vaccines (→250) (e. g., hepatitis B surface antigen). Unlike *E. coli*, yeast allows for the posttranslational modification of gene products, in particular for glycosylation (→262).

Candida utilis differs from *Saccharomyces* by forming a mycelium, but it propagates solely asexually by budding. Some *Candida* genes show noncanonical codon usage (e. g., CUG for serine instead of leucine), which has retarded their heterologous expression. *Candida* strains have been used in biotechnology for production of extracellular enzymes and generation of digestible biomass. They can be grown on unconventional substrates such as sulfite suds or alkane fractions. Some *Candida* strains, such as *Candida albicans*, are pathogenic to humans.

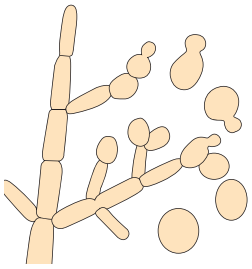
Pichia pastoris and Hansenula polymorpha are methylotrophic yeasts, which can grow on methanol as their sole carbon source. Isolated and studied in the context of the manufacture of single-cell protein (→122), they are used today as attractive host organisms in cloning experiments. Thus, diverse proteins such as lipases, β interferon, and antibody fragments have been functionally expressed in *P. pastories* in yields of several grams of recombinant products/L of culture broth. The *Hansenula polymorpha* genome (9.5 Mbp, 6 chromosomes) was sequenced in 2003, the *Pichia pastoris* genome (9.4 Mbp, 4 chromosomes) was sequenced in 2009.

Schizosaccharomyces pombe was first isolated from an East African beer variety (Swahili: pombe = beer). The genome of this ascomycete was fully sequenced in 2002 (12.6 Mbp, 3 chromosomes), and is similar in size to the *S. cerevisiae* genome. Mutant strains with reduced genome size and partial deletion of protease genes have been constructed which allow for excellent expression of foreign proteins.

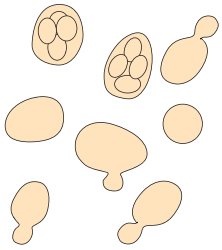
Morphology



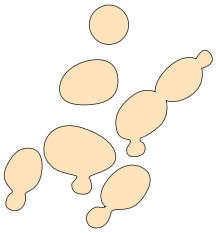
Endomycopsis
forms ascus
forms mycelium
forms buds



Candida
–
forms mycelium
forms buds



Saccharomyces
forms ascus
–
forms buds



Torulopsis
–
–
forms buds

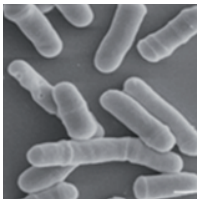
genetic

	size of haploid genome [Mbp]	chromo-somes	gene	genome-sequence
Saccharomyces cerevisiae	12.1	16	5905	1996
Candida utilis	14.6	14	8646	2012
Pichia pastoris	9.4	4	5040	2009
Hansenula polymorpha	9.5	6	5933	2003
Schizosaccharomyces pombe	14.1	3	4970	2002
for comparison: *Escherichia coli* K12	4.6	1	4145	1997

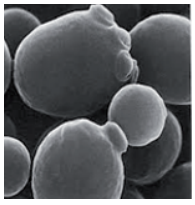
Technical applications of yeasts

Saccharomyces cerevisiae	• baker's yeast, brewers yeast • host organism for the expression of peptides, proteins and enzymes • model organism for the analysis of metabolic and gene regulation • modell organism for aging research
Candida strains	• animal feed • manufacture of biosurfactants • biotransformation reactions
Pichia pastoris, Hansenula polymorpha	• host organisms for the expression of proteins and enzymes
Schizosaccharomyces pombe	• host organism for the expression of proteins and enzymes • model organism for the analysis of gene regulation

Yeasts

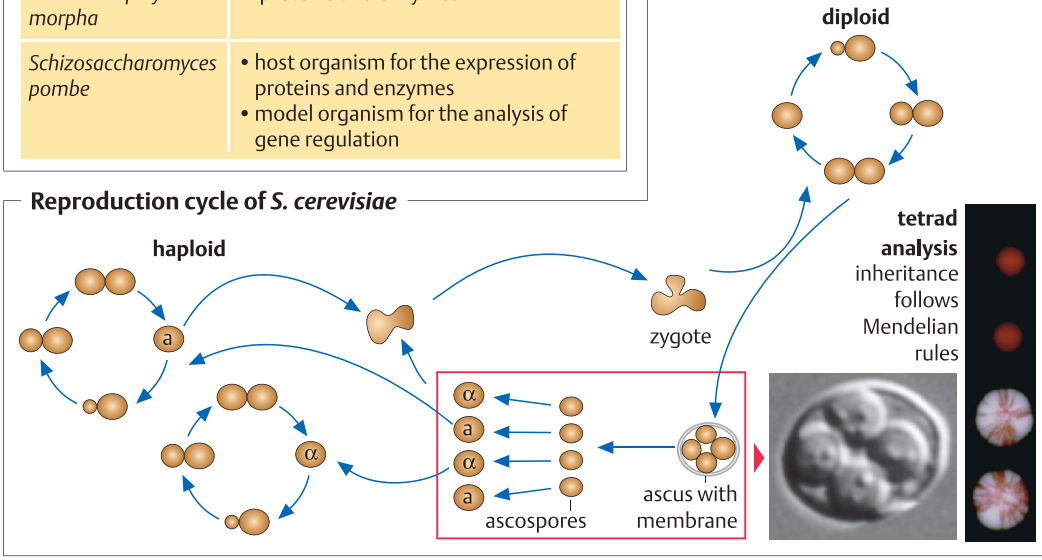


Schizosaccharomyces pombe



Saccharomyces erevisiae
= baker's yeast

Reproduction cycle of *S. cerevisiae*



Fungi

General. Fungi play a key role in the carbon catabolism of the biosphere, e. g., in the decomposition of wood and the formation of humic acids. Mycorrhizal fungi are associated with plant roots and assist in the uptake of nutrients, but other fungi, such as mildews, are dangerous plant pathogens. In biotechnology, they have an important role in the decay of food, but also in the preparation of fermented food products. Some fungi produce antibiotics or valuable enzymes. Among ca. 70,000 fungal species that have been classified, the Ascomycetes comprise ca. 20,000 species, forming the largest subgroup, which includes *Penicillium notatum* and *Aspergillus niger*. Among the lower fungi (Zygomycetes), *Rhizopus* and *Mucor* species have the greatest importance in biotechnology. Some of the ca. 12,000 stand mushrooms (Basidiomycetes) are edible (e. g., champignons, shiitake, chanterelles, ceps), and others participate in the degradation of wood (white and red rot fungi). Approximately 300 fungal species are pathogenic to humans. All fungi live heterotrophically. Their cell wall is composed of chitin and glucans.

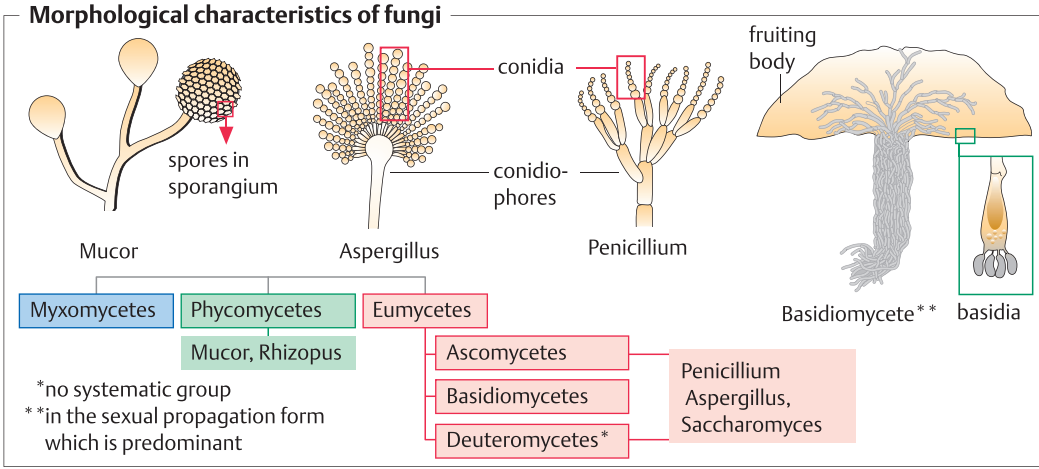
Reproduction forms. The reproduction of fungi follows highly diverse patterns, which are described here using the Ascomycetes as an example. The cell mass (thallus) consists of a mycelium that is made up of hyphae. During asexual reproduction, the conidiophores, which form at the top of the mycelium, divide and form spores (conidia), which grow into a new mycelium. Like most fungi, Ascomycetes can also propagate by a sexual mechanism. This results in a different phenotype (dimorphism). In this case, their hyphae form male and female sexual organs (antheridia and ascogonia). They fuse, during plasmogamy, into dikaryotic hyphae, which develop into an ascocarp ("fruiting body"). In the terminal cells of the dikaryotic hyphae, the dikaryotic nuclei are fused into a diploid zygote (karyogamy). Meiosis transforms the zygote into 8 haploid ascospores (or 4 basidiospores, in Basidiomycetes), which again grow into a mycelium.

Penicillium chrysogenum grows as a mycelium which forms fruiting bodies liberating spores (conidia) for asexual reproduction. Fungi like *Penicillium*, which have lost the capacity for sexual reproduction, are termed *Fungi imperfecti*. Consequently, if recombination is required during breeding in the laboratory, protoplast fusion among different types of nuclei (heterokaryosis) must be used. *P. chrysogenum* and the related fungus *Acremonium chrysogenium* are important industrial organisms, since they synthesize the lactam antibiotics (→206). Other *Penicillium* species such as *Penicillium camembertii* play an important role in the maturation of cheese (→188). The genome of *P. chrysogenum* contains ca. 32 Mbp and the sequence was published in 2008.

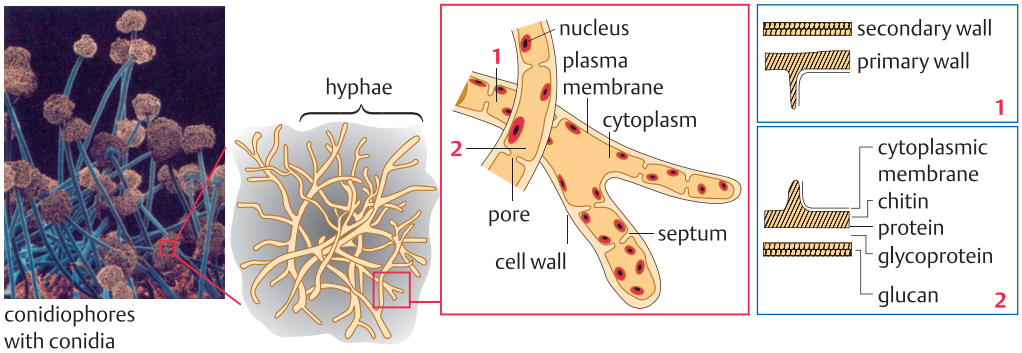
Aspergillus nidulans differs from *Penicillium* in the form of its conidia. Its genome contains 30.5 Mbp. *A. oryzae* is used for industrial production of extracellular enzymes (→172) and is a favorite host organism for producing recombinant enzymes from other eukaryotes. Various *Aspergillus* strains play a traditional role in Asian countries for the manufacture of food products such as soy sauce, miso, and sake (→86, 114), and their genetic and biochemical properties related to the production of these products have been analyzed in great detail. *Aspergilli* are also used for the production of extracellular enzymes such as proteases or amylases, and are preferred hosts for the production of recombinant fungal enzymes which they secrete. *A. niger* is the preferred production organism for citric and gluconic acid (→146, 150). Similar to *Penicillium*, strain improvement still uses protoplast fusion and selection; as the genome sequences of *A. nidulans, A. niger, A. oryzae* and eight more *Aspergillus* strains are now available (2013), targeted strain improvements based on the molecular genetic analysis of desired traits are rapidly advancing.

Rhizopus oryzae, a zygomycete, grows on rice, and *R. nigricans* is the black mold on bread. Its hyphae grow rapidly and bore their way through their substrates. Asexual reproduction proceeds by the formation of spores in differentiated mycelium (sporangia). *Rhizopus* and the closely related *Mucor* species can also grow on decaying organic materials and synthesize numerous extracellular hydrolases for this purpose. As a result, they have become important organisms for the manufacture of extracellular enzymes such as lipases and proteases. The *R. oryzae* genome is composed of 45.2 Mbp and was completely sequenced in 2009. A second *Mucor* genome sequence is available from *Mucor circinelloides*.

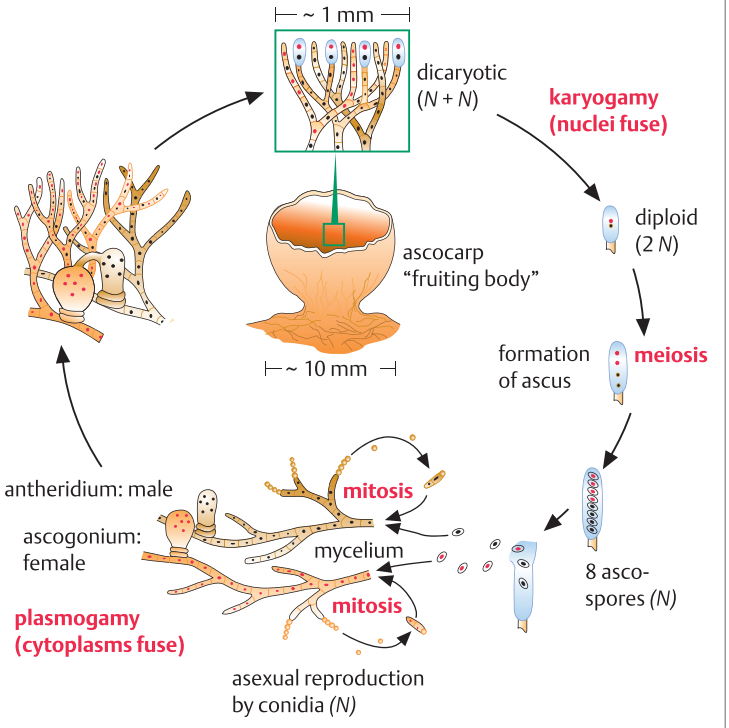
Morphological characteristics of fungi



Aspergillus niger, an ascomycete

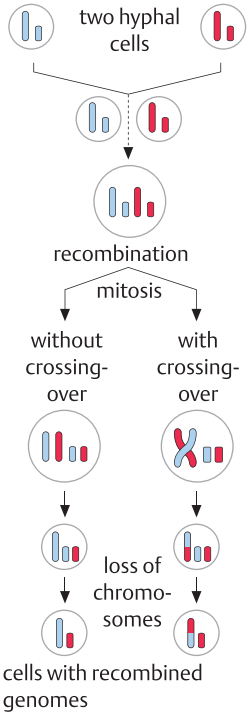


Propagation cycle of an ascomycete



Parasexual breeding

e.g., of Aspergillus



Algae

General. Most algae live in water, assimilate CO_2 and produce O_2 through photosynthesis. Unlike terrestrial plants, they do not pass an embryonic stage. Prokaryotic algae are termed cyanobacteria or "blue-green algae" and classified into about 100 genera. Eukaryotic algae form > 20,000 genera and are subdivided into green, brown, red algae, diatoms and others. Some cyanobacteria and algae form toxins (microcystins, saxitoxin), others are used for the production of food additives or specialty chemicals such as, e. g., alginate, agar-agar or astaxanthin. More recently, algae have been explored as a source of bio-energy, since, like terrestrial plants, they use CO_2 as their sole carbon-source, but without competing for agricultural land. Bio-energy may be harvested as algal biomass (formation of biogas) or lipids (triglycerides or isoprenoids), sometimes optimized in yield by metabolic engineering. Cultivation can be performed by aquaculture, in open ponds or in bioreactors. The biotechnology of algae is mainly promoted in nations with plentiful sunshine and long coasts such as the USA, Australia, Japan, Israel and China.

Eukaryotic algae comprise unicellular organisms of some µm size (Chlorella), but also large multicellular organisms up to 30 m long (kelp). Algal cells contain compartments such as chloroplasts which, in addition to chlorophyll a und b, often contain carotenoids. Some genera such as, e. g., ***Euglena***, can live both as autotrophs or complete heterotrophs. In the latter case, they lose their chloroplasts. The cell wall of many algae is composed of cellulose fibrils which are reinforced by other polysaccharides such as alginic acid. Diatoms form their cell wall from silicates built by silica deposition on a protein matrix. ***Laminaria*** and other marine brown algae are an important source of alginates (→158). The viscosity of an alginate solution depends on Ca^{2+} concentration. Alginates are used in the food industry as thickening agents, in medicine for surface wound treatments, and recently also as fibers in textile production. ***Chlorella*** are unicellular freshwater algae which propagate asexually. They contain one chloroplast and only a few mitochondria. Their cultivation is quite simple, and they are used as food additives. ***Botryococcus braunii*** is another green freshwater microalgae. Unlike *Chlorella*, it forms colonies. Under appropriate conditions, it can accumulate up to 60 % hydrocarbon content (alkanes, terpenoids, squalen). *Botyrococcus* oil is being investigated as a biofuel. ***Haematococcus pluvialis*** is a freshwater algae which forms cocci. It is able to synthesize the red-colored tetraterpene astaxanthine in high yields. Through the aquatic food chain, astaxanthine is responsible for the reddish color of salmon, shrimps etc.. It is a strong antioxidant which is well tolerated in human nutrition. As a consequence, it is used as a food additive and in cosmetics. ***Crypthecodinium cohnii*** is a marine red algae from the family dynoflagellatae. It can form up to 20 % of its dry mass as dodosahexaeoic acid (DHA), an ***ω-3***-fatty acid (→34, 162), which is used as an antioxidant food additive. ***Dunalilla*** are halophilic marine microalgae. They form high concentrations of ***ß***-carotene and glycerol, the latter as an osmoregulant. ***Neochloris oleoabundans*** is a green micro-algae which accumulates up to 30 % of its dry mass as triglyceride. This oil is being investigated as a biofuel. The genus ***Nannochloropsis*** comprises several marine phytoplankton algae, some of which store triglycerides. As they are quite easy to transform, they may have potential for producing alkanes from fatty acids, using synthetic metabolic pathways.

Cyanobacteria are prokaryotic organisms. Some of them can also grow in a heterotrophic manner. They exhibit wide morphological diversity and are divided into 5 classes. Their cell walls are composed of peptidoglycan, and their photosynthetic membranes are multi-layered and complex: besides chlorophyll a, they contain phycobiline pigments. Many cyanobacteria contain "heterocysts" for nitrogen fixation and cyanophycine, an aspartate-arginine copolymer, as a carbon-nitrogen storage compound. The genomes of about 35 cyanobacteria have been sequenced, and the molecular biology of ***Synechocystis sp.*** is most advanced. ***Spirulina*** is a 1–3 µm long cyanobacterium which grows in highly alkaline salt lakes. It forms multicellular spiral microfilaments. Spirulina biomass is produced in aquaculture and marketed as a food and feed additive.