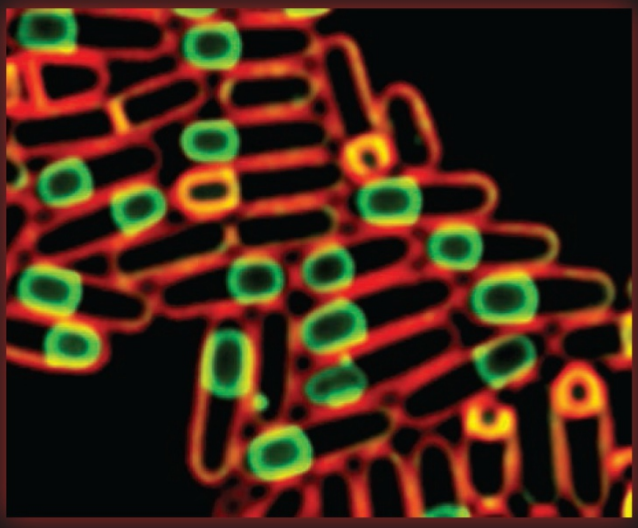
THE NEW Microbiology

From Microbiomes to CRISPR



Pascale Cossart

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Pascale Cossart
Institut Pasteur
Paris, France

WASHINGTON, DC

Cover image: *Bacillus subtilis* bacteria in the process of sporulation. The spore membrane is green and the bacterial membrane is red. Courtesy of Javier Lopez Garrido and Kit Pogliano, University of California, San Diego.

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PREFACE

For many of us, the word "microbe" still conjures a negative image, one of sickness, infection, or contamination. In general, we do not wonder "Where does this microbe come from?" except in case of an epidemic. We simply observe that its presence is inopportune and is dismantling a preestablished order, an equilibrium: this well-being that is named "health."

We now know, however, that good health depends on the presence of millions of beneficial microbes and microorganisms. These live on our skin and in different places in our bodies, such as the intestine, the mouth, and the nose, or they participate in various processes, such as the making of cheese, yogurt, and other foods, or water treatment and environment decontamination. They play a key role in maintaining the stability of our environment and the biodiversity of the flora and fauna of our planet.

Thanks to the studies of Louis Pasteur and Robert Koch at the end of the 19th century, it is well established that microbes do not spontaneously generate, that each microbe is born from another microbe, and that the smallest living organisms capable of autonomous life are called bacteria (from the Greek *baktēria*, meaning a stick or rod, named for the rod-like shape of the first observed bacteria). These bacteria, observable with simple microscopes, are single-celled organisms that can generate thousands of similar unicellular daughter cells.

Louis Pasteur and Robert Koch importantly discovered that bacteria were responsible for numerous diseases that have devastated humanity for thousands of years, such as the plague, cholera, and tuberculosis. Their studies paved the way for powerful methods of diagnosis of, and treatment for, bacterial infections, and for the development of vaccines, some of which are still being used today. Pasteur and Koch also introduced the concept of the study of bacteria in general, whatever their nature—i.e., either pathogenic, illness-generating bacteria or nonpathogenic bacteria that carry out other functions. In fact, the discoveries of Pasteur, Koch, and their collaborators were so revolutionary and so important that by the early 20th century they

triggered an immense interest, first among medical doctors and then among biologists of all sorts attracted to this new discipline: microbiology, the study of various microorganisms invisible to the naked eye, and more specifically, bacteriology, the study of bacteria.

During this flourishing period and the entire century that has followed, the field has advanced by leaps and bounds in many directions. At first, shortly after Pasteur and Koch, microbiology developed rather slowly, with the meticulous identification of all kinds of bacteria, the establishment of various collections, and diverse classifications and precise descriptions. Then things really sped up. In the early 1950s, the discovery of DNA as the basis of the genetic material of all living organisms, combined with the previous research on bacteria, quickly led to the development of concepts that applied, as Nobel laureate Jacques Monod put it, to the bacterium as much as to the elephant. These concepts included DNA replication, DNA transcription, protein translation, and protein synthesis. This in turn led to the development of molecular biology and genetic engineering: the art of manipulating genes and species.

By the end of the 20th century, technologies in DNA sequencing—the determination of the structure of genes and, soon, of complete bacterial genomes—sparked a totally unexpected acceleration in the study of bacteria, both pathogenic and not. Our understanding of infectious diseases was completely redefined by these approaches that, in association with cellular biology techniques such as imaging, started to shed light on the multiple mechanisms used by microorganisms to establish infection by interacting in various ways with the infected host and by harnessing many of the host's essential functions and fundamental mechanisms.

In parallel with this new vision on infectious diseases, research on the behavior of bacteria has shown that all bacteria without exception have a social life. They can live in small groups and diverse communities known as *biofilms* present on all kinds of surfaces. They can live in harmony with their fellow bacteria in heterogeneous, but stable, groups. When these groups grow in size and associate with other microorganisms, including parasites or viruses, they are called *microbiomes*. What was once known as the "intestinal flora" is now termed the *intestinal microbiome*. The intestinal microbiome is not the only type of microbiome; other parts of the body, and other organisms, feature their own. We now know that these microbiomes evolve and that they are unique to the individual they inhabit, based on their host's specific eating habits, genetic heritage, underlying illnesses, and even personal behavior.

Even if bacteria seem to live independently in nature, many exist in symbiotic relationships not only with humans but also with all animals, including insects, and even plants. This cohabitation sometimes produces stunning

effects on the host, such as sterility and even the eradication of males in insects. Bacteria present on plant roots can help them capture the soil nitrogen essential for the plant's growth.

Bacteria have very elaborate social lives. In addition to their ability to live in groups, and in order to do so, they can communicate using a chemical language that allows them to recognize and distinguish one another by species or family. Bacteria use these chemical languages to cooperate against a common enemy. For example, some pathogenic bacteria will not deploy their attack mechanisms unless they are numerous enough to succeed. Some bacteria can also regulate the times when they become luminescent, lighting up only once their numbers reach a certain threshold.

In order to adapt to various situations and to decide when to use their special capabilities, bacteria employ very sophisticated regulatory mechanisms. Each bacterial component, from proteins to small molecules, including vitamins and metals, participates in multiple adaptation mechanisms that bacteria put into action at various points in their lives. The molecules that participate in the controlled expression of genomes, and on which researchers have made the most progress recently, are RNA molecules. François Jacob and Jacques Monod hypothesized that RNAs could regulate gene expression, but they never imagined that RNAs could regulate gene expression in so many different ways. Bacterial RNA, considered as recently as the end of the last century to be mostly a production intermediary between DNA and proteins (hence the term *messenger RNA*), plays various and sometimes surprising roles. One of the most important recent advances in biology is the discovery that bacteria have extremely effective RNA-dependent defense strategies in place, known as CRISPR (pronounced *crisper*) for clustered regularly interspaced palindromic repeats, which they use to protect themselves from the bacteria-infecting viruses known as *bacteriophages*, or just *phages*. Specifically, bacteria remember their first encounter with a given phage and are able to put in place a kind of immunity, "vaccinating" themselves against this phage.

These bacterial systems work so well and are so adaptable that they are now the basis for a revolutionary technique, the CRISPR/Cas9 technology, that allows genome editing in all organisms that have been tested so far. This method makes genome modification quick and easy, and the mutations created allow for sophisticated studies of gene function or for the replacement of defective genes, paving the way for gene therapies. The CRISPR/Cas9 technology was recognized by a Breakthrough Prize in Life Sciences in 2015 in the United States and by numerous other prestigious international prizes that honor great scientific advances.

Bacteria defend themselves not only from viruses but also from their fellow bacteria, which are sometimes very aggressive. To do this, they produce

many kinds of toxins and antibacterial poisons for which they themselves have one or more immunity proteins. In the bacterial world, the struggle for life is continually taking place on an infinitesimal scale. But could these antibacterial poisons also be used on a much larger scale, to fight and gain better control over pathogenic bacteria? They certainly constitute a foreseeable strategy for replacing antibiotics that have become ineffective.

In fact, antibiotics have been, for decades, the most used antibacterial agents. Unfortunately, bacteria have adapted accordingly, developing resistances that have dramatic medical consequences, as in the case of the bacterium responsible for tuberculosis (*Mycobacterium tuberculosis*, or the Koch bacillus). We are no longer able to treat certain serious illnesses, and, as a result, they are coming back with a vengeance. The alarm has been sounded. The public is aware that this is a worldwide concern. Nevertheless, there are now reasons for optimism, or at least hope. Based on our recent knowledge, we are discovering new, alternative ways of fighting pathogens, raising new hopes for more effective treatments. For example, we can use our knowledge of bacterial genomes to identify inhibitors of chemical reactions or metabolic pathways that exist only in bacteria, not in humans.

Nevertheless, the threat of returning to a "preantibiotic" era is real and must be taken into account. We must therefore maintain constant vigilance when putting in place new therapies or when halting formerly obligatory vaccinations. Would it be reasonable, for example, to continue the policy in France of restricting vaccination with BCG (bacille Calmette-Guérin) against tuberculosis? Such questions should be carefully considered, especially in our global society where travel to and from countries with lower vaccination rates can be easy.

The objective in this book is to illustrate that very important discoveries and new concepts have come to light in the last few decades. These developments clearly show that the field of microbiology has undergone a *bona fide* revolution and that the amazing renaissance that is taking place can have wide-ranging consequences. This new understanding is going to change our daily lives dramatically, from our eating habits and daily routines to our way of looking at the rest of the living organisms on Earth: bacteria, plants, animals, even insects. In addition, recent discoveries will help us implement new strategies for fighting pathogenic agents and battle not only infectious diseases, but also their vectors. An example already in place in Australia is a plan to eliminate certain disease-bearing mosquitoes by releasing into the wild male mosquitoes that have been rendered sterile by infection with *Wolbachia* bacteria.

This book is limited to the rebirth of bacteriology, in part because this is my own domain of expertise. That said, virology, parasitology, and mycology are also mentioned because these areas benefit from the same technological

advances. Bacteriology, however, is the field that has been the most profoundly impacted by these advances and that consequently has benefited from the development of the greatest number of new concepts.

It was predicted that the 21st century would be the age of biology. This is indeed the case, and microbiology is at the forefront. In 2012, the French Academy of Sciences, with its sister institutions in England and Germany, the Royal Society and the Leopoldina, held a colloquium titled "The New Microbiology" that met with great success. I have used the same title for this book.

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ABOUT THE AUTHOR

After studying chemistry in Lille, France, Pascale Cossart obtained a masters degree from Georgetown University, Washington, DC. Returning to France, she obtained her Ph.D. in Paris at the Institut Pasteur, where she now heads the Bacteria-Cell Interactions unit, which is also an Inserm and an INRA unit. After studying DNA-protein interactions, in 1986 Dr. Cossart began to study the molecular and cellular basis of infections by intracellular bacteria, taking as a model the bacterium *Listeria monocytogenes*. Her research has led to new concepts in infection biology as well as in microbiology, cell biology, and epigenetics.

Pascale Cossart is considered a pioneer in cellular microbiology. Her contributions have been recognized by a number of international awards, including the L'Oreal/UNESCO Prize for Women in Science (1998), the Richard Lounsbery Prize (Académie des Sciences, Paris/National Academy of Sciences, 1998), the Robert Koch Prize (2007), the Louis Jeantet Prize for Medicine (2008), and the Balzan Prize (2013). She is a member of the French Academy of Science (2002); a foreign member of the National Academy of Sciences (United States; 2009), the German Leopoldina (2001), the Royal Society (United Kingdom; 2010), and the National Academy of Medicine (United States; 2014); and, since January 2016, Secrétaire Perpétuel de l'Académie des Sciences, Institut Pasteur. (Photo courtesy of Agnès Ullmann.)

PART I

New Concepts in Microbiology

CHAPTER 1

Bacteria: Many Friends, Few Enemies

Bacteria are unicellular living organisms that make up one of the three domains of life: *Bacteria*, *Archaea*, and *Eukaryota* (Fig. 1). This model of three branches stemming from a common ancestor was first proposed by Carl Woese in 1977. The absence of a nucleus is one major difference between prokaryotes and eukaryotes. Eukaryota or eukaryotes include animals, plants, fungi, and protozoa, which all have nuclei; bacteria and archaea are prokaryotes and do not have a nucleus. The DNA of prokaryotes is non-membrane bound, unlike in eukaryotes. But do not assume that bacteria are merely small sacks full of disorderly contents. Their "interior" is in fact very well organized.

Archaea, like bacteria, are unicellular organisms but differ from bacteria in that they have lipids that are not found in bacteria and an ensemble of compounds that are similar to those of eukaryotes, in particular the machinery that regulates gene expression. When they were discovered, archaea were thought to exist only in extreme environments, such as very

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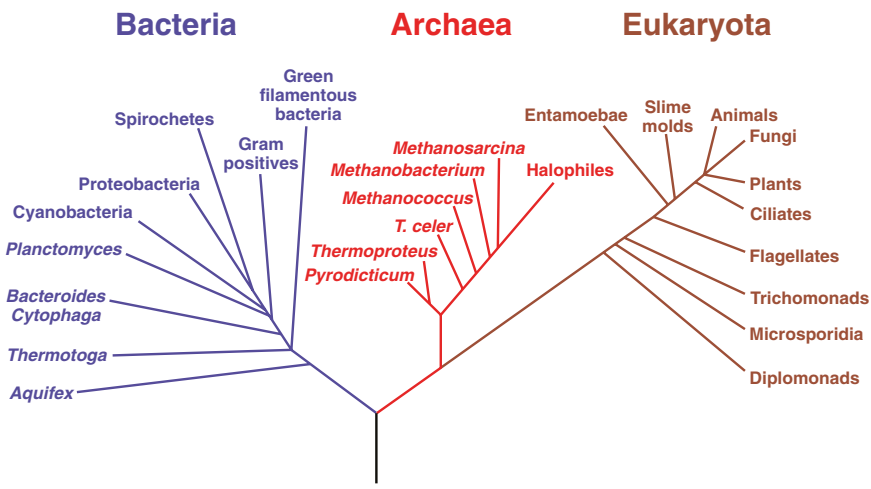

Figure 1. The three large domains of life. *Bacteria*, *Archaea*, and *Eukaryota* have a common ancestor.

hot water springs, but we now know that they are present everywhere, including in our gut.

Bacteria are extremely varied and make up the most diverse domain of life. They have been on Earth for billions of years and have evolved to survive in a great variety of conditions. There are more than 11,500 known species of bacteria in more than 2,000 genera (groupings of species). These numbers have so far been based only on gene comparisons, particularly the 16S RNA genes, and they keep rising. Classification methods are changing too. Now that we can compare entire genome sequences, the definition of "species" itself is evolving.

Bacteria may have different shapes (Fig. 2). There are four main categories: cocci, or spheres; bacilli, or rods; spirals; and comma-shaped, or curved bacteria. All bacteria divide, regardless of their shape. One bacterium splits into two, via an asexual reproduction. Nevertheless, genetic material can be exchanged between two bacteria by means of mechanisms described as *horizontal gene transfer*. We will come back to this topic later on.

Bacteria are present everywhere in the environment; they are in all habitats on earth, including hot springs and seawater, even in very high salinity. Many live in humans—there are an estimated 10^{10} bacteria on our skin, 10^{10} in our mouths, and 10^{14} in our intestines. That's 10 times more bacterial cells in our bodies than human cells! However, a recently published article investigated this number and concluded that it was actually overestimated by a factor of 10. Whatever the count, in our intestine—which contains tens

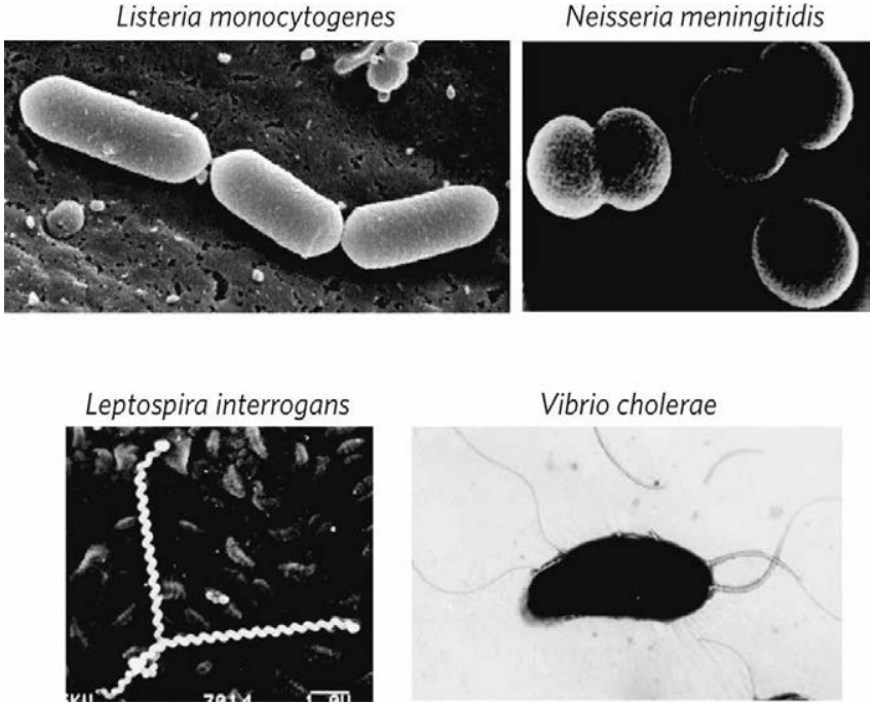

Figure 2. The four main types of bacteria: bacilli (*Listeria monocytogenes*), cocci (*Neisseria meningitidis* or meningococci), spirals (*Leptospira interrogans*), and comma-shaped (*Vibrio cholerae*).

of billions of bacteria—there are more than a thousand different species. Sometimes I like to think that bacteria are our constant companions, generally friendly hitchhikers that we carry around with us everywhere!

Bacteria first appeared more than 3 billion years ago—that's 2 billion years before animals—and have since lived more or less undisturbed in the biosphere. We do not know for sure how the first organism with a nucleus was born, but it was probably from the fusion of a bacterium with an archaeon. Indeed, genes from both of these domains are present in animals; it is clear that an ancestor of all modern eukaryotes must have "swallowed" a bacterium, leading to the stable symbiotic relationship that produced the energy-producing compartments called mitochondria in all of our cells. These small organelles somewhat resemble bacteria and are indispensable to the formation of thousands of compounds, most notably ATP, a chemical compound that temporarily stores energy and is used for many chemical reactions in cells. One could say that the first animals

started out as bacterivores before they became herbivores, carnivores, or omnivores!

Many bacteria live free in natural environments. There they live, grow, and feed and by doing so contribute to the equilibrium and characteristics of the specific ecosystems in which they grow. For example, bacteria from the *Streptomyces* family are responsible for the so refreshing smell of the woods after a rain.

Many other bacteria are not alone and are associated with a partner. They establish long-lasting relationships that are mutually beneficial, or "symbiotic," within humans, animals, and even plants. Additionally, as we will soon see, bacteria of several species can assemble in very large communities, called *microbiomes*, which become integral part of organisms. These combinations of organisms and microbiomes are referred to as *superorganisms*.

It is important to realize that of all the bacteria on Earth, pathogenic bacteria (those responsible for disease) are in the minority. Among those, a few produce very powerful toxins and always induce disease. An example is *Vibrio cholerae*, the water-transmissible *Vibrio* species responsible for cholera, which produces a toxin that can cause fatal diarrhea and dehydration. Another example is *Corynebacterium diphtheriae*, which causes diphtheria, a disease nearly forgotten in developed countries due to the efficacy of mandatory vaccinations. Also in this group is *Clostridium tetani*, which produces the toxin for tetanus, and *Clostridium botulinum*, which produces the botulinum toxin.

However, illnesses caused by a single toxin are extremely rare. As a general rule, bacteria create disease by means of an arsenal of strategies and tools called *virulence mechanisms* and *virulence factors*. The combination of different virulence factors allows a bacterium to enter an organism, evade the host's defense mechanisms, and multiply and invade different parts of the body such as the throat (*Streptococcus*), the lungs (*Legionella*), the intestines (*Salmonella*), or the nose and pharynx (*Pneumococcus*). Bacteria often can establish an infection only if the host's immunity is weakened by fatigue by a viral or other infection (such as pneumococcal infections that often follow respiratory tract inflammation due to influenza), by medical treatment (such as immune suppression due to chemotherapy), or by a genetic mutation.

The importance of the genetic context with regard to a host's susceptibility to infection is now the subject of extensive studies by scientists around the world. The laboratory led by Jean-Laurent Casanova is a strong proponent of the hypothesis that disease can be or is linked to the host's genetics. These investigators have found evidence for genetic predisposition to several illnesses, for example, susceptibility to infection with low-pathogenicity mycobacterial strains including BCG, the live attenuated strain of *Mycobacterium bovis* used to vaccinate against tuberculosis. Some children have a defect