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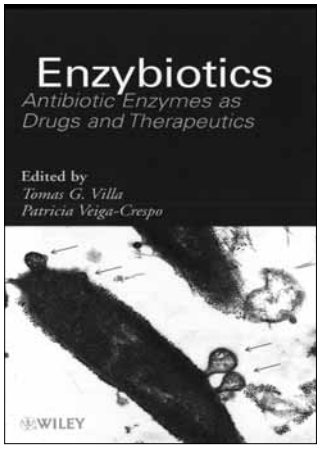

Enzybiotics. Antibiotic enzymes as drugs and therapeutics

TOMAS G. VILLA, PATRICIA VEIGA-CRESPO (EDS)

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In the 1930s, the war against disease was aimed mostly at infectious illnesses, such as pneumonia, meningitis, syphilis, and tuberculosis. However, in most cases, physicians could only alleviate their patients' suffering and hope that they—the patients—would be strong enough to overcome the infection. With the advent of antibiotics, the initial aim was the treatment of infectious diseases in people, but the miraculous effects of these drugs soon led to their use in other animals and in plants. In less than 30 years, dozens of effective and relatively cheap antibiotics were discovered and prescribed, saving millions of people from death caused by infections. Some "experts" even thought that, finally, the battle against pathogenic microorganisms had been won. Thus, in 1967, the USA Surgeon General, William H. Stewart, solemnly declared, "The time has come to close the book on infectious diseases. We have basically wiped out infection in the United States." Unfortunately, Stewart's optimism was unfounded and today the health of the world's population continues to be threatened not only by new but also by re-emerging infectious diseases.

Antibiotics control bacterial infections, thereby maintaining the health of people and animals as well as preventing the destruction of agricultural crops. But while over the last 70 years antibiotics have altered the relationship between bacteria and people, today we are witnessing another significant change, this time among the bacteria themselves. At present, a high proportion of pathogenic bacteria are functionally resistant to most of the antibiotics that were used extensively during the "antibiotic era." Indeed, almost as soon as it was established that microorganisms could be killed by certain substances, it was also recognized that some microbes could survive normally lethal doses of these same agents.

Staying ahead of resistant bacteria is a continuous challenge to our know-how, and we remain both optimistic and enthusiastic that the challenge posed by drug-resistant bacteria can be met, by discovering and developing effective antibacterial agents through a combination of different approaches. The book *Enzybiotics. Antibiotic enzymes as drugs and therapeutics*, with contributions from several experts in the field, including the book's editors T.G. Villa and P Veiga-Crespo, represents the state-of-the-art in the emerging field of "enzybiotics" (from the words enzymes and antibiotics). These agents are believed to offer the best hope of controlling bacterial and fungal diseases, either alone or in combination with traditional forms of antibiotics.

The term "enzybiotics" was used for the first time in 2001. It refers specifically to the antibacterial potential of bacteriophage enzymes actively produced during the phage lytic cycle and capable of degrading the bacterial cell wall. Recently, it was suggested that "enzybiotics" should refer to all enzymes displaying antibacterial and/or antifungal activity. In the literature, they are also called "lytic enzymes" or "peptidoglycan hydrolases," as bacterial cell-wall peptidoglycans represent the major target of enzybiotics and their enzymatic cleavage results in lysis of the bacterial cell. Peptidoglycan hydrolases comprise different enzymes from different sources. They are introduced in Chapter 1, which describes the major groups of enzybiotics, including lysins (from phages), bacteriocins and autolysins (produced by the bacteria themselves), and lysozymes (from different organisms).

Phage-based therapies consist of the administration of phage lytic enzymes or/and whole phages. Soon after the discovery of phages, their potential in the treatment of disease was proposed and analyzed. Thus, Félix D'Herelle (one of the co-discoverers of bacteriophages, he suggested this name to describe them) applied phage therapy to treat bacterial dysentery in Paris as early as 1918, although many scientists for decades afterwards remained skeptical regarding his apparent success. Later on, in the 1930s, other investigators worked with phages targeting *Staphylococcus* infections. However, the antibiotic era, which began in the 1940s, brought a halt to the phage therapy approach. Today, with the recent dramatic increase in antibiotic resistance, the therapeutic use of phages has gained renewed and intense interest. Phages are already used as food preservatives; for example, the FDA approved the utilization of phages in cheese to control *Listeria monocytogenes*, categorizing these phages as "generally recognized as safe" (Chapter 2).

The capacity of phage-encoded peptidoglycan hydrolases to specifically kill bacteria provides a tool that, together with disinfectants or mild physical treatments, can be applied to the control and decontamination of areas polluted by particular pathogenic or toxic bacteria (Chapter 3).

In the viral lytic cycle, after replication inside its bacterial host, the phage must lyse the bacterial cell to disseminate its progeny. Double-stranded DNA phages have evolved a lytic system to disrupt the bacterial cell wall. Generally, newly synthesized lysins do not have signal sequences, nor are they translocated through the cytoplasmic membrane to attack their target, the peptidoglycan. Instead, the ability of lysins to penetrate the membrane is controlled by another phage product, the "holins." During phage development in the infected bacterium, lysins accumulate in the cytoplasm at a specific time, while holins are inserted in the cytoplasmic membrane, forming patches that result in membrane disruption. This provides the lysins with access to the peptidoglycans and thus the ability to initiate cell lysis. Moreover, the binding between a given peptidoglycan hydrolase, e.g., lysin, and the bacterial cell wall is not a random event: the enzyme attacks one of the five major bonds in the peptidoglycan. This mechanism of action is reflected in the fact that members of the lysin family typically are chimeric proteins, with a well-conserved catalytic domain fused to a largely divergent binding domain. Thus, precise knowledge of the sequences and of the implications of the different motifs is necessary to develop an appropriate phage therapy (Chapters 4–6).

Human mucosal membranes are reservoirs (and sometimes the only reservoir) for many pathogenic bacteria, some of which include strains resistant to antibiotics (e.g., *Staphylococcus*). It is well recognized that by reducing this human reservoir, both in the community and in hospital or nursing home environments, the incidence of *Staphylococcus*-mediated disease will be reduced as well. Antibiotics are not indicated to control the host carrier state, because of the risk of increasing antibiotic resistance. However, enzybiotic treatment could prevent infection by specifically destroying pathogenic bacteria on mucous membranes. Lytic enzymes (i.e., lysostaphin, staphylococcal phage lysins) can also kill the widely spread methicillin-resistant *Staphylococcus aureus* (MRSA) as well as *S. aureus* strains with reduced susceptibility to vancomycin (Chapter 7).

Membrane-targeted enzybiotics could serve as an alternative anti-infectious therapy. Antimicrobial host defense peptides, which act shortly after microbial infection to neutralize a broad range of pathogens, are a critical component of innate immunity, with microbicidal, endotoxin-neutralizing, and immunostimulatory properties. These peptides kill bacteria primarily through non-specific membrane lysis and are therefore less likely to provoke resistance. More than 800 antimicrobial peptides have been isolated, purified, and characterized structurally and functionally. Extensive structure-activity relationship studies have revealed that net charge, amphipathicity, hydrophobicity, and structural propensity are among the most important physicochemical and structural variables that dictate the ability of these peptides to interact with and disrupt membranes. Despite the insights achieved thus far, the potential of antimicrobial peptides to combat undesired infections is still under debate. Challenges to their successful development are related to the limits of our knowledge (Chapter 8).

A major concern regarding the use of phage therapy in the treatment of infectious diseases is the development of phage-resistant bacteria. The susceptibility of a bacterium to phage infection depends on whether the phage can attach to receptors on the cell. Many constituents of the bacterial cell surface are receptors for phage, such as pili, flagella, capsule, and lipopolysaccharide. Thus, phage cocktails must be designed such that they take into account the possibility of bacterial resistance through receptor mutation. In addition, they must infect all bacterial strains within a given species. Chapter 9 describes several approaches to the design of phage cocktails and provides examples of their use for diagnostic purposes, e.g., RapidChekSELECT*Salmonella*.

Phages constitute a great reservoir of genetic information. Indeed, it has been estimated that there are 10^{31} phage particles in the biosphere; however, only a very small fraction of the total lysins encoded by global phage have been recombinantly expressed and functionally tested. Chapter 10 offers several general experimental strategies that could be employed in the search for new enzybiotics.

Along with the study of phage gene products that lyse bacterial cells, genetically modified phages are being developed. These recombinant phages have been modified to encode lethal genes, which are then delivered to the host bacteria. Expression of the lethal gene gives rise to a gene product that inactivates the target cell. Alternatively, phages can be labeled with toxic molecules that destroy the bacterium after phage binding. These are among the many approaches that will contribute to improving the potential of phage therapy (Chapter 11).

Enzybiotics. Antibiotic enzymes as drugs and therapeutics is an exceptional review and update of information on alternative therapy solutions to the increasing problem of bacterial and fungal antibiotic resistance. The book thoroughly explores not only the pros but also the cons of this emerging therapeutic approach. The wisdom underlying this long ignored strategy remains sound: combating pathogenic bacteria with viruses, which unlike antibiotics, are their true sempiternal enemies

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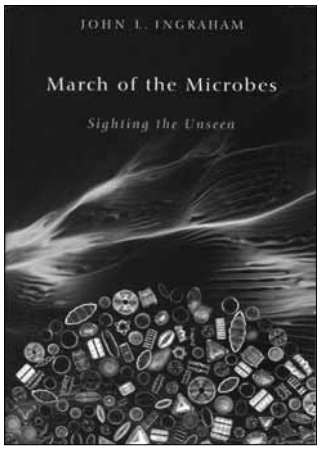

March of the microbes. Sighting the unseen

JOHN L. INGRAHAM

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What do snowmaking machines, marine mudflats, limestone caverns, coral reefs, red tides, luminous fish, broad-bean plants, bright-colored hot Springs in Yellowstone Park, obesity, wood-eating termites, Château d'Yquem wine, the holes in Emmenthal cheese, and linen table cloths have in common? The answer is "microbes." John L. Ingraham, ASM former President and Professor Emeritus of Microbiology at the University of California, Davis, has devoted a whole life to the study of these tiny yet powerful organisms. A more than sixty year career has allowed him to do research in various fronts and to meet microbiologists that are currently myths of this science. He has also witnessed the development of technologies and new specialties that have helped microbiologists—and reasearchers in all fields of biology—to broaden the knowledge on the functioning of life, the links and relationships among living beings and between them and their environment, as well as the role of microorganisms in the biosphere.

Without the burden of academic duties, Ingraham can now handle microbiology as if it were only a hobby—which surely has been for him and for other microbiologists that have made of her careeer a hobby. One has the feeling that Ingraham has enjoyed himself very much writing *March of Microbes*. Since 1975, Ingraham has coauthored various editions of *The Microbial World* as well as other microbiology textbooks, one of them—*Introduction to Microbiology*—written with his daughter, Catherine Ingraham Vigran. Those that are acquainted with *Introduction to Microbiology* will surely remember how Ingraham used stories to introduce many topics and make them more comprehensible. He has used this tactics also in *March of Microbes*: by telling stories in which microorganisms are the main characters, he has brought microbiology closer to lay audiences. Even if one knows the book is about microbes, to learn what bizarre roles microbes can play either on their own or when humans tame them for their own behalf can be a surprise. And so can be to know that microbes have been the only inhabitants in the Earth for almost 85 per cent of the history of life on the planet and that, as Ingraham himelf states, they "did much more than arrive first." For microbes have shaped the planet and have made it as it is now, quite different from the Earth's nearest neighbours—Venus and Mars.

An introductory chapter ("The Microbial Landscape") provides the basis to become familiar with the various kinds of organisms that the word *microbe* comprises, to learn what kinds of living beings—or not alive, because many microbiologsts do not consider viruses to be alive—make up the world of microbes, and how they grow and reproduce. From the very beginning we realize that microbes have been the most innovative and diverse living beings in their metabolisms although they do not look morphologically so diverse. Their wide range of nutrients and ways to obtain energy, as well as the range of growth temperature, pH, salinity, and hydrostatic pressure limits found in the microbial world account for their ubiquity. Many microbes can only be perceived through the changes they cause in the evironment when they acquire energy. The book devotes a chapter ("Acquiring metabolic energy") to describe, with examples, various ways microbes have to obtain their energy.

The chapter "Food and drinks" goes far beyond describing the typical roles of microbes in the production of cheese, wine, etc. How do Swiss cheese get its holes? What makes a wine such as Portuguese *vinho verde* to be *pétillant*? What makes a Bordeaux wine—Château d'Yquem—acquire its unique flavor, taste, color and thickness? Why do raw eggs spoil more slowly than boiled eggs? What do microbes and thick salad dressings have in common? These are questions that find their answers in this chapter. Some kinds of symbiotic relationships including lichens, microbes in the guts of many animals, as well as light-emitting microbes that dwell in the light organ of various marine animals, are discusssed in "Living together." Symbiosis between nitrogen-fixing bacteria and plants are included in the chapter "Cycling nitrogen", one of the three chapters that deal with the cycling of elements—the other two being "Cycling sulfur" and "Cycling carbon"—along with several environmenal issues related to the cycling of those elements. The limits of microbial life, which are the limits of all kind of life, are discussed in "Hostile environments". The author, however, has kept for the last chapter ("Survivors") one kind of extremophiles: those like *Deinococcus radiodurans*, which endures intense radionuclide radiations.

Non-prokaryotic microorganisms have their own chapters: "Fungi, hostile and benign", "Viruses", and "Closer to us" (protists, "a sort of microbial leftover"), and so have pathogenic bacteria ("Felonious bacteria"). Probably because

much has already been written about the typical pahogenic bacteria, and concentrating in a 30-page chapter what takes hundreds of them in medical microbiology books would not have had sense, Ingraham focuses only on some aspects of these bacteria and their pathogenicity, and on the fight against infections. “Shapers of the planet” deals with global issues, and describes some roles microbes have played in the history of the Earth that have made our planet as we know it. Without mentioning Gaia, this chapter provides a Gaian vision of the Earth.

Most chapters of *March of Microbes* are easy reading, while others need some general microbiology knowledge that not all readers may have. To solve this problem, a glossary at the end includes definitions of many terms found throughout the text. In addition, the author uses metaphors to describe some molecules, processes and phenomena hard to understand by lay people. For example, ATP is described as “the indivisible unit currency of metabolic energy”; it is suggested that ATP synthase “could be viewed as a water wheel, generating mechanical energy from a flow of water”; and protons that accumulate outside the cell are shown “as a bank account in which metabolic energy is saved.”

In contrast with Ingraham’s—and other author’s—microbiology textbooks, which usually have plenty of full-color illustrations and pictures, *March of Microbes* includes only very simple black and white drawings and diagrams. In this book, it is the text—the stories told— that catches the reader’s attention and is worth thousand pictures.

Maintaining biodiversity has become a priority for many international institutions, and many projects are being carried out worldwide to study the diversity of life. The United Nations even declared 2010 to be the International Year of Biodiversity. Yet, in most programs to investigate and preserve biodiversity, the invisible biodiversity is ignored despite the fact that microorganisms have been the only inhabitants of the Earth for most of the history of life on the planet. As John Ingraham states at the beginning of the book, “[m]icroorganisms—microbes—are our progenitors, our inventors, and our keepers.”

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