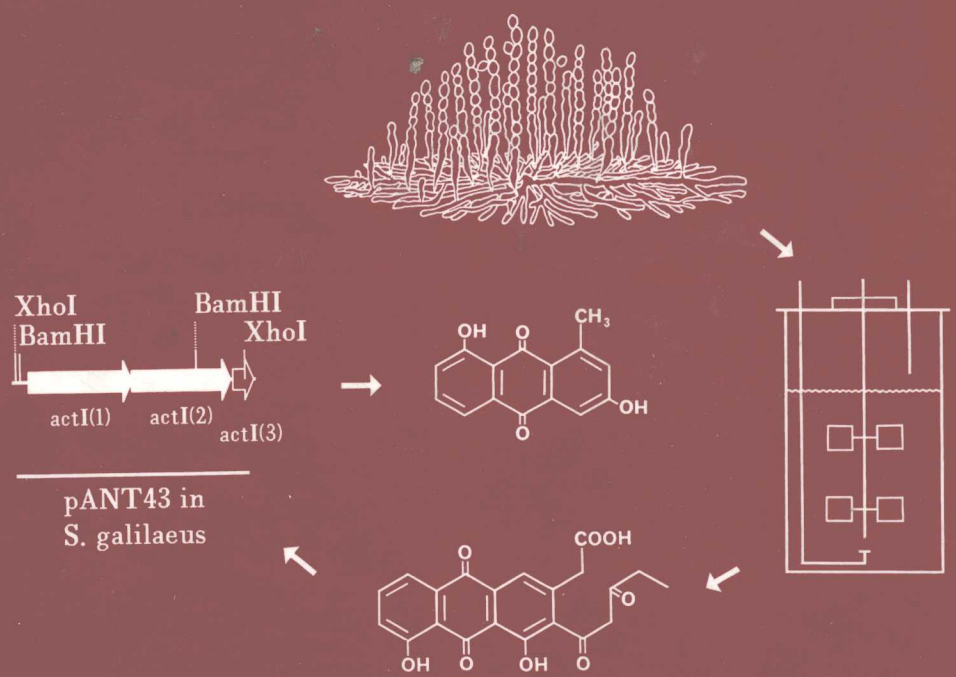
DRUGS AND THE PHARMACEUTICAL SCIENCES VOLUME 82

Biotechnology of Antibiotics

Second Edition, Revised and Expanded



edited by
William R. Strohl

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PREFACE

In the preface to the first edition, Professor Erick Vandamme indicated that the book originated in part from frustration, in the attempt to keep abreast of current literature and recent advancements in the field of antibiotics. He began his Preface by listing many of the processes in which microorganisms are used to produce chemicals of vital use to mankind. Professor Vandamme included the production of antibiotics, antitumor agents, vitamins, biopolymers, vaccines, and amino acids, as well as the bioconversion of steroids, antibiotics, vitamins, amino acids, and carbohydrates. Yet these cover only a small, albeit important, fraction of the vast biotechnological processes in which microorganisms are used to produce economically important molecules. The same applies to this second edition, as we can focus on only a small fraction of the products of the rapidly expanding field of modern biotechnology.

In the decade or so since the first edition, the field of antibiotic research has changed considerably, as have the challenges facing society. In 1983, acquired immunodeficiency syndrome (AIDS) was still in its infancy, and there was little knowledge of the devastation it would cause. Tragic outbreaks of diseases caused by exotic viruses such as Ebola were still only in the minds of science fiction writers. Multiple-antibiotic-resistant *Mycobacterium tuberculosis*, vancomycin-resistant enterococci, *E. coli* O157-H7 and invasive staphylococci were essentially unknown, and the extraordinary promiscuity with which bacteria transfer resistance genes was only whispered in academic circles. Today, we face all these challenges and more. Unfortunately, it is all too likely that we have only scratched the surface and that new challenges to the discovery, production, and use of antibiotics will continue to mount at ever increasing rates.

Thirteen years ago we had only the first glimpses of the utilization of modern industrial biotechnology (i.e., genetic engineering and computerized process control of fermentations) for making new antibiotics or assisting in the overproduction of existing antibiotics. At that time the first antibiotic biosynthesis genes from *Streptomyces* had just been cloned, and expression in heterologous strains and the first fungal antibiotic biosynthesis gene to be cloned, was still a year away from being reported in *Nature*. A decade ago, we could only dream of finding regulatory genes that conferred the overproduction of some antibiotics. Producing novel metabolites via interspecies cloning and cloning genes that conferred specific biochemical modifications on existing structures such as a sterol oxygenase were also far from being realized. In the past 14 years, all these breakthroughs and considerably more have been accomplished.

And so the questions now become: Where has biotechnology of antibiotics gotten us? Where will it lead us into tomorrow?

Modern biotechnology has given us several new products: human insulin, human and bovine growth hormones, and granulocyte colony stimulating factors are just a few examples. Adaptation of genetic engineering and process control has also yielded better

methods in the production of traditional products, with new generations of β-lactam antibiotics and enzyme inhibitors, such as pravistatin. In the field of antibiotic production, however, we see only a few examples where genetically engineering and modern biotechnology processes have already been brought into practice. To date, there are no publicly known genetically modified antibiotics or hybrid antibiotics on the market. Similarly, it does not appear that there are any commercial antibiotics currently being overproduced using genetic engineered microorganisms, although this would not necessarily be public knowledge. Does this imply that genetic engineering and sophisticated process control methodologies have little or no future in the antibiotic industry? I believe not. It is the contention of this book that since the first edition was published, the badly needed foundation and data base, from which a new golden era of biotechnology of antibiotics will emanate, has been established. Although the foundation still has some significant cracks and holes, these are being filled, in ever increasing numbers by the industrial and academic research groups who recently have entered the field. This suggests that the maturation of genetic engineering for antibiotic production has begun. This is an essential prerequisite for the eventual and imminent industrial production of genetically engineered antibiotics via biosynthesis pathways.

How will "antibiotic" be defined in this volume? According to Webster, an antibiotic is "a substance produced by a microorganism and able in dilute solution to inhibit or kill another microorganism." Strictly speaking, this definition is still largely accurate. As we face the new millennium, however, it seems that perhaps Webster's definition, introduced by Waksman in 1942, is in need of a practical update. Even the editor of the *Journal of Antibiotics*, Dr. Morisama Yagisawa, has realized the diversity of antibiotics in our modern world, by including in his journal articles covering virtually all areas of bioactive natural products. So, where should the line be drawn between antibiotics and non-antibiotic bioactive metabolites? Certainly, antitumor drugs are antibiotics. They kill not only tumor cells, as well as healthy tissue, but also certain microorganisms. Are cholesterol-lowering agents antibiotics? Again, some of these inhibit or kill microorganisms, but their clinical use has nothing in common with their ability to inhibit the growth of microorganisms. Are β-lactamase inhibitors antibiotics? Their function, presumably in nature and certainly in medicine, is as helper molecules to assist the efficacy of β-lactam antibiotics. Are veterinary growth-promoting natural products antibiotics? Some are known coccidiostats, while the exact function of others is still only marginally understood. Yet, since they function to fatten livestock, they are used in great quantities. Are nisin and related peptides actually antibiotics, or are they just bacteriocins with convenient activities? Are aflatoxins antibiotics? They are microbial secondary metabolites, like most traditional antibiotics, and they kill or inhibit biological entities. Nevertheless, they are not utilized commercially as antibiotics. What about delta-endotoxins of *Bacillus thuringiensis* and *Bacillus sphaericus* antibiotics? Certainly, they kill insects, which are biological entities. They too are produced commercially. In the same light, are algal toxins, *Clostridium botulinum* toxins, and other such proteinaceous toxins antibiotics? Perhaps the definition would be stretched too far to include these. Should immunosuppressive agents be considered? Some exhibit antibiotic activity although it is usually poor; they nevertheless carry out antimetabolic functions. But if immunosuppressants such as cyclosporin and rapamycin are included, why not immunomodulatory proteins such as granulocyte colony–stimulating factors? In this book, the line between antibiotics and other pharmaceutically active natural products has been drawn between those non-protein products that are microbially derived or produced, and those that are derived from plants or animals. While this may

appear to be an arbitrary distinction, it does serve the purpose of defining limits. Therefore, while it was very tempting to include a chapter on taxol biosynthesis, its inclusion would have required chapters on vincristine and vinblastine biosynthesis. Moreover, even a single chapter on production of recombinant human protein, tissue plasminogen activator (tPA), for example, would have begged the question of including all of the now thirty or so recombinant proteins on the market. Thus, this book is specific to microbially derived, commercially important bioactive products that are currently available or appear to have a bright future in early 21st-century markets.

Finally, the question of choice must be addressed. Why include two chapters concerning β-lactam antibiotics and another two relating to peptide antibiotics while leaving out many others that are significant to human health but which occupy small niche markets, such as pseudomonic acid (mupirocin). Why include, in some chapters, basic biochemistry and molecular genetics, while in others emphasize industrial scale-up and fermentation processes? Why not include chapters on chemically synthesized antibiotics, or antibiotics such as chloramphenicol, originally produced by *Streptomyces* fermentations but now chemically synthesized? The answer is that, within the confines of twenty-six chapters, this book endeavors to place itself at the leading edge of the antibiotic biotechnology field, while striking a delicate balance between proven, traditional ("old") technologies and those modern technologies which promise to yield new products and processes as we enter the 21st century. The future of antibiotic production promises to be just such a mixture, with the use of novel biological approaches to solve traditional chemical problems, the development and use of novel screening methods to discover new biological activities, and the application of traditional approaches to genetically engineered microorganisms to overproduce existing or new generations of antibiotics. The contributing authors for this book include several academics, who are often at the leading edge of science and novel concepts but may have never set foot in a production facility; and industrial scientists, who are diverse in their backgrounds and whose areas of expertises range from the genetic engineering of new generations of antibiotics to applying traditional methods to the production of new and unusual molecules.

No book with this scope can be assembled by a single person—particularly one like me, who resides in academia—without the thoughtful assistance and advise of others, particularly those who are associated with industrial antibiotic production. I owe considerable thanks to Dean Taylor for endless hours of fruitful discussion on industrial antibiotic production and potential authors and chapters, and to Arnold Demain for his thoughtful suggestions and gentle reminders to stay close to schedule. I also wish to thank each of the contributors for their considerable efforts and patience with me as I prodded them for updates and new information. I would especially like to thank my wife, Lila, and two sons, Joshua and Justin, for their patience and understanding during the period of this endeavor.

Since entering the field of antibiotic biosynthesis in the early 1980s, I have been inspired by a great number of outstanding scientists. A few have had an enormous influence on my career, whether they know it or not. Thus, it is with great pleasure that I dedicate this book to Heinz Floss and Arnold Demain, colleagues who have taken the time to show me the light and give me much needed encouragement over the past decade, and to David Hopwood, who, through his generosity and kindness, made it possible for me and so many others to delve into the exciting field of streptomycete molecular biology as it unfolded.

William R. Strohl

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