THE BACTERIA

A TREATISE ON STRUCTURE AND FUNCTION

Consulting Editor
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VOLUME IX

Antibiotic-Producing *Streptomyces*

Volume Editors
STEPHEN W. QUEENER
LAWRENCE E. DAY

The Bacteria

VOLUME IX: ANTIBIOTIC-PRODUCING *STREPTOMYCES*

THE BACTERIA

A TREATISE ON STRUCTURE AND FUNCTION

Volume I: Structure (I. C. Gunsalus/R. Y. Stanier, eds.)

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Volume VII: Mechanisms of Adaptation (J. R. Sokatch/L. N. Ornston, eds.)

Volume VIII: Archaebacteria (C. R. Woese/R. S. Wolfe, eds.)

Volume IX: Antibiotic-Producing *Streptomyces* (S. W. Queener/L. E. Day, eds.)

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PREFACE

"The Bacteria" is a venerable treatise that has, through the years, attracted a talented array of contributors who have described and summarized important structure/function relationships in the living cell as represented in bacteria. By devoting Volume IX to *Streptomyces,* we recognize the importance of these bacteria to mankind. We are also afforded the opportunity to emphasize the rapid progress being made in the understanding of how these gram-positive filamentous bacteria produce a broad array of antimicrobial substances.

The fundamental process of secondary metabolism is a key and highly developed attribute of *Streptomyces*. The great chemical diversity of secondary metabolism in *Streptomyces* has made this genus the object of intense study in university and industrial laboratories. This volume emphasizes the biology related to antibiotic production and, because of the use of *Streptomyces* in the antibiotic fermentation industry, allows a cross-sectional, multidisciplinary view of research as practiced in the antibiotics industry. All the authors in Volume IX have made important contributions to that industry either through pioneering fundamental studies impacting the industry and/or by direct participation in *Streptomyces* research that has resulted in the establishment and maintenance of economically viable fermentations that produce, on mass scale, products useful to society. Thus the reader may be assured of the practical relevance of the material presented in this book.

As editors of Volume IX, we are indebted to the contributors who have taken time from demanding research schedules to share with the reader important aspects of current research on *Streptomyces* and their antibiotics. We also wish to thank Dr. Satoshi Ōmura for providing his incisive overview of the subject of this book, an overview drawn from a long and distinguished career in antibiotics and *Streptomyces* research. We appreciate the opportunity to serve as editors of this volume, and are indebted to Drs. L. N. Ornston, J. R. Sokatch, and I. C. Gunsalus for providing us this opportunity.

STEPHEN W. QUEENER
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The Bacteria

A TREATISE ON STRUCTURE AND FUNCTION

VOLUME VI: BACTERIAL DIVERSITY

VOLUME VII:
MECHANISMS OF ADAPTATION

VOLUME VIII:
ARCHAEBACTERIA

INTRODUCTION

Actinomycetes, represented by *Streptomyces,* include more than thirty genera of gram-positive bacteria that show branching, filamentous, or irregularly rod-shaped cell morphology. The term *Actinomyces* appeared in the literature as early as 1887, a time when L. Pasteur and R. Koch were still active in research. This name means "ray-fungus" in Greek, and suggests that these microorganisms were observed, cultured, and studied as fungi in the early days. However, their taxonomic position is well established within the kingdom Procaryotae. This classification is based on the conceptual proposal of Chatton, who, in 1937, classified microorganisms according to their general cellular organization, a method which is supported by an abundance of biochemical, physiological, and microscopic evidence that has accumulated since then. The essential features of the prokaryotic nature of the genus *Streptomyces* are as follows: the lack of a membranous boundary which separates the nucleus and the cytoplasm as seen in fungi; the lack of unit membrane-bound cytoplasmic organelles; and ribosomes of the 70 S type in contrast to the 80 S type found in fungi. Also, peptidoglycan, a cell wall constituent shared by almost all, but not every, bacterial strain is another distinguishing feature of these prokaryotes.

In spite of such unequivocal classification as bacteria, *Streptomyces* are boundary organisms from physiological and metabolic points of view. Streptomycete strains grow well under aerobic conditions, with lag, log, and stationary phases, as typical bacteria do. During growth on agar, however, they differentiate into two types of long and branching cells, i.e., substrate and aerial mycelia, the latter of which subsequently fragment and/or sporulate. This is similar to the growth and differentiation of fungi.

Primary metabolism in this group of organisms also appears to possess boundary characteristics. For example, higher fatty acids in the cells of *Streptomyces erythreus,* an erythromycin producer, are the same as those found in the lipid fraction of typical bacteria. Palmitic acid is the major component. On the other hand, the fatty acid-condensing enzyme, or 3-ketoacyl-[acyl-carrier-protein] synthetase, of this *Streptomyces* is a multienzyme complex type typical of yeasts and filamentous fungi.

The classification of actinomycetes (see Dietz, Chapter 1, this volume, for classification within the genus *Streptomyces*) is based on morphology, physiological characteristics, the composition of cell constituents such as cell walls, and the presence of characteristic lipids, sugars, and quinones. The GC content of DNA of actinomycetes is generally in the range of 65–75%, although certain thermophilic actinomycetes have a low GC content (44–54%). The GC content of *Streptomyces* is high (70–75%) and characteristic of that genus. For mycobacteria and nocardiae GC content is 60–70% and for most other genera of actinomycetes GC content varies between 65 and 70%. The broad range of GC

content in the various genera of actinomycetes suggests that this is a rather heterologous order of microorganisms; also the narrow, high range for *Streptomyces* suggests close homology among the microorganisms in this genus.

The outstanding property of the actinomycetes, particularly those belonging to the genus *Streptomyces*, is their capability of elaborating a variety of antibiotics. It is not unusual to observe during antibiotic screening studies that more than 50% of a set of soil isolates produce antibiotics. It is for this reason that actinomycetes attracted great attention from applied microbiologists. Actinomycin, the first antibiotic from a streptomycete, was discovered by Waksman and Woodruff in 1940, followed by the first antituberculotic antibiotic, streptomycin, by Waksman *et al.* in 1944. These events, and the earlier discovery of penicillin, marked the beginning of the antibiotic era and served to initiate this broad field of natural science in which actinomycetes have played such a prominent role. Since then, continual efforts have been directed toward the isolation and characterization of various types of antibiotic-producing actinomycete strains. It is impossible to describe here the details of individual strains. A large number of them have not been made available due to their commercial value.

To date, some 6000 antibiotics of microbial origin have been characterized, and about 60% of them are produced by actinomycetes. The rest are fungal and bacterial metabolites. Among these antibiotics, about seventy compounds, 90% of which originate from streptomycetes, have found practical application in human and veterinary medicine, in agriculture, and in the fishery industry. β-Lactams, originally found as products of filamentous fungi, are now known to be elaborated also by streptomycetes and by certain genera of gram-negative bacteria. Antibiotics from actinomycetes include almost all known structural classes of commercially important antibiotics; for example, aminocyclitols, ansamycins, anthracyclines, β-lactams, macrolides, glutarimides, nucleosides, peptides, peptidolactones, polyenes, polyethers, tetracyclines, and other important antibiotics such as chloramphenicol (see Crandall and Hamill, Chapter 10, this volume). These antibiotics have had great influence on human and animal health care, the fermentation industry, and natural science.

Tuberculosis, a serious disease caused by *Mycobacterium tuberculosis* with a high mortality rate in the 1940s, is of far less consequence today owing to the use of streptomycin. Enterotyphus, then a disease with about a 50% mortality rate, is now curable due to the use of chloramphenicol and ampicillin. Syphilis, caused by *Treponema pallidum* and other pathogens, and bacillary dysentery, with *Shigella dysenteriae* as the causative pathogen, are now encountered only rarely. The benefit of such a drastic reduction of serious infectious diseases is best illustrated by the lengthened life span of man. From 1960–1980 the average life span in Japan increased from 66 to 73 years for males and 71 to 78 years for females, while in the United States this span increased from 67 to 71 for males and 74 to 78 for females. The increases during earlier years, 1946–1951, were

even more significant in Japan: from 43 to 61 years for males and 51 to 65 for females. The infant mortality rate has declined to some 1% of the level of thirty years ago. There is no doubt that antibiotics, together with improved food and sanitary conditions, have contributed extensively to the promotion of human health.

The successful use of antibiotics has resulted in a greatly expanded fermentation industry. The research, development, and production of antibiotics is now a multibillion dollar industry worldwide. The antibiotic industry today accounts for nearly 25% of the total market for pharmaceuticals in Japan. Of this volume, the β-lactams have the biggest share, followed by the aminocyclitols and the macrolides. The chemical industry has also prospered from the antibiotics. Most of the β-lactams currently on the market are semisynthetic or synthetic. Noticeable is the recent contribution to this area by research groups in Japan, the United States, and Germany as exemplified by the number of semisynthetic antibiotics (mostly β-lactams) put into use from 1981 to 1983: 34 from Japan, 27 from the United States, and 18 from Germany. Switzerland, Italy, and France contributed 14, 12, and 11, respectively, to a worldwide total of 138.

In addition to medical uses, antibiotics have proved useful in controlling animal, fish, and plant diseases and in promoting animal growth. Antibiotics are also utilized in research as biochemical tools with specific modes of action. For example, penicillin, puromycin, cerulenin, and tunicamycin have brought about marked progress in the understanding of the biosynthetic mechanisms of bacterial cell walls, proteins, fatty acids, polyketide-derived secondary metabolites, and glycoconjugates, to cite just a few.

The above discussion emphasizes those areas in which antibiotics have contributed significantly to progress; however, the science and application of antibiotics have many unsolved problems. For example, existing antibiotics, although excellent as antibacterial agents, do not exhibit sufficient activity for the control of fungi such as *Candida,* viruses such as *Herpes* or hepatitis, or protozoa. They are not very effective against many kinds of tumor cells.

In association with antibiotic therapy, new problems have evolved; for example, the occurrence of drug-resistant microorganisms; opportunistic infections caused by normally nonpathogenic organisms, especially in patients with defects in their immune response due to aging or to the use of immunosuppressive agents such as antitumor compounds; and superinfection in patients given broad spectrum antibiotics. The increase in chronic diseases, replacing acute ones, is a recent trend which suggests that the use of antibiotics plus changes in our social life (with which food and stress are also concerned) have resulted in changes in the etiology of infectious disease. It would seem prudent to reinvestigate the merits of narrow-spectrum antibiotics.

Additionally, basic studies on the microbiology of antibiotic-producing organisms have progressed more slowly than the chemistry of the products. While a

huge number of actinomycetes have been isolated in various university and industrial laboratories, much more interest has been focused on the compounds they produce, rather than on the organisms themselves. In fact, we have very little knowledge of the major pathways that supply energy and building units for the biosynthesis of cell constituents in actinomycetes. The structures of cell wall peptidoglycan and the mechanisms of DNA, RNA, and protein synthesis and their regulation are less understood in the streptomycetes than in *Escherichia coli*. Although fundamental features of the two bacteria are identical, many details are likely to be different, e.g., the boundary characteristics.

Knowledge of streptomycetes microbiology is rapidly increasing because these organisms are attracting new attention from the standpoint of recombinant DNA technology. The new technology promises unparalleled progress in pure and applied microbiology. The techniques now available include recombination by conjugation and protoplast fusion; transformation, transduction, and transfection; and gene cloning using host–vector systems. Worldwide research efforts in the molecular biology of actinomycetes have included the following: studies of the biochemistry, physiology and genetics of established antibiotic-producing streptomycetes and of the related antibiotic synthetic mechanisms; application of gene technology to improving the efficiency of antibiotic biosynthesis; improved screening methodology for rare and unusual antibiotic-producing actinomycetes; and the construction of hybrid organisms capable of producing hybrid antibiotics with improved properties. Microbial conversion using modified organisms is also beneficial for the biological modification of known antibiotics.

This new technology is also stimulating progress in the biochemistry, physiology, and genetics of streptomycetes. A detailed genetic map for a streptomycete (see Rhodes, Chapter 2, this volume) and new methods for genetic analysis have become available. Studies of enzymes, mechanisms of self-resistance, and new inducers involved in antibiotic biosynthesis, as well as mutational analysis (see Baltz, Chapter 3, this volume) in *Streptomyces* have increased rapidly. The time has come when the initiation and regulation of antibiotic biosynthesis are being studied at the molecular level. Current curiosity focuses on the location, structure, and function of genes determining antibiotic biosynthesis, regulation, and resistance. Available evidence favors a chromosomal location for the determinant of many antibiotics, although the biosynthesis of and/or resistance to a few antibiotics such as methylenomycin are specified by plasmids (see Hopwood *et al.*, Chapter 6, this volume). The results of these studies, covered in several chapters of this book, are not only useful for a better understanding of streptomycete physiology, but are also helpful as a basis for applying gene technology to streptomycetes. A recent report of Hopwood *et al.* on the cloning and expression of the actinorhodin biosynthesis genes and the elaboration of new hybrid antibiotics suggests, as discussed in this book, that many of these goals are being reached step by step. These studies require ver-

satile but systematized information of both basic and practical aspects, and it is a purpose of this book, by the collection and review of accumulated knowledge in these areas, to meet this need.

It should be noted, however, that gene technology will never create an organism that is capable of producing antibiotics with entirely novel structures and unexpected activities; in other words, novel DNA, which is unrelated to either the parent or the vector DNA, will not be created, although it will be possible to produce organisms harboring modified or unmasked genes. Even though we are witnessing exciting achievements by this new technology, the importance of screening for new antibiotic-producing actinomycetes cannot be overemphasized. The organisms thus selected can serve not only as sources of antibiotics for chemical, biochemical, microbiological, and taxonomic studies, but also as a source of new genes for recombinant DNA studies as well as for new gene banks.

It is more difficult now than ever to discover new antibiotics from natural sources. The producers of cephamycin, olivanic acid, and, more recently, of monobactams and of the novel antiparasitic macrolide, avermectin, were discovered with the aid of specialized techniques for the isolation and fermentation of cultures and for the detection and purification of the products. It is hoped that the advances in basic knowledge of the biochemistry and physiology of antibiotic-producing streptomycetes will suggest more rational ways for culture isolation and identification. In addition, ecological studies suggest that we have examined only a small fraction of the total population of soil microorganisms. A large number of actinomycetes and antibiotics await discovery.

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