THE BIOLOGY OF THE ACTINOMYCETES

EDITED BY

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Preface

Actinomycetes are a successful and widely distributed group of bacteria which have a number of properties that favour them in competition with other saprophytic microorganisms. They are best known for their economic importance as producers of antibiotics, vitamins and enzymes and are certain to have a significant role in the future of biotechnology. Some are causal agents of important human and animal diseases, others are plant pathogens but most are involved in turnover of organic matter. Given their industrial, ecological and medical importance it is not surprising that actinomycetes have been the subject of several recent books. Most of these have originated from the proceedings of symposia and, while useful to the specialist, they do not, by their very nature, always present a comprehensive view of the properties and biological importance of actinomycetes. The purpose of the present book is to give readers a balanced survey of the current knowledge of actinomycete biology.

All of the chapters have been written by specialists so that the book as a whole constitutes a unique collection of information on an interesting, but all too often neglected, group of bacteria. There are valuable and detailed reviews on ecology, genetics, morphology, pathogenicity, systematics, wall envelope composition and on the clinically significant actinomycetes. Specialist texts should be consulted for additional consideration of important pathogens, such as *Corynebacterium diphtheriae*, *Mycobacterium leprae* and *Mycobacterium tuberculosis*, and on the principles and methods of antibiotic production. We hope that the book will find a place in advanced courses of microbiology, will provide a useful general background to all those who work with, and try to unravel the nature of, actinomycetes, whether this be in industry, the health service or institutes of higher education.

We would like to take this opportunity to thank all of the contributors for bearing with us and for providing such excellent and well researched chapters. We would also like to thank many colleagues who have assisted us in a variety of ways and last but not least we extend our gratitude to Judith Gaigy and Dorothy Lewis whose help in typing was invaluable. Throughout, we have been greatly encouraged and assisted by Academic Press.

July, 1983

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Contents

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Introduction to and Importance of Actinomycetes

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1. Introduction

The existence of the actinomycetes has been recognized for over a hundred years. For much of this time they were regarded as an exotic group of organisms with affinities to both bacteria and fungi. However, determinations of their fine structure and chemical composition, initiated in the 1950s, confirmed their prokaryotic nature (see Chapters 3 and 7). They now constitute the order *Actinomycetales* (Buchanan, 1917) and their removal from the mycologist's sphere of influence has been completed. Their change of status paralleled that of the blue-green algae to the cyanobacteria but it was accepted more rapidly and less acrimoniously. It is not easy to give a short, accurate definition of an actinomycete. They are frequently described as bacteria which have the ability to form branching hyphae at some stage of their development. However, this attribute is tenuous and it often requires imagination to believe in it (Gottlieb, 1973). The exact composition and boundaries of the order *Actinomycetales* are still open to question and modification by the application of new taxonomic techniques which have also led to improvements in the classification and identification of

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actinomycete genera and species (see Chapter 2). Despite their relegation to a single order of the kingdom *Prokaryotes*, their biological attributes, their importance to man and their history have ensured that actinomycetes are still generally studied as a group distinct from other bacteria.

Since Waksman's volumes on the actinomycetes (Waksman, 1950, 1959, 1961, 1967; Waksman and Lechevalier, 1962), most books on their biology have originated from the proceedings of symposia (e.g. Prauser, 1970; Arai, 1976; Goodfellow *et al.*, 1976; Lloyd and Sellers, 1976; Freerksen *et al.*, 1978; Mordarski *et al.*, 1978; Schaal and Pulverer, 1981). Although such publications are particularly useful to the specialist, they do not, by their very nature, always present a balanced coverage of the subject. There have been many advances in actinomycete biology since Waksman's publications, so that it is now appropriate to stand back and present a balanced survey of current knowledge. Although the actinomycetes have been the subject of an extensive literature in recent years, many aspects of their nature, physiology, and especially their role in natural ecosystems, have still to be understood. Clearly much remains to be done but an exciting array of powerful molecular and microbiological methods are available to those with the responsibility for developing actinomycete biology further.

The development of knowledge on the biology of the actinomycetes over the last hundred years is summarized below. Detailed accounts of early studies of actinomycetes can be found elsewhere (Lieske, 1921; Henrici, 1930; Krasilnikov, 1941; Waksman, 1959).

2. Causal Agents of Disease (from 1875)

As with many microbes, the study of actinomycetes was initiated in the late 19th century by workers examining diseased material from humans, animals or plants. The first unambiguous description of an actinomycete was probably that of Cohn (1875) who observed filamentous growth in concretions from lachrymal ducts and named the organism *Streptomyces foersteri*, but this generic name had been used by Corda (1839) for a group of fungi and was invalid. Shortly after this, an organism seen in a specimen of 'lumpy jaw' of cattle was described as *Actinomyces bovis* (Harz, 1877). Other observations of actinomycete-like microbes associated with human or animal infections soon followed, but their taxonomy and pathogenicity were confused due to the lack of pure cultures. The first plant pathogen *Streptomyces* (née *Oospora*) *scabies*, was isolated from potato scab by Thaxter (1891).

Subsequently, actinomycetes have proved to be causal agents of many human and animal infections. These include some widespread and intensively studied diseases, such as diphtheria (*Corynebacterium diphtheriae*),

tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*), but it must be noted that the inclusion of such microbes in the actinomycetes has been a matter for debate (see Chapter 2). Consideration of the medical aspects of such diseases is beyond the scope of this book, but the relevant information can be found in recent detailed reviews (e.g. Saragea *et al.*, 1979; Ratledge and Stanford, 1982; Kubica and Wayne, 1983).

However, there is a wide range of actinomycete infections which are less widely known (Slack and Gerencser, 1975; Lloyd and Sellers, 1976). Many of these are proving to be more clinically significant than previously thought, partly due to improvements in procedures for their diagnosis (see Chapters 8, 9 and 10). It is also becoming increasingly evident that *Actinomyces* sp. can play a role in the aetiology of caries and periodontal disease.

3. Ecology and Physiology of Saprophytes (from 1900)

One of the first truly saprophytic actinomycetes to be detected was *Streptothrix chromogena* which was isolated from soil by Beijerinck (1900). The widespread occurrence of actinomycetes (particularly streptomycetes) in soil was demonstrated by Krainsky (1914) and Waksman and Curtis (1916, 1918). Over the next 20 years, knowledge of the ecology of actinomycetes in soil, composts and other habitats was considerably extended by Waksman, Jensen and other soil microbiologists. These studies also provided basic information on the isolation, cultivation, identification and physiology of saprophytic actinomycetes; indeed, many of the concepts and techniques originated in this period are still accepted today. This work also proved to be a valuable prelude to the exploitation of the actinomycetes as producers of antibiotics.

Subsequent studies of actinomycete ecology have sometimes consisted of little more than elaboration or repetition of the results of these pioneering workers. However, there has also been increasing emphasis on their roles in extreme environments and in many natural processes such as nitrogen fixation, decomposition of ligno-celluloses and the control of root pathogens (see Chapter 11). An impressive array of procedures are now available for the selective isolation of specific actinomycete taxa from natural habitats (Williams and Wellington, 1982; Cross, 1982).

4. Production of Antibiotics and Other Useful Secondary Metabolites (from 1940)

The first purified antibiotic to be obtained from an actinomycete was actinomycin (Waksman and Woodruff, 1940). This heralded a new era in

which the potential medical and commercial value of these microbes was realized; this in turn influenced all aspects of research on the group. The discovery of actinomycin was soon superceded by that of streptomycin (Schatz *et al.*, 1944) which is probably best known for its use in the control of tuberculosis. Many other medically useful antibiotics followed, most of which are still in use. Although most of these antibiotics originated from streptomycetes, other genera such as *Actinoplanes*, *Actinomadura* and *Micromonospora*, also produce useful or potentially useful antibiotics. Although the rate of return has decreased in recent years, new antibiotics and other useful metabolites from actinomycetes are still being discovered. This is well illustrated by β-lactamase inhibitors, such as clavulanic acid from *Streptomyces clavuligerus* (Reading and Cole, 1977), which have recently been commercially produced to overcome bacterial resistance to existing β-lactam antibiotics. The ability of actinomycetes to produce useful secondary metabolites remains unsurpassed, although the reasons for this and the biological significance of such products are still not clear (see Chapter 11).

The importance of actinomycetes in industrial biosynthesis has undoubtedly stimulated many aspects of basic research on these microbes. However, it inevitably led to concentration on the detection and selection of potentially useful strains (mainly streptomycetes) and the optimization of their fermentations. Therefore, research on some basic aspects of the biology of actinomycetes was somewhat retarded and overshadowed by their practical exploitation. Thus, most research from 1940–1970 was concentrated on the streptomycetes, to the comparative neglect of other saprophytic actinomycetes. In recent years, however, there has been an increasing recognition of the mutual interests of pure and applied microbiologists working with actinomycetes.

The principles and methods of antibiotic production have been extensively reviewed and will not be covered here. However, the use of microbes to transform known organic compounds into novel, useful agents is increasing, and the potential of actinomycetes in this field is also considerable (see Chapter 6). In addition, the genetic (see Chapter 5) and ecological (Chapter 1) aspects of antibiotic production are of theoretical and practical importance.

5. Genetics (from 1955)

Until the prokaryotic nature of actinomycetes was recognized, knowledge of their genetics was extremely limited. Since the 1950s, research on actinomycete genetics has generally paralleled that on other bacteria, most attention being paid to *Streptomyces coelicolor* and other *Streptomyces*

species (see Chapter 5), and to *Nocardia* and *Rhodococcus* species (see Chapter 4).

The first report of recombination in streptomycetes (Sermonti and Spada Sermonti, 1955; Hopwood, 1957) initiated a period of study of the location of genes on the chromosome which facilitated study of its behaviour during conjugation. In the 1970s, the role of plasmids in controlling fertility and the genetic control of differentiation, primary metabolism and antibiotic biosynthesis in streptomycetes were elucidated by Hopwood and his co-workers (see Chapter 5).

These studies and others provided a basic model of actinomycete genetics, which has and will facilitate the application of the techniques of 'genetic engineering', such as gene cloning and protoplast fusion. Hopefully these will lead to still further exploitation of actinomycetes for their useful metabolites. Such developments, allied to their natural biochemical, physiological and ecological diversity, should ensure that actinomycetes have a role in the future of biotechnology which is at least as significant as their current one.

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2

Classification

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1. Introduction

The actinomycetes have been traditionally considered to be prokaryotic bacteria with elongated cells or filaments that usually showed some degree of true branching. Although the morphology of these organisms ranges from simple to complex, most strains of most species can be assigned to one of two broad morphological groups, nocardioform- and sporo-actinomycetes (Prauser, 1970, 1976a, 1978, 1981). Nocardioform bacteria form hyphae which eventually fragment into coccoid or rod-like elements that give rise to new mycelia (Locci, 1976, 1978, 1981). The genera *Caseobacter*, *Mycobacterium* and *Rhodococcus* are generally included in this group, though all of them contain strains that exhibit little, if any, branching, growing merely as rod or coccoid elements. The sporoactinomycetes encompass a greater morphological complexity that includes the formation of spores in or on definite parts of the mycelium (Locci, 1976; Williams *et al.*, 1976; Williams and Wellington, 1980). A third level of organization is presented by *Dermatophilus* and *Geodermatophilus* which form a substrate mycelium that

divides both transversely and longitudinally to give a primitive multilocular sporangium. Coccoid elements are released which may gain motility and eventually germinate into filaments or hyphae (Cross and Goodfellow, 1973). *Frankia* shows some of the morphological traits associated with dermatophili and geodermatophili (Callaham *et al.*, 1978; Becking, 1981).

Actinomycetes have for many years been grouped together solely on morphological grounds even though they have never been satisfactorily distinguished from coryneform bacteria on this basis (Bousfield and Goodfellow, 1976; Goodfellow and Minnikin, 1981a). It is now beyond dispute that innumerable morphological transitions exist between nocardioform actinomycetes like *Actinomyces*, *Nocardia* and *Rhodococcus* and coryneform bacteria, such as *Arthrobacter*, *Cellulomonas* and *Corynebacterium*, that have a tendency to form branched elements (Locci, 1976, 1978, 1981; Williams *et al.*, 1976; Prauser, 1978, 1981). Recent morphological studies help to explain why early workers (eg. Lehmann and Neumann, 1920, 1927; Lieske, 1921; Ørskov, 1923; Jensen, 1953) found it impossible to distinguish between corynebacteria, mycobacteria, nocardiae and related bacteria simply on morphological features. In the light of current knowledge it is clear that many of the earlier classifications of coryneform and nocardioform bacteria were artificial, had a narrow data base, and were consequently unreliable vehicles for the identification of unknown isolates (Goodfellow and Minnikin, 1977, 1981a, b, c, 1982). A tentative step towards remedying this situation was taken in the current edition of *Bergey's Manual of Determinative Bacteriology* (Buchanan and Gibbons, 1974) where actinomycetes and coryneform bacteria were considered together in a section entitled '*Actinomycetes and related organisms*'.

Bacterial systematics has undergone revolutionary change in the last 20 years. The application of new and reliable biochemical, chemical, genetical, numerical and molecular biological techniques have been responsible for rapidly changing views on how bacteria ought to be classified and identified (see Goodfellow and Board, 1980; Berkeley and Goodfellow, 1981). These techniques have generally been applied to greatest effect on taxa where dependence on form and function proved most unsatisfactory and they have provided a framework for revised classification of both coryneform (Bousfield and Callely, 1978; Stackebrandt *et al.*, 1980a, b; Goodfellow and Minnikin, 1981a; Keddie and Bousfield, 1980; Keddie and Jones, 1981; Döpfer *et al.*, 1982) and nocardioform bacteria (Bradley and Mordarski, 1976; Lechevalier, 1976; Minnikin and Goodfellow, 1976, 1980, 1981a; Goodfellow and Minnikin, 1977, 1978, 1981b, c, 1983; Mordarski *et al.*, 1977, 1978a, b, 1980a, b, 1981a, b; Stackebrandt and Woese, 1981a, b). The newer methods are now being applied to sporoactinomycetes with interesting results (Stackebrandt and Woese, 1981a, b; Stackebrandt *et al.*, 1981, 1982; Williams *et al.*, 1981, 1983a, b; Goodfellow and Pirouz, 1982).

Most of the new taxonomic techniques only serve to detect affinities between closely related taxa; they are of limited value in determining relationships among distantly related species, genera and families. Suprageneric and evolutionary relationships of bacteria can, however, be detected using the powerful techniques of DNA–(ribosomal r) RNA association and 16S rRNA cataloguing. Base sequences of rRNA cistrons are more highly conserved than most of the genes forming the bacterial genome (Doi and Igarashi, 1965; Dubnau *et al.*, 1965; Moore and McCarthy, 1967), a fact that allows comparisons to be made between nucleotide sequences of rRNA preparations from representatives of diverse taxa (De Smedt and De Ley, 1977; De Smedt *et al.*, 1980; Gillis and De Ley, 1980). Ribosomal RNA cistron similarity data show that the acid-fast fast actinomycetes are phylogenetically close (Mordarski *et al.*, 1980a, 1981b) and indicate that the sporoactinomycetes fall into at least three major homology groups: *Actinoplanes*, *Amorphosporangium*, *Ampullariella* and *Micromonospora*; *Planobispora*, *Planomonospora* and *Streptosporangium*; and *Chainia*, *Elytrosporangium*, *Kitasatoa*, *Microellobosporia*, *Streptomyces* and *Streptoverticillium* (Stackebrandt *et al.*, 1981). These groupings are in good agreement with the current trends in the taxonomy of these organisms.

Ribosomal RNA cataloguing provides an even more exacting way of detecting phylogenetic relationships amongst prokaryotes (Fox *et al.*, 1977a, b; Woese and Fox, 1977; Stackebrandt and Woese, 1981a, b). In this method, purified RNA is digested by TI ribonuclease, the oligonucleotides separated by two-dimensional electrophoresis are sequenced by a combination of endonuclease digestion procedures which yield a catalogue of sequences characteristic of the strain under study. The oligonucleotide catalogues of any two strains are compared one with another and oligonucleotides, of six residues or larger, common to any two catalogues, are scored to produce a 'S_{AB} value' characteristic of that pair of organisms. The function S_{AB} is equivalent to twice the total number of residues in sequences common to a pair of catalogues, divided by the total number of residues in all of the sequences in the two catalogues. S_{AB} values are analysed using standard clustering algorithms and data presented as dendrograms or, more appropriately, as evolutionary trees (Stackebrandt and Woese, 1981a).

16S rRNA cataloguing data show that Gram-positive bacteria form a distinct phyletic line that can readily be divided into two branches on the basis of DNA base composition (Stackebrandt and Woese, 1981a). The actinomycete–coryneform line includes bacteria with a guanine (G) plus cytosine (C) content above about 55 mol% and can be separated from the low G+C content (below 50 mol%) *Clostridium-Bacillus-Streptococcus* branch. Several taxa previously associated with the actinomycetes clearly belong to this second evolutionary branch. The genus *Eubacterium* is phy-

logenetically related to *Clostridium*, *Kurthia* to the lactic acid bacteria and, perhaps most surprisingly of all, *Thermoactinomyces* to the *Bacillaceae* (Ludwig *et al.*, 1981; Tanner *et al.*, 1981; Stackebrandt and Woese, 1981a).

The thermophilic genus *Thermoactinomyces* has long been regarded as a 'good actinomycete' because of the gross appearance of its powdery white or yellow colonies on agar, and branching hyphae which carry lateral spores on both substrate and aerial hyphae. Several taxonomic studies have shown that the genus can be clearly distinguished from actinomycete genera with single spores, for example *Micromonospora*, *Saccharomonospora* and *Thermomonospora*, and the present authors earlier suggested that it should be placed in a distinct family because of its ability to produce endospores (Cross and Goodfellow, 1973). However, the reclassification of thermoactinomycetes in the *Bacillaceae* is a revolutionary move which requires some explanation and justification.

Wall analyses showed that the peptidoglycan of *Thermoactinomyces* species contained *meso*-diaminopimelic acid (*meso*-DAP) with no other characteristic amino acids or sugars (Becker *et al.*, 1965) and the genus was therefore included in the actinomycete wall chemotype III classification of M. P. Lechevalier and Lechevalier (1970a, b). This peptidoglycan, namely the directly cross-linked *meso*-DAP-D-alanine type, is also found in the majority of *Bacillus* species (Schleifer and Kandler, 1972) and is not restricted to actinomycetes. The DNA base composition of *Thermoactinomyces* species show that they have a much lower mol% G+C content than is found in actinomycete genera (see Table 1). Values of 52.0 (Fritzsche, 1967), 53.4–54.4 (Craveri and Manachini, 1966), 54.1 and 54.8 (Craveri *et al.*, 1966) are much nearer those found in some mesophilic and thermophilic *Bacillus* species (Priest, 1981). When detailed studies on the fine structure of *Thermoactinomyces* spores showed that they had the typical structure of bacterial endospores and contained dipicolinic acid (Cross, 1968; Cross *et al.*, 1968a; Dorokhova *et al.*, 1968) the relationship was even more apparent.

Further evidence for the relationship between *Bacillus* and *Thermoactinomyces* has recently come from a study of their isoprenoid quinones. Both genera typically contain major amounts of unsaturated menaquinones with seven or nine isoprene units (Collins and Jones, 1981a; Collins *et al.*, 1982g; Minnikin and Goodfellow, 1981b) in contrast to the vast majority of actinomycetes which contain complex mixtures of partially saturated or hydrogenated menaquinones (Collins and Jones, 1981a). It is also interesting to note that the purified malate dehydrogenases from *Team. sacchari* and several *Bacillus* spp. were found to be tetramers which exhibited immunochemical homology and differed from the dimeric enzymes present in *Temo. fusca* and other non-endospore bacteria (Sundaram *et al.*, 1980).

It is clear that the mere possession of branching hyphae should not automatically place a bacterium within the actinomycetes, and that the