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ACTINOMYCETALES

Characteristics and Practical Importance

edited by
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Preface

THIS second volume in the Symposium series of the Society for Applied Bacteriology publications is concerned with a review of the characteristics and significance of the Actinomycetales. The Symposium was held at the Loughborough University of Technology, England in July 1972. Each chapter has been contributed by a specialist in his field and so the book as a whole constitutes a unique collection of information on this group of micro-organisms which has long been recognized as having many unusual and interesting features.

As might be expected of such a diverse group of organisms there is much to be learned of their physiological characteristics and several chapters are devoted to such studies. Under this heading there are valuable and comprehensive contributions on the taxonomy and genetics of the group and on the production and germination of their spores. In other chapters the occurrence and significance of the actinomycetes in water, soil and the human digestive tract, and their role in causing potato scab disease is discussed.

It is relevant to stress the medical importance of the actinomycetes both in causing and curing disease. The realization that some streptomycetes could produce antibacterial substances stimulated great interest in the group, especially in the years immediately following the Second World War, and led to the development of the present impressive array of antibiotics of clinical value. The pathogenic properties of the anaerobic actinomycetes have been known for a long time but more recently attention has been directed to the activities of the aerobic types as allergens in pulmonary conditions such as Farmer's Lung disease.

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1. Introduction

THE ACTINOMYCETES have always been a strange group of organisms to the bacterial taxonomists. The number of investigators studying this group of microbes has always been small and for the most part these organisms have been neglected by medical bacteriologists, physiologists and biochemists. Such affairs were to be expected when one recalls that until recently not even their taxonomic position was certain (Breed, Murray & Smith, 1957). Bacteriologists considered them as bacteria and mycologists generally considered them as fungi. The diseases caused by them have been described in books on medical mycology (e.g. Conant *et al.*, 1954). This era is now over and the actinomycetes are generally accepted as bacteria. They have no nuclear membrane, are sensitive to lysozyme for the most part and to the common antibacterial agents; there is a similarity to the type of bacterial flagella when these organelles are present and the types of cell wall resemble those of bacteria. The hyphal diameters are much smaller than those of fungi and are close to those of the bacteria.

Genera in actinomycetes consist of varied groups of bacteria whose common feature is the formation of hyphae at some stage of development. This tendency is tenuous and often requires imagination to believe in it. In some species of the genus *Mycobacterium* hyphae are never seen, whereas other species have incipient hyphae only in young cultures. An even worse situation exists in the various genera of the Actinomycetaceae; these form microcolonies which have very transitory filaments that are extremely difficult to see and the dominant forms are diphtheroid, bifid or even rod shaped. Hyphal development is more pronounced in the nocardias but they, too, fragment. The time at which this occurs varies with the species and it determines the types of colony which form. Colonies in which the filaments break up early tend to be soft or mucoid whereas those whose filaments break up after a few days have time for a branched mycelium to form and develop a leathery texture: they later fragment to bacillary or coccoid elements. In the Streptomycetaceae, filament development is strong and fragmentation is rarely found. Examples are in the *Streptomyces, Micromonospora* and *Actinoplanes* spp.

Reproduction is usually asexual, though sexual processes have been shown to occur by genetic analyses. In the nonhyphal forms, asexual reproduction is by fragmentation or perhaps even by the usual fission of single cells. Where stable hyphae are produced, vegetative reproduction is by well formed spores resembling fungal arthrospores, borne either free or in sporangia, as in the Actinoplanaceae. The free spores are in the form of sporophores and may consist of 1, 2 or many spored chains arising singly or verticillate from primary hyphae. The long chains are straight, looped or spiral. Except for the Actinomycetaceae, which contain aerobic or micro-aerophilic genera, the actinomycetes are generally aerobic.

2. Habitat

Members of the actinomycetes which live a saprophytic existence can be found with greater or less frequency in most ecological settings, soil having the greatest population density. Numerically they are less dominant than the other bacteria and more prominent than the fungi. The numbers vary a great deal but it is not uncommon in fertile soils from many different parts of the world to find -5×10^6 propagules/g of soil (Taber, 1960; Gottlieb, unpublished). They occur in greatest numbers in the top few inches of the soil and decrease with depth (Waksman & Purvis, 1932). The sensitivity of *Streptomyces* spp. to acids at pH 5·0 or less precludes high populations in such soils (Taber, 1960). Similarly, the writer has not found them in soils from the Arctic tundra of northern Sweden or in soils of the Great Salt Desert of the U.S.A. High densities of spores can also be found on potato or sweet potato that have been infected by pathogenic *Streptomyces* spp. *Streptomyces* spp. are also common on most plant parts and, being dustborne, can be isolated from almost all artifacts and natural materials (Grein & Meyer, 1958). They are found in marine littoral and associated with *Micromonospora* spp., especially in fresh water lakes and in bottom muds. By their nature thermophilic forms, are found commonly in composts of manures. Other types are favoured by particular oxygen environments; *Actinomyces israeli*, for example, is anaerobic, whilst a number of other species are micro-aerophilic.

The streptomycetes have 3 important roles in the soil. One is their function in decomposing the organic matter of the soil (Waksman & Lomanitz, 1925;

Reynolds, 1954; Williams, 1966). Another is their effect on soil structure in binding clay particles by the hyphal threads to impart a granular viable structure that is conducive to crop production. The actinomycetes are undoubtedly responsible in part for the earthy odour of soil (Gerber & Lechevalier, 1965).

3. Soil Competition

The third interesting role of actinomycetes in soil is their ability to produce antibiotics which are inimical to other soil microbes. This is on the assumption that antibiotics are produced in normal untreated soils in the same way as they are produced in special laboratory media. This results in an intriguing hypothesis to explain some of the competitive effects in soil, resulting in the increase of some species and loss of other species among the microbial components of the soil. Competition certainly occurs between organisms in soil, but it is difficult to demonstrate that the actinomycetes produce in untreated soils antibiotics at levels high enough to inhibit other soil organisms. There are data to show that actinomycetes, especially members of the genus *Streptomyces,* can produce antibiotics in some sterilized soils, with or without added metabolites, and even in nonsterile soils, but the question remains: which of these conditions represents the natural state of the soil? Two main considerations are (1) natural soils do not have the necessary metabolites in concentrations necessary to stimulate and allow antibiotic production and (2) the necessary metabolites are normal in soil and are part of the natural life cycles in soils. An added difficulty is that of assessing the 'clinical' level of antibiotic concentration in soil.

A few examples clarify the situation. The addition of *S. griseus* and *Bacillus subtilis* to normal or treated sterile soil resulted in a decrease in the population of the *Bacillus* but there was no detectable amount of streptomycin. Moreover if streptomycin had been produced it would have been adsorbed by the negatively charged clay component of the soil to render it inactive (Siminoff & Gottlieb, 1951). On the other hand *S. venezuelae* produces chloromycetin, a neutral molecule. This actinomycete produced the antibiotic in trace amounts in sterile, but not in nonsterile soil, and the amounts were increased by adding green alfalfa hay or tryptone to the soil (Gottlieb & Siminoff, 1952). The antibiotic was not found in field plots that had been inoculated with the streptomycete nor in any of the 91 soils that were collected in field surveys, including areas from which the organism had originally been isolated (Ehrlich, Anderson, Coffey & Gottlieb, 1952, 1953). Actidione, another neutral antibiotic produced by *S. griseus* gave similar results (Gottlieb, Siminoff & Martin, 1952). The results of studies with *S. rimosus* and *S. aureofaciens,* which produce the amphoteric compounds terramycin and aureomycin, respectively, are different (Martin & Gottlieb, 1952). The actinomycetes inhibited *B. polymyxa* in soil even though relatively high concentrations of the antibiotics did not. In this case the inactivation or removal from soil of the antibiotics was presumably due to the pH value of the soil.

There appears to be no correlation between the ability of an organism to inhibit the growth of other organisms in soil and its ability to produce an antibiotic that inhibits them. As a part of one of our studies, 15 actinomycetes were isolated that produced an antibiotic which inhibited *Rhizoctonia solani* and 15 others that did not. The actinomycetes were separately added to sterile soil 2 weeks before, at the same time as, and 2 weeks after, the *Rhizoctonia.* After 1 month in pots the relative populations of *Rhizoctonia* in the soils were determined by the numbers of radish and pea seedlings that did not emerge or were damped off, and there were no significant differences between groups. Some isolates that produced antibiotics antagonized the fungus, whereas others did not. Similarly some that did not produce antibiotics inhibited the test fungus whereas others did not.

4. Antibiotics

It is because of their ability to produce antibiotics that the actinomycetes are perhaps best known. Except for a few that are produced by fungi, e.g. penicillin and cephalosporin, and a few that are produced by bacteria, e.g. bacitracin and polymyxin, all other antibiotics that are medically useful and have a wide application are synthesized by actinomycetes. A compilation of the microbial sources of antibiotics discovered in the U.S.A. and Japan between 1953 and 1970 reveal that *c.* 85% are produced by actinomycetes, 11% by fungi and 4·5% by bacteria. These differences may not reflect the actual proclivities of the different groups to produce antibiotics for similar ratios might be obtained if more effort was spent on the last groups. Yet the decision, if it were so made, to spend most time on the actinomycetes is in my experience the result of the fact that the actinomycetes have shown the greatest potential for producing new and useful antibiotics. The exact number of antibiotics that are known is difficult to even estimate. About 2700 substances have been reported but I would estimate that *c.* 50% of them are so poorly characterized that their uniqueness is suspect. The most commonly reported new antibiotics are those with antibacterial activity, the proportion in Japan being 48% and in the U.S.A. 56%; next are antifungal agents, in Japan, 32% and in the U.S.A. 24%. Antitumour antiviral and antiprotozoal antibiotics comprise the remainder. These figures probably reflect not so much the innate ability of microbes to produce such agents but the amount of research effort that has been placed in the search for antibiotics of different therapeutic abilities.

Another means of determining the importance of antibiotic production is by the amount and value of the product that is produced. With these data one can get into ethical difficulties such as the value of saving one life against the value of an entire industry. Putting aside the ethical aspect, such figures indicate the amount of effort that is being spent in antibiotic production. The total value of antibiotics produced throughout the world has been estimated to be $>10^9$ dollars/year. For the U.S.A. alone, the total production of units which make >100,000 lb of antibiotics/year was 13,199,000 lb valued at $115,555,000. It is interesting that when the figures are broken down into medical and nonmedical uses, the amounts used for the different purposes are roughly the same. On the other hand, if calculated on a dollar value, the portion that is used for medical purposes is >3 times that for nonmedical purposes.

There is a belief current that actinomycetes have already been well screened for antibiotic production and that few if any additional ones of therapeutic value remain to be found. Consequently, one hears constant rumours that a number of institutions are cutting back on their actinomycete screening programmes. Certainly very few new materials of therapeutic value are being released by the industries engaged in the search, but examination of the number of new antibiotics that have been reported in the literature from 1953 to 1969 showed no definite downward trend, the number of new antibiotics reported for each year ranged from 34 to 63. In 1971 the figure dropped to 12 which may reflect either the greater difficulty in finding new materials or the lower effort being expended in this field.

5. Species Pathogenic to Man

Among the Actinomycetales are species that are pathogenic to man or animals. It is interesting that this is the same Order that contains species that produce agents active against other bacterial and fungal diseases. Only brief mention is made of the pathogens here since they are discussed in more detail in other papers in the symposium. The genus *Mycobacterium* contains many species that are pathogenic to man or animals, among which *Myco. tuberculosis* is perhaps the best known. Here is one of the rare examples in which 2 members of an order are in direct conflict with each other. Streptomycin is produced by *S. griseus* and the antibiotic in turn is used to control the growth of the mycobacterium and plays a large part in the therapeutic regimen for the disease. The family Nocardiaceae also contains a number of presumed species that are especially prevalent or pathogenic in the tropical regions. Among them are *N. asteroides, N. brasiliensis, N. madurae, N. pelletieri* and *N. paraguayensis* (Conant *et al.*, 1954). The Actinomycetaceae have 2 pathogenic species, *A. bovis*, causing lumpy jaw of cattle, and *A. israeli*, inciting a similar disease in humans (Thompson, 1950). Despite the large number of species in the Streptomycetaceae only one organism has been shown to be pathogenic, *S. galtieri*, to guinea pigs and rabbits; other species have also been isolated from diseased tissue but their pathogenicity has not been proved (Breed *et al.*, 1957).

Another area of medical concern is the ability of some thermophilic actinomycetes to produce allergic respiratory disorders. They proliferate on natural products which heat during storage and shipment, and then affect the handlers of these products to cause farmer's lung and bagassosis (Ehrlich *et al.*, 1953; Gregory & Lacey, 1963; Lacey, 1971).

6. Plant Diseases

This aspect is discussed in detail by Lapwood in this volume (p. 253). A few streptomycetes are important as agents producing plant diseases. *Streptomyces scabies* produces the common scab of potato and *S. ipomoea* the scab of sweet potato (Walker, 1952). Both diseases can be controlled by taking advantage of the susceptibility of these organisms to pH values. Sulphur is usually added to soil to bring the pH value down to 5·0 or less, thus preventing the development of the pathogen (Gillespie, 1918). Another method is to turn green manures into the soil. Apparently, this increases the numbers of other organisms in the soil to the disadvantage of the *Streptomyces* (Millard & Taylor, 1927).

The ability of the streptomycetes to produce antibiotics is also important in plant disease control. Though many antibiotics have been shown to be effective in the control of different diseases under experimental conditions only few are used in agricultural practice. Perhaps most widely used is streptomycin applied as a spray to prevent fireblight of apples or pears caused by *Erwinia amylovora.* Cycloheximide is used as an application for diseases of turf such as dollar spot, helminthosporium leaf disease and powdery mildew grass. Also important is the use of blastocidin S for the control of rice blast caused by the fungus *Piricularia oryzae*.

7. Animal Nutrition

Another area in which the production of the secondary metabolites by actinomycetes has proved important is their incorporation into animal feeds (Lucky, 1959). This is done by adding either the mycelium from a fermentation or a crude preparation of the antibiotic to a standard feed. The primary effect is to increase the rate of growth in animals such as poultry, pigs and cattle; it does not affect the final weight of the animal. Concentrations of 0·1–25 p/m of antibiotic are commonly used. Among the compounds that have been used in feeds are streptomycin, the tetracyclines and chloramphenicol. Antifungal agents are less commonly used. These effects are not a property of actinomycete-produced materials alone for penicillins, which are produced by fungi, also have similar growth promoting effects.

The nature of the growth-promoting mechanism is still uncertain. The concept now held is that the antibiotics control low levels of infection in the