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*STRAIN SPECIFICITY AND PRODUCTION OF ANTIBIOTIC SUBSTANCES. VII. PRODUCTION OF ACTINOMYCIN BY DIFFERENT ACTINOMYCETES**, †

BY SELMAN A. WAKSMAN, WALTON B. GEIGER AND DONALD M. REYNOLDS

NEW JERSEY AGRICULTURAL EXPERIMENT STATION, RUTGERS UNIVERSITY

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The ability of organisms belonging to different species or even different genera to produce the same antibiotic has been definitely established for the fungi. This is true of penicillin production by different species of *Penicillium* and *Aspergillus;* of clavacin, produced by a variety of species belonging to these two as well as other genera; of citrinin; and of other antibiotics. In the case of spore-forming bacteria, which received considerable attention during the last few years, the problem is more difficult because of the greater complexity of the substances produced. It is difficult to say as yet whether some of the products described in the literature, such as subtilin and bacitracin, are chemical individuals or mixtures. Likewise it is difficult to say whether such mixtures may contain one or more of the recognizably pure substances such as gramicidin, tyrocidine, or gramicidin S along with other active and inactive polypeptides; tyrothricin, for example, has been shown to contain gramicidin and tyrocidine, along with other less well-defined substances.

The identity of an antibiotic produced by different species of actinomycetes is complicated by the difficulty of recognizing distinct species. It is believed, however, that the data presented in this paper tend to suggest that different species, or at least what may be considered as such, may produce the same type of antibiotic. The differences in yield and especially in the impurities accompanying the particular antibiotic tend to emphasize further the differences in species, or at least in strain specificity.

In 1940, the isolation from the soil of an actinomyces that had strong antibiotic properties was reported.[1] This substance was designated as actinomycin and the organism described as *Actinomyces* (*Streptomyces*) *antibioticus*.[2] Since then some 10,000 cultures of actinomycetes have been isolated from soils, composts, and other substrates, and tested for anti-

biotic properties, and in only two other instances was the production of this antibiotic definitely established.

The production of actinomycin can be detected by its antibiotic spectrum, by its characteristic pigmentation, and by its chemical properties, since it can be isolated from the medium and crystallized.[3] Its antibacterial properties comprise a very high activity against gram-positive bacteria and rather low activity against gram-negative organisms.

In a search for substances possessing activity against viruses,[4] a culture of actinomyces was obtained and found to produce the typical red substance described originally as actinomycin A. The new culture (S-4) resembled *S. antibioticus* in some of its morphological and cultural properties, although it was not identical with it. It produced on synthetic media, for example, a deep black zone in the aerial mycelium. The exact significance of this zone has not yet been determined.

TABLE 1

BACTERIOSTATIC ACTION OF THREE ACTINOMYCIN PREPARATIONS

CRYSTALLINE ANTIBIOTIC	BACTERIOSTATIC ACTION BY AGAR DILUTION METHOD, DILUTION UNITS PER GRAM		
	ACTINOMYCIN FROM CULTURE S-4	ACTINOMYCIN A FROM *S. antibioticus*	ACTINOMYCIN FROM 36-G
E. coli	<10,000	<5,000	<30,000
A. aerogenes	<10,000	<10,000	<30,000
S. aureus	>20,000,000	>20,000,000	>3,000,000
B. subtilis	>20,000,000	>20,000,000	>3,000,000
B. mycoides	>20,000,000	>20,000,000	>3,000,000
S. lutea	>100,000,000	>100,000,000	.....
	Bacteriostatic action by cup method, diameter of zone in mm.		
0.1 mg./ml.	31.4	31.6	
0.01 mg./ml.	27.2	27.5	

By following the original procedure[3] for isolating actinomycin A, a crystalline red solid was obtained which had a melting point of 252°C. and which gave no depression in the melting point when mixed with an authentic specimen of actinomycin A. The antibiotic spectra of the two substances and the quantitative concentration of pure actinomycin, as measured by the cup method against *B. subtilis*, were found to be identical, as shown in table 1.

In the course of isolation of the actinomycin from S-4, some material soluble in petroleum ether was also obtained. This fraction was previously designated actinomycin B; it was a mobile yellow oil and was produced by either strain. The bacteriostatic spectra of the two fractions (after repurification) were also similar, both giving 20,000 to 60,000 units per gram, against gram-positive bacteria. Since activity of this magnitude could result from an admixture of inert material with as little as 0.5 per cent of

actinomycin A, it is probable that the activity of this fraction is due entirely to some contamination with actinomycin A. Since even this limited activity gave exactly the same type of spectrum as actinomycin A, the second fraction may, therefore, be considered as an impurity of the latter. Because of this and in order to avoid future confusion, it is proposed to abandon the term "actinomycin B" and to change the name "actinomycin A" to "actinomycin."

The amounts of actinomycin produced by the newly isolated S-4 strain as well as by the original *S. antibioticus* 3435 strain, grown both in shaken and in stationary cultures, were then investigated. Starch tryptone medium was used, with 0.25 per cent agar for stationary cultures. The results, summarized in table 2, show that strain S-4 produces nearly 10 times as much actinomycin as the original *S. antibioticus*. The substance produced by S-4 can also be isolated more readily. The actinomycin produced by the two strains differed also in another respect: the substance formed by

TABLE 2

PRODUCTION OF ACTINOMYCIN BY 3 DIFFERENT CULTURES OF STREPTOMYCES

STRAIN S-4		*S. antibioticus* 3435		STRAIN 36-G
SHAKEN	STATIONARY	SHAKEN	STATIONARY	STATIONARY
Activity of culture filtrate, dilution units (*B. subtilis*)				
3,000	3,000	200	300	300
Yield of isolated crude actinomycin, milligrams per liter				
200	170	114	100	66
Total activity of crude actinomycin produced, dilution units				
1,200,000	1,000,000	140,000	120,000	250,000

S-4 was readily crystallized from acetone-ether mixtures after the removal of the B-fraction, whereas that produced by *S. antibioticus* could not be crystallized until after chromatographic separation because of the presence of tarry impurities.

In the course of this work, three methods of extraction of the actinomycin were used: (1) extraction of cultures with ether in stationary flasks; (2) continuous extraction with ether; (3) extraction with ethyl acetate. Methods 2 and 3 proved to be about equally satisfactory and were superior to method 1 on the basis of completeness of removal of the substances from the culture filtrate. The nature of the product seemed to be independent of the method of extraction.

More recently, another culture was isolated which produced actinomycin when grown on a glucose-tryptone medium, both in stationary soft agar cultures and in a submerged state. This culture, 36-G, was isolated from soil. It was markedly different from both *S. antibioticus* and S-4. It was non-chromogenic and did not form the typical sporulating aerial mycelium

characteristic of *S. antibioticus*. The straight conidiophores were arranged irregularly on the aerial mycelium. The yield of actinomycin (66 mg. per liter) given by this culture was less than that of the other 2 cultures. The activity of the culture filtrate (300 *B. subtilis* units per ml.) was similar to that of *S. antibioticus*. The antibiotic spectrum of the culture filtrate and of the crystalline product was that typical of actinomycin, as shown in the tables.

Summary.—Actinomycin is produced by different species of the genus *Streptomyces*. The yield and purity of the antibiotic depend upon the nature of the culture. One organism yielded about 10 times as much actinomycin as the original *S. antibioticus*. Another culture gave a lower yield than *S. antibioticus*, but a purer product was obtained. The nature and activity of the second fraction accompanying the actinomycin, namely actinomycin B, also varied for the different cultures; however, its antibiotic spectrum was similar to that of actinomycin. Because of the insignificant yields of the B fraction, and because of the suggestion that its activity is due to traces of actinomycin A present as impurities, it is proposed to abandon the name of "actinomycin B" and to change the name of "actinomycin A" to "actinomycin."

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GROWTH REQUIREMENTS OF VIRUS-RESISTANT MUTANTS OF ESCHERICHIA COLI STRAIN "B"

BY E. H. ANDERSON*

DEPARTMENT OF BIOLOGY, VANDERBILT UNIVERSITY

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The isolation of a number of virus-resistant mutants from a virus-sensitive strain of *Escherichia coli* (strain *B*) has been described in a previous publication.[1] The mutant strains were obtained by using, as selective