BIOCHEMICAL STUDIES ON SOME LOCALLY ISOLATED STRAINS OF ACTINOMYCETES

A THESIS

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By

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This thesis has not been submitted for a degree at this or any other University.

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ABBREVIATIONS USED

AS	Ain Shams
SHK	Shibin El Kanater
B. subtilis	*Bacillus subtilis*
S.	*Streptomyces*
U.V.	Ultraviolet light
I.R.	Infrared light
r.p.m.	Round per minute
m.p.	Melting point
b.p.	Boiling point
LD_{50}	Lethal dose killing 50 % of the number of test animal
M.I.C.	Minimum inhibitory concentration

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P R E F A C E

A research project was started more than a decade to isolate actinomycetes from the soil of A.R.E. and other Arabian countries. The isolated organisms were then subjected to a screening program to appraise their antagonistic properties against microbial growth. Subsequently, trials to isolate the antimicrobial agents and to determine their physicochemical characteristics as well as biological properties were conducted.

Among the various workers engaged in this project the present author could isolate 192 pure Streptomyces ultures from soil samples of "Shibin El Kanater". The potentialities of these isolates as antibiotic producers were examined. Streptomyces AS-SHK-15 showed the most notable antagonistic properties against Gram-positive bacteria and Gram-negative bacteria.

Streptomyces AS-N-7a which produce the antibiotic AS-N-7a and S. AS-SHK-15 showed great similarity to each other. The two antibiotics produced by Streptomyces AS-SHK-15 and Streptomyces AS-N-7a were rather identical in their physical, chemical and biological properties [illegible] the author considered them the same.

Culture studies were conducted to examine the effects exerted by changes of incubation periods, different concentrations of the medium constituents, supplementation of some natural products and by-products on the antibiotic production. These trials could elevate the yield of the antibiotic AS-SHK-15 from 16.5 mg/100 ml. to 44.6 mg/100 ml.

PART I

INTRODUCTION

MATERIALS AND METHODS

Part I

SECTION A

INTRODUCTION

I N T R O D U C T I O N

The discovery of penicillin by Fleming in 1929 initiated an era of unusually rapid advancement in searching for new antibiotics. At present, about 3000 antibiotics are known, of which 2300 are produced by microorganisms, and their number is continually increasing (1). According to Kurylowicry in 1966 (2), less than 1 % of known antibiotics are produced by lichens, about 2 % by animal tissues, about 6 % by fungi of the classes Ascomycetes and Basidiomycetes, 10 % by fungi imperfecti and more than 10 % are produced by true bacteria, while actinomycetes are responsible for the production of about 56 % of the known antibiotics.

Vuillemin (3) was the first to use the term antibiosis in 1889 to describe a type of association in which one living creature was destroying another one inorder to sustain its own life. Papacostas and Gate (4) restricted the significance of the word by differentiating the in vitro injurious effect of one organism upon another, a type of association called "Antibiosis". The same effect when occurring in vivo is referred to as "antagonism".

Subsequently, the term antibiotic was introduced by Waksman (5) in 1942 to designate a chemical substance of microbial origin which has the property to inhibit the growth of microorganisms,"bacteriostatic". Waksman (6) in 1947 added that an antibiotic might also destroy bacteria and other microorganisms, i.e. bactericidal. Benedict and Langlykhe (7) modified the definition to comprise substances which act upon certain organisms at least in very dilute solutions. Mascherpa (8) proposed the following difinition, "Antibiotics are substances spontaneously produced by living organisms (or synthetically obtained) but possessing analogous structure of that of natural products endowed with selective antibacterial action through antimetabolic mechanism".

Umezawa (9) suggested the inclusion among antibiotics not only of substances of microbial origin but also of those produced by higher forms of life, as well as those of anti-tumour activity. The definition thus becomes:
"Antibiotics are chemical substances that are produced by living organisms and can inhibit or destroy the growth of microorganisms or other living cells in highly diluted solutions".

The first comprehensive survey concerning the occurrence of antagonistic forms among actinomycetes was carried out in Russia (10) during 1937. Out of 80 cultures of actinomycetes isolated and tested, 47 were able to exert antagonistic effects but only 27 produced active substances.

Welsch (11) in 1939, isolated a chemical substance called actinomycetin from a Streptomyces culture. This substance showed interesting antibacterial properties, but it was too toxic for therapeutic use. Waksman and Woodruff (12) in 1940, isolated actinomycin from a culture of Actinomyces antibioticus. This active substance was found highly toxic to animals and thus did not offer any chemotherapeutic potentialities (13).

The first antibiotic that appeared to show distinct promise as a chemotherapeutic agent was streptothricin isolated by Waksman and Woodruff (14). This was followed by the isolation of numerous antibiotics which were found to have extensive medical applications in control of bacterial and fungal diseases as well as certain forms of cancer cells (15-19).

Numerous trials were devoted by different workers to isolate antagonistic cultures of actinomycetes especially of the genus Streptomyces. Routien and Finlay (20) screened thousands of soil samples from widely scattered geographical areas. They found that some organisms which produce certain antibiotics were extremely common. Actinomycetes elaborating streptomycin, streptothricin, chloramphenicol, actinomycin, and xanthomycin-like antibiotics have a world-wide distribution. The tetracycline producing cultures are more scarce. Similar results were recorded by other investigators (21-25).

Microorganisms with antimicrobial activities varied in number from one soil to another. Pope and Strutry (22) found that 25 % of the isolated cultures, secreted anti-bacterial metabolites. Gaveri et al., (24) observed that half of the isolates had the capacity to produce anti-microbial agents on solid media, while the number decreased to one-third when grown in shaken cultures.

Of the 1,879 actinomycetes that were isolated by Tarikova et al., (25) from the soil of the Soviet Union, 1,262 proved promising as antibiotic producers.