BIOCHEMICAL STUDIES ON NEW ANTIBIOTICS

THESIS

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

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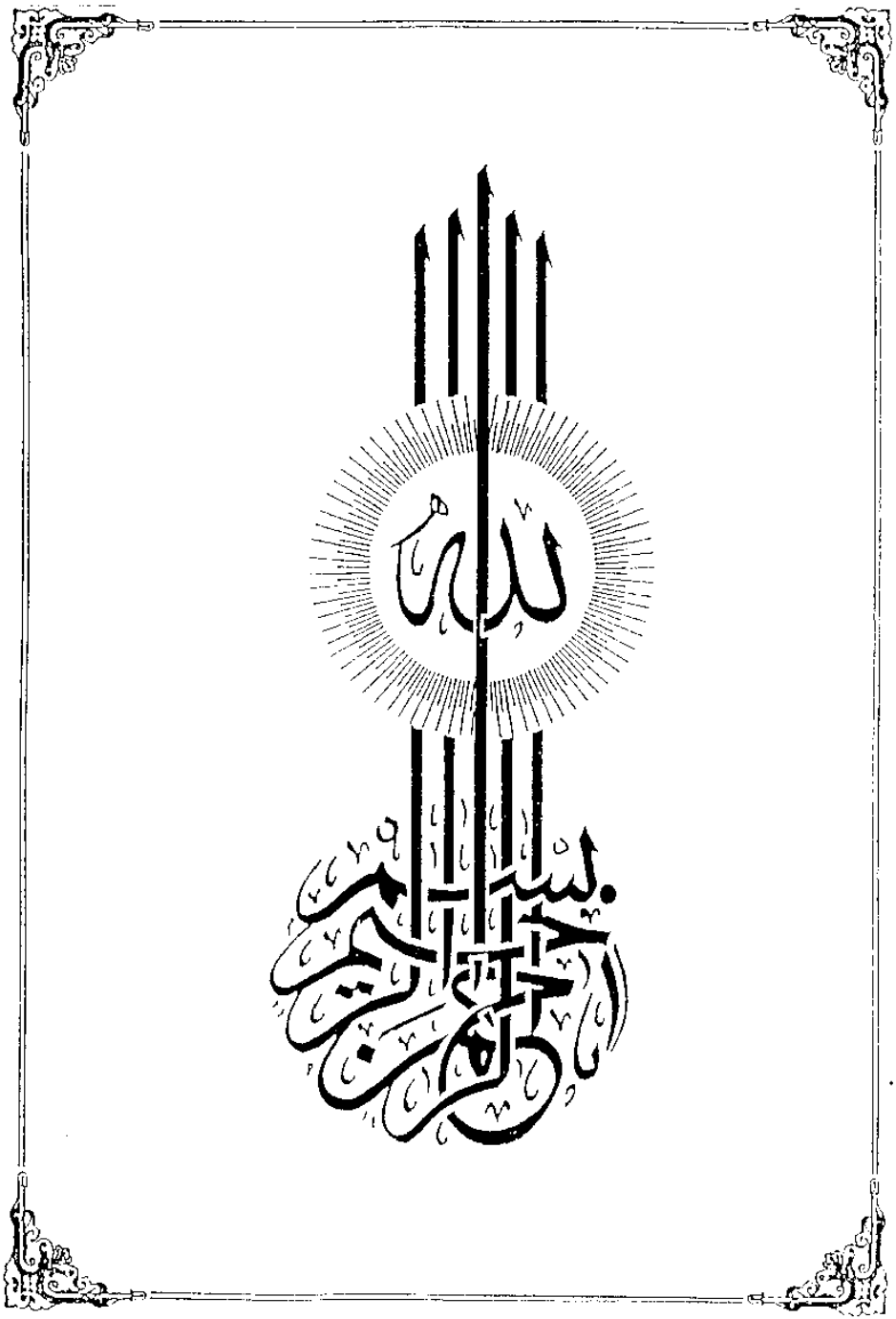

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ABBREVIATIONS USED

AS	Ain Shams University.
S.	*Streptomyces.*
r. p. m.	round per minute
B.subtilis	*Bacillus subtilis*
U.V.	Ultraviolet
IR.	Infrared
T.L.C.	Thin layer chromatography.
M.I.C.	Minimum inhibitory concentration
Log.	Logarithm.
V.	Volume.
PPt.	Precipitate
O.D.	Optical density.
L.	Litre
DNA	Deoxyribonucleic acid.
RNA	Ribonucleic acid.
m-RNA	Messenger ribonucleic acid.
r-RNA	Ribosomal ribonucleic acid.
t-RNA	Transfer ribonucleic acid.

C O N T E N T S

P R E F A C E

A research project was started more than a decade to isolate actinomycetes from the soil of Egypt 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 physico-chemical characteristics as well as biological properties were conducted. The following new antibiotics were isolated in our laboratory polychlorosubtilin, gluconimycin, ferramido chloromycin (FACM), polyketoacidomycin (PKAM), negabillin, AS-K-753, hodydamycin, yemenimycin, kuwaitimycin, nilamycin and cairomycin B.

Among the various workers engaged in this project the present author could isolate nearly 500 pure Streptomyces cultures from soil samples of "Egypt Arab Republic". The potentialities of these isolates as antibiotic producers were examined. Out of this collection fifty isolates which possessed the highest antagonistic properties were selected and were given different numbers from 101 to 200. Morphological, biochemical and culture characteristics for isolate

No. 133 which proved to be the most potent were studied. Then the antibiotic produced by this isolate was separated and purified. On chemical analysis the antibiotic was found to contain C, H and N. On acid hydrolysis, the antibiotic splitted into 3 identified amino acids.

Next, culture studies were conducted to examine the effects exerted by changes of incubation periods and different concentrations of the medium constituents on the antibiotic production. These trials could elevate the yield of the antibiotic from 3 to 5 mg/100ml·of the medium.

Moreover, the mechanism by which AS-M-133 inhibited the growth of Bacillus subtilis cells was examined.

PART I

INTRODUCTION

MATERIALS AND METHODS

Part I

SECTION A

INTRODUCTION

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

Before the discovery of penicillin by Fleming, L. Pasteur[1] was the first to observe antagonistic interrelations between microorganisms. He recorded the rapid death of anthrax bacteria in mixed cultures with putrefying bacteria and characterized this phenomenon as a struggle for existence.

Gosio[2] in 1896 mentioned that antibacterial substances could be produced by various microorganisms. He isolated mycophenolic acid from Penicillium brevi-compactum which inhibited Bacillus anthracis but it was too toxic for use as a therapeutic agent.

The discovery of penicillin by Fleming in 1929 initiated an era of unusually rapid advances in the studies searching for new antibiotics. Since then, more than 3000 new antimicrobial agents could be recorded. Actinomycetes were responsible for the production of nearly 500 compounds and preparations that possess antimicrobial properties. Among these, about 50 compounds have proved to possess therapeutic potentialities.[3]

Waksman and Woodruff[4] in 1940 isolated the first pure crystalline antibiotic from soil actinomycetes and gave it the name actinomycin, they also isolated in 1942 a new antibiotic which was named streptothricin[4]. Waksman reported that actinomycin was a toxic substance for animals and did not offer, therefore, any chemotherapeutic potentialities, but streptothricin was the first substance that appeared to show distinct promise as a therapeutic agent.

Schatz[5] et al. in 1944 isolated streptomycin, which exhibited antibiotic activity against Gram-positive and Gram-negative bacteria. The group of tetracycline antibiotics[6-8] were then discovered and isolated in pure form. During the same time several other antibiotics were isolated from bacteria, actinomycetes, fungi and from higher plants as well[9].

Vuillemin[10] 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 in order to sustain its own life. Papacostas and Gate[11] limited the meaning 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 occuring

in vivo is referred to as "antagonism". Subsequently, the term antibiotic was introduced by Waksman[12] in 1942 to designate a chemical substance of microbial origin which has the property to inhibit the growth of microorganisms; "bacteriostatic." Waksman[13] in 1947 added that an antibiotic might also destroy bacteria and other microorganisms, i.e. bactericidal. Benedict and Langlykhe[14] modified the definition to comprise substances which act upon certain organisms at least in very dilute solutions. Maschera[15] proposed the following definition, "Antibiotics are substances spontaneously produced by living organisms (or synthetically obtained) but having analogous structure to that of natural products endowed with selective antibacterial action through antimetabolic mechanism". Umezawa[16] 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 antitumour activity. The definition thus becomes "Antibiotics are chemical substances that are produced by living organisms and that have the capacity to inhibit or destroy the growth of microorganisms or other living cells in highly diluted solutions".

Numerous trials were devoted by different workers to isolate antagonistic cultures of actinomycetes especially