# GENETICAL STUDIES ON SOME ANTIBIOTICS PRODUCING STREPTOMYCES

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## THESIS

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# I. INTRODUCTION

Streptomycetes are known to be producers of many valuable biologically active substances. Species of this genus showed the ability to produce numerous antibiotics, many of these substances found wide pharmaceutical and clinical applications. Preparations as streptomycin, oleandomycin, tetracyclines, refamycins etc., saved the life of millions of human beings. Antibiotics have various other nonmedical applications.

Because of the great value of these substances, laboratories in many countries carryout screening programs for the isolation of producers of new valuable antibiotics from different natural substrates as soils, composts, water, air etc. The soils of Egypt insufficiently studied in this aspectes.

The subject of this thesis is to study a group of 113 isolates of Streptomyces belonging to the Griseus group which is suitable for genetic studies. It has a short life cycle and a great diversity of inherited biochemical and morphological dissimilarities which can be induced by various mutagens. The 113 studied isolates were collected from different soils of Egypt. The present work deals with the morphological, cultural and antimicrobial characteristics of isolates. The taxonomic identification of these cultures is also concerned. The antimicrobial potentialities of antibiotic producing isolates are tested as affected by different nutritional conditions. Therefore it was designated through these investigations to determine the effect of UV-irradiation on the induction of mutations in the highest wild strain of this group in producing streptomycin. It was also intended to correlate between each of the obtained mutants, and its antibiotic production, and to elucidate the possibility of the applied genetical procedures on antibiotic production of these mutants.

## II. REVIEW OF LITERATURE

### Historical background

Microorganisms living in natural substrates exhibit different associations which may be positive or negative. In the positive ones, the growth of certain microorganisms is enhanced or stimulated by the growth of the adjacent microbial colonies. This effect may be due to the production of stimulating substances such as amino acids, vitamins, or any other chemical compounds of vital importance. Such relationships may be mutual, i.e., adjacent colonies exchange necessary metabolities and this type of relationships is called symbiotic. In the negative relationships the growth of neighbouring microorganisms is inhibited or delayed by the metabolites produced by adjacent microorganisms. This effect may be due to the production of antimicrobial substances which may be antibiotics, toxins, acids or any other antimetabolites. This type of relationships is called antagonism. Different types of microbial antagonism is discussed in detail in the review of Waksman (1949). The most important type of antagonism is that which is due to the formation of antibiotic substances. One of the glorious achievements of science in the present century is the discovery of antibiotics. These substances have saved the lives of millions of peoples whose life could be in danger if antibiotics were not available as pharmaceutical preparations.

Antibiotics have been defined as chemical substances of microbial origin Waksman (1959). Later these substances were found to be produced by higher plants and animals. Thus the antibiotic allicin was extracted from Allium sativium; rafanin from Raphanus sativus, and crepin from Crepis traxcifolia (Gause. et al)(1957).The antibiotic erythrin was obtained from the liver of rabbits (Konikova et al. 1944); and ecmolin from fish tissues (Ermolova et al.,1943). However the main producers of antibiotics are still microorganisms. According to Kurylowicz (1967) less than 1% of the known antibiotics are produced by higher plants, 2% by animals and 97% by microorganisms.

Of the antibiotics produced by microorganisms 10% are produced by fungi, 20% by bacteria, while the majority of these substances are produced by Streptomycetes.

Scientists were able to extract from the culture filtrate of various species of this genus more than 2700 antibiotics of which more than 40 substances had found clinical applications. (Gottlieb 1973).

Such interesting ability of this genus led many scientists in different laboratories to carry out screening programs for the isolation of producers of new antibiotics or obtaining active producers of known valuable antibiotics.

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This great value of the genus Streptomyces induced numerous studies which gave valuable informations on the biology of species belonging to this genus.

A - Biological Characteristics of the genus Streptomyces

This genus represents a large and heterogenous groups of microorganisms comprising numerous species (Waksman 1961). The first acquantance of man with Actinomycetes dates back to the year 1875 when Cohn described a branching filamentous organism which he found in the tear duct of a human eye.

With the start of the 20th century Streptomycetes were found to occur in the soils, compost and many other natural substrates (Waksman 1916; Krainsky 1914; and Krassilnikov, 1938). The types of Streptomycetes in soil are greatly affected both quantitatively and qualitatively by the physical and chemical properties of soil, temperature, manures and plant cover (Waksman, 1959).

The abundance and distribution of Streptomycetes in soil of Egypt was studied by Krassilnikov & Hussein (1965) and Naguib et al., (1973).

1- Morphological, physiological and cultural properties

a- Morphological characteristics

The spores of Streptomyces species when transfered to a fresh medium germinate after 8-12 hours, giving one or more germ tubes. The latter grow in length to give a net like filamentous branching mycelium. The hyphae of the mycelium are of two types, one prostrate forming substrate mycelium, and the other erect forming aerial mycelium. The spores are formed inside special hyphae of the aerial mycelium. These spore-bearing hyphe differ greatly in their form. Some are straight, long or short, others are curved or spiral in shape with various degrees of curvature. The number of whorls or turns of the spiral sporophores may be from 1 to 20 (Pridham et al., 1958) and (Waksman, 1959). The spores are produced inside the spore bearing-hyphe in chains, a detailed study of the nature of spores and spore formation in the genus Streptomyces had been made by Jensen (1930) and more recently by Flaig, et al. (1952) and Vernon (1955). The spores may be spherical, oval, or cylindrical. The shape, size, and colour of spores are characteristics of the species. Kriss et al., (1945) were the first to use electron microscope for the study of spores of Streptomyces species. These were followed by the work of many authors and these studies showed that the surface of Streptomyces spores may be smooth, spiny, hairy or wurty, (Flaig and Kutzner, 1958; Grein, 1955; and Ettlinger *et al.*, 1958 a,b).

b- Cultural characteristics

Streptomyces species form on the surface of agar media colonies which are usually covered with aerial mycelium; this may be long or short. Short aerial mycelium gives the colony powdery appearance, while long gives velvety or cottony appearance. The majority of species of the genus *Streptomyces* produce coloured aerial mycelium. This colouration ranges from white, yellow, red, rose, lavender, gray, blue, green, brown or black (Waksman, 1959; Pridham *et al.*, 1958). Many species produce coloured substrate mycelium. The colouring substances may be water soluble, diffusible into the agar colouring it in corresponding shades, or water insoluble, retained in the cells.

The colour of growth and production of pigments are of great significance in the characterization of species of the genus *Streptomyces*.

Since streptomyces species vary greatly in their nutritional requirements, media which are suitable for the growth of a group of species may be unsuitable for other species. Different sets of media were suggested for culture descriptions. (Krassilnikov 1968; Gause *et al.*, 1957;

Pridham et al., 1957 and Waksman, 1959). Kudrina et al., 1964 carried a comparative study of the suitability of 5 nutrient media for the description of cultural properties of 496 cultures. They came to the conclusion that the applied media were nonequivalent for these studies and the most favourable media were Gause No.1 (Starch-nitrate agar) and Krassilnikov $SR_1$ agar (glucose-nitrate agar).

c- Physiological characteristics

The species of the genus Streptomyces represent a fairly heterogenous system, differing greatly in their physiological and biochemical activities. These differences together with other characters are used for the determination of species of this genus (Waksman, 1919; Liesk , 1921 and Krassilnikov, 1938). The formation of melanin pigments is characteristic of many Streptomyces species. These pigments are often produced on proteinaceous media, however few species produce such pigments on synthetic media (Krassilnikov, 1970). Some authors tend to use this reaction as an important criterion in species differentiation (Waksman, 1959; Krassilnikov, 1960; Gause et al., 1957 and Hütter, 1962). The ability of some species of Actinomycetes to produce $H_2S$ on iron-peptone media was also suggested for description or characterization (Tresner and Danga, 1958).

2- Taxonomic identification of Streptomyces

With the recognition of Streptomyces as producers of antibiotic, intensive efforts have been made for isolating new antibiotic. This trials resulted in the accumulation of thousands of isolates and the claim of hundreds of newly described species.

Hesseltine et al. (1954) suggested for species differentiation, the study of the morphology of sporophores, colour of sporulating aerial mycelium; some cultural and physiological properties.

Gause et al., (1957) divided the genus Streptomyces into a number of series on the basis of the colour of aerial mycelium. Waksman (1961) divided this genus into 15 series on the basis of several characters such as the colour of aerial and substrate mycelium, morphology of sporophores, melanin formation and relation to temperature.

Krassilnikov (1970) divided this genus into two big sections : (a) with pigmented substrate mycelium, (b) with non-pigmented substrate mycelium. Sections were further subdivided into series according to the colouration of the substrate and aerial mycelia. Species were further characterized by the colour of aerial mycelium, morphology of sporophore and spore surface, physiological and antagonistic properties were greatly considered in species characterization. In a trial to solve the problem of the diversity of criteria and technique applied in the taxonomy of species more than 40 collaborating laboratories representing 18 nations were joined in an international effort to assemble and redescribe authentic type strains or new type strain for the named species in the genera Streptomyces and Streptoverticillium. Uniform descriptive criteria and standarized methods and media were developed under the supervision of the Subcommittee on Taxonomy of Actinomycetes of the Society of Microbiology and the Subcommittee on Bacteriological Nomenclature (U.S.A.). In our studies taxonomical work was carried out according to these methods.

i- Griseus series of Streptomyces

One of the most abundant series of the genus Streptomyces is the Griseus series. One species of this series "Streptomyces griseus" offered mankind a highly valuable and unreplacable antibiotic "Streptomycin". Probably no other drug in the history of medical science has had such a phenomenal rise as Streptomycin. The griseus series is characterized by certain morphological and cultural properties that make possible its differentiation from other series : (a) Sporophores are straight produced in tufts, spores are oval with smooth surface.