STUDIES ON THE BIOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL ACTIVITIES OF VIOLET STREPTOMYCES STRAINS ISOLATED FROM EGYPTIAN SOILS

A Thesis Submitted for the
Degree of Philosophy Doctor of Science

By

AMIRA AHMED ABDEL RAHMAN EL-GAMMAL
Assistant Research Worker
Laboratory of Chemistry of Microorganisms

National Research Centre
Cairo, Egypt

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C O N T E N T S

PART VI

TAXONOMICAL STUDIES

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A C K N O W L E D G E M E N T

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P R E F A C E

Streptomycetes are known to be producers of many valuable biologically active substances. More than 2000 antibiotics have been isolated from streptomycetes and a good number of these antibiotics have found wide pharmaceutical and clinical application. Species of this group of microorganisms produce different enzymes, pigments, vitamins, growth promoting substances and toxins.

The search for producers of new antibiotics led to the isolation and description of many new species of the genus Streptomyces. Certain interest was directed towards the study of pigmented isolates and species.

The subject of this thesis is the study of a group of 53 isolates of violet streptomycetes isolated from Egyptian soils. The study deals with the morphological, cultural, physiological and antimicrobial characteristics of this group. Taxonomical studies of the cultures and identification of their pigments are included.

PART I

INTRODUCTION

Historical Backgrounds

The actinomycetes are generally recognized to represent a large and heterogenous group of microorganisms comprising several families, genera and numerous species (Krassilnikov, 1970; Waksman, 1959). 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. Later many reports were made on the isolation of similar organisms from infected tissues of animals and man (Harz, 1878; Israel, 1879, 1884).

With the start of the 20th century actinomycetes were found to occur in the soil,and several cultures were isolated from soil, compost and many other natural substrates (Globig, 1888; Beijerinck, 1900; Waksman, 1916; Krainsky, 1914; Krassilnikov, 1938). The abundance and distribution of actinomycetes in Egyptian soils was studied by Krassilnikov and Hussein 1965 ; Naguib, Hussein and Zahran, 1973.

Waksman et al.(1943) created the genus Streptomyces to separate certain aerobic saprophytic aerial mycelium producing actinomycetes from the rest of the genera of the order Actinomycetales. Starting from the year 1940 microbiologists succeeded to observe that Actinomycetes and especially species

of the genus Streptomyces are capable of producing extremely valuable biologically active substances of which the most important are antibiotics, enzymes, vitamins, growth stimulators, pigments, toxins etc., (Krassilnikov, 1938;Waksman, 1959).

A review of the available literature including biological characteristics, biochemical activities, as well as taxonomy and identification of species of the genus streptomyces is given in the following pages.

I. BIOLOGICAL CHARACTERISTICS OF SPECIES OF THE GENUS STREPTOMYCES

1) Morphological Characteristics :

The spores of species of the genus Streptomyces when transferred 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 this mycelium are of two types, one prostrate forming substrate mycelium, the other erect forming aerial mycelium. The sporophores of streptomyces differ greatly in their structure. Some are straight, long or short, others are curved or spiral with various degrees of curvature. The number of whorls or turns of the spiral sporophores may be from 1 to 20. The branching of the sporophores is commonly monopodial , however certain species show typical verticillate type of branching of sporophores (Krassilnikov, 1970; Pridham, Hesseltine and Benedict, 1958; Waksman, 1959).

A detailed study of the nature of the spores and spore formation in the genus Streptomyces had been made by Jensen (1930) and more recently by flaig, Beutelspacher, Kästner, and Segler-Holzweissig (1952), Flaig and Kutzner (1954), Vernon (1955). The spores may be spherical , oval or cylindrical. The shape, size and colour of spores are characteristic of the species. Kriss, Rudina and Isachenko

[illegible], were the first to use the electron microscope in the study of the spores of Streptomyces species. Those studies were followed by the work of Carvajal (1946); Bringman (1951), Flaig et al. (1952, 1955) and Flaig and Kutzner (1958), Baldacci and Grein (1955), Grein (1955), Vernon (1955), Corbaz, et al. (1957), Ettlinger et al. (1958 a;b), Enghusen (1955), Nikitina (1956); Preobrajenckaya, Kudrina, Maksimova, Sveshnikova and Bioarkaya (1960). These studies showed that the surface of Streptomyces spores may be smooth, spiny, denty, warty or covered with long or short hairs.

2 2) Cultural Characteristics :

According to Henrici (1947), Streptomyces species form surface colonies on solid media that are almost cartilagenous in consistency and adhere strongly to the substratum. The colonies are usually covered with aerial mycelium which may be long or short.

The majority of species of the genus Streptomyces produce coloured aerial mycelium. The colouration ranges from white, grey, yellow, orange, red, rose, lavender, blue-green, brown and black. Many species produce coloured substrate mycelium. Colouration is usually due to the formation of one or more coloured substances which are called

pigments. Such substances may be water insoluble-endocellular-colouring only the cells, or water soluble-exocellular-colouring both cells and media (Krassilnikov, 1968).

It is impossible to find a universal medium which would suffice the requirements of all Streptomyces species as they vary greatly in their nutritional requirements. Consequently media which are suitable for a group of species may be unsuitable for the growth of other species.

Different sets of media were suggested for culture descriptions (Krassilnikov, 1968; Gauze et al., 1957; Pridham, Anderson, Foley, Lindenfelser, Hesseltine and Benedict, 1957; Waksman, 1959). The lack of universal set of media led to the accumulation of uncomparable descriptions of cultures and species which, in turn led to great confusion in the taxonomy of Streptomyces species. Kudrina et al (1964) carried a comparative study of the suitability of 8 nutrient media for the description of cultural properties of 496 Streptomyces cultures. They came to the conclusion that the applied media were non-equivalent for cultural studies , and the most favourable media were Gauze No. 1 agar and Krassilnikov SNI agar.

3) Physiological Characteristics

Species of the genus Streptomyces represent a fairly

heterogeneous system differing greatly in their physiological and biochemical activities. These differences are used together with other characters - for the determination of the species of the genus (Waksman, 1919; Lieske, 1921 ; Krassilnikov, 1938).

The property of Streptomycetes to hydrolyse proteins was mentioned by early investigators (Nadson, 1903; Münter, 1913; Waksman, 1919; Lieske, 1921; Krassilnikov, 1938). The ability of some species of Streptomycetes to undergo certain chemical changes while growing on milk-media (coagulation, peptonization, pH changes, or formation of colours) or on gelatin media (liquefaction , formation of pigments) were used in the identification of Streptomyces species (Waksman, 1916; Lieske, 1921; Jensen, 1930; Krassilnikov, 1938; Stapp, 1953; Gordon & Smith, 1955; Kutzner, 1956).

Some species of Streptomycetes can reduce nitrate to nitrites and this property was used also in species identification (Krainsky , 1914; Münter, 1913; Waksman, 1919 ; Lieske, 1921; Krassilnikov, 1938).

The property of some species of Streptomycetes to grow on paper strips decomposing its cellulose fibers was suggested as a criterion in species differentiation (Krainsky, 1914; Pousek, 1912; Waksman, 1919; Lieske, 1921; Krassilnikov, 1938).

The formation of melanin pigments is characteristic of many Streptomyces species. These pigments are often produced on proteinaceous media, however few species produced such pigments on synthetic media (Krassilnikov, 1970).Some authors tend to use this reaction as an important criterion in species differentiation (Waksman, 1959; Krassilnikov, 1962; Gauze et al., 1957; Hütter, 1962).

The ability of some species of actinomycetes to produce H_2S, on iron-peptone media was also suggested for species description and characterization (Tresner and Danga, 1958).

4) Antagonistic Characteristics

Actinomycetes as all other microorganisms are found in natural substrates in mixed populations and among these populations numerous associations and antagonism occur. The antagonistic properties of actinomycetes against bacteria , yeasts and fungi were reported by many early investigators (Gaspirini, 1890; Müller, 1908; Gratia and Dath, 1924; Rosenthal, 1925).

The results of the study of the antagonistic properties of microorganisms depend greatly on two factors : a) medium constitution, and b) type of test organisms (Krassilnikov, 1970). Certain species may fail to show inhibitory effect when cultivated on synthetic media but they succeed to do so on a