TAXONOMIC STUDY OF FAMILY MICROMONOSPORACEAE

THESIS

Submitted for the Degree of

DOCTOR OF PHILOSOPHY

in

BOTANY

By

NAGWA AHMED ABD-ALLA

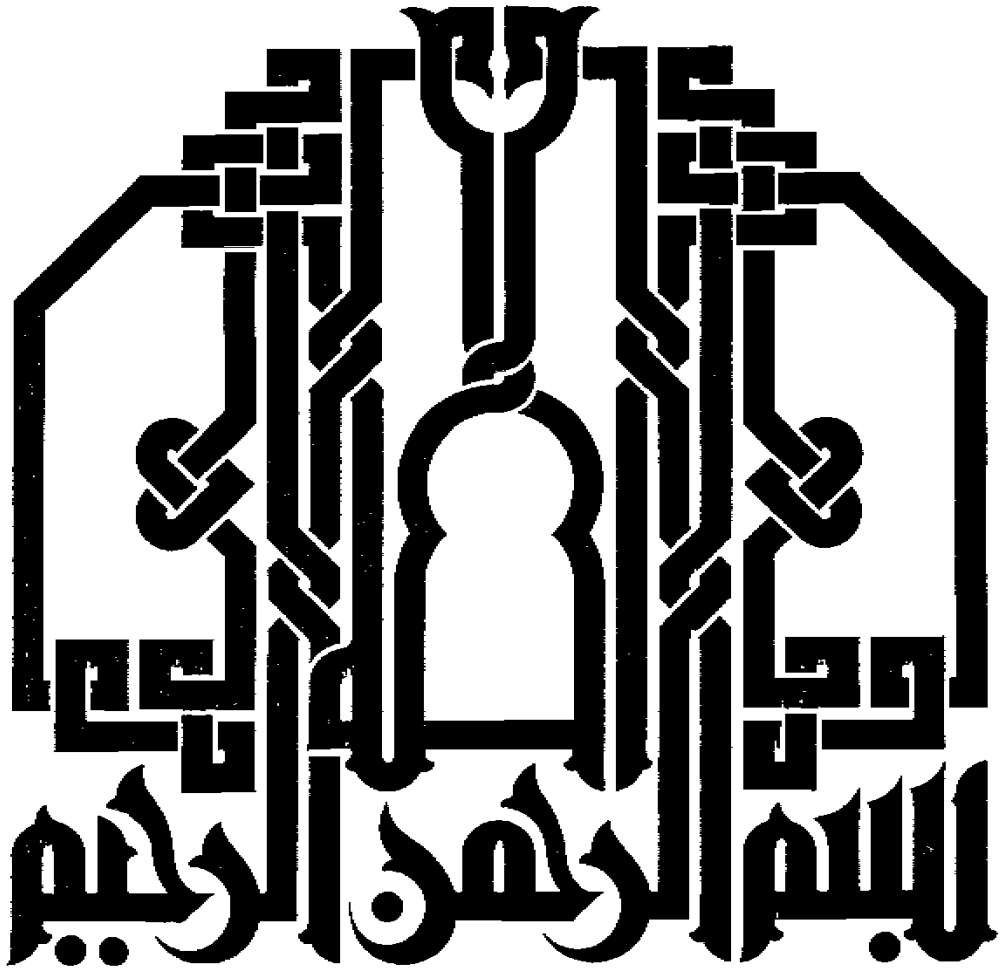
(B. Sc. & M. Sc.)

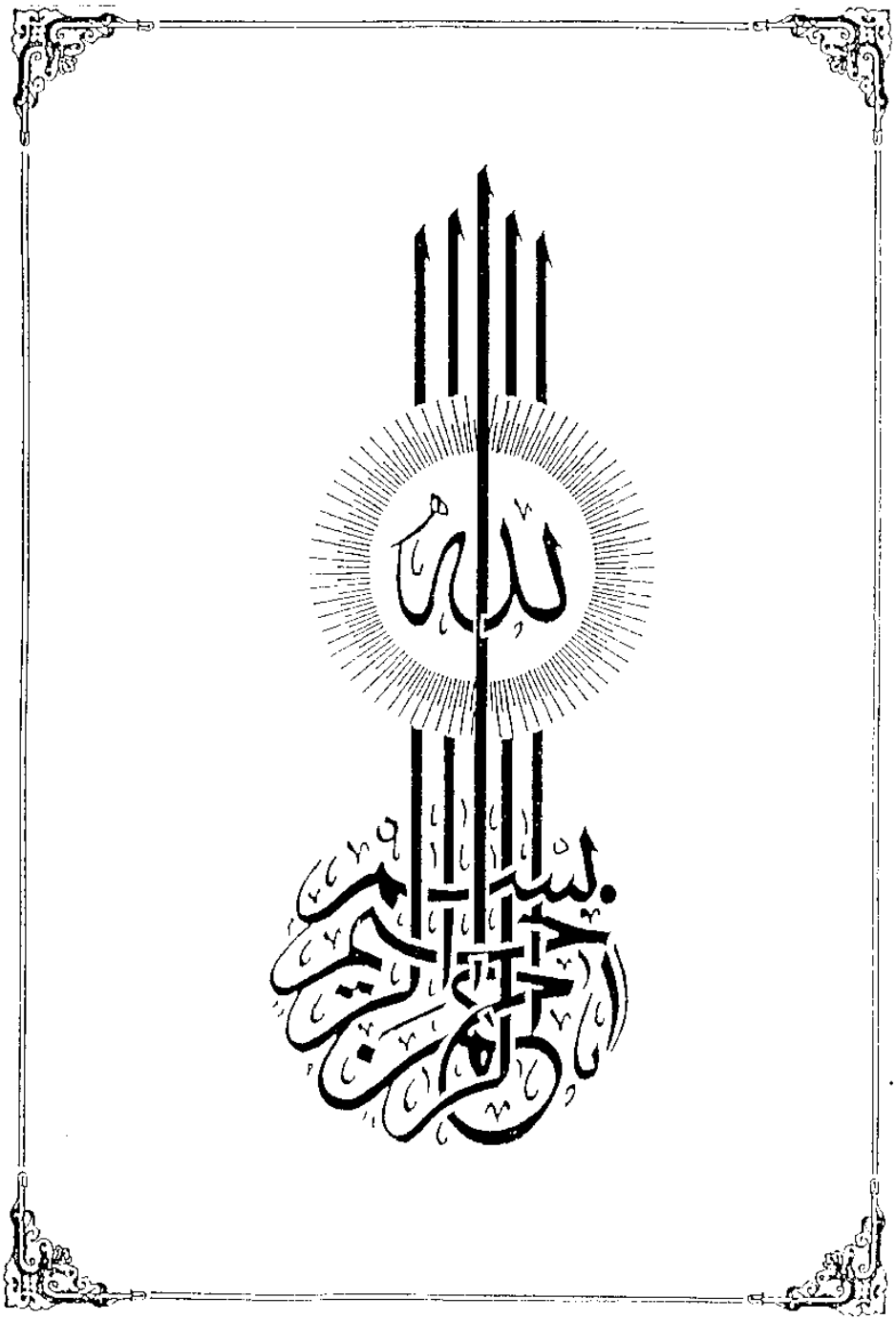
Ain Shams University

Faculty of Science

Botany Department

1989





ACKNOWLEDGEMENT

The author wishes to express her thanks and gratitude to Prof. Ahmed Dewedar, Vice presedent, Suze Canal University for suggesting and supervising this work.

Sincere thanks are due to Prof. A.M. Shaaban for his help and advice. The members of Bacteriology laboratory at Suze-Canal University are greatly aknowledged for their help.

My thanks are due to Prof. A.S.Al-Nowaihi, Head of Botany Department, Faculty of Science, Ain Shams University for his encouragement and kind asistance.

This

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

The literature cited shows how far I have availed my self of the work of the others.

Nagwa Ahmed Abd-Alla

CONTENTS

Page

Part III

INTRODUCTION

INTRODUCTION

The exestance of the actinomycetes has been recognized for over a hundred years. For much of this time they were regarded as an exotic group of organisms with affinities to both bacteria and fungi. However determination of their fine structure and chemical composition confirmed their prokaryotic nature. They now constitute the order Actinomycetales and their removal from the mycologist's sphere of influence has been completed (Buchanan, 1917).

The study of actinomycetes was initiated in the late 19th century by workers examining diseased material from humans, animals, or plants. Cohn (1875) first description of an actinomycete was based upon his study of an organism found in concretions of the lachrymal ducts and which he named Streptothrix foersteri. Shortly after this, an organism seen in a specimen of "Lumpy jaw" of cattle was described as Actinomyces bovis (Harz, 1877). Other observation of actinomycete- like microbes associated with human or animal infections soon followed, but their taxonomy and pathogenicity were confused due to the lack of pure cultures. Subsequently, actinomycetes have proved to be causal agents of many human and animal infections (Nocard, 1888).

One of the first truly saprophytic actinomycetes to be detected, was Streptothrix chromogena which was isolated from soil by Beijerinck (1900). The widespread occurrence of

actinomycetes in soil was demonstrated by Krainsky (1914) and Waksman and Curtis (1916, 1918). Over the next 20 years, knowledge of the ecology of actinomycetes in soil, composts and other habitates was considerably extended.

Still up till now the taxonomic possition of most genera of micromonosporous actinomycetes is not settled. Bassing on spore formation Jensen (1931) classified actinomycetes into two families, possessing the first identified monosporous actinomycetes, Micromonospora, in family Actinomycetaceae and the second family was Proactinomycetaceae.

Waksman and Umbreit (1940) proved the above system and divided actinomycetes into four families depending on mycelial formation and number of spores. They classified Micromonospora among family Micromonosporaceae. Considerable confusion was created when Waksman and Co-workers included all actinomycetes with single spores in the genus Micromonospora.

Krassilnikov (1941) also included the monosporic actinomycetes forming aerial mycelium in genus Micromonospora so ignoring the initial descriptions of Thermoactinomyces by Tsiklinsky (1899), and Micromonospora Orskov (1923)

Waksman and Corke (1953) proposed to reintroduce the generic name Thermoactinomyces to include the thermophilic forms that show growth at 50°C and above. The majority are also

able to grow at lower temperatures, down to 37°C and in some cases 30°C and relatively few have a restricted growth range in the higher temperature.

Henssen (1957) proposed to include the monosporous actinomycetes among family Streptomycetaceae taking into consideration the thermophilic forms. He classified the genera, Micromonospora, Streptomyces, Thermomonospora, Thermopolyspora, Thermoactinomyces, and Pesudonocardia among family Streptomycetaceae depending on presence or absence of spores on aerial or substrate mycelium, number of spores, presence of septation on substrate mycelium as well as temperature limite.

Lechevalier et al. (1971) classified actinomycetes by using a combination of morphological, physiological and chemical criteria into eight families. According to their proposed system they classified the monosporous group among three families; family Micromonosporaceae, cell wall type II formed no sporangia to include genus: Micomonospora, family Thermoactinomycetaceae, cell wall type III formed no sporangia, to include the genera Thermoactinomyces and Microbispora and family Nocardiaceae, cell wall type IV, no mycolic acid but nocardiomycolic acid may present, to include the genera Thermomonospora and Micropolyspora.

Cross and Goodfellow (1973) classified the actinomycetes into ten families, where the monosporous group was classified into four families depending on presence or absence of aerial

mycelium, type of spores (heat sensitive or heat resistant endospores), length of sporophore and number of spores as well as wall chemotype. They were in coincedence with Lechevalier et al.(1971)in the classification of family Micromonosporaceae to include the single genus Micromonospora and family Nocardiaceae to include Micropolyspora. They proposed that family Thermoactinomycetaceae, originally enunciated by Baldacci and Locci (1966) and later modified by Lechevalier and Lechevalier (1970), be limited to those actinomycetes forming endospores, Thermoactinomyces. They also proposed family, Thermomonosporaceae to contain certain genera previously classified in the families Nocardiaceae and Thermoactinomycetaceae. They suggested this family to include the genera Actinomadura, Microbispora, Microtetraspora, Saccharomonospora, and Thermomonospora. Cross and Goodfellow considered family Thermomonosporaceae to be a family of convenience as it includes organisms with relatively few similarities.

Kuster (1974) in Bergey's Manual of Determinative Bacteriology proposed family Micromonosporaceae to include all the monosporic actinomycetes bassing on myclelial filaments remain intact, spores formed singly, in pairs or short chains on either or both aerial and substrate mycelium and cell wall Type (II, III or IV which varies with the genus). This family which originally proposed by Krasilnikov (1938)was monogeneric, later Krasilnikov (1964) added other genera. The genera, Micromonospora, Thermoactinomyces, Actinobifida, Thermomonospora, Microbispora and Micropolyspora, were included in Kuster system,

although they have few features in common, and further study may necessitate a change in the taxonomic position of one or more genera and several species (Kuster, 1974).

These suggested systems depended mainly on morphological, physiological and biochemical characteristics as well as wall chemotype. Some of these criteria were considered by some authers as generic criteria and by others as species or even family criteria, this explain the confusion found in classifying actinomycetes generally and monosporous actinomycetes specially . In the following pages we will review at these criteria.

1- Morphological characteristics

Until recently morphological characteristics were widely used in identification of actinomycetes. Actinomycetes growth is made up of branching filaments, producing a mycelium. This may be of two types, one prostrate, forming a vegetative growth, sometimes referred to as substrate mycelium; the other, erect or aerial mycelium. The nature of substrate mycelium growth represents one of the main characteristics of actinomycetes. This mycelial growth habit has been related to their ability to break down insoluble organic materials by extracellular enzymes (Chater and Merrick, 1979). Hyphal growth and branching determine the appearance of the mycelium (Prauser, 1976 , 1978). The production of an aerial mycelium from substrate

mycelium is influenced by the composition of the growth medium, the incubation temperature and the presence of specific stimulating compounds (Kalakoutskii and Agre , 1976;Williams et al., 1976; Chater and Merrick, 1979; Pogell, 1979). The two mycelia are different ontogenetically, morphologically, structurally and physiologically (Ensign, 1978). In general aerial growth appears to be less branched than the substrate mycelium (Higgins and Silvey, 1966). Aerial mycelium development usually ceases with the onset of sporulation, lytic processes also take place in ageing colonies and may play a role in spore liberation of some actinomycetes (Locci, 1971, 1976).

Among the stable morphological properties essential for purpose of characterization and classification, the structure and subsequent changes in the substrate mycelium and nature of aerial mycelium, the nature of the sporulating branches or sporophores, the size, the shape, and surface of the spores. These criteria are very important on order or even genus levels. Actinomycetes was classified among order Actinomycetales within kingdom Prokaryota into seven families, on morphological basis, as this order includes all prokaryotic organisms which form true branching hyphae (Prauser, 1976, 1978; Clark, 1979; Locci et al., 1982). All monosporous actinomycetes were classified under one family, family Micromonosporaceae. Morphological characters as presence or absence of aerial mycelium, number and position of spores as well as length and branching of sporophores

play an important role in seprating this family into genera (Kuster , 1974).

Similarities in the morphology of Thermomonospora and Thermoactinomyces strains, having a white aerial mycelium and single spore on both aerial and substrate mycelium increase the possibility of misidentification which is of a particular important problem (McCarthy and Cross , 1984) . Kuster and Locci (1963, 1964) noted the occurance of pairs of spores in strains of Thermomonospora viridis and of Thermoactinomyces vulgaris. Lechevalier and Lechevalier(1967) noted the observation of those strains that morphologically could be assigned to either the genus Micropolyspora or Microbispora.

There has been much confusion concerning the production of spores on the primary mycelium. The genera Thermoactinomyces and Thermomonospora may be taken as an example. Henssen (1957) did not mention the presence of spores on the primary mycleium in either genera. Kuster and Locci (1963, 1964) consider the production of spores on the primary mycleium as typical character of the genus Thermoactinomyces and lacking in strains of Thermomonospora.

The differentiation of genera on the basis of production of short or long chains of spores is simple in typical cases , but intermediate cases may cause problems (Kalakoutskii, 1964).