Ain Shams University
Faculty of Science
Botany Department

STUDIES ON CERTAIN NITROGEN-FIXING BACTERIA IN SOME EGYPTIAN SOILS

M.Sc. Thesis

Submitted by

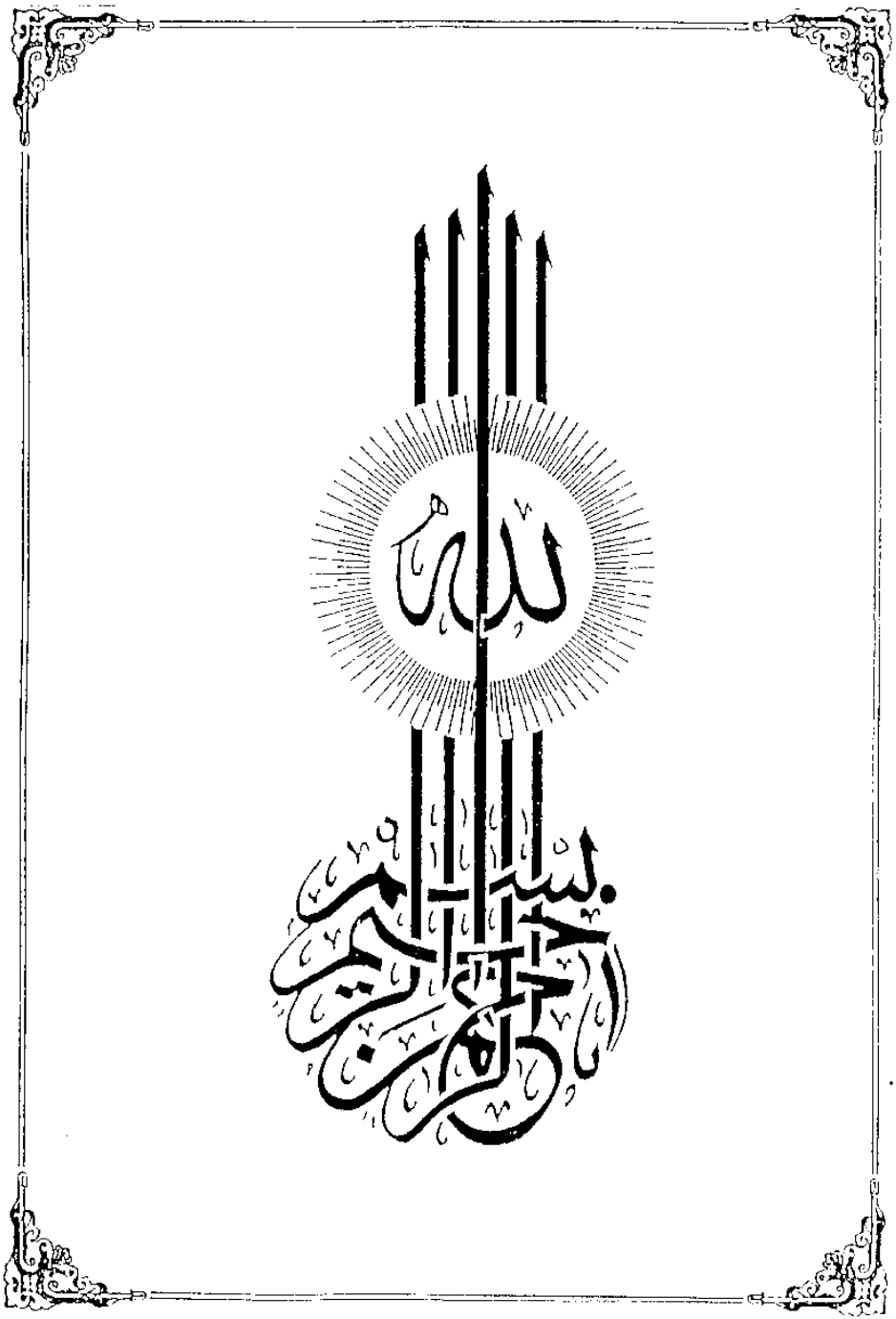
MOHAMMED MOHAMMED EL-HOSEINY
B. Sc.

1972

This thesis has not been previously submitted to this or any other University.

The references mentioned show how far I have availed myself to the work of other researchers.

M.M. El-Hoseiny



CONTENTS

A - General Preface

On the counting plates of Azotobacter chroococcum, small or large transparent mucoid bacterial colonies are usually encountered with a distribution frequency higher than that of Azotobacter (Sasson, 1960; Elsan et al.,1969) This group of bacteria which can develop in supposedly nitrogen-free agar media was suggested by Bouisset & Breuilland (1958), Fedorov & Kalininskaya (1959), Sasson (1960), and Mal'tseva & Izzheurova (1967) to form a general physiological group of "oligonitrophilic" microorganisms. The nitrogen-free agar medium might have contained very small amounts of combined nitrogen which probably contribute for their ability to develop. These bacteria probably belong to the group of weak fixers of atmospheric nitrogen which are reported by a number of investigators (e.g. Mishustina, 1953; Bouisset & Breuilland, 1958; Fedorov & Kalininskaya, 1959; Sasson, 1960; and Mal'tseva & Izzheurova, 1967).

The presence of such bacteria in various soils has already been repeatedly reported or cited by a number of workers during about 70 years (Beijerinck, 1900; Waksman, 1920; Omelyanskii, 1923; Lipman, 1938; Plotho, 1940; Panosyan, 1945; Krasil'nikov, 1945; Vinogradskii, 1952;

Therefore, this thesis was planned to study their distribution in various localities of Egyptian soils (Fig.1), in relation to soil physical & chemical characteristics, and their ability to fix atmospheric nitrogen. Attempts were also made to increase their potency of nitrogen fixation of certain amendments.

B - Introduction

Since the object of this thesis is the study of the aerobic non-Azotobacteriaceae group of soil bacteria which can fix atmospheric nitrogen (oligonitrophilic, according to Bouisset & Breuilland, 1958; Fedorov & Kalininskaya, 1959; Sasson, 1960; and Mal'tseva & Izzheurova, 1967), the review of the work on all nitrogen fixing microorganisms including algae, fungi, and Azotobacter family, seems not required in the present introduction. Accordingly, the present historical review will deal only with work on aerobic non-Azotobacteriaceae nitrogen fixing soil bacteria. This will be presented according to the following sequence :

1. Distribution and Identity
2. Nitrogen fixation.

1. Distribution and Identity :

Some authors (Bouisset & Breuilland, 1958; Fedorov & Kalininskaya, 1959; Sasson, 1960; and Mal'tseva & Izzheurova, 1967) have used the term "oligonitrophiles" to designate the bacteria which do not belong to Azotobacter & Clostridium and which can grow and develop on a medium containing a traces of combined nitrogen. Bouisset and

Breuilland (1958) have mentioned that "oligonitrophilic" bacteria of the soil are active in soil formation. This was evidenced by the benificial symbiosis with Azotobacter in agar slants or in solution cultures. These bacteria were found by these authors to be of millions per gram soil.

Numerous bacteria of this type were found by Fedorov & Kalininskaya (1959) among the microflora of sod-podzol soil.

Mal'tseva & Izzheurova (1967) have studied the distribution of oligonitrophiles in 4 types of the Ukrainian soils (Sodsly podzolic, grey forest podzolized loam, dark chestnut weak solonets, south slight humus chernozem), representing the main climatic zones of the republic. Oligonitrophilous microorganisms are widely distributed in the mentioned types of soils. Considerable quantities of them are found in soils without plants, in the rhizosphere and on the roots of winter wheat and maize. As a rule, quantity of oligonitrophiles in the rhizosphere is higher than in the soil without plants. These authors found that the distribution of "oligonitrophiles" in the Ukrainian soils depends on soil climatic conditions, plants and

their growth phase. Non-sporiferous bacteria predominate among the oligonitrophilous ones in both the soil and the zone of plant root system. In the rhizosphere and on the plant roots oligonitrophiles in most cases outnumber saprophytic microorganisms capable of growing on beef extract agar.

Elwan et al.,(1969) have studied the distribution of "oligonitrophiles" in various regions of Saudi Arabia Peninsula. They found that nearly all the localities studied contained these bacteria in numbers ranging from 100 to 2366 cells per gram oven dry soil. These bacteria were found less abundant than actinomycetes.

With regard to earlier reports on the occurrence of this group of bacteria, Fedorov & Kalininskaya(1959) have mentioned a number of investigators who reported on the presence of such bacteria in various soils(Beijerinck, 1900; Waksman, 1920; Omelyanskii, 1923; Lipman, 1938; Plotho, 1940; Panosyan, 1945; Krasil'nikov, 1945;Vinogradskii, 1952; Mishustina, 1953; Hamilton et al., 1953; Armi Kaila, 1954; Metcalfe et al., 1954; Anderson, 1955; Jensen, 1955; Fedorov & Ernandes, 1956; Fedorov & Kudryasheva,1956;and Fedorov & Kalininskaya, 1956).

Paul & Newton (1961) reported on the occurrence of aerobic, non-symbiotic, nitrogen fixing bacteria in samples of soil collected in the various soil zones of Alberta & Saskatchewan. They isolated smaller aerobic nitrogen fixing bacteria which developed as 1-3 mm circular, convex, unpigmented colonies, which were classified as Pseudomonas.

Florenzano & Materassi (1966) have studied the identities of 19 aerobic nitrogen fixing bacterial strains, not related to Azotobacter and hence may be called oligonitrophiles, isolated from acid forest soil. Their cells are straight or slightly curved rods, gram negative, asporogenous, and motile by polar flagella. All strains produce fluorescin. They utilize (in addition to atmospheric nitrogen), various organic and inorganic nitrogenous compounds. They are, according to these and other identities suggestive of being Pseudomonas fluorescens. However, since they fix nitrogen, and are also distinguished within the species by other biochemical characteristics, these authors suggested to segregate them into 2 new varieties, namely P. fluorescens var. indologenes & P. fluorescens var. Vallis umbrosae. These organisms

were found abundant in the soil microflora showing a remarkable adaptability to unfavourable conditions.

Mal'tseva & Izzheurova (1967) have related their studied "oligonitrophiles " to the species Bacillus megaterium, B. mycoides & B. subtilis.

Meiklejohn & Weir (1968) have reported on very numerous small transparent colonies on plates of nitrogen-poor agar (hence may be called oligonitrophiles) inoculated with soils from various places in Rhodesia. Eighty-three colonies were studied. All but 2 of them were small Gram-negative rods. Two colonies contained Gram positive cocci. The gram negative isolates are not all alike. Their identity is uncertain, but some might be Pseudomonas sp., some Achromobacter (or Acinetobacter), and one good fixer might be a Flavobacterium.

Abdel-Wahad (1969) found appreciable numbers of aerobic non-Azotobacteriaceae bacteria from sand dunes harbouring Ammophila arenaria. These bacteria belonged to the genus Bacillus and were able to fix nitrogen.

2. Nitrogen Fixation :

Aerobic bacteria-particularly soil ones, which do not belong to the family Azotobacteriaceae, and can fix

atmospheric nitrogen will be the subject of this section. These bacteria might be considered as "oligonitrophilic". This is because the supposedly nitrogen-free liquid medium might have scavenged an "oligo" amount of atmospheric ammonia which may act as "starter" in the process of cell proliferation, which might be further oriented towards proliferation through utilizing nitrogen instead of the combined atmospheric ammonia nitrogen. In other words, the nitrogen-activating enzyme (nitrogenase) might be synthesized after the exhaustion of the scavenged ammonia. However the term " oligonitrophilic" might be designated to indicate a weak minute desire of utilizing molecular nitrogen. In this sense the following non-Azotobacteriaceae bacteria, will be considered as "oligonitrophilic", such bacteria were isolated in pure form and identified as e.g. Bacterium azophile, which was claimed to fix 3-5mg nitrogen per 100 ml. manure extract liquid medium at 28°C (Flumer & Fred, 1916), Aerobacter aerogenes was confirmed to fix nitrogen by using $^{15}N_2$ technique (Hamilton et al., 1953; Hamilton & Wilson 1955 and Jensen, 1956). The strains of Hamilton and co-workers required strictly anaerobic conditions for nitrogen fixation, but the supply of oxygen had no influence on the growth and

nitrogen fixation of the two strains studied by Jensen. Similar results under anaerobic condition were also found by Pengra & Wilson (1958) whereas Johnstone and Pfeffer (1959) have reported results on fermentation of whey in aerobic conditions. Pseudomonas azotocolligans was found to fix nitrogen by using Kjeldahl method and was confirmed by $^{15}N_2$-technique (Anderson, 1955). Other Pseudomonas resembling Anderson's type in some respects was also described to fix nitrogen (Roy & Mukherjee, 1957). Proctor & Wilson (1958) have isolated six different species of Pseudomonas which all were confirmed to fix nitrogen. The nitrogen-fixing enzyme system seemed to be adaptive in these microorganisms (Virtanen & Meittinen,1963). Chloropseudomonas ethylicum, was also reported to fix nitrogen (Evans & Smith, 1971).

Other genera, mostly anaerobic bacteria have been shown to possess the power of nitrogen fixation, such as Achromobacter (Jensen,1958), which was isolated from water. Proctor & Wilson (1958) demonstrated aerobic nitrogen fixation in eight strains of Chromobacter, of these only Jensen's strains fixed nitrogen anaerobically. Bacillus hydrogenes (Belayaeva, 1954), and B. polymyxa (Hino & Wilson, 1958) which was reported to fix about 0.85-2 mg.

nitrogen per gram sugar consumed, was found to contain hydrogenase, and its nitrogen fixation is sensitive to molecular oxygen. 1% of oxygen significantly reduced nitrogen fixation, but its growth on nitrate nitrogen required oxygen (Hino & Wilson, 1958). Also Thermobacillus azotofigens (Ross, 1963), Bacillus circulans and Enterobacter aerogenes (Line & Loutit, 1971), Flavobacterium sp.(Meiklejohn & Weir, 1968) were found to fix nitrogen.

C - Materials and Methods

I. Soil Samples :

Soil samples were taken from different localities (Fig.1) and listed hereafter. For collecting soil from any region, the surface soil (up to 5 cm. depth) was firstly removed and about 2 kg. was collected from 3 spots at the same region, then mixed together and transferred to the laboratory in plastic bags, ready for analysis. In the laboratory the soil samples were dried in air, sieved through a sieve of 0.2 mm and stored in brown bottles for future chemical and microbiological analysis. Soils used in this investigation were collected from the following regions :

1. Mansourah: The soil was collected from a field cultivated with cotton at Bani-Ebaid, 29 km far from Mansourah in 21/7/1969.

2. Fayoum: This soil was obtained from pea-nut field, K 75, road in 25/8/1969.

3. Wadi-El-Natrun : Two types of soils were collected from Wadi-El-Natrun. Both from uncultivated areas, but they differ in their natural flora, and colour.