STUDIES ON THE DEVELOPMENT AND ACTIVITY OF CERTAIN MICROBES IN RELATION TO SALINITY

A THESIS

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BY
FATMA ABDEL-WAHAB HELEMISH
M. Sc. B. Sc.

Botany Department
University College for Women
Ain Shams University

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بسم الله الرحمن الرحيم

This thesis has not been submitted by me for a degree to this or to any other University. The literature mentioned hereafter shows how far I have availed myself of the work of the others.

Signed

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GENERAL PREFACE

Salinity in the soil and consumption of common salt by human and animal represent a particular physiological condition in which the living cell would find itself either resisting stress, initiating adaptation or full filling a "requirement". Although it is generally agreed that Na^+ is not required by most procaryotes, yet some strains of bacteria (e.g. Bacillus circulans) proved to "require" certain level of Na^+ (Elwan, Khodair and El-Hoseiny, 1975) to fix atmospheric nitrogen determined both by the conventional Kjeldahl and the acetylene reduction techniques. The requirement of Na^+ by eucaryotic cells is well known and recognized.

Azotobacter cells represent a good system with particular behaviour (molecular nitrogen fixation) which would help follow the response of the living cell to Na^+ under controlled conditions. Investigations made on the physiological response of Azotobacter cells to sodium chloride (e.g. Blinkov, 1955,1959 & 1963; Iswaran et al., 1966) have taken the ability to fix nitrogen as "criterion" for the "effect". The role of the "energy" source consumption, the growth factors, the Na^+ uptake, the ammonia-amino acid transformation were not taken in these investigations as criteria for elucidating the role of sodium chloride in the physiology of the cell. There were indications in certain experiments (Elwan, unpublished) that certain concentrations of the organic matter in the medium would nullify-in part - the harmful effect of sodium chloride in the cell. Certain organic "functional" substances (e.g. gibberellins and water soluble vitamins) that proved to be synthesized by Azotobacter (e.g. Vancura, 1961; Hennequin and Blachère, 1966; [illegible]asson, 1967; Elwan and El-Naggar, 1972) have exerted stimulatory effect on the process of nitrogen fixation (e.g. Okuda and Kobayashi, 1963; Elwan, Khodair and El-Hoseiny, 1971 and Elwan and El-Naggar, 1972).

Keeping in mind that "model systems" are the best way to get precise knowledge regarding the role of a "factor" in operating a "process" by a living cell, the work in this thesis has been suggested and planned to elucidate the role of NaCl in presence of gradient concentrations of sucrose, gibberellic acid and cyanocobalamin in operating the "process" of nitrogen fixation by Azotobacter chroococcum. Elucidations might be imperfect if one criterion was considered. Thus integrative determinations were taken as criteria viz total fixed nitrogen, ammonia content in culture and amino acids conjugated in synthesized cell protein.

This thesis will - therefore - deal with the role of NaCl in the process of nitrogen fixation by *Azotobacter* according to the following sequence.

I. The role of sodium chloride in the process of nitrogen fixation by *A. chroococcum* as manifested by total fixed nitrogen, in response to gradient concentrations of sucrose, gibberellic acid, and cyanocobalamin.

II. The role of salinity in the process of nitrogen fixation as manifested by the ammonia content in *A. chroococcum* culture, in response to gradient concentrations of sucrose, gibberellic acid and cyanocobalamin.

III. The role of salinity in the process of nitrogen fixation as manifested by the amino acids composition of cell-protein hydrolysate of *A. chroococcum*, in response to gradient concentrations of sucrose, gibberellic acid, and cyanocobalamin.

It will be seen that the integrative criteria and the "model systems" used proved adequate to reveal masked effects which would have both fundamental and practical importance.

INTRODUCTION

INTRODUCTION

The aim of this thesis was primarily to elucidate the physiological responses of <u>A. chroococcum</u>, n., to increasing concentrations of salinity as manifested by its ability to fix molecular nitrogen in view of Na^{+} and energy supplement uptake. Indications in previous work (Elwan, unpublished) gave favour to the role of organic matter in nullifying the deleterious effect on <u>Azotobacter</u> of relatively higher concentrations of sodium chloride. On the other hand, indoles vitamins amino acids, and gibberellic acid were found to contribute to a certain extent for the potency of <u>A. chroococcum</u> to fix atmospheric nitrogen (Elwan, Khodair and El-Hoseiny, 1971). It was felt that some of these additives (as functional organic substances) would nullify the harmful effect of NaCl. In this thesis, therefore, responses of <u>A. chroococcum</u> to salinity levels in culture in presence of a particular vitamin cyanocobalamin (B_{12}) and gibberellic acid (GA_3) were investigated in an endeavour to evaluate the potential introduction of the strain investigated in certain saline soils of soil reclamation programmes.

Keeping in mind, the aim mentioned above, this introduction will deal with the following:

a) Salt tolerant nitrogen fixing micro-organisms other than <u>Azotobacter</u>.

b) *Azotobacter* as a [illegible] nitrogen-fixing micro-organism.

c) Growth factors affecting *Azotobacter* growth.

a) Salt tolerant nitrogen-fixing micro-organisms other than *Azotobacter*:

Saline soils and sea-water represent the most abundant naturally occurring salty habitats. Estimations of global biological N_2-fixation (Hardy, 1972) indicate that 20% of the overall fixation of 175 x 10^6 metric tons per year takes place in the sea. These data for the oceans were based primarily on knowledge of the activity of blue-green algae in the marine environment (e.g. Allen, 1963; Dugdale *et al.*, 1965; Stewart, 1965 & 1967; Bunt *et al.*, 1970; and Goering & Parker, 1972).

Stewart (1962, 1963 & 1964) reported on the basis of long-term growth analysis that the two blue-green algae *Calothrix scopulorum* and *Nostoc entophytum* isolated from marine environments fix atmospheric nitrogen and liberate a proportion of the nitrogen fixed into the medium. Rate of fixation was rather faster in natural sea water than in artificial sea-water free from combined nitrogen. He observed that fixation rates were not markedly affected by large variations in salinity of the medium and *Calothrix* was more resistant to changes in salinity than was *Nostoc*

Moreover, N_2-fixing fresh water blue-green algae were found to tolerate high salinity levels. Allen (1956) reported that the fresh water blue-green algae *Anabaena cylindrica*, *Calothrix parietina* and various *Nostoc* species fixed nitrogen at salinities approaching those of natural sea-water. Fay and Fogg (1962) reported similar results for *Chlorogloea fritschii*.

Relatively, little data are available on the magnitude of bacterial N_2-fixation in the sea (Brown *et al.*, 1972). The first evidence of bacterial N_2-fixation in the marine environment in situ was obtained by Brooks *et al.* (1971) and by Sugahara *et al.* (1971) using the acetylene-reduction test. Sister and Zobell (1951) reported N_2-fixation by sulfate reducing bacteria, which are known to be widely distributed in the sea. N_2-fixing clostridia were also isolated from the sea (e.g. Hotchkiss, 1936; Pshenin, 1959). Werner *et al.* (1974) investigated the distribution of N_2-fixing bacteria in marine habitat and stated that N_2-fixing facultatively anaerobic bacteria may be quite common in the coastal environment. They