STUDIES ON CERTAIN HALOTOLERANT BACTERIA

Thesis

Submitted for the Degree of

Master of Science

(Microbiology)

By

Monira Mouhamed Rifky Khater

B.Sc.

Ain Shams Univeresity

Women's College

Botany Departement

Under the supervision of

Dr. Sohair A. Moustafa

Prof. of Microbiology

Women's College Ain Shams University

Dr. Sawsan M. El - Gammal

Prof. of Microbiology

Women's College Ain Shams University

1993

Board of Scientific Supervision

Professor ***Dr. Sohair A. Moustafa***

Professor of Microbiology, Botany Department,

Women's College Ain Shams University Egypt .

Professor ***Dr . Sawsan M. El- Gammal***

Professor of Microbiology, Botany Department,

Women's College Ain Shams University Egypt .

S.M. El-Gammal

16.3.1993

بسم الله الرحمن الرحيم

This thesis has not been Previously submitted for a degree at this or any other university

signed

Monira Mohamed Rifky

ACKNOWLEDGEMENT

I would like to express my deepest gratitude and thanks to Prof. Dr. Sohair A. Moustafa Professor of Microbiology, University College for Women. Ain Shams University for suggesting the problem of this study, also for her encouragement, valuable advice, and her interest in this work.

I would like to declare my deepest thanks and sincere gratitude to Prof. Dr. Sawsan M. El-Gammal Professor of Microbiology, University College for women. Ain Shams University, for her supervision and valuable suggestions.

Many thanks are presented to the Head of the Botany Dept. Prof. Dr. Amal Shehab for her kind encouragement.

I would also like to thank all the members of Botany Dept. of University College for women, Ain Shams University for their cooperation and encouragement.

TABLE OF CONTENTS

II.MATERIALS AND METHODS

III.RESULTS AND DISCUSSION

PREFACE

An attempt to investigate the Gram-positive and Gram- negative halotolerant bacteria isolated from the gills of salted fish has been done. The halotolerant bacteria have been less studied than extremely and moderately halophilic bacteria. Though the halotolerant bacteria can grow in media containing no added NaCl and in media containing high NaCl concentration. Novitsky and Kushner (1975), Matheson et al. (1976) and Vreeland et al. (1980) have characterized halotolerant bacteria as that which has the ability to grow easily in a medium containing as litle as 0.045 M NaCl or as much as 5.5 M NaCl. This bacterium was considered as halotolerant bacteria. Such salt tolerant organisms present interesting questions regarding the physiological mechanisms and nutritional requirements behind adaptation to such a wide variety of NaCl concentrations. The purpose of this work was studying the effect of physical factors and some nutritional requirements on the salt tolerance of halotolerant isolates. Also a comparison between the behaviour of a Gram-positive and a Gram- negative halotolerant bacteria was also intended.

The halotolerant isolates grew in salted fish, so the proteolytic enzyme presumably occurs in these isolates. The isolate with a relatively high proteolytic activity was chosen to study the parameters which affect the proteolytic activity and its production by the organism.

REVIEW OF LITERATURE

1 1.HALOPHILIC BACTERIA

Water in living cells serves as a medium of interactions of small and large molecules between themselves and with each other. The solutes dissolve in cell water and the structure of this water control all the vital processes: enzyme action and regulation, assembly and disassembly of organells, membrane structure and function. Most microorganisms (as well as plant cells) living in high solute concentrations do not seem able to keep a much more dilute cytoplasm. Indeed, as pointed out by Brown (1964a, 1976) it would be very difficult for them to do so since this would involve impermeability to water or the continued active excretion of solutes. Non are known to maintain over all more dilute conditions within their cells. Microorgansims which can or must live in the presence of high salt orothersolutes are known to halophilic microorganisms, most of them are bacteria which constitute the most distinctive organisms in this group.

CLASSIFICATION:

The classification of the halophilic bacteria has been reviewed several times in the past, including Flannery (1956) who classified the halophilic bacteria into two main groups: obligate halophils that will grow in media containing more than 0.3MNaCl and facultative halophiles that grow in media containing less than 0.3MNaCl but grow best above this salt level. Obligate halophiles may be further classified as moderate, growing in the range from 0.5 to 3.5 MNaCl or as extreme, growing in the range from 3.0 M to saturated NaCl Kushner (1968).

The upper and lower salt tolerence limits of halophilic bacteria are considerably in excess of those for most other life forms. It has ben possible to construct a rough classification scheme for halophilic bacteria Larsen (1962) by utilizing the upper and lower limits of NaCl tolerence as guidelines Under this scheme one can find marine bacteria (slight halophiles) which grow best between 2.5% and 5% NaCl, moderate halophiles growing between 5% and 15% NaCl and extreme halophiles which grow in NaCl solutions of 15% or higher. The bacteria within these groupings have been extensively studied and have been found to be remarkably well adapted to their various lifestyles.

Some reports have appeared describing bacteria whose NaCl tolerence are considerably different from those listed above. Colwell et al (1979) described a number of new strains of Halobacterium cutirubrum which have the ability to grow in as little as 8% NaCl. Other authors; Novitsky and Kushner (1975), Matheson et al (1976), and Vreeland et al (1980) have chracterized bacteria that have the ability to grow easily in media containing as little as 0.045 Mor asmuch as 5.5 MNaCl (0.26%-32%w/v). The new bacterial genus Halomonas has been established to encompass these halotolerant microorganisms Vreeland et al. (1980).

HABITAT

Halophilic bacteria are mostly isolated from different environments such as. hypersaline water. salted food and fish. and hypersaline soils. MacLeod (1965) and Cheng and Costerton (1973) isolated halophilic bacteria with different salt tolernce from oceans and hypersaline ponds respectively.

The bacterial populations in salt lakes and bacteriology of salted food have both been extensively studied. Brisou et al. (1973). Nissenbaum (1975). and Post (1977). However, hypersaline waters derived from sea water are widespread throughout the world. and they probably represent the normal habitat of halophilic bacteria Brock (1979). Few attempts have been made to describe the bacterial flora of these environments. The dead sea has several as a source for some halophilic strains used by Israeli scientists Volcani (1940).

Extremely halophilic bacteria spoiled foods and discolored hides and induced striking changes in the Indscape by imparting various shades of red to natural salterms. Dussault and LaChance (1952), Larsen (1962), and Kushner (1968).

The microbial ecology of salted fish products is influenced markedly by the water activity of the product. The normal Gram- negative spoilage biota is not halotolerant and is not halotolerant micrococci. yeasts. spore-formers and lactic acid bacteria and moulds. (Hobbs and Hodgkiss. 1982). Extremely halophilic bacteria may cause a spoilage condition referred to as pink in more completely dried products (aw-0.75). (Hobbs and Hodgkiss. 1982). These bacteria are actively proteolytic and produce a variety of spoilage compounds such as hydrogen sulphide and indole as well as discoloration of the product. On the other hand several food-spoilage organisms have been classified as moderate halophiles by Baxer and Gibons (1956).

Other food products provide rich media for the growth of salt and solute tolerant microorganisms. Soy sauce and miso paste (which contain approximately 18% and 7 to 20% NaCl respectively, Onishi (1963) have provided many salt and solute tolerant bacteria and yeasts which were studied by Japanese workers. Fortunately for the consumers. these food products contain salt tolerant microorganisms, but not any extreme halophiles.

Several studies have been made of the microbiology and microbial ecology of hypersaline water Volcani (1940). Brisou et al. (1973). Nissenbaum (1975) and Post (1977). Little information is available. however. about the microbiology of hypersaline soils. Quesada et al (1982) studied types and properties of some bacteria isolated from hypersaline soils. They found that most isolates appeared to be typical moderate halophiles. Extreme halophiles were rare but this may have been due to an insufficient incubation period.

2.SALT TOLERANCE:

Bacterial halophilism involves two phenomena, which are not necessarily related: tolerance of salt below a certain maximum and a requirement for salt above a certain minimum(Baxter and Gibbons. 1956). Most halophilic bacteria can tolerate a wide range of NaCl concentration especially moderately halophilic and salt-tolerant bacteria which can grow in high salt concentrations as well as in much lower ones. On the other hand salt-tolerant bacteria can grow without NaCl at all. The cultures of Micrococcus halodenitrificans and Vibrio costicolus are homogeneous in its salt response. (Forsyth and Kushner. 1970), that is, each cell can grow over the entire salt range in which the culture grows in a liquid medium. The situatior became more complex in solid medium especially at high salt concentration (3.0-3.5M). The same authors found that some of the cells precultured in 0.6MNaCl failed to form colonies at 3.0 NaCl.

They explained that by some cells are damaged on being transferred from a lower to a higher salt concentration, but not on being transferred from a higher to a lower. They concluded that any changes in the properties of these bacteria on growth at different salt concentrations are probably not due to selection of more or less salt resistant cells. Most microorganisms that can grow in high salt concentrations can also grow in much lower ones (Forsyth et al., 1971). They observed that most of marine bacteria isolated on agar containing 3% NaCl could grow in up to 20% NaCl, and some could grow in 30% NaCl. Such bacteria may be considered moderately halophilic or at least very salt-tolerant microorganisms. They concluded that the particular salt concentration did not select population of cel ls having different salt responses; rather, each cell could grow over the entire range of NaCl concentration.

Matheson et al.(1976) reported that, an unidentified halophilic isolated from plates of a complex agar medium containing NaCl showed optimum growth in broths containing 0.5- 1.CMNaCl but exhibited a wide range of growth from 0.045- 4.5MNaCl. The organism can be classified as a facultative halophile with wide salt tolerance. This bacterium grew over a wide range of NaCl concentration. This was true whether the inoculum was grown in 4.25MNaCl or 0.5MNaCl. The 0.5M and 2.0M cultures gave growth curves similar to 1.0MNaCl cultures. The optimum growth rate occurred in 1.CMNaCl media. Javor (1984) tested the strains of moderate halophiles for growth potential with respect to NaCl concentration in SG (Sehgel and Gibbons (1960) medium). Most of the strains showed the greatest growth rate in >3.5MNaCl. The moderate halophiles grew best in SG with 2.5 to 3.5MNaCl.

Many authors suggested explanations for salt tolerance. Smithies and Gibbons (1955) observed that the majority of enzymes of extreme halophiles are most active at high salt concentrations. This may represent partial explanation of salt tolerance of the organism. Other macromolecular constituents of the organism may be similarly adapted to high salt concentrations. The chromatin material in particular may well differ from that of non halophiles. which has been shown to be very sensitive to changes in the ionic environment (Whitfield and Murray, 1956). Baxter and Gibbons (1954) reported that the increase in cytochrome oxidase activity Micrococcus halodenitrificans with increasing salt concentration may perhaps be regarded as a feedback mechanism tending to maintain a relatively constant internal environment. This also seems to be a reasonable explanation for the halophilic behavior of the cytochrome oxidase from a halotolerant organism (Yamada and Asano, 1954). which would not be expected to contain a high concentration of salt.

Another explanation for salt tolerance is the adaptation of most microorganisms to high external solute concentrations. This adaptation can not be achieved by maintaining low internal solute concentration. (Shindler et al., 1977). Those authors reported that the NaCl concentration of the medium did not alter the internal potassium ion concentration but had a large effect onthe internal Na^{+} concentration in moderately halophilic Vibrio costicola. They also observed that the internal Na^{+} concentration is close to K^{+} in the medium containing NaCl concentration which supports the optimal growth. Also Unemoto and Hayashi (1979) reported that the mode of adaptation of marine bacteria Vibrio alginolyticus was quite different from that of extremely halophilic or nonhalophilic bacteria. Thus the internal K^{+} concentration increased in response to the increase in medium-osmolality. This was true especially with extremely halophilic bacteria where at 3.5mNaCl an enormous amount of K^{+} accumulated reaching 4 to 5m. On the other hand at low salt concentrations the permeability of the cell wall or cytoplasmic membrane of moderate halophiles is greatly increased allowing leakage of cytoplasmic constituents, (Smithies and Gibbons, 1955). Certain members of the extreme halophiles, particulaly rod-shaped forms, appear to dissolve completely at low salt concentration. These observations provide at least a partial explanation of the salt requirement of halophiles.

3.PHYSICAL FACTORS AFFECTING SALT TOLERANCE:

3.1.TEMPERATURE:

Several authors have reported that the optimal salt concentrations for growth and the