بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها على هذه الأفلام قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ١٥-٥٠ مئوية ورطوية نسبية من ٢٠-٥٠%. To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%



بعض الوثائـــق الاصليـة تالفـة

بالرسالة صفحات لم ترد بالاصل

EFFECT OF STRESSED ENVIRONMENTAL CONDITIONS ON THE VIABILITY OF ESCHERICHIA COLI AND ENTEROCOCCUS FAECALIS

Вy

HABIBA MOSTAFA AHMED

B.Sc. (Agric. Biochemistry),
Fac. Agric., Ain Shams Univ. 1977
M.Sc. (Agric. Microbiology),
Fac. Agric., Ain Shams Univ. 1988

A thesis submitted in partial fulfilment

Of

the requirements for the degree of DOCTOR OF PHILOSOPHY

 $\Im n$

Agricultural Science (Agricultural Microbiology)

Department of Agric. Microbiology Faculty of Agriculture Ain Shams University



Approval Sheet

EFFECT OF STRESSED ENVIRONMENTAL CONDITIONS ON THE VIABILITY OF ESCHERICHIA COLI AND ENTEROCOCCUS FAECALIS

By

HABIBA MOSTAFA AHMED

B.Sc. (Agric. Biochemistry), Fac. Agric., Ain Shams Univ. 1977 M.Sc. (Agric. Microbiology), Fac. Agric., Ain Shams Univ. 1988

This thesis for Ph.D. Degree has been approved by:

Prof. Dr. F.M. Thabet
Prof. of Agric. Microbiology, Fac. Agric., Minufiya
Univ.

Prof. Dr. Fatma Refaat Nassar

Prof. of Agric. Microbiology, Fac. Agric., Ain ShamsUniv.

Prof. Dr. W.A. Mashhoor

Prof. of Agric. Microbiology, Fac. Agric., Ain Shams
Univ. (Supervisor)

Date of examination: / /1997

EFFECT OF STRESSED ENVIRONMENTAL CONDITIONS ON THE VIABILITY OF ESCHERICHIA COLI AND ENTEROCOCCUS FAECALIS

Ву

HABIBA MOSTAFA AHMED

B.Sc. (Agric. Biochemistry),
Fac. Agric., Ain Shams Univ. 1977
M.Sc. (Agric. Microbiology),
Fac. Agric., Ain Shams Univ. 1988

Under the supervision of:

Prof. Dr. W.A. Mashhoor
Prof. of Agric. Microbiology, Ain Shams Univ.

Prof. Dr. Rawia F. Gamal
Prof. of Agric. Microbiology, Ain shams Univ.

Dr. M.N.I. Abou-Seada

Assoc. Prof. of Agric. Microbiology, Ain Shams Univ.

Habiba Mostafa Ahmed. Effect of Stressed environmental conditions on the viability of *Escherichia coli* and *Enterococcus faecalis*. Unpublished Ph.D., University of Ain Shams, Faculty of Agriculture, Department of Agricultural Microbiology, 1997.

The stress of some environmental conditions usually practised for water treatment or food processing, such as exposure to heating, freezing, preservatives or disinfectants, leaves some of surviving bacterial cells damaged. These injured cells are still viable, though their recovery by normal enumeration procedures is negatively affected. Thus, bacterial populations may be seriously underestimated when a selective medium is used for enumeration of specific bioindicators, causing error and consequently health hazard.

The aim of the present work is to study the effect of some stressed environmental conditions on the death and injury of two universal bioindicators; *Escherichia coli* and *Enterococcus faecalis*, and recovery of the injured cells by different methods.

Liquid cultures of the tested organisms were subjected to several stress conditions including heating (at 60 C for 4-10 min), freezing (-20, -40, -78C for 1 hr), high concentrations of sugar (10-60%) or salt (5-15%), sodium benzoate, sodium nitrite (0.02-0.1%), acetic acid, lactic acid (0.25 - 1.0%), hypochlorite (2-10 ppm chlorine). Data revealed that the lethality percentages of both tested organisms were directly proportional to treatment intensity, meanwhile injured cells were induced in most treatments and their rates were not corresponding with stress intensity. Consequently convenient method should be used for repairing such injured cells to grow on the selective media.

Effect of exposing *E. coli* and *Enterococcus faecalis* to sublethal stress on some metabolic properties was also studied. It was found that stress conditions led to decrease the activities of dehydrogenase and dissimilatory nitrate reductase enzymes in both tested organisms. Furthermore, the reactions of IMVIC tests were found to be slower in the stressed *E. coli* cells than in the normal ones. The type of cellular damage was determined to be to protein synthesis, RNA or cell wall by using specific antibiotics such as chloramphenicol, actinomycin D and penicillin. The plasma membrane leakage was determined by measuring the 260 & 280 nm absorbing materials.

Effect of processing steps of carbonated beverage production on the bioindicators was examined, as a model of food processing. Results exhibited very high rate of lethality, meantime a ratio of injured cells was recorded in all treatments.

Different nutritional combinations were tried as recovery suspending media to repair the injured cells to find out the best one. A simple method for recovery was suggested. It was performed by applying membrane-filter technique with a recovery suspending medium consisting of pyruvate, glucose and phosphate for 3 hr at 25 C; then adding trypticase soy-yeast desoxycholate broth for counting *E. coli* or adding KF broth for counting *Enterococcus faecalis*.

Key words: Escherichia coli, Enterococcus faecalis, stressed bacteria, damaged bacteria, injured bacteria, recovery of injured bacteria, carbonated beverage.

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to Prof. Dr. Wagdy A. Mashhoor, Prof. Dr. Rawia F. Gamal and Assoc. Prof. Dr. Mohamed Nageeb I. Abou Seada, Agricultural Microbiology Dept., Fac. of Agriculture, Ain Shams University, for suggesting the problem, supervision, guidance, keeping interest and progressive criticism.

I am indebted to all members of Agric. Microbiology Dept., Ain Shams Univ., for their kind cooperation and encouragement.

Grateful appreciation is extended to the authorities of Pepsi-Cola factory for the offered facilities during the course of this work.

CONTENTS

I. INTRODUCTION	Page 1
2. REVIEW OF LIFERATURE	3
2.1. Effect of different stress environmental conditions on viability of <i>Escherichia coli</i>	
and Enterococcus faecalis	3
2.1.1. Heating	3
2.1.2. Freezing	7
2.1.3. Sodium chloride or salinity	9
2.1.4. pH and organic acids	10
2.1.5. Nitrite and Nitrate salts	13
2.1.6. Chlorination	14
2.2. Structural and physiological changes in in-	
jured bacterial cells	15
2.3. Repair of injured bacterial cells	18
3. MATERIAL AND METHODS	22
3.1. Tested organisms	22
3.1.1. Escherichia coli	22
3.1.2. Enterococcus faecalis	22
3.2. Microbiological determinations	22
3.2.1. Determination of total population	22
3.2.2. Determination of injured cells	23
3.2.3. Determination of total survivors	23
3.2.3.1. Repair of injured cells by	
solid-repair technique	23
3.2.3.2. Repair of injured cells by	
membrane filteration technique	24
3.2.4. Viability parameters and calcula-	
tions	24

	Page
3.2.5. Indole production test	25
3.2.6. Methyl red test	25
3.2.7. Voges-Proskauer test	25
2.2.8. Citrate utilization test	25
3.2.9. Eijkman test	26
3.3. Media used	26
3.3.1. Trypticase soy yeast extract broth, TSY	26
3.3.2. Trypticase soy yeast extract Desoxy-	
cholate agar, TSYDA	26
3.3.3. Endo agar	27
3.3.4. Violet red bile agar, VRBA	27
3.3.5. Eosin methylene blue, EMB	27
3.3.6. MacConky-Neutral red broth	28
2.3.7. MacConky purple broth	28
2.3.8. Forget fredett agar	28
2.3.9. Buffered Azide glucose glycerol broth,	
BAGG	28
3.3.10. Azide Maltose agar, KF streptococcal	
agar	29
3.3.11. Minimal medium	29
3.3.12. Glucose phosphate peptone broth	30
3.3.13. Tryptone broth	30
3.3.14. Koser's citrate medium	30
3.3.15. Nutrient broth	30
3.4. Physio-chemical analysis	31
3.4.1. pH	31
3.4.2. Total titratable acidity (TTA)	31
3.4.3. Chlorine determination	31
3.4.4. Sugar determination	31
3.4.5. CO ₂ measurement	31
3.4.6. Determination of membrane leakage	
substances	32

	Page
3.5. Enzymatic determinations	32
3.5.1. Dissimilatory nitrate reductase activity.	32
3.5.2. Dehydrogenase activity	32
3.6. Exposure of bacterial cells to stressed condi-	
tions	33
3.7. Statistical analysis	33
4. RESULTS AND DISCUSSION	34
Part 1 4.1 Determination of the lethality and injury	
rates of Escherichia coli and Enterococ-	
cus faecalis exposed to different stressed	
conditions	34
4.1.1. Effect of heating on the percentages	•
of death and injury of Escherichia	
coli and Enterococcus faecalis	34
4.1.2. Effect of freezing on the percent-	
ages of death and injury of Esche-	
richia coli and Enterococcus	
faecalis	38
4.1.3. Effect of different concentrations	
of sucrose on the percentages of	
death and injury of Escherichia	
coli and Enterococcus faecalis	41
4.1.4. Effect of different concentrations of	
sodium chloride on the percentages	
of death and injury of Escherichia	
coli and Enterococcus faecalis	45
4.1.5. Effect of different concentrations of	
sodium benzoate on the percenta-	
ges of death and injury of Escheri-	
chia coli and Enterococcus faeca-	48
lis	4 ለ

.

	Page
4.1.6. Effect of different concentrations of	
acetic acid on the percentages of	
death and injury of Escherichia	
coli and Enterococcus faecalis	51
4.1.7. Effect of different concentrations of	
 lactic acid on the percentages of 	
death and injury of Escherichia	
coli and Enterococcus faecalis	54
4.1.8. Effect of different concentrations of	
sodium nitrite on the percentages of	
death and injury of Escherichia	
coli and Enterococcus faecalis	54
4.1.9. Effect of different concentrations of	
hypochlorite on the percentage of	
death and injury of Escherichia	
coli and Enterococcus faecalis	59
Part 2	
4.2. Effect of exposing Escherichia coli and	
Enterococcus faecalis to sublethal stress	
on some metabolic attributes	62
4.2.1. Dehydrogenase activity in Escheri-	
chia coli and Enterococcus faecalis	
as influenced by different sugar con-	
centrations	63
4.2.2. Dehydrogenase activity in Escheri-	
chia coli and Enterococcus faecalis	
as influenced by different nitrate	
concentrations	66
4.2.3. Dehydrogenase activity in Escheri-	
chia coli and Enterococcus faecalis	
as influenced by different nitrite	
concentrations	66