

378  
EFFECTS OF CHANGING THE CARBON SOURCE ON THE  
PHOSPHOLIPIDS COMPOSITION OF E. COLI

by

KAWKAB ABDUL-GANI AHMAD

B.S., University of Baghdad, 1974

---

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Graduate Biochemistry Group  
Department of Biochemistry

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1980

Approved by:

  
Major Professor

Spec. Coll.  
LD  
2668  
.T4  
1980  
A37  
c-2

TABLE OF CONTENTS

	Page
LIST OF FIGURES . . . . .	iii
LIST OF TABLES . . . . .	iv
ACKNOWLEDGMENT . . . . .	v
INTRODUCTION . . . . .	1
ABBREVIATIONS . . . . .	2
LITERATURE REVIEW . . . . .	3
Intracellular Location of the Phospholipids . . . . .	3
Phospholipid Composition and Structure . . . . .	7
Phospholipid Species . . . . .	7
Phosphatidylethanolamine . . . . .	7
Phosphatidylglycerol . . . . .	8
Cardiolipin . . . . .	8
Phosphatidic Acid . . . . .	8
Phosphatidylserine . . . . .	10
Other Phospholipids . . . . .	10
The Fatty Acids of the Phospholipids . . . . .	11
Saturated Fatty Acids . . . . .	11
Unsaturated Fatty Acids . . . . .	12
Cyclopropane Fatty Acids . . . . .	14
Miscellaneous Fatty Acids . . . . .	15
Effect of Culture Conditions . . . . .	16
Effect of Temperature . . . . .	16
Effect of Growth Phase . . . . .	16

	Page
Changes in Energy Metabolism . . . . .	17
Thin Layer Chromatography of Lipids . . . . .	26
MATERIALS AND METHODS . . . . .	32
Growth of Cells . . . . .	32
Phospholipid Extraction . . . . .	33
Thin Layer Chromatography . . . . .	34
Quantitative Determination of Phospholipids Using Densitometry . . . . .	34
Thin Layer Chromatography for Methyl Ester Preparation . . . . .	36
Thin Layer Chromatography for Phosphorus Determination . . . . .	36
Phosphorus Determination . . . . .	37
Fatty Acid Methylation . . . . .	38
Methylation of Chloroform Extracts . . . . .	38
Methylation of Silica Gel-Lipid . . . . .	39
Gas Liquid Chromatography of Methyl Ester . . . . .	39
CALCULATIONS . . . . .	40
Gas-Liquid Chromatography Peak Areas . . . . .	40
Weight Per Cent Phospholipids . . . . .	40
Phospholipid Per Gram of Cells . . . . .	41
Densitometry Peak Area . . . . .	42
RESULTS AND DISCUSSION . . . . .	43
SUMMARY . . . . .	76
REFERENCES . . . . .	78

LIST OF FIGURES

	Page
Figure 1 -- The Davson-Danielli Membrane Model . . . . .	6
Figure 2 -- The Structure of the Phospholipids of <u>E. coli</u> . . . . .	9
Figure 3 -- A Representative One Dimensional Separation of Phospholipids by TLC From a Mutant of <u>E. coli</u> 29 Grown on Oleic + Aspartic Medium . . . . .	47
Figure 4 -- A Representative One Dimensional Separation of Phospholipids by TLC From a Mutant of <u>E. coli</u> 29 Grown on Oleic Medium . . . . .	47
Figure 5 -- A Representative One Dimensional Separation of Phospholipids by TLC From <u>E. coli</u> Grown on Aspartic + Oleic Medium . . . . .	49
Figure 6 -- A Representative One Dimensional Separation of Phospholipids by TLC From <u>E. coli</u> Grown on Glucose Medium . . . . .	49
Figure 7 -- The Structure of the Phospholipids of <u>E. coli</u> and its Mutant 29 From Densitometry Study . . . . .	59
Figure 8 -- The Fatty Acid Compositions of the <u>E. coli</u> Grown on Aspartic Medium . . . . .	61
Figure 9 -- The Fatty Acid Compositions of the <u>E. coli</u> Mutant 29 Grown on Oleic Medium . . . . .	63

LIST OF TABLES

	Page
Table 1 -- The Structure of the Fatty Acids of <u>E. coli</u> . . . . .	13
Table 2 -- The Standard Doubling Time of <u>E. coli</u> 15T <sup>-</sup> and its Mutant 29 . . . . .	33
Table 3 -- Color Reactions and R <sub>f</sub> Values of Phospholipids . . . . .	44
Table 4 -- Analytical Data of the Phospholipids From <u>E. coli</u> . . . . .	50
Table 5 -- Fatty Acid Composition of Phospholipids From <u>E. coli</u> Grown on Various Media . . . . .	66
Table 6 -- Ratios of the Main Fatty Acids to Palmitic Acid of the Phospholipids From <u>E. coli</u> Grown on Various Media . . . . .	67
Table 7 -- Fatty Acid Composition of Phosphatidyl- ethanolamine From <u>E. coli</u> Grown on Various Media . . . . .	68
Table 8 -- Ratios of the Main Fatty Acids to Palmitic Acid of PE From <u>E. coli</u> Grown on Various Media . . . . .	69
Table 9 -- Fatty Acid Composition of the Phospho- lipids From a Mutant of <u>E. coli</u> 29 Grown on Various Media . . . . .	70
Table 10 -- Fatty Acid Composition of the Phosphatidyl- ethanolamine From an <u>E. coli</u> Mutant 29 Grown on Various Media . . . . .	71
Table 11 -- Ratios of the Main Fatty Acids to Palmitic Acid of Phospholipid From a Mutant <u>E. coli</u> 29 Grown on Various Media . . . . .	72
Table 12 -- Ratios of the Main Fatty Acids to Palmitic Acid of PE Fraction From a Mutant <u>E. coli</u> 29 Grown on Various Media . . . . .	72

ACKNOWLEDGMENT

The author is greatly indebted to Dr. W. E. Klopfenstein for his continuous encouragement and guidance, without which this study would never have been completed. Sincere thanks is hereby extended to Dr. R. Clegg, Dr. J. Urban for serving on the author's committee and to Dr. T. Roch for his advices.

Most of all I should thank the members of my family: my father and my mother who helped provide encouragement, my husband, Walid, and my child, Zaynab, who gave me purpose.