

CONTENTS

	PAGE
ENGLISH ABSTRACT	ii
THAI ABSTRACT	iv
ACKNOWLEDGEMENTS	vi
CONTENTS	vii
LIST OF TABLES	ix
LIST OF FIGURES	xii
LIST OF SYMBOLS	xv
CHAPTER	
1. INTRODUCTION	1
1.1 Introduction	1
1.2 Objectives	3
1.3 Scopes	3
1.4 Expected Benefits	3
2. THEORY AND LITERATURE REVIEWS	4
2.1 Thailand's Exported Food Products	4
2.2 Food-Borne and Waterborne Diseases	5
2.3 Morphology and characteristic of Yeast & Mold	5
2.4 Impact of Yeast & Mold infection	7
2.5 Thai FDA Revising Yeast and Mold Level in Foods	8
2.6 Enumeration of Yeasts and Mold in Food	14
2.7 Pathogenic Escherichia coli	15
2.8 Microbial Detection Methods	24

	PAGE
3. MATERIALS AND METHODS	34
3.1 Bacterial Strains	34
3.2 Equipment and Instruments	34
3.3 Media and Chemical Reagent	34
3.4 Experimental Design	35
3.5 Sample Preparation	35
3.6 <i>E. coli</i> , Yeast and Mold Cell Enumeration	38
3.7 Growth Characteristic Determination Using Logistic Model	39
3.8 Statistical analysis	40
3.9 Swabbing Production Line Equipment	40
4. RESULTS AND DISCUSSION	42
4.1 Development of TPC enumeration concept	42
4.2 Protocol improvement to detect industrial hygienic microbial Contaminant	52
4.3 Improvement of industrial yeast/mold detection	61
5. CONCLUSIONS AND RECOMMENDATIONS	71
5.1 Conclusions	71
5.2 Recommendations	73
REFERENCES	74
APPENDIX	79
A. Experimental Data	79
CURRICULUM VITAE	94

LIST OF TABLES

TABLE	PAGE
2.1 The new proposed level of yeast and mold allowed to be found in foods	10
2.2 Bacteria That Cause Acute Bacterial Diarrheas and Food Poisonings	21
2.3 Pros and cons of conventional and rapid methods in bacterial detection	33
4.1 Detail comparisons of different TPC procedures including pour plate, spread plate and MIC techniques	43
4.2 Comparison of the total cell count of <i>E. coli</i> at different background color of the medium using plate count agar (PCA)	47
4.3 TPC enumeration in food samples using various techniques (pour plate, spread plate and MIC techniques)	50
4.4 TPC enumeration in production lines using various techniques (pour plate, spread plate and MIC techniques)	51
4.5 Comparison of the total cell count of <i>E. coli</i> at different incubation temperatures using Chromocult [®] Coliform Agar	53
4.6 Growth kinetics of <i>E.coli</i> on Chromocult [®] Coliform Agar and relative coefficient of logistic model at different incubation temperatures	55
4.7 Comparison of key kinetic parameters in term of color development (delta blue)	58
4.8 Colony count on culture plates using different concentrations of CCA comparing to the original concentration	69
4.9 Comparison of alternative media for the enumeration of yeast/mold when perform on pure culture	63
4.10 Viable cell counts of yeast and mold comparing pure dextrose to PDB control media using pure cultures	65
4.11 Final colony enumeration of yeast and mold culturing by nitrogen-supplement PDB and control PDB	67

TABLE	PAGE
4.12 Comparison of test kit and MIC for the enumeration of yeast and mold when perform on plasticine samples	70
4.13 Comparison of test kit and MIC for the enumeration of yeast and mold when perform on dough clay samples	70
A.1 Comparison of the total cell count of <i>E. coli</i> at different background color of the medium using plate count agar (PCA)	79
A.2 TPC enumeration in food samples using various techniques (pour plate, spread plate and MIC techniques)	80
A.3 TPC enumeration in production lines using various techniques (pour plate, spread plate and MIC techniques)	81
A.4 Comparison of the total cell count of <i>E. coli</i> at different incubation temperatures using Chromocult® Coliform Agar	82
A.5 Growth kinetics of <i>E.coli</i> on Chromocult® Coliform Agar and relative coefficient of logistic model at different incubation temperatures (μ_{\max})	83
A.6 Growth kinetics of <i>E.coli</i> on Chromocult® Coliform Agar and relative coefficient of logistic model at different incubation temperatures (X_{\max})	84
A.7 Comparison of key kinetic parameters in term of color development (delta blue) (μ_{\max})	85
A.8 Comparison of key kinetic parameters in term of color development (delta blue) (X_{\max})	86
A.9 Colony count on culture plates using different concentrations of CCA comparing to the original concentration	88
A.10 Comparison of alternative media for the enumeration of yeast/mold when perform on pure culture	89
A.11 Viable cell counts of yeast and mold comparing pure dextrose to PDB control media using pure cultures	90
A.12 Final colony enumeration of yeast and mold culturing by nitrogen-supplement PDB and control PDB	91

TABLE	PAGE
A.13 Comparison of test kit and MIC for the enumeration of yeast and mold when perform on plasticine sample	92
A.14 Comparison of test kit and MIC for the enumeration of yeast and mold when perform on dough clay sample	93

LIST OF FIGURES

FIGURE	PAGE
2.1 Thailand's Exported Food Products, 2008 –2011	4
2.2 Gram stain of <i>Candida albicans</i> cells isolated from the blood culture of a patient. At left the yeast cells are budding, and at right, they have formed long, filamentous, irregularly staining hyphae	6
2.3 Colonies of three <i>Aspergillus species</i> . Some molds may be recognized by the color of their spores (conidia). Clockwise from left: <i>A. flavus</i> (yellow), <i>A. fumigatus</i> (smoky gray-green), <i>A. niger</i> (black)	7
2.4 Unstained cells of <i>E. coli</i> viewed by phase microscopy. about 1000X magnification. CDC	16
2.5 Unstained cells of <i>E. coli</i> O157:H7	18
2.6 Presumptive tests for coliforms, fecal coliforms and <i>E. coli</i>	27
2.7 Confirmed test for coliforms	28
2.8 Confirmed test for fecal coliforms and <i>E. coli</i>	29
2.9 Completed test for <i>E. coli</i>	30
2.10 Chromogenic culture media products available for the isolation and identification of yeasts and mold in food products	31
2.11 <i>E. coli</i> /coliform Selective Agar is a chromogenic medium for the detection and enumeration of <i>E. coli</i> and other coliforms (important hygiene indicators) from food and water samples. <i>E. coli</i> colonies turn a distinctive cherry red color whereas coliforms colonies turn blue	32
3.1 All types of raw materials to prepare alternative media for yeast/mold growth	37
3.2 24-microwell plate format	38
3.3 Micro Inoculation Technology Scope	39
3.4 Swabbing test kit	41
4.1 Bacterial analytical techniques of BAM standard (a) pour plate (b) spread plate (c) petrifilm TM and (d) MIC	43

FIGURE	PAGE
4.2 Photographs of the MIC equipment used to perform miniaturized TPC experiment	45
4.3 Correlation between estimates of TPC obtained by Pour- Spread plate (Δ) and Pour plate- MIC (X)	46
4.4 Photographs of colony formed on plate count agar using different techniques (a) pour plate, (b) spread plate and (c) MIC	47
4.5 Contrast difference between colony and background color at different culture period using plate count agar (PCA)	48
4.6 Photographs of colony formed on plate count agar using different background color of the medium (a) control; (b) green dye pigment; (c) red dye pigment and (d) yellow dye pigment	49
4.7 Profiles of colony growth in five different incubation conditions (red line: 30°C; green line: 35°C; yellow line: 37°C; blue line: 40°C and pink line: 45°C)	54
4.8 Photographs of colony formed on plate count agar using different incubation conditions (a) 30°C; (b) 35°C; (c) 37°C; (d) 40°C and (e) 45°C	56
4.9 Profiles of color change using different incubation temperatures (a) delta R; (b) delta B; (c) delta G and (d) overall color change or delta E	57
4.10 Digitized images of E. coli colony from pure culture after 12 h of incubation at 35 \pm 2°C	60
4.11 Profiles of color change (delta blue) of E. coli colonies as a result of vary in CCA concentrations	61
4.12 Comparison of growth models fitted to variable count data of yeast grown at 30° C between PDB and alternative media	64
4.13 Comparison of growth models fitted to variable count data of mold grown at 30° C between PDB and alternative media	64
4.14 Comparison of growth models fitted to variable count data of yeast grown at 30° C between PDB and alternative media	65
4.15 Comparison of growth models fitted to variable count data of mold grown at 30° C between PDB and alternative media	66
4.16 Alternative media as nitrogen sources agent	66

FIGURE	PAGE
4.17 Comparison of growth models fitted to variable count data of yeast grown at 30° C between PDB and alternative media	68
4.18 Comparison of growth models fitted to variable count data of mold grown at 30° C between PDB and alternative media	68
4.19 The equipment of each technique was used to perform yeast and mold count (a) Easicult® Combi test kit (B) MIC technique	69

LIST OF SYMBOLS

SYMBOL

μ_{max}	=	Maximum Specific Growth Rate (min^{-1})
X_{max}	=	Maximum colony area / Maximum blue color intensity