



**Resistance of acid-adapted *Escherichia coli* O157:H7 to  
lactoperoxidase and heat in goat milk**

**By**

**Angela Araba Parry-Hanson**

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## DECLARATION

I declare that the thesis herewith submitted for the degree of PhD (Food Science) at the University of Pretoria, has not been previously submitted by me for a degree at any other institution of higher education.

Angela Araba Parry-Hanson



## **DEDICATION**

To my heavenly Father

and

To my parents (Hector and Irene) and  
my siblings (Freda, Michael and Jocelyn Parry-Hanson)

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## Abstract

### **Resistance of acid-adapted *Escherichia coli* O157:H7 to combined treatments in goat milk**

By

Angela Araba Parry-Hanson

Supervisor: Professor Elna M. Buys

Co-Supervisor: Professor Piet J. Jooste

Degree: PhD Food Science

Survival of *Escherichia coli* O157:H7 in fermented dairy products has been attributed to acid-adaptation. Acid-adaptation enhances resistance to extreme acid pH and confers cross-protection to heterologous stresses. This study sought to investigate whether acid-adaptation confers cross-protection to lactoperoxidase (LP) system and lactic acid in Tryptone Soy Broth (TSB), and to determine the mechanism of cross-protection. Subsequently, cross-protection of acid-adapted *E. coli* O157:H7 to the combination of LP activation, heat and lactic acid treatments was determined in fresh goat milk. Finally, the effect of LP activation and *E. coli* O157:H7 survival on acid production during fermentation of traditional and commercial goat milk was investigated with indigenous cultures and single strain lactic acid bacteria (LAB) respectively.

Acid-adapted *E. coli* O157:H7 strain UP10 showed high acid-resistance at pH levels 4.0 and 5.0 for up to 24 h in TSB at 25 °C compared to non-adapted *E. coli* O157:H7. Acid-adaptation also conferred cross-protection against activated LP system and lactic acid challenge at pH 4.0 and 5.0. Results from fatty acid analysis and quantitative real time Polymerase Chain Reaction (RT-PCR) indicated that sigma S (RpoS)-independent systems were responsible for acid-resistance and cross-

protection in TSB. Increase in the saturation of fatty acids, increased expression of outer membrane porin, OmpC, and activation of the glutamate decarboxylase system contributed to acid-resistance and cross-protection.

Growth of acid-adapted *E. coli* O157:H7 strains UP10 and 1062 were inhibited in fresh goat milk compared to the non-adapted cells. Nonetheless, strain 1062 showed better growth and resistance to activated LP in fresh goat milk compared to strain UP10. LP activation alone did not significantly inhibit either acid-adapted or non-adapted *E. coli* O157:H7, but it sensitized *E. coli* O157:H7 cells to sub-lethal heat treatment at 55 and 60 °C. The combination of heat treatment at 60 °C, LP activation and lactic acid at pH 5.0 had a greater inhibitory effect on both acid-adapted and non-adapted *E. coli* O157:H7, but the acid-adapted strains displayed cross-protection against combined treatments. This indicates that non-adapted *E. coli* O157:H7 can survive a certain threshold of stresses unscathed. Below that threshold, acid-adaptation may be detrimental to survival.

LP activation did not inhibit growth and acid production by single strain and indigenous LAB in the processing of commercial and traditional fermented goat milk products. LP activation however inhibited *E. coli* O157:H7 in both the commercial and traditional goat milk products although *E. coli* O157:H7 had become acid-adapted during the fermentation process. *E. coli* O157:H7 inhibition could be due to the combination of LP activation, low pH, fermentation time and antimicrobial compounds present in the milk or produced by the LAB during milk fermentation.

Results from this study suggest that while acid-adaptation protects *E. coli* O157:H7 under harsh conditions, it can sensitize *E. coli* O157:H7 to sub-lethal stresses that does not require acid-adaptation for survival. On the other hand, non-adapted *E. coli* O157:H7 could become acid-adapted in food at mild acid pH which may enhance prolonged survival in such foods.



## TABLE OF CONTENTS

<b>DECLARATION .....</b>	<b>II</b>
<b>DEDICATION .....</b>	<b>III</b>
<b>ACKNOWLEDGEMENTS .....</b>	<b>IV</b>
<b>Abstract.....</b>	<b>V</b>
<b>LIST OF TABLES .....</b>	<b>I</b>
<b>LIST OF FIGURES .....</b>	<b>III</b>
<b>LIST OF ABBREVIATIONS .....</b>	<b>VI</b>
<b>Chapter 1: INTRODUCTION.....</b>	<b>1</b>
1.1 Introduction.....	1
1.2 Problem statement.....	1
<b>Chapter 2: LITERATURE REVIEW.....</b>	<b>4</b>
2.1 Goat milk production .....	4
2.1.1 Anti-allergenic properties of goat milk.....	5
2.1.2 Nutritional properties of goat milk .....	5
2.1.3 Goat milk products.....	6
2.1.4 General bacterial quality of goat milk .....	7
2.2 Significance of <i>Escherichia coli</i> O157:H7 as a foodborne pathogen.....	8
2.2.1 Virulence factors and pathogenesis .....	10
2.3 Preservation technologies applied in dairy processing .....	13
2.3.1 Pasteurization of milk .....	13
2.3.2 Fermentation .....	14
2.3.2.1 Traditional fermented milk .....	17
2.3.2.2 Lactic acid bacteria used for amasi fermentation .....	17
2.4 The lactoperoxidase system .....	18
2.4.1 Characterization of the LP enzyme.....	19
2.4.2 LP activities in milk .....	19

2.4.3 Other components of the LP system .....	20
2.4.4 Antimicrobial action of LP system .....	21
2.5 <i>E. coli</i> general stress response .....	24
2.5.1 Properties and functions of RpoS .....	25
2.5.2 Regulation of RpoS.....	26
2.6 <i>E. coli</i> response to acid stress .....	29
2.6.1 Acid tolerance response .....	32
2.6.2 Acid habituation.....	33
2.6.3 Acid resistance of <i>E. coli</i> .....	34
2.6.3.1 The oxidative acid resistance system.....	35
2.6.3.2 The pH homeostasis systems .....	35
2.6.3.2.1 Glutamate decarboxylase acid resistance system .....	36
2.6.3.2.2 Arginine decarboxylase acid resistance system.....	37
2.6.4 Contribution of other macromolecular components to acid resistance.....	38
2.6.4.1 Outer membrane fatty acids .....	38
2.6.4.2 Cyclopropane fatty acid .....	39
2.6.4.2.1 Biosynthesis of CFAs .....	40
2.6.4.2.2 Mechanism of action of CFAs .....	42
2.6.4.2.3 Regulation of CFA synthesis .....	43
2.6.4.3 Outer membrane proteins.....	44
2.7 <i>E. coli</i> tolerance to lactoperoxidase system .....	47
2.8 Cross-protection of acid adapted <i>E. coli</i> .....	48
2.9 Hypotheses .....	50
2.10 Objectives .....	52
<b>Chapter 3:RELATIVE GENE EXPRESSION IN ACID-ADAPTED</b>	
<b><i>ESCHERICHIA COLI</i> O157:H7 DURING LACTOPEROXIDASE</b>	
<b>AND LACTIC ACID CHALLENGE IN TRYPTONE SOY BROTH..</b>	<b>53</b>
3.1. Introduction.....	55
3.2 Materials and Methods.....	56





3.2.1 Bacterial cells and culture conditions .....	56
3.2.2 Acid-resistance assay and viability of <i>E. coli</i> O157:H7 .....	56
3.2.3 Fatty acid analysis.....	57
3.2.4 RNA extraction and cDNA synthesis .....	58
3.2.5 Quantitative real-time PCR.....	58
3.2.6 Statistical analysis.....	59
3.3 Results.....	60
3.3.1 Resistance of <i>E. coli</i> O157:H7 to lactoperoxidase in combination with lactic acid.....	60
3.3.2 Effect of acid- adaptation on the fatty acid profile of <i>E. coli</i> O157:H7.....	61
3.3.3 Relative expression levels of lactoperoxidase and acid-inducible genes in <i>E. coli</i> O157:H7.....	63
3.4 Discussion .....	65
3.5 Acknowledgements.....	69
<b>Chapter 4:THE INFLUENCE OF LACTOPEROXIDASE, HEAT AND LOW PH ON SURVIVAL OF ACID-ADAPTED AND NON-ADAPTED <i>ESCHERICHIA COLI</i> O157:H7 IN GOAT MILK.....</b>	<b>70</b>
4.1 Introduction.....	72
4.2 Materials and Methods.....	73
4.2.1. <i>E. coli</i> O157:H7 strains and acid-adaptation .....	73
4.2.2 Milk source .....	73
4.2.3 Inoculation of milk with <i>E. coli</i> O157:H7 .....	74
4.2.4. Activation of the lactoperoxidase system .....	74
4.2.5 Heat treatment.....	75
4.2.6 Microbial analyses .....	75
4.2.7. Statistical analysis.....	76
4.3 Results.....	76
4.3.1 Lactoperoxidase activity.....	76
4.3.2 The effect of combined treatments of activated lactoperoxidase and low pH on	



survival of <i>E. coli</i> O157:H7 .....	77
4.3.3 The effect of combined treatments of activated lactoperoxidase, low pH and heat on survival of <i>E. coli</i> O157:H7 .....	79
4.4 Discussion .....	82
4.5 Conclusion .....	84
4.6 Acknowledgement .....	84
<b>Chapter 5: EFFECT OF LACTOPEROXIDASE SYSTEM AND <i>ESCHERICHIA COLI</i> O157:H7 GROWTH ON ACID-PRODUCTION BY SINGLE STRAIN AND INDIGENOUS LACTIC ACID BACTERIA IN GOAT MILK .....</b>	<b>85</b>
5.1 Introduction.....	87
5.2 Materials and Methods.....	89
5.2.1 Milk Source.....	89
5.2.2 Cultures .....	89
5.2.3 Inoculation and fermentation .....	90
5.2.4 Acid challenge .....	91
5.2.5 Chemical analyses.....	91
5.2.6 Microbiological analyses .....	92
5.2.7 Statistical analyses .....	92
5.3 Results.....	93
5.3.1 Quality of raw and pasteurized Saanen goat milk .....	93
5.3.2 The effect of LP activation on single strain LAB in goat milk.....	94
5.3.3 The effect of LP activation on single strain LAB in goat milk in the presence of <i>E. coli</i> O157:H7 .....	96
5.3.4 The effect of the activated LP system during processing of a traditional fermented product in the presence of <i>E. coli</i> O157:H7 .....	99
5.4 Discussion .....	102
5.5 Conclusion .....	105
5.6 Acknowledgement .....	105



<b>Chapter 6: GENERAL DISCUSSION .....</b>	<b>106</b>
6.1 Review of Methodology .....	106
6.1.1 Acid-resistance assays for <i>Escherichia coli</i> O157:H7 .....	106
6.1.2 Choice of hurdles .....	110
6.1.3 Quantitative Real-Time PCR (qRT-PCR) .....	111
6.1.4 Microbiological analyses .....	113
6.1.5 Biochemical analysis .....	115
6.1.5.1 Fatty acid profile .....	115
6.1.5.2 Activation of the lactoperoxidase system .....	116
6.1.5.3 Lactic acid determination.....	116
6.2 Comparative acid-resistance of <i>E. coli</i> O157:H7 in Tryptone Soy Broth, goat milk and fermented goat milk .....	117
6.2.1 Acid resistance of acid-adapted <i>E. coli</i> O157:H7 in Tryptone Soy Broth.....	117
6.2.2 Effect of acid-adaptation on outer membrane components of <i>E. coli</i> O157:H7.	120
6.2.3 Survival and growth of acid-adapted <i>E. coli</i> O157:H7 in TSB versus goat milk	123
6.2.4 Lactoperoxidase activity in goat milk.....	123
6.2.5 Cross-protection of acid-adapted <i>E. coli</i> O157:H7 in broth .....	124
6.2.6 Cross-protection of acid-adapted <i>E. coli</i> O157:H7 in goat milk .....	126
<b>Chapter 7: CONCLUSIONS AND RECOMMENDATIONS.....</b>	<b>129</b>
<b>Chapter 8: REFERENCES .....</b>	<b>131</b>

## LIST OF TABLES

---

Table 1:	Average essential amino acid and fatty acid composition (g/10g milk) in proteins and lipids of goat and cow milk .....	6
Table 2:	EHEC isolated from milk, milk products or contaminated milk contact surfaces .....	10
Table 3:	The minimum pasteurization temperature and time combination 14	
Table 4:	Oligonucleotide primers used for quantitative real time-PCR .....	59
Table 5:	Outer membrane fatty acid profile of acid-adapted and non-adapted <i>Escherichia coli</i> O157:H7 challenged to lactic acid pH levels 4.0, 5.0 or 7.4 or activated lactoperoxidase at pH 7.4 .....	62
Table 6:	Chemical and microbiological quality of raw and pasteurized Saanen goat milk .....	93
Table 7:	The effect of single strain lactic acid bacteria (LAB) on LAB counts, pH and titratable acidity in goat milk fermented at 30 °C for 6 h .....	94
Table 8:	Changes in the mean values (†standard deviation) of pH, titratable acidity and lactic acid bacteria counts in pasteurized and lactoperoxidase (LP) activated Saanen goat milk fermented at 30 °C .....	95
Table 9:	The effect of single strain lactic acid bacteria (LAB) on LAB counts, <i>E. coli</i> O157:H7 counts and titratable acidity in goat milk fermented at 30 °C for 24 h .....	96
Table 10:	Changes in the mean values (†standard deviation) of titratable acidity, lactic acid bacteria (LAB) and <i>Escherichia coli</i> O157:H7 counts in pasteurized and lactoperoxidase activated Saanen goat milk fermented by single strain lactic acid bacteria at 30 °C .....	97
Table 11:	Changes in pH, titratable acidity and counts of <i>Escherichia coli</i>	



	O157:H7 and indigenous lactic acid bacteria during processing of traditional Madila at 30 °C .....	100
Table 12:	Effect of single lactic acid bacteria (LAB) strains, lactoperoxidase system (LP) and time on pH, titratable acidity and counts of lactic acid bacteria and <i>E. coli</i> O157:H7 in commercial and traditional fermented goat milk. ....	101

## LIST OF FIGURES

---

Figure 2.1:	Genes involved in pathogenicity of Enterohaemorrhagic <i>Escherichia coli</i>	11
Figure 2.2:	(A) The invasion pathway and diseases caused by Enterohaemorrhagic <i>Escherichia coli</i> (EHEC) ;	12
	(B) Interaction of EHEC with the mucosal cells in the large intestine	12
Figure 2.3:	Glucose fermentation in homofermentative and heterfermentative lactic acid bacteria	16
Figure 2.4:	RpoS regulation is differentially affected by various stress conditions. An increase in cellular levels of RpoS is modulated by activating <i>rpoS</i> synthesis at transcriptional level from the <i>rpoS</i> gene or at the translational level from the <i>rpoS</i> mRNA. Stabilization of the RpoS protein by inhibition of proteolysis (which occurs rapidly under optimal conditions) is another method used to increase cellular RpoS levels.	27
Figure 2.5:	Interaction of weak acids in the microbial cell. (A), Exterior of the cell favours undissociated weak acid; (B), On entering the cell, the interior of the cell favours dissociated molecule and acid dissociates; (C), Proton pumps use ATP to remove excess H <sup>+</sup> ions	30
Figure 2.6:	Structures of CFA synthase substrates and products. The phospholipids shown (phosphatidylethanolamines) are typical components of membrane lipids of Gram-negative bacteria. AdoMet, S-adenosyl-L-methionine; AdoHme, S-adenosyl-L-homocysteine	41
Figure 2.7:	Probable mechanism for Cl addition to double bonds for sterol methyltransferases	42
Figure 2.8:	The OmpF porin of <i>Escherichia coli</i> . (A) View of the trimer from the top, i.e., in a direction perpendicular to the plane of the membrane. Loop 2, colored blue, plays a role in interaction of the monomer with its neighboring unit. Loop 3, colored orange, narrows the channel. (B)	

- View of the monomeric unit from the side, roughly in the direction of the arrow in panel A. Loops 2 and 3 are colored as in panel A 45
- Figure 2.9: Proposed model for mechanisms of *Escherichia coli* survival under low pH stress. Glut-glutamine, GABA- $\gamma$ -amino butyric acid; Arg-arginine; AGM-agmatine; green circle is the transmembrane glutamate:  $\gamma$ -aminobutyric acid antiporter; orange circle is the arginine: agmatine antiporter; RpoS-alternative sigma factor  $\sigma$ ; ASPs-acid shock proteins; CFA-cyclopropane fatty acids; OmpC-outer membrane porin C; OmpF-outer membrane porin F 47
- Figure 3.1: The effect of lactic acid and activated lactoperoxidase (LP) system on survival of acid-adapted (A, B) and non-adapted (C, D) *Escherichia coli* O157:H7 inocula in Tryptone Soy Broth. (A, C) Lactic acid challenge only, (B, D) Activated LP in combination with lactic acid challenge. 61
- Figure 3.2: Expression of lactoperoxidase (LP) and acid inducible genes in *Escherichia coli* O157:H7 challenged against LP system and lactic acid for 6 h in Tryptone Soy Broth for 6 h at 25 °C. The bars represent the expression ratio of genes compared to untreated non-adapted *E. coli* O157:H7 cells. Error bars represent one standard error of the mean. 65
- Figure 4.1: The lactoperoxidase activities of fresh goat milk (pH 6.9) before and after heat treatment at 55 °C, 60 °C and 72 °C for 15 s 77
- Figure 4.2: The effect of activated lactoperoxidase (LP) on acid-adapted (AA) and non-adapted (NA) *E. coli* O157:H7 strains UP10 and 1062 in fresh (pH 6.9) and acidified (pH 5.0) goat milk incubated for 6 h at 25 °C. Error bars represent one standard error of the mean 78
- Figure 4.3: The survival of acid-adapted (AA) and non-adapted (NA) *Escherichia coli* O157:H7 strains UP10 and 1062 to the combined effect of heat (55°C or 60°C for 15 s, excluding time taken to reach target temperature) in activated lactoperoxidase (LP) goat milk at pH 6.9. 80
- Figure 4.4: The survival of acid-adapted (AA) and non-adapted (NA) *Escherichia*

*coli* O157:H7 strains UP10 and 1062 to the combined effect of heat (55°C or 60°C for 15 s, excluding time taken to reach target temperature), activated lactoperoxidase (LP) and low pH (pH 5.0) treatments in goat milk. 81

Figure 5.1: The percent increase in acid production by lactic starter cultures after 6 h fermentation of goat milk inoculated with *Escherichia coli* O157:H7 compared to 6 h fermentation of goat milk that had no *E. coli* O157:H7 present 98

Figure 6.1: The effect of acid challenge at pH levels of 4, 5 and 7 on survival of acid-adapted and non-adapted *E. coli* O157:H7 in Tryptone Soy Broth incubated for 6 h at 37 °C 119

Figure 6.2: The effect of acid challenge at pH levels of 4, 5 and 7 on survival of acid-adapted and non-adapted *E. coli* O157:H7 in Tryptone Soy Broth incubated for 6 h at 25 °C 119



## LIST OF ABBREVIATIONS

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AA	Acid-adapted
ABTS	2,2'-Azino-bis-3-ethyl-benzthiazoline-6-sulphonic acid
AdiA	Arginine decarboxylase
AdiC	Arginine: agmatine antiporter
AdoHme	S-adenosyl-L-homocysteine
AdoMet	S-adenosyl-L-methionine
AGM	Agmatine
AH	Acid habituation
ANOVA	Analysis of variance
AR	Acid resistance
ASP	Acid shock proteins
ATA	Arginine tetrazolium agar
ATP	Adenosine triphosphosphate
ATR	Acid tolerance response
BHI	Brain Heart Infusion
CAC	Codex Alimentarius Commission
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary Deoxyribonucleic Acid
CDSC	Communicable Disease Surveillance Centre
CFA	Cyclopropane Fatty Acid
<i>cfa</i>	Cyclopropane fatty acid synthase gene
ClpXP	Serine protease complex responsible for ATP-dependent degradation of proteins
CorA	Magnesium transporter
CRP	Cyclic adenosine monophosphate receptor protein
DNA	Deoxyribonucleic acid
DnaK	<i>Escherichia coli</i> chaperone protein
E $\sigma^S$	RNA polymerase holoenzyme

EHEC	Enterohaemorrhagic <i>Escherichia coli</i>
EIIA(Glc)	Glucose specific EII component of the phosphotransferase system
FAO	Food and Agriculture Organization
Fur	Transcriptional repressor of iron-regulated promoters
GAD	Glutamate decarboxylase
GABA	Gamma aminobutyric acid antiporter
GroE, GroEL, GroES:	A group of chaperone proteins required for high temperature growth/ viability
HC	Haemorrhagic colitis
Hfq	host factor 1
HNS	Histone-like DNA binding proteins
HPLC	High performance liquid chromatography
HST	Heat shock proteins
HTST	High temperature short time
HU	Major DNA binding protein of <i>Escherichia coli</i>
HUS	Haemolytic uremic syndrome
IDF	International Dairy Federation
LA	Lactic acid
LAB	Lactic acid bacteria
LamB	Maltoporin of <i>Escherichia coli</i>
LEE	Pathogenicity island in <i>Escherichia coli</i> O157:H7 genome
LP	Lactoperoxidase
LTLT	Low temperature long time
MarA	Transcriptional activator in <i>Escherichia coli</i>
MOPS	Morpholinemethanesulfonic acid
MRS	de Mann Rogosa Sharpe
mRNA	Messenger ribonucleic acid
MTC	Medium chain triglycerides
MviA	Mouse virulence gene in <i>Salmonella</i> ; plays a central role in facilitating sigma S degradation by ClpXP

MUFA	Monounsaturated fatty acid
NA	Non-adapted
Omp	Outer membrane porins
OmpR/EnvZ	Regulator proteins for the <i>Escherichia coli</i> outer membrane
PCR	Polymerase chain reaction
PhoP	Protein that phosphorelates and regulates the expression of a large collection of genes in enteric bacteria
ppGpp	Guanosine tetraphosphate
ppm	Parts per million
PUFA	Polyunsaturated fatty acid
RNA	Ribonucleic acid
RNAP	Ribonucleic acid polymerase
RpoS	Alternative sigma factor S
RSA	Republic of South Africa
RssB	<i>Escherichia coli</i> response regulator
qRT-PCR	quantitative real time polymerase chain reaction
SFA	Saturated fatty acid
SH	Sulfhydryl group
SMAC	Sorbitol MacConkey agar
STEC	Shiga toxin producing <i>Escherichia coli</i>
<i>stx</i>	Shiga toxin gene
TSA	Tryptone soy agar
TSB	Tryptone soy both
TSBG	Tryptone soy broth supplemented with 1 % glucose
WHO	World Health Organization