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

By

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Submitted in partial fulfilment of the requirements for the degree

PhD (Food Science)

In the

Department of Food Science
Faculty of Natural and Agricultural Science
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Republic of South Africa

August 2009

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)

ACKNOWLEDGEMENTS

I would like to acknowledge the following people and institutions:

My supervisor, Professor Elna M. Buys for her guidance, insights and dedication to my research, the freedom to explore my project and believing in my capabilities. I am particularly appreciative of her hospitality, and the opportunities and challenges she sent my way to make this academic experience an interesting one.

My co-supervisor, Professor Piet J. Jooste for his supervision, gentle criticisms, inquisitions and suggestions that made the thesis interesting.

Third World Organization for Women in Science (TWOWS) and University of Pretoria for awarding me a fellowship and a bursary respectively for my PhD study. The National Research Fund (NRF) for sponsoring my research.

Staff and postgraduate students of the Department of Food Science for the support, discussions, suggestions and encouragement throughout my research.

Dr. Antoinette van Schalwyk for her untiring assistance with the molecular aspects of my research.

My parents, Hector and Irene, and my siblings, Freda, Michael and Jocelyn for the moral and spiritual support.

Friends, Ubomba-Jaswa family that made me feel at home, KK Arthur, BC Dlamini and EK Ndungu for their companionship, support and encouragement.

Abstract

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

By

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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.

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LIST OF ABBREVIATIONS

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
cfa	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	*Escherichia coli* chaperone protein
$E\sigma^s$	RNA polymerase holoenzyme

EHEC	Enteroheamorrhagic *Escherichia coli*
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 *Escherichia coli*
HUS	Haemolytic uremic syndrome
IDF	International Dairy Federation
LA	Lactic acid
LAB	Lactic acid bacteria
LamB	Maltoporin of *Escherichia coli*
LEE	Pathogenicity island in *Escherichia coli* O157:H7 genome
LP	Lactoperoxidase
LTLT	Low temperature long time
MarA	Transcriptional activator in *Escherichia coli*
MOPS	Morpholinemethanesulfonic acid
MRS	de Mann Rogosa Sharpe
mRNA	Messenger ribonucleic acid
MTC	Medium chain triglycerides
MviA	Mouse virulence gene in *Salmonella*; 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 *Escherichia coli* 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	*Escherichia coli* 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 *Escherichia coli*
stx	Shiga toxin gene
TSA	Tryptone soy agar
TSB	Tryptone soy both
TSBG	Tryptone soy broth supplemented with 1 % glucose
WHO	World Health Organization