Relative gene expression in acid-adapted *E. coli* O157:H7 during lactoperoxidase and lactic acid challenge

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Introduction

Advancements in food processing technology have led to improved food safety. However, recent incidents have highlighted the challenges in maintaining a high standard of food safety. The introduction of new pathogens and the resilience of existing ones pose significant threats to public health.

One example is the threat from the ‘new E-coli’. A superbug that scientists believe has been brought into Britain through the food chain is infecting about 30,000 people a year, according to government experts. One million bars of chocolate were withdrawn from British shops over the last decade, yet research has not found the source of the contamination.

Talking Point

What are the implications of such incidents for the food industry and public health?
Escherichia coli O157:H7

• An emerging food pathogen
  – Diarrhoea, HUS and HC

• Adapt to mild acidic environments
  – Acid-resistance (pH 1.5 to 4.5)

• Stationary phase AR systems in *E. coli*
  – Glutamate decarboxylase
  – Arginine decarboxylase
  – Oxidative system
Acid resistance

- Oxidative system
  - RpoS-dependent
  - Cross-protection

- Changes in membrane components
  - Outer membrane proteins (OmpC and OmpF)
  - Outer membrane fatty acids
    - cyclopropane fatty acid
Mechanism of acid-resistance in *Escherichia coli* (adapted from Lin *et al.*, 1994; Lin *et al.*, 1995; Grogan and Cronan, 1997; Chung *et al.*, 2006)
Problem statement

• RpoS-dependent oxidative system
  – Acid-resistance and Cross-protection
  – Glucose repressed

• Food systems are complex and in many cases contain glucose

• If RpoS is glucose repressed in stationary phase cells, then what system is responsible for acid resistance (pH 4.0) and cross-protection in complex media?
Methodology

• Acid-adaptation of *E. coli* O157:H7 in TSB supplemented with 1% glucose

• Acid-resistance assay
  – Lactic acid (pH 4.0 and 5.0)

• Cross-protection assay
  – Activated lactoperoxidase and lactic acid challenge

• qRT-PCR
Does acid-adaptation of *E. coli* O157:H7 confer cross-protection against lactoperoxidase system in combination with low pH in rich media?

The effect of combined activated lactoperoxidase and acid challenge at pH levels 4 and 5 on non-adapted *E. coli* O157:H7 at 25 °C (Parry-Hanson *et al.*, 2009)
The effect of combined activated lactoperoxidase and acid challenge at pH levels 4 and 5 on acid-adapted *E. coli* O157:H7 at 25 °C (Parry-Hanson *et al.*, 2009)
Fatty acid profile of acid-adapted (A) and non-adapted (N) *E. coli* O157:H7 treated at pH levels of 4.0, 5.0 and 7.4 or with activated lactoperoxidase (LP) for 6 h at 25 °C (Parry-Hanson *et al.*, 2009) (SATFs, Saturated fatty acids; MUFAs, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acid; FAME, Fatty acid methyl esters)
qRT-PCR

- Reference gene (mdh, 16SrRNA)
qReal Time PCR

• Acid-resistance genes
  – *rpoS* (Alternative sigma factor; Cross-protection)
  – *gadA* (Glutamate decarboxylase; pH homeostasis)
  – *ompC, ompF* (outer membrane proteins)
  – *cfa* (cyclopropane fatty acid synthase)

• LP inducible gene
  – *corA* (Magnesium transporter)

• REST

Expression ratio = \[
\frac{(E_{\text{target}}^\Delta CT_{\text{target}})^{\text{Control-Sample}}}{(E_{\text{reference}}^\Delta CT_{\text{reference}})^{\text{Control-Sample}}}
\]

(Pfaffl, 2001)
Results: Non-adapted *E. coli* O157:H7 (LP)

Relative gene expression in non-acid adapted *Escherichia coli* O157:H7 challenged to lactoperoxidase system for 6 h at pH 7.4 in tryptone soy broth.
Results: Acid-adapted *E. coli* O157:H7 (LP)

Relative gene expression in acid adapted *Escherichia coli* O157:H7 challenged to lactoperoxidase system for 6 h at pH 7.4 in tryptone soy broth
Relative gene expression in acid adapted *Escherichia coli* O157:H7 challenged to lactic acid at pH 4.0 for 6 h in tryptone soy broth
Results: Acid-adapted *E. coli* O157:H7 (LP, pH 4.0)

Relative gene expression in acid adapted *Escherichia coli* O157:H7 challenged to lactoperoxidase activation in combination with lactic acid at pH 4.0 for 6 h in tryptone soy broth
Conclusions

• RpoS-independent AR systems confer cross-protection of acid-adapted *E. coli* O157:H7 against LP system and low pH

• In complex media that has glucose present, the glutamate decarboxylase system protects acid adapted *E. coli* O157:H7 at least in part against cellular damage at pH 4.0

• Activation of acid-adaptation may repress the expression of *corA* in *E. coli* O157:H7 cells

• Acid resistance and cross-protection genes are expressed during acid-adaptation and not during exposure to environmental stress
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