

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



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Introduction

- AC

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From The Sunday Times

September 23, 2007

Threat from 'new E-coli'

Sarah-Kate Templeton, Health Editor

A new superbug that scientists believe is 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- One million bars of chocolate were withdrawn from British shops soon after the last decade, research has found.

Talking Point

Country Profiles

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1908 - 2008



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Escherichia coli O157:H7

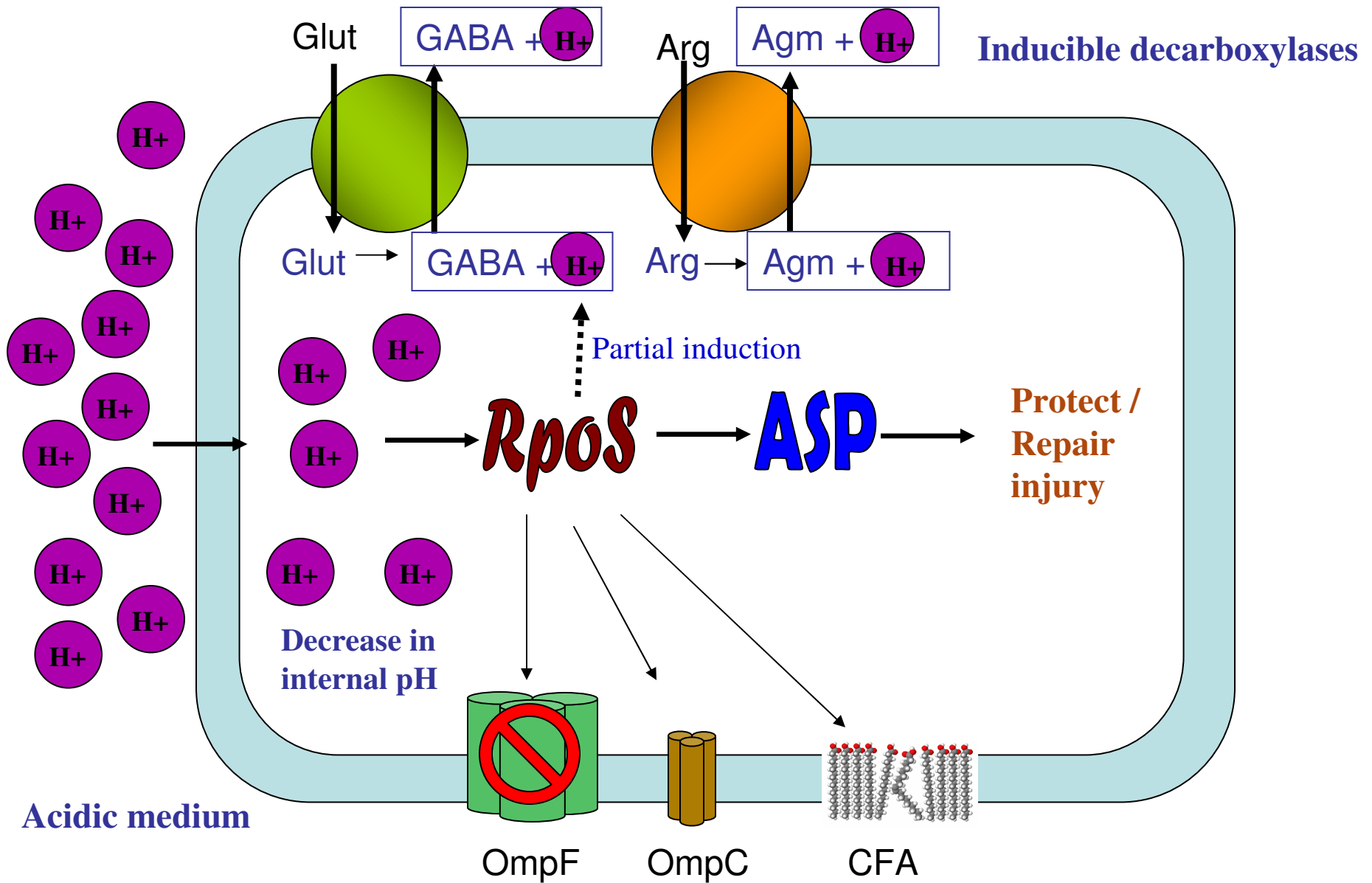
- An emerging food pathogen
 - Diarrhoea, HUS and HC
- Adapt to mild acidic environments
 - Acid-resistance (pH 1.5 to 4.5)
- Stationary phase AR systems in *E. coli*
 - Glutamate decarboxylase
 - Arginine decarboxylase
 - **Oxidative system**



E. coli O157:H7

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)

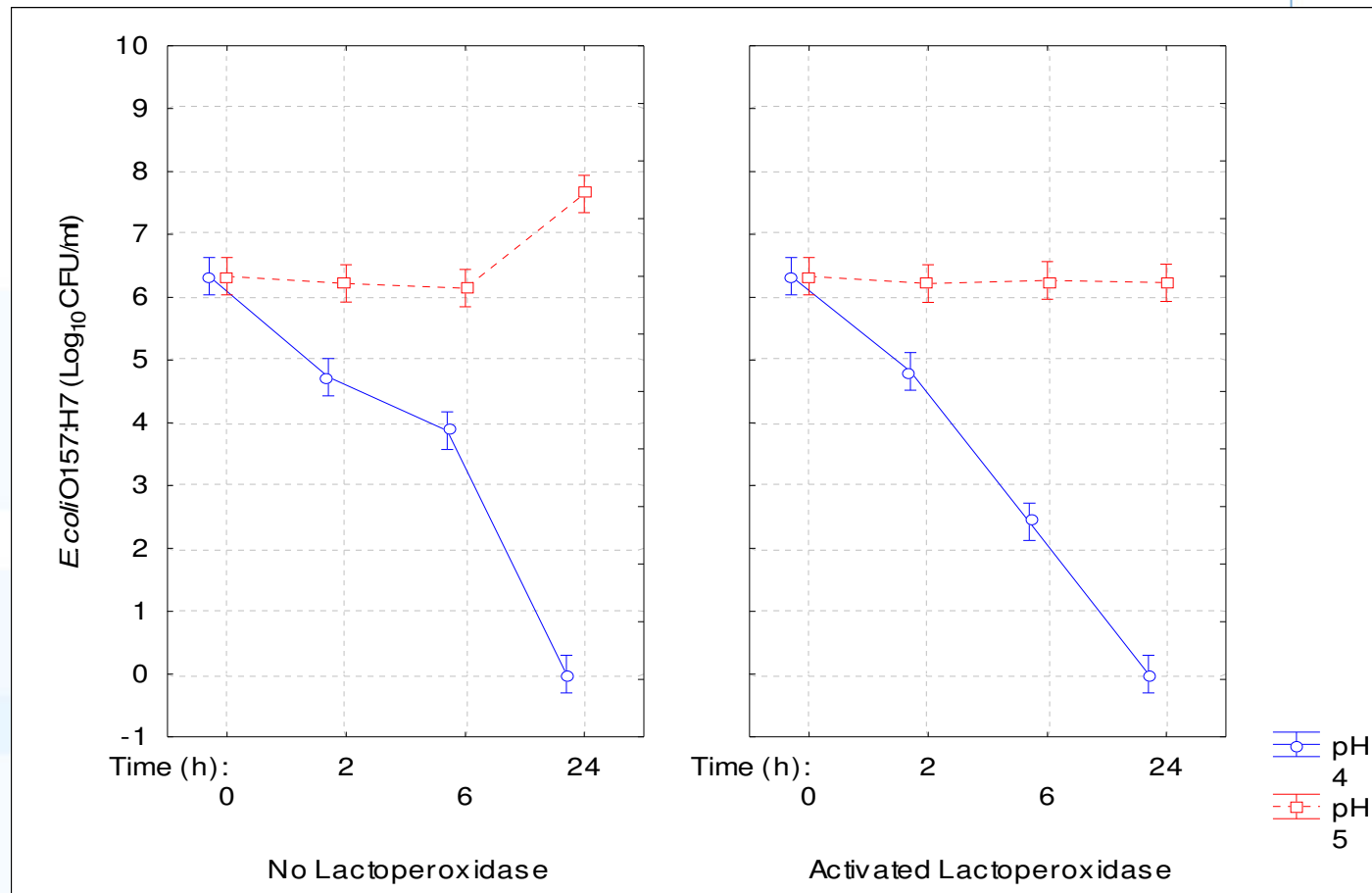
Problem statement

- 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?

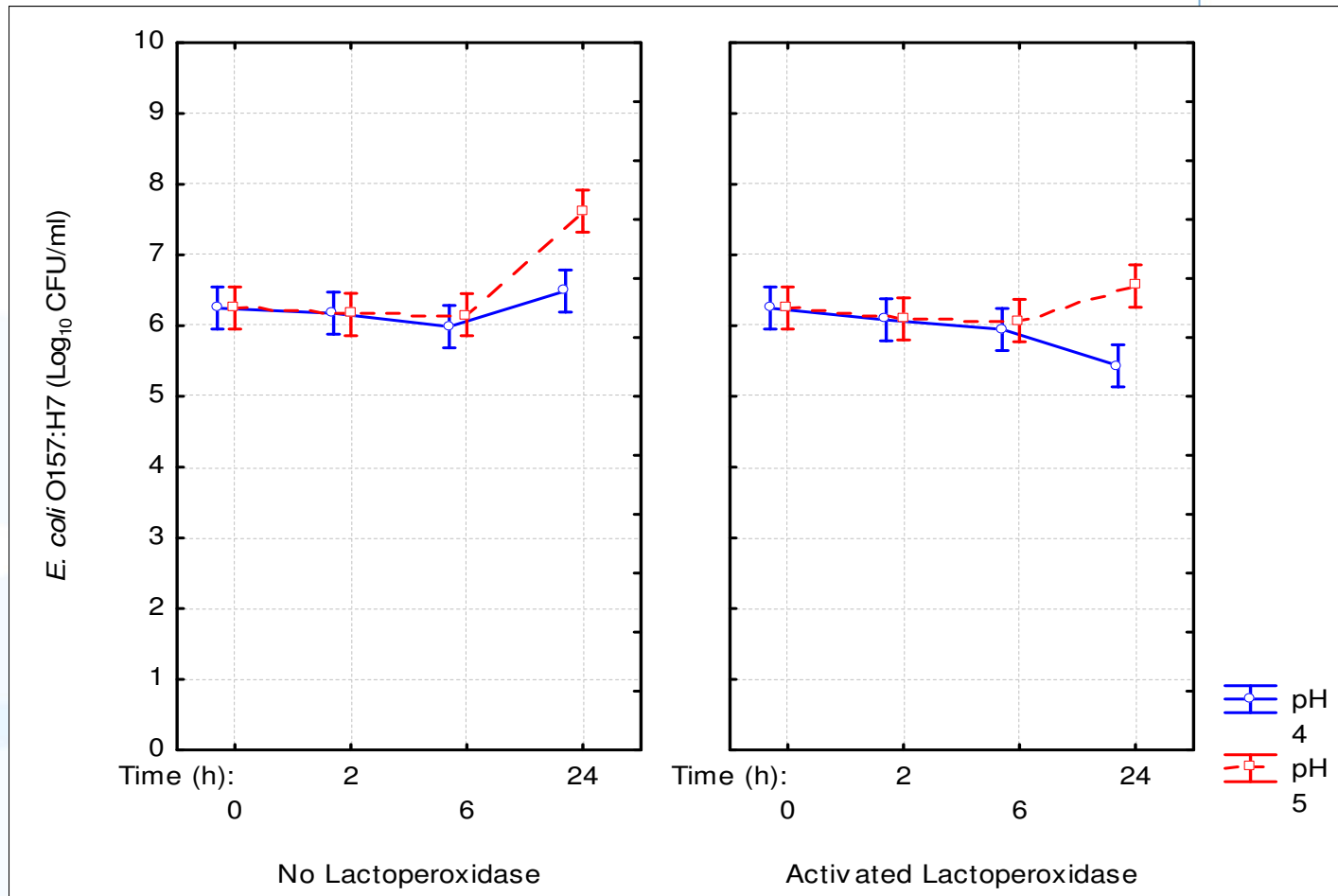
Methodology

- 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?

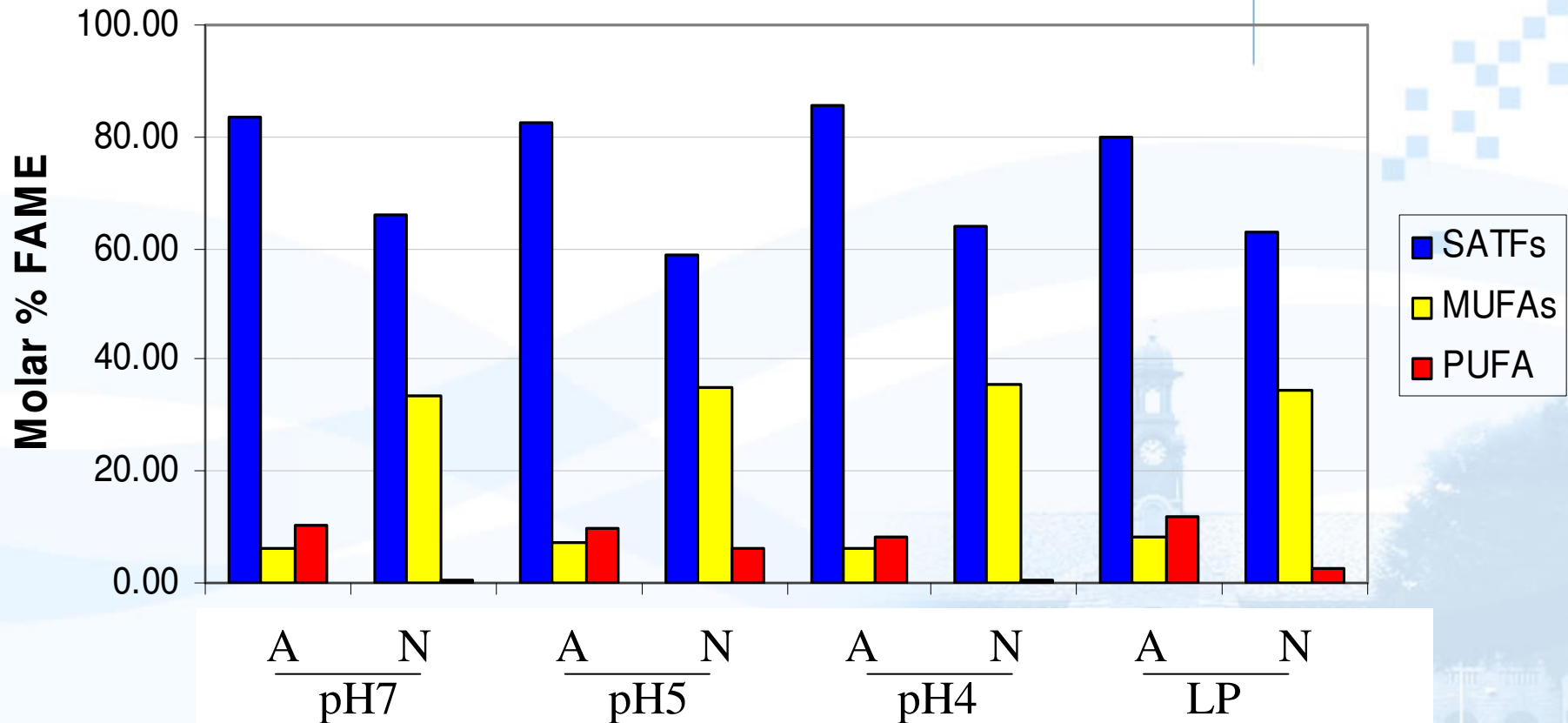


The effect of combined activated lactoperoxidase and acid challenge at pH levels 4 and 5 on non-adapted *E. coli* O157:H7 at 25 °C (Parry-Hanson *et al.*, 2009)



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)

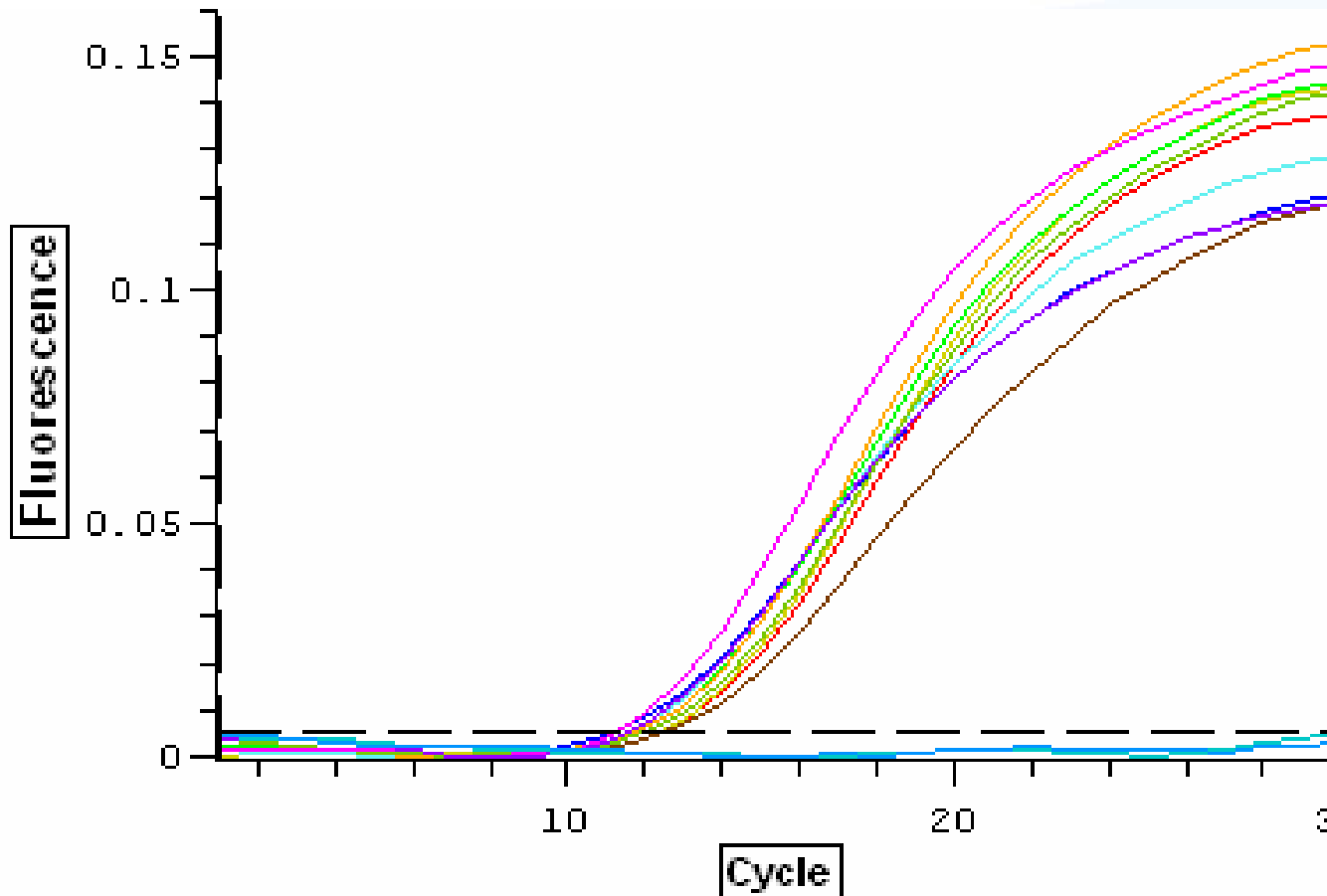
Outer membrane fatty acids



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)

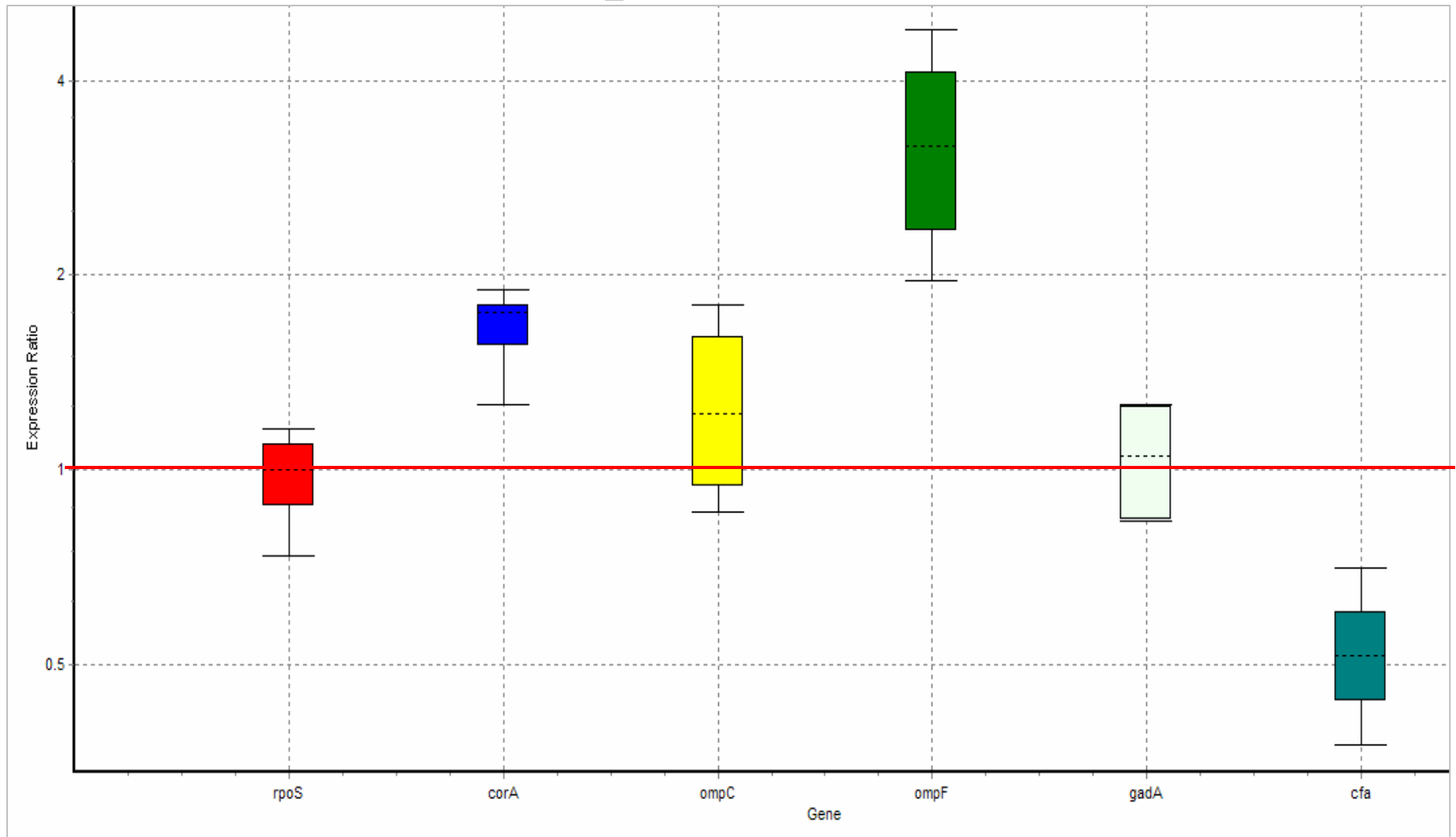


qReal Time PCR

- 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

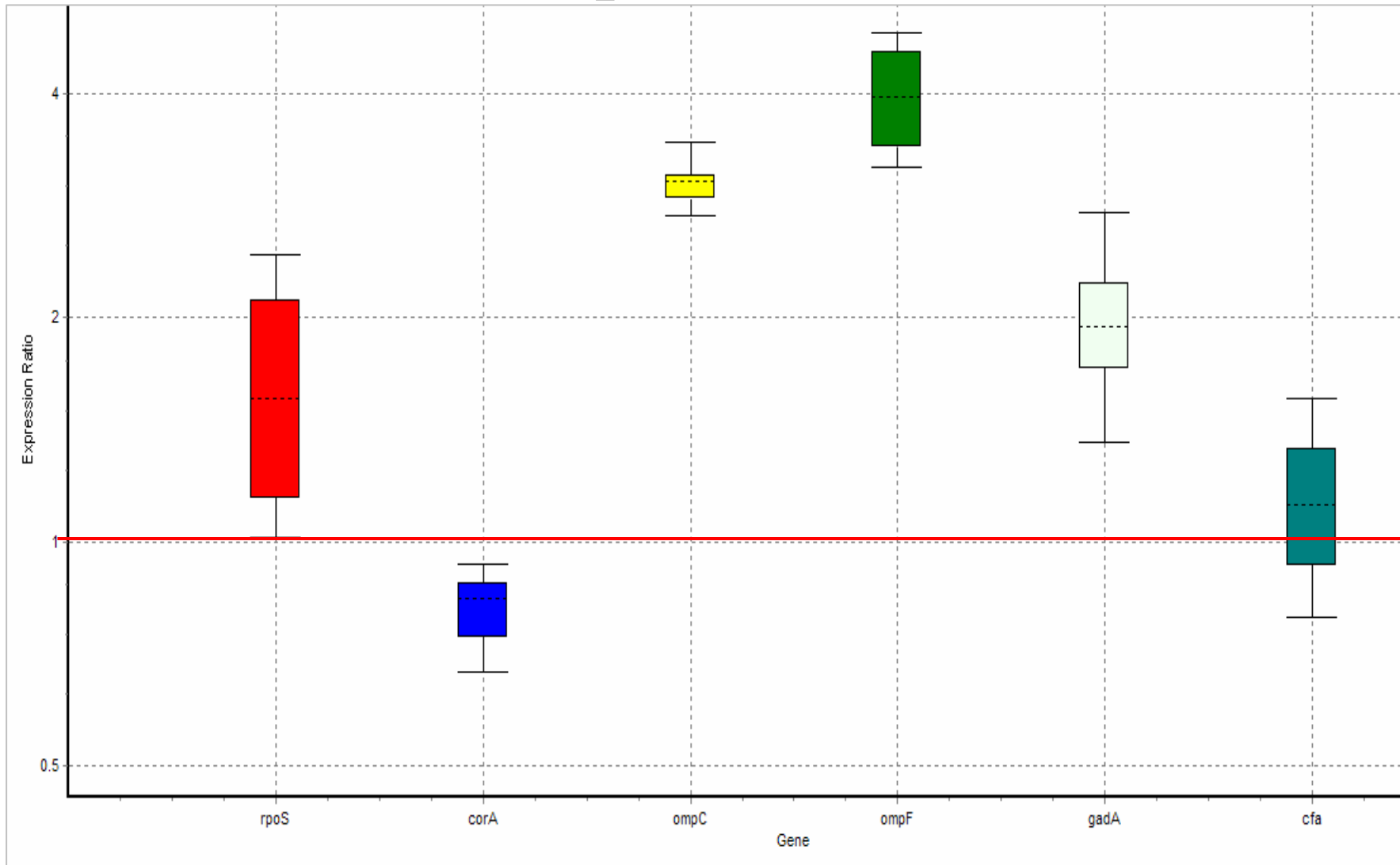
$$\text{Expression ratio} = \frac{(E_{\text{target}})^{\Delta\text{CT target (Control-Sample)}}}{(E_{\text{reference}})^{\Delta\text{CT reference (Control-Sample)}}} \quad (\text{Pfaffl, 2001})$$

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



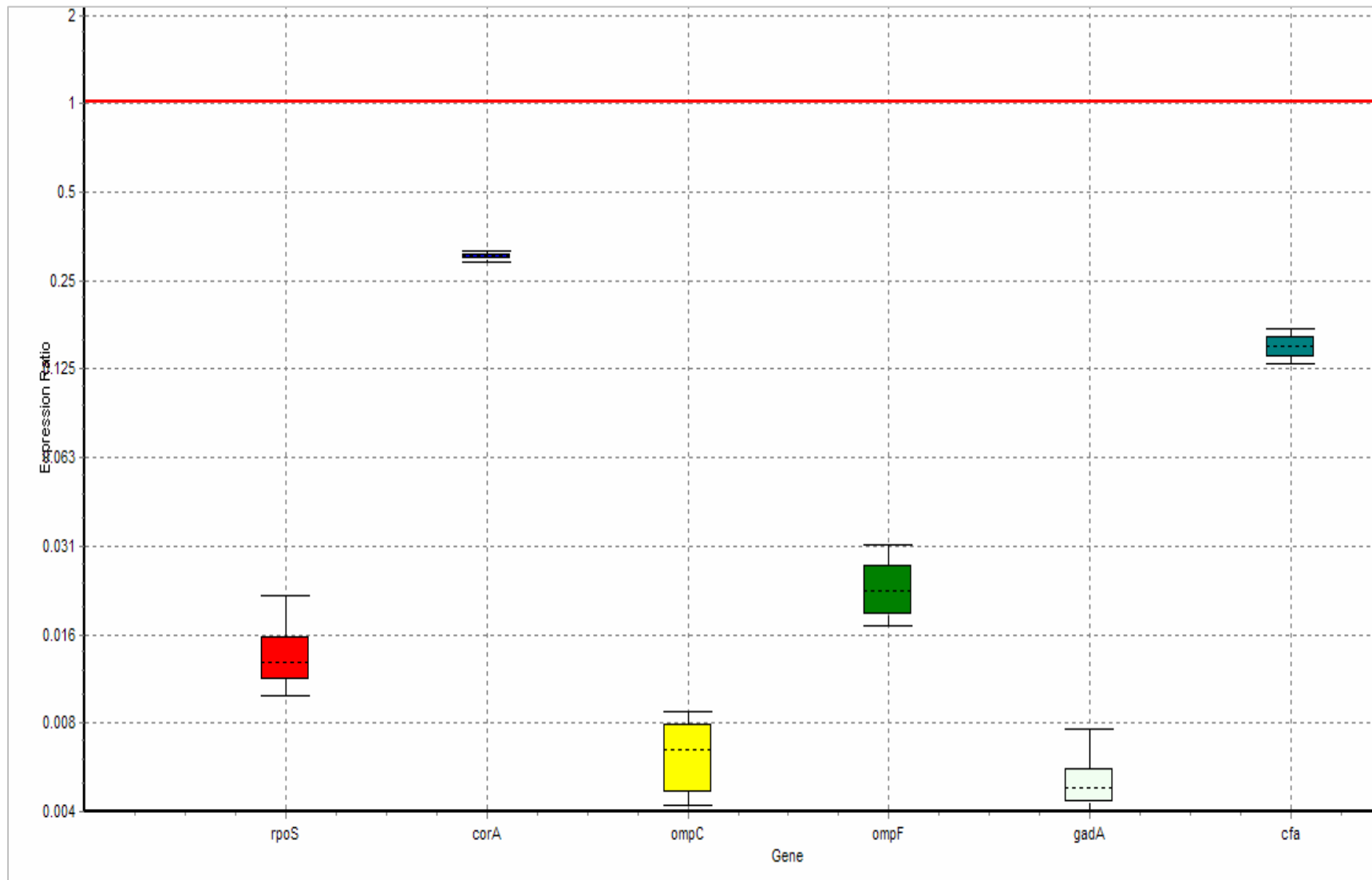
Relative gene expression in non-acid adapted *Escherichia coli* O157:H7 challenged to lactoperoxidase system for 6 h at pH 7.4 in tryptone soy broth

Results: Acid-adapted *E. coli* O157:H7 (LP)



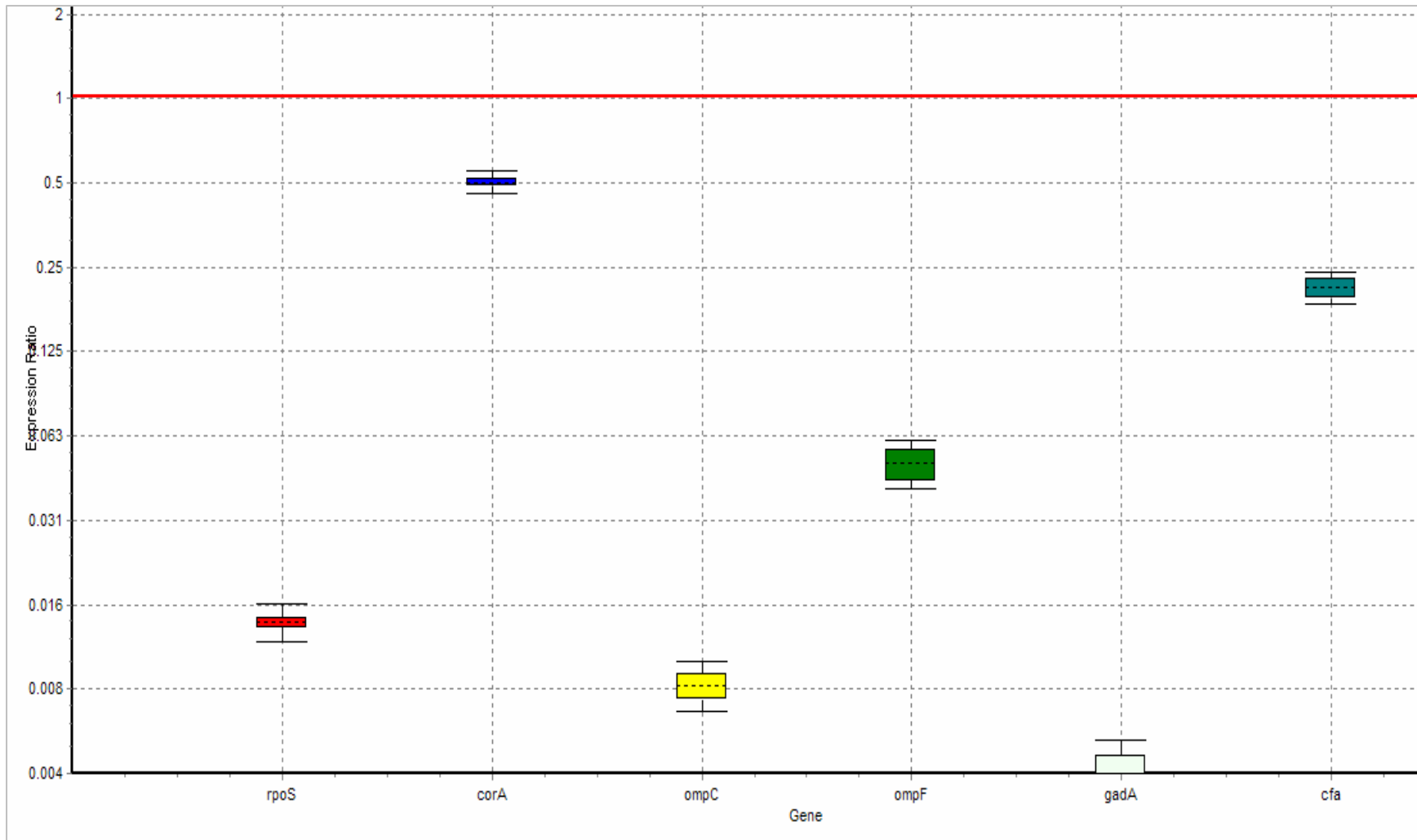
Relative gene expression in acid adapted *Escherichia coli* O157:H7 challenged to lactoperoxidase system for 6 h at pH 7.4 in tryptone soy broth

Results: Acid-adapted *E. coli* O157:H7 (pH 4.0)



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

Acknowledgement

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