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Survival of *E. coli* O157 under acidic conditions

2001

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Survival of *E. coli* O157 under acidic conditions

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EXECUTIVE SUMMARY

E. coli 0157: H7 is an important foodborne pathogen and much work has been undertaken to better understand this organism and its ability to survive in food. Acid tolerance has been identified as being an important factor in the virulence of E. coli 0157: H7 because an enhanced acid tolerance would allow this organism to survive in the acidic regions of the gastrointestinal tract. Many studies have been carried out to assess the acid tolerance of E. coli 0157: H7 and the genetic basis of this acid tolerance.

The aim of this study was to investigate the adaptation of E. *coli* 0157: H7 to different acid conditions and the effect of adaptation techniques on subsequent inactivation kinetics.

Decimal reduction values (D values) or the time taken for a log decrease were calculated for cells inoculated into 1% acetic acid, pH 2.8 broth. There was considerable variation in the acid resistance between the three strains of E. *coli* O157:H7 studied. However, it was found that adapted cells survived better than non-adapted cells when acid challenged. It was also found that the use of citric acid or lactic acid adjusted broths to adapt the cells increased the D value compared to the use of acetic or hydrochloric acids.

Variability was observed within replicates. Two trials were carried out to establish if this was due to culture differences or media batch variation. In the first trial two different cultures were acid challenged using the same batch of media and one culture was used to inoculate broths from separate media batches. It was found that replicates of each condition were similar, although there was a difference between each batch and culture type.

In the second trial, one culture was used for each batch of media in order to keep variability to a minimum. There was little difference between any of the treatments in this trial.

D values were also obtained for *Salmonella* Typhimurium (acid adapted) and non-0157 E. *coli* (acid adapted and non-acid adapted) when they were acid challenged. The D value for *Salmonella* Typhimurium was lower than that of non-0157 E. *coli* (adapted and non-adapted) or E. *coli* 0157 (non-adapted). The acid resistance of the E. *coli* (non O157:H7) strain was within the range of the E. *coli* 0 157 strains tested.

In conclusion, E. *coli* 0157 is more resistant to an acid challenge at pH 2.8 when grown in an acid environment prior to the acid challenge. Different strains of this organism vary in their ability to survive in acidic environments.

The resistance of E. *coli* 0157 to three different acid types commonly used throughout the food industry is of concern. Pre-adaptation to acid conditions enhances the resistance observed. Such conditions may well be prevalent in the factory / manufacturing environments or ecological niches within acidic products. The large variation seen in the acid resistance of E. *coli* 0157 shows the need for continued verification that the acid conditions used to achieve product safety are reviewed against current literature and challenged tested using organisms which are adapted to the relevant conditions. Failure to do this could result in an underestimation of the risks from E. *coli* 0157 within acidic products.

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1. INTRODUCTION

Escherichia coli is a microorganism that occurs naturally in the human intestine and the intestine of other warm-blooded animals. This organism is also able to survive in the environment and can be present in water, soil and on plant surfaces.

The majority of *E. coli* are harmless; however, certain serotypes of this organism are pathogenic. *E. coli* 0157: H7 is one of the **most** pathogenic of *E. coli* serotypes, causing a serious, sometimes life-threatening, illness.

E. coli 0157: H7 is an important foodborne pathogen and much work has been undertaken to better understand this organism and its ability to survive in food. Acid tolerance has been identified as being an important factor in the virulence of E. coli 0157: H7 because an enhanced acid tolerance would allow this organism to survive in the acidic regions of the gastrointestinal tract (Duffy et al, 2000). Many studies have been carried out to assess the acid tolerance of E. coli 0157: H7 (Duffy et al, 2000, Buchanan and Edelson, 1996) and the genetic basis of this acid tolerance (Cheng and Kaspar, 1998; Choi et al, 2000).

This acid resistance also has implications for the food industry as organic acids are often added to foods to enhance the flavour or safety of a product. Sometimes organic acids are produced during processes such as fermentation. These organic acids lower the pH of the product, which will increase the safety and stability of the product. However, some of these products with a low pH have still been implicated in outbreaks of E. *coli* 0157: H7 food poisoning. Fermented meat (Clavelo and Larry, 1996) and apple "ciders" (Zhao *et al*, 1993) have been associated with E. *coli* 0157: H7 outbreaks. In such cases it is thought that *E. coli* 0157: H7 was able to adapt to low pH conditions whilst present in these products. The foodstuff was then consumed and the E. *coli* 0157: H7 cells, having become more acid tolerant, were able to survive the low pH conditions of the stomach.

The aim of this study was to investigate the adaptation of E. *coli* 0157: H7 to different acid conditions and the effect of adaptation techniques on subsequent inactivation kinetics.

2. MATERIALS AND METHODS

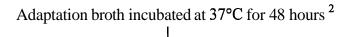
2.1 Adaptation trials

The main purpose of this study was to investigate the effect of adaptation conditions, i.e. to establish whether pre-adaptation of E. *coli* 0 157 cells to a range of acidic conditions affected their subsequent survival characteristics at a lower pH. The types of acid and pH level were investigated. In addition, the effects of batch-to-batch variation in the pH 2.8 broth and also culture to culture variations on the survival characteristics of E. *coli* 0 157 were studied.

Figure 1 summarises the main experimental protocol and the stages at which conditions were varied are indicated.

Figure 1: Evaluation of *E.* coli 0157 acid resistance

E. coli O157 cultures inoculated into relevant adaptation broths ¹



Adapted cells (lnil) inoculated into pH 2.8 acetic acid broth and survival rate determined³. This procedure was replicated up to 5 times on each occasion.

¹ Experiments used either a single culture for all replicates or separate cultures set up at the same time to assess the effect of different cell populations on resistance characteristics.

² The usual incubation time for adaptation was 48 hours, although some tests were done to evaluate the effect of incubation for 72 hours.

³ The replicate pH 2.8 acetic acid broths were either taken from one large batch of broth or from separate broths made to the same specifications to evaluate the effect of minor variations in broth composition.

2.2 Preparation of adaptation media

A range of broths were prepared and acidified with various different acid types. These were used as adaptation broths (Table 1). The basal broth was nutrient broth (NB) (Oxoid CM1); the relevant acid was added to the broth to achieve the correct pH value and the broths were then autoclaved. The final pH levels for acetic, lactic and citric acid broths were chosen as these were the minimum pH values of growth (Gonzalez et *al* 1999). For example, acetic acid is generally more antimicrobial than other organic acids and growth is limited below the level of 5.0 used in this work. Acid was added until the appropriate pH value was achieved. The pH was re-checked after autoclaving. The broths were made in 10ml volumes.

The broths were as follows:

TABLE 1
Adaptation broths

Acid type	pH	Amount of acid added (ml)	Amount of undissociated acid (ml)
Acetic	5.0	0.29	0.10 (35%)
Lactic	4.2	1.28	0.137 (11%)
Citric	4.5	NT	NT
Hydrochloric	4.0	1.37	-
Non adapted	7.0	-	-

2.3 Organisms

Three strains of E. *coli* 0157 were used in this trial:

E. coli 0157: H7 CRA 8263 isolated from apple "cider".

E. coli 0157: H7 CRA 6353 isolated from salami.

E. *coli* 0 157: H7 CRA 5183 isolated from ground beef.

These strains were taken fkom the CCFRA culture collection. The cultures are stored on beads at -80°C. Beads were transferred to NB and incubated at 37°C overnight. Nutrient agar slopes (NA, Oxoid, CM3) were prepared from this suspension. Fresh NA slopes were prepared on a regular basis.

2.4 Acid resistance studies

Each of the three strains was inoculated separately fkom NA slopes into the adaptation broths described in Table 1. These adaptation broths were then incubated at 37°C for 48-72 hours. After this time period 1ml of the adaptation broth was inoculated into 100ml NB containing 1% acetic acid and adjusted to a final pH of 2.8, using HCl. These broths were then incubated at 22°C.

The level of E. *coli* 0157 cells in the 1% acetic acid broth was then enumerated at regular time intervals up to 100 hours using 1ml duplicate NA pour plates. The plates were poured, allowed to set, inverted and incubated at 37°C for 24 hours. All resultant colonies were then counted, and the log₁₀ of surviving cells was plotted against time. A linear regression line was fitted and the decimal reduction (D) value calculated, i.e. the time taken for a one-log decrease in numbers. All adaptation conditions were evaluated in duplicate and it was apparent that there was considerable variation between replicate D values obtained.

2.5 Adaptation time

Due to the variation observed between replicates (2.3) a further trial was carried out to assess if the incubation time of the inoculum was significant as some of the experiments had used 72 instead of 48h. NB acidified with lactic acid to pH 5.0 was used. It was decided to use pH 5.0 as, at pH 4.2, growth was very slow and the high inoculum level required was not reached whilst at pH 5, a high inoculum level was reached. These broths were then inoculated with strain 8263 at a level of 10⁵ cfu/ml and incubated for either 2 or 3 days at 37°C. The D value for acid resistance was then assessed. This was carried out in quintuplicate for each incubation time.

2.6 Variation in cultures and batch of pH 2.8 broth

Further trials were carried out to assess whether variability in D values could be due to batch to batch variation in the media or due to the variability in cultures. Two trials were set up using NB acidified with lactic acid to pH 5.0. In the first trial, two different batches of the NB lactic acid broth and the 1%, pH 2.8, acetic acid broths were prepared (batches B1 & B2), and two separate cultures were used (cultures C1 & C2, strain 8263). D values were determined in triplicate.

In the second, trial two separate batches of NB acidified with lactic acid to pH 5.0 and 1%, pH 2.8, acetic acid broths were prepared and two separate cultures of strain 8263 were used. However, on this occasion each of the cultures was used to inoculate each of the batches, i.e. culture 1 was inoculated into batch 1 & 2. This minimised variation. The variables were therefore, culture 1 batch 1, culture 1 batch 2, culture 2 batch 1, culture 2 batch 2. This was carried out in triplicate.

2.7 Acid resistance of other enteric pathogens

Preliminary investigations had shown that (i) adapted cells of E. *coli* 0157 were resistant to pH 2.8 acetic acid broth and that (ii) there was considerable variation in the resistance characteristics (D values) determined under these conditions. Trials were done to determine whether similar effects were observed for other enteric organisms.

NB was prepared as in 2.1 and adjusted to pH 5.0 using lactic acid. *Salmonella* Typhimurium (CRA 1005) and non-0157 E. *coli* (NCTC 12241) were inoculated into these broths and incubated at 37°C for 48 hours. These broths were then used to inoculate NB containing 1% acetic acid adjusted to pH 2.8 by HCl. The survival of these two organisms was then monitored by enumerating levels using NA as in previous trials. The resistance of non-acid adapted E. *coli* was also evaluated.

3. RESULTS

3.1 Adaptation using different acid types

The results of the initial adaptation trials are given in Table 2 and in the Appendix (Figures 2-11). It can be seen that adapted cells survive better than non-adapted cells as the D value for non-adapted cells was lower than the D values for adapted cells. It can also be seen that the use of citric acid or lactic acid adjusted broths to adapt the cells increased the subsequent D value compared to the use of acetic or hydrochloric acids.

These data also show a large difference in the survival characteristics between each of the 3 strains tested. Strain 5183 was the most acid resistant, followed by strain 8263 and then strain 6353.

Statistical analysis (one way analysis of variance, ANOVA) was carried out on this data to assess whether there was a difference between the D values obtained by using the various broths acidified with the different acid types. This data is given in Table 3. As can be seen there are differences between some of the treatments, in particular the non-adapted cells compared to the other treatments such as citric and lactic acids. This is seen more so for strains 5183 and 6353. Strain 8263 illustrates little difference between any of the treatments; however, this is not surprising as there was considerable variability in the duplicate values obtained for any individual adaptation broth.

TABLE 2

Effect of different adaptation treatments on the sum val of three strains of *E. coli* 0157: H7. Non acid adapted and acid adapted strains of *E. coli* 0157: H7 strains were inoculated into NB + 1% acetic acid (pH 2.78) and the D values (in hours) are given below

	Strain 5183			Strain 6353		Strain 8263			
			OVERALL*		THE REAL PROPERTY.	OVERALL*			OVERALL*
Non-acid adapted	3.93	5.70	5.0	1.04	0.88	0.98	4.19	13.25*	4.2
Adapted with HCl (pH 4.0)	7.42	7.79	7.52	2.89	3.52	2.91	6.28	11.17	10.33
Adapted with acetic acid (pH 5.0)	7.89	8.71	9.09	2.63	2.39	2.51	5.78	8.05	7.63
Adapted with lactic acid (pH 4.2)	11.31	12.74	11.99	6.62	5.18	5.56	6.97*	12.6	12.58
Adapted with citric acid (pH 4.5)	13.33	13.04	13.33	3.33	3.33	3.33	10.74	6.96*	10.68

^{*,} regression line not well fitted, therefore not used in subsequent calculations

^{+,} calculated as the regression of all data points in both replicates

TABLE 3
A comparison of each treatment type for each strain indicating P values

STRAIN 5183	Non	HCl	Acetic	Lactic
Non	-	-	-	-
HC1	0.076	-	-	-
Acetic	0.070	0.383	-	-
Lactic	0.024**	0.028**	0.046*	-
Citric	0.011**	0.001***	0.008***	0.253

STRAIN 6353	Non	HCl	Acetic	Lactic
Non	-	_	-	-
HC1	0.020**	_	-	-
Acetic	· 0.009***	0.175	-	-
Lactic	0.021**	0.076	0.043*	-
Citric	0.001***	0.730	0.021**	0.070

STRAIN 8263	Non	HCl	Acetic	Lactic
Non	-	-	-	-
HC1	0.999	-	-	-
Acetic	0.736	0.571	-	-
Lactic	0.860	0.803	0.444	-
Citric	0.981	0.971	0.473	0.809

*** = significant at 99.9% confidence level

** = significant at 99% confidence level

* = significant at 95% confidence level

3.2 Varying incubation length

The results **from** this trial are given in Table **4.** They indicate that there is greater variation between replicates compared with incubation length.

TABLE 4

D values (hours) obtained using 2 different incubation lengths

	Replicate one	Replicate two
2 day	16.75	9.62
	16.47	11.55
	17.73	11.71
	16.23	9.53
	15.65	10.01
MEAN	16.57	10.48
3 day	11.76	19.31
	10.73	21.89
	11.44	21.51
	11.86	25.77
	11.95	21.32
MEAN	11.55	21.96

Statistical analysis, one way ANOVA, was also carried out on this data to assess the difference between the log reductions at a given time points, e.g. 0-5 hours, between the two replicates at each incubation length. The data used is given in Table 5. The analysis (ANOVA) indicated that there was a statistically significant difference between each of the replicates at all given incubation periods at either a 99% or 95% confidence level for all time points except for 0-5 hours for the 2-day incubation (Table 6). This analysis also indicated that there were significant differences between the incubation lengths.

TABLE 5
Log reductions at various time points for each of the two replicates at both incubation lengths

Time period (hours)		3 d	ays	2 days	
		Replicate one	Replicate two	Replicate one	Replicate two
0-5	A	1.07	0.53	0.56	0.81
_	В	1.03	0.53	0.46	0.52
-,-	C	0.88	0.48	0.49	0.39
	D	0.78	0.40	0.49	0.24
	E	0.91	0.33	0.19	0.33
5-24	A	0.90	0.28	0.364	0.81
	В	0.92	0.53	0.32	1.1
-	C	0.98	0.68	0.29	0.96
1000	D	0.90	0.56	0.34	1.19
	E	0.70	0.69	0.55	1.31
0-24	A	1.97	0.81	0.90	1.61
	В	1.95	1.1	0.78	1.60
	C	1.86	1.2	0.78	1.35
	D	1.69	0.96	0.83	1.42
	E	1.61	1.02	0.74	1.31
24-51	A	3.01	2.25	2.54	3.86
	В	3.1	1.54	0.39	2.65
-	C	2.74	1.43	0.37	2.95
-	D	2.98	1.13	2.41	3.62
	E	2.57	1.57	2.66	3.81
0-51	A	4.98	3.06	3.44	5.48
	В	5.05	2.60	3.18	4.25
	C	4.60	2.60	3.15	4.3
-	D	4.66	2.01	3.24	5.04
	E	4.18	2.60	3.41	5.12

TABLE 6
ANOVA results

3-day incubation period replicates one versus replicate two

Times	P value
0-5	0.000***
5-24	0.060*
0-24	0.000***
24-51	0.000***
0-51	0.000***

2-day incubation period replicates one versus replicate two

Times	P value
0-5	0.881
5-24	0.000***
0-24	0.000***
24-51	0.032*
0-51	0.000***

Comparison of 3 and 2 day incubation

Times	P value
0-5	0.000***
5-24	0.000***
0-24	0.0166**
24-51	0.001**
0-51	0.000***

*** = significant at 99.9 %

** = significant at 99 %

* = significant at 95 %

3.3 Media batch and culture variations

Trial 1

The D value results of the first of these trials to establish if there was a batch-to-batch media variation and a difference in the cells of different inoculation media are given in Table 7. As can be seen there were differences between each of the 4 different conditions and a difference between the 2 cultures and 2 media batches. However, each of the replicates within a set of conditions is very similar. ANOVA also illustrated that there were statistically significant differences between these variables at between **99** and 95% confidence levels (Table **8**).

TABLE 7

D Values (hours) of replicate acid challenge studies (cultures 1 and 2) using pH **5.0** (lactic acid) adaptation broth using different cultures of strain 8263 with the same batch of media

Culture 1	Culture 2
17.18	18.31
18.34	19.88
18.24	20.08

D Values (hours) of replicate acid challenge studies using pH **5.0** (lactic acid) adaptation broth using different batches of media and the same culture of strain 8263

Batch 1	Batch 2
14.51	14.12
12.97	16.6
13.75	15.63

TABLE 8
P Values of replicates using different batches of media and different cultures

Conditions	P Value
C1, C2, B1, B2	0.000***
C1, C2	0.019*
B1, B2	0.041*
C1, B1	0.000***
C1, B2	0.005***
C2, B1	0.000***
C2, B2	0.001***

C = Culture

B = Batch

= significant at 99.9%

** = significant at 99%

* = significant at 95%

Trial 2

This second trial was more tightly controlled than the first trial and the same culture was used for each batch of media in order to keep variability to a **minimm.** This further trial to investigate the difference between media batch variation and culture difference again illustrated that there were smaller differences between media batch and culture batch when only one variable was changed, e.g. batch type. These results are given in Table 9. The P values **from** the ANOVA showed that these small differences were not statistically significant (Table 10).

TABLE 9
Acid challenge D values (hours) of media and culture variation

Condition	D value
C1 B1	17.92
	15.95
	15.46
MEAN	16.44
C1 B2	17.18
	16.86
	18.15
MEAN	17.40
C2 B1	16.78
	15.87
	16.86
MEAN	16.50
C2B2	14.80
	15.77
	17.86
MEAN	16.14

C = Culture

B = Batch of media

TABLE 10
P values of culture and media batch variation trial

Conditions	P Value
C1B1, C1B2, C2B1, C2B2	0.571
C1B1, C1B2	0.322
C1B1, C2B1	0.942
C1B1, C2B2	0.814
C1B2, C2B1	0.147
C1B2, C2B2	0.272
C2B1, C2B2	0.726

C = Culture

B = Batch

The results indicate that there are no statistically significant differences between some of these conditions, indicating that variation in D values is minimised when the effects of batch errors and culture variation is controlled.

3.4 Significance of experimental procedure on variability in resistance characteristics

ANOVA (one way) was also carried out on all (44) of the D values obtained for the lactic acid adapted broths in order to establish whether the culture inoculum used, media batch and incubation time were significant. The D values are summarised in Table 11.

The ANOVA analysis (Table 12) showed that the different starting cultures had a **significant** effect at the 95% confidence level and that media batch (P value of 0.00) was significant at the 99.9% confidence level. Adaptation time (2/3 days) was shown to be **not** significant (P value of 0.424).

TABLE 11
Summary table of all the D values obtained using pH 5 lactic acid adaptation broth (2-day incubation) and an acid challenge (1% acetic acid, pH 2.8 adjusted by HCl)

D value (h)
16.75
16.47
17.73
16.23
15.65
9.62
11.55
11.71
9.53
10.01
17.18
18.34
18.24
14.51
12.97
13.75
18.31
19.88
20.08
14.12
16.6
15.63
17.9
15.9
15.5

TABLE 11 (cont.)

Trial	D value (h)
Culture 1 / batch 2	17.18
	16.87
	18.14
Culture 2 / batch 1	16.78
	15.87
	16.86
Culture 2 / batch 2	14.79
	15.77
	17.86
3 day incubation	11.76
	10.73
	11.44
	11.86
	11.95
	19.31
	21.89
	21.51
	25.77
	21.32

TABLE 12
P values of culture, media batch and incubation time for all replicates

Conditions	P Value
Culture	0.05*
Batch	0.00***
Incubation length	0.424 N/S

3.5 Survival of other enteric pathogens

Table 13 indicates the D value obtained when *Salmonella* Typhimurium (acid adapted) and non-0157 E. *coli* (acid adapted and non-acid adapted) were acid challenged. As can be seen, the D value for *Salmonella* Typhimurium was lower than that of E. *coli* (adapted and non-adapted) or E. *coli* 0157 (non-adapted). *E. coli* was seen to be approximately 2.5 times more acid resistant when acid adapted than the non-adapted E. *coli* strains, but was still not as resistant as two of the acid adapted E. *coli* 0157 strains (5183, 8263). However, the E. *coli* strain tested showed similar D values to the third strain of 0157 tested (6353).

TABLE 13 $\label{eq:table_eq} D \ values \ (hours) \ for \ Salmonella \ Typhimurium \ and \ E. \ coli \ when \ acid \ challenged \ in$ broth at pH 2.8 with 1% acetic acid

Organism	D value (h)
Lactic acid adapted S. Typhimurium	0.50
	0.55
	0.75
MEAN	0.60
Lactic acid adapted E. coli	5.78
	5.71
	5.38
MEAN	5.62
Non-adapted E. coli	2.15
	2.11
	2.07
MEAN	2.11
Non-adapted E. coli O157 (strain 5183)	5.7
	3.93
MEAN	4.82
Non-adapted <i>E. coli</i> O157 (strain 6353)	1.04
	0.88
MEAN	0.96
Non-adapted <i>E. coli</i> O157 (strain 8263)	4.19
MEAN	4.19

TABLE 14 Summary table D values (hours) for Salmonella Typhimurium and E. coli when acid challenged in broth at pH 2.8 with 1% acetic acid

Organism	Non Adapted	Adapted
E. coli	2.1	5.6
E. coli O157 (5183)	4.8	12
E. coli O157 (6353)	1.0	5.6
E. coli O157 (8263)	4.2	12.6
Salmonella Typhimurium	-	0.6

4. CONCLUSIONS

This study set out to evaluate the effect of acid adaptation on survival of E. *coli* 0157 under acidic conditions, i.e. pH 2.8 adjusted by acetic acid. Three strains of E. *coli* 0157 and 4 adaptation conditions were evaluated. There are several important conclusions that can be drawn from this work.

- (i) It is clear that E. *coli* 0157 is more resistant to an acid challenge at pH 2.8 when grown in an acid environment prior to the acid challenge. Furthermore, the technique used for acid adaptation affects the subsequent resistance characteristics to varying degrees. In this study, the subsequent resistance characteristics were placed in the following order froni highest to lowest lactic > citric > HCl > acetic. However, the concentration of acid varied.
- (ii) Different strains of this **organism** vary in their ability to survive in acidic environments. Two of the three strains (5183 and 8263) tested showed similar high resistance characteristics, e.g. D values of >7.0 hours at pH 2.8 (Table 2), whereas the third strain showed D values of < 3 hours (Table 2) for most of the conditions tested.
- (iii) This work has also illustrated that the D value of E. *coli* 0157 in pH 2.8 acetic acid broth obtained by using the same technique on different occasions can vary markedly. However, the variation between replicates carried out at the same time is usually smaller. It is not clear from this work what causes this variation, but this needs careful consideration in future studies.
- (iv) The resistance of an acid adapted non-pathogenic E. *coli* was similar to that found for one of the E. *coli* 0157 strains but it showed lower D values than the other two strains.
 - It would appear that E. *coli* (pathogenic / non-pathogenic) could be more resistant to acidic conditions following acid adaptation than some other members of the Enterobacteriaceae. For example, a strain of *Salmonella* was 6-fold to 20-fold less

resistant to pH 2.8 acetic acid broth than E. *coli* (D value of 0.6 hours). Similar data have been reported for other enteric pathogens, e.g. *Shigella flexneri* where the *D* values obtained at pH 3.5 with propionic acid were <5 minutes (Tetteh and Beuchat, 2001).

The resistance of E. *coli* 0157 to three different acid types commonly used throughout the food industry is of concern. Pre-adaptation to acid conditions enhances the resistance observed. Such conditions may well be prevalent in the factory / manufacturing environments or ecological niches within acidic products. The large variation seen in the acid resistance of E. *coli* 0157 shows the need for continued verification that the acid conditions used to achieve product safety are reviewed against current literature and challenged tested using organisms which are adapted to the relevant conditions. Failure to do this could result in an underestimation of the risks from E. *coli* 0157 within acidic products.

5. REFERENCES

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APPENDIX

