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ORIGINAL ARTICLE

Characterization of an extremely heat-resistant *Escherichia coli* obtained from a beef processing facility

E.A. Dlusskaya, L.M. McMullen and M.G. Gänzle

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada

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acid resistance, beef processing, *Escherichia coli*, heat resistance, pathogen interventions.

Correspondence
Lynn M. McMullen, Department of Agricultural, Food and Nutritional Science, University of Alberta, 4-10 Ag/For Centre, Edmonton, AB, T6G 2P5 Canada.
E-mail: lynn.mcmullen@ualberta.ca

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Abstract

Aims: This study aimed to determine the survival of *Escherichia coli* strains during steam and lactic acid decontamination interventions currently used by the beef-processing industry, and to determine their heat resistance.
Methods and Results: Strains were grouped into cocktails of five strains each differing in their RAPD patterns for subsequent identification. Steam and lactic acid treatments on meat reduced cell counts of *E. coli* strain cocktails by 90–99%. The 20 slaughter plant isolates exhibited only minor variation in their resistance to steam and lactic acid treatments but were more resistant than reference strains (three strains) or isolates from live cattle (seven strains). D_{60} values of strains from live cattle, and reference strains ranged from 0·1 to 0·5 min, in keeping with literature data. However, D_{60} values of current slaughter plant isolates ranged between 15 for *E. coli* DM18.3 and 71 min AW 1.7. Cell counts of *E. coli* AW 1.7 were reduced by <5 $\log_{10}$ CFU g^{-1} in ground beef patties cooked to an internal temperature of 71°C.
Conclusions: Strains of *E. coli* that survive cooking of ground beef to the recommended internal temperature of 71°C can be isolated from beef-processing facilities.
Significance and Impact of the Study: Pathogen interventions in current commercial beef slaughter may select for extremely heat-resistant strains of *E. coli*.

Introduction

Pathogen intervention treatments are widely used in beef processing to reduce the cell counts of pathogens on beef carcasses. Heat treatments are the most widely used interventions in beef processing and common methods of application include hot water rinses, pasteurization with saturated steam, and steam vacuuming (Morgan *et al.* 1996; Nutsch *et al.* 1997; Castillo *et al.* 1998; Gill and Bryant 2000; Minihan *et al.* 2003; Sheridan 2004; Corantin *et al.* 2005; Rajic *et al.* 2007). Carcass washes with 2–4% lactic acid are also used for beef carcass decontamination in North America. The combined use of steam pasteurization and lactic acid washes reduces bacterial counts on the surface of beef carcasses by 90–99% (Smulders and Greer 1998).

Despite the current pathogen intervention techniques used in beef processing, enterohaemorrhagic *Escherichia coli* (EHEC) remain a major concern to the meat industry and represent a potential public health risk. EHEC are etiological agents of haemorrhagic colitis, haemolytic uremic syndrome, and thrombotic thrombocytic purpura (Riley *et al.* 1983; Doyle and Schoeni 1984; Karmali 1989). The US Centre for Disease Control and Prevention estimates that 73 000 cases of illness annually are caused by Shiga toxin–producing *E. coli* in the US, resulting in 2000 hospitalizations and 60 deaths (Frenzen *et al.*, 2005). The intestines of ruminant animals, including beef and dairy cattle, are the primary source of EHEC contamination of food or water (Montenegro *et al.* 1990). Contamination of muscle tissues with EHEC during the beef slaughter process occurs primarily in the dehiding and evisceration steps (Barkocy-Gallagher *et al.* 2001;. Aslam *et al.* 2004).

The presence of generic *E. coli* on beef is considered to be an indicator for pathogenic strains in the species

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(Anonymous, 1996; Castillo *et al.* 1998). Aslam *et al.* (2004) demonstrated that strains of *E. coli* survive decontamination interventions during slaughter process and persist on processing equipment. The objectives of this research were to determine the survival of *E. coli* during interventions currently used by the beef-processing industry, and to compare the heat resistance of slaughter plant isolates of *E. coli* with *E. coli* isolated from live cattle and with laboratory collection strains.

Materials and methods

Bacterial strains and culture conditions

Bacterial strains used in this study are listed in Table 1. Twenty strains of *E. coli* were received from Agriculture and Agri-Food Canada, Lacombe Research Centre, Alberta. They were isolated from a commercial beef slaughter plant processing cattle at a line speed of 250 head per h (Aslam *et al.* 2004). Seven strains of *E. coli* were isolated from the vagina or from rectal samples of dairy cows in the University of Alberta experimental farm; strain *E. coli* GGG10 was isolated from a commercial slaughter plant about 15 years ago, prior to the commercial use of decontamination interventions. *Escherichia coli* K-12 and *E. coli* ATCC 25922 served as reference strains. The taxonomic position of all strains was established or confirmed by a combination of biochemical and molecular methods (Table 1).

Prior to the experiments, bacterial strains were cultured for 24 h on mEndo agar LES (Endo agar; Difco, Becton Dickinson, Sparks, MD, USA) subcultured for 24 h on Luria Bertani (LB) agar [LB broth (Difco) + 15 g l^{-1} of agar], inoculated into 50 ml of LB broth in 250-ml Erlenmeyer flasks and incubated overnight at 37°C with shaking (120 rev min^{-1}). All experiments were performed with overnight cultures in the stationary phase of growth.

DNA isolation

Genomic DNA was used in all PCR-based methods. Cells from 1 ml of overnight cultures were centrifuged at 6800 ***g*** for 7 min. DNA was extracted using DNeasy Tissue kit (Qiagen, Mississauga, ON, Canada) according to instructions of the manufacturer.

Species-specific PCR and detection of gene coding for shiga-like toxins

To confirm the taxonomic position of presumptive *E. coli*, primers ECP79F-ECR620R were chosen (Sabat *et al.* 2000). The thermal cycling programme was carried out as follows: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 45 s, and extension at 72°C for 1·5 min, final extension at 72°C for 5 min. PCR analyses for shiga toxin genes (*stx*1 and *stx*2) were carried out as described previously (Gannon *et al.* 1992).

Random amplified polymorphic DNA analysis

Strain typing was performed by RAPD analysis with the DAF4 primer (Vogel *et al.* 2000). The thermal cycling programme was carried out as follows: 94°C for 3 min; 3 cycles at 94°C for 5 min, at 35°C for 5 min, at 72°C for 5 min; 32 cycles at 94°C for 30 s, at 45°C for 2 min, at 72°C for 3 min; final extension at 72°C for 7 min.

Sequencing

PCR for sequencing was performed in total volume of 150 μl (3 × 50 μl). The reaction mixture (50 μl) contained: 1× *Pfu* buffer (*Pfu* Turbo polymerase; Stratagene, La Jolla, CA, USA), primers (30 pmol each), 200 mmol l^{-1} each of dNTPs, 0·3 μl of Taq/Pfu Polymerases mixture (4 : 1), 1 μl of template DNA, and MQ water to 50 μl. PCR products were purified using QIAquick PCR Purification kit (Qiagen) and sent to the DNA Core Services Lab (Department of Biochemistry, University of Alberta) for sequencing. For 16S rDNA sequencing, primers 616F and 630R were used (Ehrmann *et al.* 2003). The *rpoH* genes were sequenced with primers RpoH2F (5′-ATTCCTACATCCGGGCAGCTAACG) and RpoH2R (5′-TTTTTTCATCGCGTTCTTTTTCCAGC). Gene sequences were analysed with Basic Local Alignment Search Tool (BLAST http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Preparation of cocktails

Cocktails that contained five strains of *E. coli* with easily distinguishable RAPD patterns were created. Strains were incubated overnight, the $OD_{600\ nm}$ of each culture was measured and an aliquot of each strain was added to the cocktail to obtain equal densities for each strain in the cocktail. To confirm recovery of all strains, 100 μl of the cocktail was serially diluted in sterile buffered peptone water (BPW) and plated onto LB agar. After 18 h of incubation, DNA from randomly chosen colonies was isolated and RAPD PCR was performed as described earlier. Twenty-six isolates were obtained from each series of the experiment, 13 each from LB agar and Endo agar, and experiments were run in duplicate. If all five strains are represented in equal cell counts in the cocktails, the probability of picking one colony of each strain at least once in 26 isolations is >99·5%.

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Table 1 Strains of *Escherichia coli* used in the research

Source	Strain no.	Identified as *E. coli* on		Taxonomic position confirmed by		
		Endo agar	TSI agar	Species-specific PCR	*rpo*H gene sequence*	16S rDNA sequence
Slaughter plant environment	MB 1.3†	+	+	+	n.d.	n.d.
	MB 2.1	+	+	+	n.d.	n.d.
	MB 3.3	+	+	+	n.d.	n.d.
	MB 3.4	+	+	+	n.d.	n.d.
	MB 10.1	+	+	+	n.d.	n.d.
	AW 1.3	−	−	+	n.d.	+
	AW 1.7	+	+	+	+	+
	AW 12.2	+	+	+	n.d.	n.d.
	GM 9.5	+	+	+	n.d.	n.d.
	GM 9.8	+	+	+	n.d.	n.d.
	GM 11.3	+	+	+	n.d.	n.d.
	GM 11.5	+	+	+	n.d.	n.d.
	GM 11.9	+	+	+	+	n.d.
	GM 12.6	+	+	+	n.d.	n.d.
	GM 16.2	+	+	+	n.d.	n.d.
	GM 16.6	+	+	+	n.d.	n.d.
	GM 16.7.2	−	−	+	n.d.	+
	GM 18.4	+	+	+	n.d.	n.d.
	GM 18.6	+	+	+	n.d.	n.d.
	DM 18.3	+	+	+	+	n.d.
Live cows (rectum)	FUA 1040	+	+	+	n.d.	n.d.
	FUA 1041	+	+	+	+	n.d.
	FUA 1044	+	+	+	n.d.	n.d.
	FUA 1045	+	+	+	n.d.	n.d.
	FUA 1048	+	+	+	n.d.	n.d.
Live cows (vagina)	FUA 1050	+	+	+	+	n.d.
	FUA 1051	+	+	+	n.d.	n.d.
Reference strains	GGG10	+	n.d.	n.d.	+	+
	ATCC 25922	+	+	+	+	n.d.
	K-12	+	+	+	100%	n.d.

n.d. not determined.

*+ indicates sequence identity of 99% or higher to the *rpoH* sequence or 16S rDNA sequence to *E. coli* K-12. For all other columns: +, typical appearance for *E. coli* on agar; − atypical appearance for *E. coli* on agar (no lactose fermentation in strains AW 1.3 and GM 16.7.2).

†The abbreviation refers to origin of strains as follows: MB, manufactured beef; AW, carcass after washing; GM, ground meat; DM, drive mechanisms (belts, rollers, *etc.*) (Aslam *et al.* 2004). FUA – strain collection of the Laboratory of Food Microbiology of the University of Alberta. GGG10, slaughter plant isolate obtained about 15 years before steam and lactic acid interventions became commercial practice.

Survival of *Escherichia coli* after decontamination treatment on meat

Aseptically cut cylinders of lean meat (surface area 4·9 cm^2) were inoculated with 100 μl of a cocktail of *E. coli* and allowed to dry at room temperature for 15 min. Samples were treated with saturated steam (98°C) for 8 or 15 s. Exposure to steam increased the surface temperature to 80°C within 2 s and to 90°C within 8 s. After steam treatment, samples were allowed to cool at room temperature for 1 min and sprayed with 2·5 ml of 4·5% (w/w) lactic acid. Samples were held at room temperature for 5 min, stomached (Stomacher Lab-Blender 400, Seward; Brinkman Instruments, Worthing, UK) for 1 min in 90 ml of BPW, and kept on ice <15 min before making serial tenfold dilutions in BPW and plating. Control samples were not subjected to steam or lactic acid treatments and were kept on ice prior plating. Samples were plated onto LB agar to count all surviving cells and onto Endo agar to count cells that were not sublethally injured (Mackey 2000) and the bactericidal effect was calculated as [log (CFU g^{-1} untreated control samples)]/[log (CFU g^{-1} treated sample)]. Experiments were done in quadruplicate. Inoculated agar plates

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were incubated overnight at 37°C prior to enumeration. Statistical analysis was performed using Student's *t*-test. To identify surviving cells, 26 isolates were subcultured from each cocktail, 13 each from LB agar and Endo agar, and typed on strain level by RAPD analysis with the DAF4 primer.

Determination of the heat and acid resistance of *Escherichia coli*

A preliminary evaluation of thermal resistance was performed with ten isolates of *E. coli* from the slaughter plant (MB 2.1; MB 3.4; AW 1.3; AW 1.7; GM 11.5; GM 11.9; GM 16.7.2; GM 18.4; GM 18.6; DM 18.3). Three 200-μl aliquots of an overnight culture for each strain were placed into 1·5-ml Eppendorf tubes (Thermo Fisher Scientific, Edmonton, AB, Canada) and heated in dry bath incubator (Thermo Fisher Scientific) at 58°C. Eppendorf tubes were removed after 5, 15 and 30 min of incubation and kept on ice before plating in triplicate. Serial dilutions in BPW were plated onto LB agar and Endo agar, plates were incubated at 37°C for 18 h and cell counts were calculated. On the basis of the preliminary evaluation of thermal resistance of slaughter plant isolates, *E. coli* strains AW 1.7 and DM 18.3 were chosen for subsequent experiments as the most heat-resistant and one of the most heat-susceptible strains among slaughter plant isolates, respectively.

To compare the heat resistance of different strains of *E. coli*, D_{60} values were determined with overnight cultures of *E. coli* strains AW 1.7, DM 18.3, K-12, GGG10, FUA 1041, and FUA 1044, grown in LB broth. Cultures were placed into sterile bags (Nasco Whirl Pak, Fort Atkinson, WI, USA), 1 ml of culture per bag per each sampling time, and the air was squeezed out prior to closing with metal tabs. Bags were fully submerged in a water bath maintained at 60°C. Control samples were not subjected to heat. Samples were removed at intervals between 0·5 and 30 min and kept on ice before plating as described earlier. Serial dilutions were plated in triplicate. The heat resistance of *E. coli* AW 1.7 was determined at 65 and 70°C. *D*-values were calculated from the slope of semi-logarithmic plots of survival curves for which at least four data points were available and were expressed as mean ± standard deviation of two independent experiments analysed in duplicate. All correlation coefficients (r^2) were >0·9. The *Z*-value for *E. coli* AW 1.7 was calculated from semi-logarithmic plots of the *D*-values *vs* temperature ($r^2 = 0{\cdot}91$).

To determine the acid resistance of *E. coli* AW 1.7 and *E. coli* GGG10, cells from overnight cultures were centrifuged at 5000 ***g*** for 15 min and resuspended in 400 mmol lactic acid in LB broth (pH 2·3). Samples were incubated at 37°C for 5 and 10 min. Decimal dilutions were immediately prepared in BPW and plated as described earlier.

Cooking of beef patties

Overnight cultures of *E. coli* AW 1.7 and *E. coli* GGG10 were centrifuged at 5000 ***g*** for 15 min, resuspended in the supernatant to 1/10 of the initial volume, and mixed with lean ground beef at a ratio of 1 ml cell suspension per 100 g meat. Ground beef patties (200 g) were made with Single Hamburger Press (Weston Brand Pragotrade, Strongsville, OH, USA). Patties were grilled on a 170°C grill and flipped every 2 min. The internal temperature was measured with Barnant t/c K thermocouple thermometer (Barnant, Barrington, USA). Patties were grilled to an internal temperature of 63°C or 71 ± 0·3°C and half of the patty was stomached in 100 ml of BPW. Decimal dilutions were made in BPW and plated on LB agar and Endo agar. Quadruplicate independent experiments were performed and samples were analysed in triplicate.

Results

Survival of *Escherichia coli* after decontamination treatment on meat

The taxonomic position of all strains of *E. coli* used in this study was established or confirmed by a combination of biochemical and molecular methods. *Escherichia coli* AW 1.3 and GM16.7.2 did not ferment lactose but were identified as *E. coli* on the basis of species-specific PCR and partial sequencing of the 16S rRNA genes (Table 1). *Escherichia coli* FUA1041, isolated from the rectum of a dairy cow, harboured the *stx*2 gene. Treatment of cocktails of *E. coli* with steam and lactic acid on meat was performed to identify individual strains with high resistance to intervention treatments. Treatment of meat inoculated with cocktails 1, 2, 3 or 4, all composed of slaughter plant isolates, with 8 s of steam, followed by spraying with 2% lactic acid reduced cell counts by about 90% and all strains in the respective cocktails were re-isolated after treatment (Table 2 and data not shown). After steam treatment for 15 s and spraying with 4·5% lactic acid solution, cell counts of cocktails 1 and 2 decreased by about 90% (Fig. 1). Cell counts on LB agar (non-selective medium) and on Endo agar (selective medium preventing growth of sublethally injured cells) were not different. All strains in cocktails 1 and 2 were re-isolated after treatment, indicating that their resistance to heat and acid treatments on meat did not differ substantially (Table 2). To compare the resistance of slaughter plant isolates with other strains of *E. coli*,

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Table 2 Frequency of recovery of strains of *Escherichia coli* from lean meat tissue after application of strain cocktails on meat and steam and lactic acid treatments

Cocktail no. and strains in the cocktail		Treatment: 8'' steam and 2% lactic acid — Incidence of recovery of each of the strains on LB agar (*n* = 13)*	Endo agar (*n* = 13)	Cocktail no. and strains in the cocktail		Treatment: 15'' steam and 4·5% lactic acid — Incidence of recovery of each of the strains on LB agar (*n* = 13)	Endo agar (*n* = 13)
No.1	MB 3.4	2	2	No.1	MB 3.4	3	1
	AW 1.3	2	3		AW 1.3	3	5
	GM 11.9	4	5		GM 11.9	2	1
	GM 16.7.2	2	1		GM 16.7.2	1	2
	GM 18.4	3	1		GM 18.4	4	4
No.2	MB 2.1	2	1	No.2	MB 2.1	1	2
	AW 1.7	1	4		AW 1.7	1	5
	GM 11.5	1	3		GM 11.5	1	1
	GM 18.6	4	2		GM 18.6	6	4
	GM 18.3	5	2		GM 18.3	4	1
No.3	GM 9.5	3	1	No.5†	ATCC 25922	0	0
	GM 11.3	5	4		GGG10	2	3
	GM 12.6	1	2		FUA 1050	6	5
	GM 16.2	2	1		FUA 1051	3	3
	GM 16.6	2	5				
No.4	MB 1.3	1	4	No.6	FUA 1040	3	1
	MB 3.3	2	2		FUA 1041	3	4
	MB 10.1	3	4		FUA 1044	1	1
	GM 9.8	3	2		FUA 1045	2	4
	AW 12.2	4	1		FUA 1048	4	3

*Cell counts of *E. coli* on meat were determined after treatment on LB and Endo agars and *n* colonies from each agar were randomly selected for determination of strain identity by RAPD typing.
†For cocktail 5, *n* = 11.

treatments were performed with cocktails 5 and 6. Cocktails 5 and 6 consisted of isolates from live cows and collection strains and their cell counts decreased by 99% and >90% respectively (Fig. 1). Surviving cells grew on Endo agar and were thus not sublethally injured (Fig. 1). The reduction of cell counts was higher ($P \leq 0{\cdot}01$) in cocktail 5 compared to cocktails 1 and 2. One strain in cocktail 5, *E. coli* ATCC 25922, was not re-isolated after treatment, indicating a high susceptibility to treatment compared to other strains in the cocktail (Table 2).

Heat and acid resistance of slaughter plant isolates of *Escherichia coli*

To determine whether the heat resistance of slaughter plant isolates contributes to their survival after steam and lactic acid treatments of meat, a preliminary evaluation of the heat tolerance of ten strains was carried out. Incubation for 5 min at 58°C reduced their cell counts by <99% (data not shown). Two strains of *E. coli* were chosen for further experiments: the most heat-resistant strain, *E. coli* AW 1.7, and one of the least heat-resistant strains, *E. coli* DM 18.3. After incubation of *E. coli* AW 1.7 and DM 18.3 at 58°C for 30 min, cell counts were reduced from 1×10^{10} and 9×10^{9} CFU ml^{-1} to 2×10^{7} and 5×10^{4} CFU ml^{-1}, respectively.

The survival of *E. coli* AW 1.7 and DM 18.3 at 60°C was compared to reference strains and isolates from live animals (Fig. 2), and the thermal death time data were used to estimate *D*-values. The *D*-value of *E. coli* K-12 was <0·2, which is in agreement with literature data (Chung *et al.* 2007). Intermediate values were obtained for isolates from live animals, and a slaughter plant isolate, *E. coli* GGG10, which was obtained prior to the introduction of steam pasteurization as intervention treatment (D_{60} 2·2 ± 0·2, <0·8, and 0·65 ± 0·01 for *E. coli* FUA1041 (STEC), FUA1044, and GGG10, respectively). D_{60} values for *E. coli* AW 1.7 and DM 18.3 were calculated as 71 ± 2 min and 15 ± 2 min, respectively. The *Z*-value for thermal inactivation of *E. coli* AW 1.7 was based on the D_{60}, D_{65} (1·1 ± 0·1 min) and D_{70} values (0·27 ± 0·04) and estimated to be 4·2°C.

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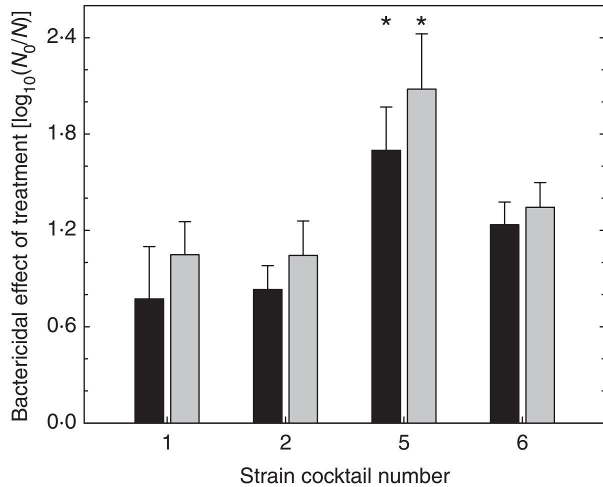

Figure 1 Bactericidal effect of pathogen intervention treatment (15 s saturated steam, followed by spray with 4·5% w/w lactic acid solution) on survival of cocktails of *Escherichia coli* strains inoculated on meat. Black bars, cell counts determined on LB agar; grey bars, cell counts determined on Endo agar. Cocktails 1 and 2 are composed of isolates from the slaughter plant environment; cocktails 5 and 6 are composed of reference strains and isolates from live animals. Refer to Table 2 for composition of each cocktail. Data are mean ± standard deviation of four independent experiments. Asterisks indicate significant difference from cocktails 1 and 2 ($P < 0{\cdot}01$).

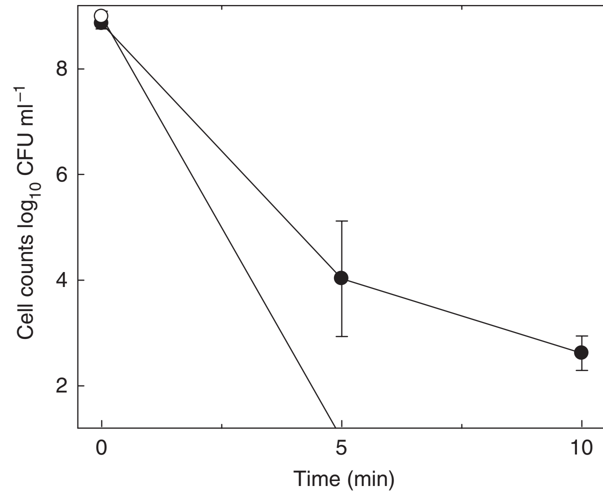

Figure 3 Cell counts of *Escherichia coli* AW 1.7 (○) and GGG10 (•) during incubation at 37°C in LB with 4% (w/w) lactic acid, resulting in a pH of 2·3. Data are mean ± standard deviation of three independent experiments. Lines dropping below the *x*-axis indicate cell counts below the detection limit (10 CFU ml^{-1}).

To determine whether the heat resistance of strains is maintained at the expense of the resistance to lactic acid, *E. coli* strains AW 1.7 and GGG10 were challenged in LB supplemented with 400 mmol l^{-1} lactic acid (pH 2·3). *Escherichia coli* AW 1.7 was less acid resistant than *E. coli* GGG10 and its cell counts were reduced to levels below the detection limit after 5 min of incubation (Fig. 3).

Survival of *Escherichia coli* after grilling of beef patties

The heat resistance of *E. coli* AW 1.7 indicated that the strain may survive in ground beef patties grilled to the internal temperature of 71°C as recommended by CFIA (Anonymous, 2009). Grilling ground beef patties inoculated with *E. coli* AW 1.7 or GGG10 revealed that both strains survived when patties were heated to internal temperature of 63°C (Fig. 4). Cell counts of *E. coli* GGG10 were two orders of magnitude lower than cell counts of *E. coli* AW 1.7. Cooking to internal temperature of 71·05 ± 0·3°C reduced cell counts of *E. coli* GGG10 to

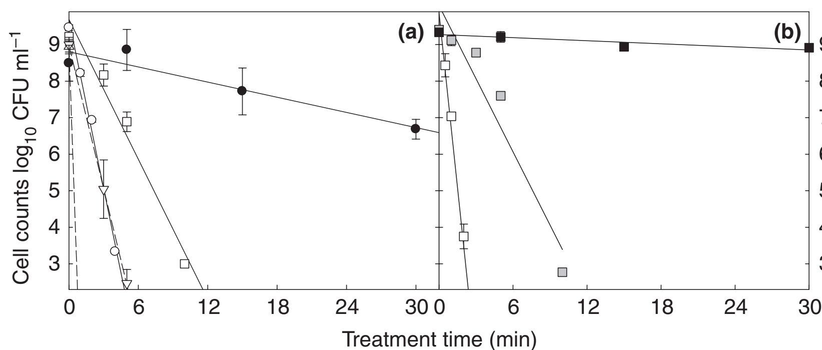

Figure 2 Cell counts of strains of *Escherichia coli* during heat treatment at 60, 65 or 70°C. Panel a: Treatment at 60°C. Strains of *E. coli*: (●) DM 18.3, (□) FUA 1041 (VTEC), (○) GGG10, (∇) FUA1044. Cell counts of *E. coli* K-12 (△) were below detection limit after 1 min of treatment. Data are mean ± standard deviation of two independent experiments analysed in triplicate. Panel b: Treatment of *E. coli* AW 1.7, isolated from the slaughter plant environment, at: (■) 60, (▩) 65 and (□) 70°C. Solid lines represent regression lines used for calculation of the *D*-values (survivor curves with four data points), dashed lines represent linear regression for survivor curves with fewer than 4 data points. Data are mean ± standard deviation of two independent experiments analysed in duplicate. Lines dropping below the *x*-axis indicate that cell counts were below the detection limit (300 CFU ml^{-1}) at the time of sampling.

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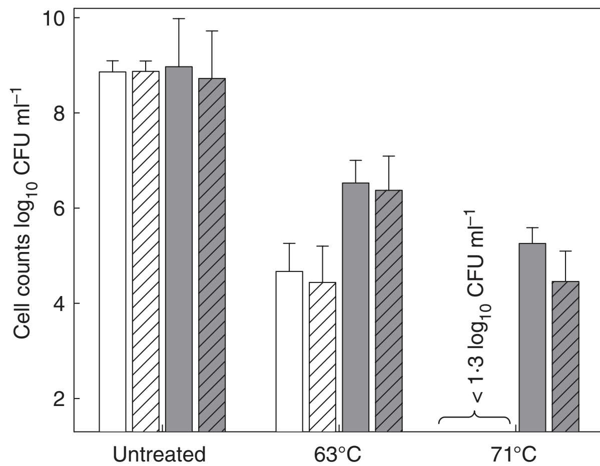

Figure 4 Cell counts of *Escherichia coli* in grilled ground beef patties heated to an internal temperature of 63°C or 71 ± 0·3°C. Data are means ± standard deviation of four independent experiments. White bars, *E. coli* GGG10; grey bars, *E. coli* AW 1.7. Solid bars, cell counts on LB agar, hatched bars, cell counts on Endo agar. Cell counts below the detection limit of 20 CFU g^{-1} are indicated.

levels below detection limit but cell counts of *E. coli* AW 1.7 were reduced by <5 $\log_{10}$ CFU g^{-1}.

Discussion

This study aimed to determine the susceptibility of *E. coli* strains to pathogen intervention treatments commonly used in the North American beef industry. Cell counts of *E. coli* were reduced by 90–99% in a laboratory-scale steam and lactic acid treatment on lean beef. Slaughter plant isolates exhibited exceptional heat resistance, and their D_{60} values were more than ten times higher than literature data for *E. coli*. *Escherichia coli* AW 1.7 survived in hamburger patties grilled to an internal temperature of 71°C.

Cocktails of *E. coli* that were used in this study encompassed isolates from a slaughter plant, isolates from live animals, reference strains and one strain that was isolated before steam and lactic intervention became commercial practice in North America. One shiga-toxin producing *E. coli* (STEC) was included in the cocktails. A polyphasic taxonomic approach that was based on biochemical characterization, species-specific PCR and sequencing of 16S rRNA genes and *rpoH* of selected isolates identified all strains unambiguously as *E. coli*.

The composition of the cocktails of *E. coli* allowed the strain-specific identification of all isolates from a cocktail on the basis of DAF4 RAPD patterns. After 15 s steam and 4·5% lactic acid treatments, cell counts were reduced by 1–2 $\log_{10}$ CFU ml^{-1}. This reduction in cell counts is in agreement with other studies on the laboratory scale and conforms to results obtained in processing plants (Prasai *et al.* 1991; Stopforth *et al.* 2004). The effects of treatments with 8 s steam and 2% lactic acid, or 15 s steam and 4·5% lactic acid were not substantially different. Similar results were reported for *E. coli* O157:H7 ATCC 43895 where cell counts on beef were reduced by 2 and 3 log units after 10 and 60 s of steam pasteurization, respectively (McCann *et al.* 2006). The resistance to steam and lactic acid treatment of the strains of *E. coli* isolated from slaughter plant environment was comparable, and all strains in the cocktails were re-isolated after treatment. The resistance of some of the reference strains and strains from live animals was significantly lower, and particularly *E. coli* ATCC 25922 was not recovered after intervention treatments, indicating a low resistance to steam and lactic acid treatment.

With exception of *E. coli* GGG10, slaughter plant isolates of *E. coli* had been exposed to steam and lactic acid interventions prior to isolation. The D_{60} value of *E. coli* AW 1.7 was more than 100 times than the D_{60} value of *E. coli* K-12 and exceeded *D*-values of strains isolated from live cows, reference strains, and *E. coli* GGG10 more than tenfold (Table 3). In comparison of *D*-values generated in this study with literature data, it must be taken into account that data compiled in Table 3 were generated with different conditions with respect to generation of precultures (stationary cells *vs* acid-adapted cells, e.g. Riordan *et al.* 2000), different heating media (e.g. phosphate buffer, Hauben *et al.* 1997; LB media, this study; and food systems, Juneja and Marmer 1999; or Weiss and Hammes 2005), as well as different heat treatments (heating in glass capillaries or heating in sealed plastic bags). However, the D_{60} value for *E. coli* K-12 reported in this

Table 3 Comparison of D_{60} values of *Escherichia coli* AW 1.7 with data reported in the literature for other strains of *E. coli*

D_{60} (min)	Strain no. (serotype)	References
0·11–0·32	K-12	This study, Chung *et al.* (2007), Jin *et al.* (2008)
0·4–1·5	LTH 5807 (O157:H⁻; Stx⁻)	Weiss and Hammes (2005)
0·47	204P (O157:H7)	Ahmed *et al.* (1995)
1·9	EDL-931, A 9218-C1, 45753-35, 933 (all O157:H7)	Juneja and Marmer (1999)
2·3	LMM1020	Hauben *et al.* (1997)
3·2	204P, 505B, 933, 932, F 501, F 585	Murfy *et al.* (2004)
4·2	ATCC 25922	Pereira *et al.* (2006)
6·7	380-94	Riordan *et al.* (2000)
15	DM 18.3	This study
71	AW 1.7	This study

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study is well in agreement with the range of *D*-values reported in the literature (Table 3), confirming exceptional heat resistance of *E. coli* AW 1.7. It is well established that the tolerance of *E. coli* and related organisms to pathogen interventions such as heat treatment, high-pressure treatments, and low pH differs substantially between strains (Metrick *et al.* 1989; Benjamin and Datta 1995; Hauben *et al.* 1997; Benito *et al.* 1999; Erkmen and Doğan 2004; Large *et al.* 2005; Tahiri *et al.* 2006). The resistance of *E. coli* O157:H7 and other shiga-toxin producing *E. coli* to heat or other environmental insults is highly variable and not generally different from that of generic *E. coli* (Table 3, Large *et al.* 2005). Remarkably, heat resistance of *E. coli* AW 1.7 was maintained at the expense of acid resistance. Pathogen intervention treatments in commercial practice include heat treatment, lactic acid sprays and fast chilling. Our data on the heat and acid resistance of *E. coli* AW 1.7 indicate that such hurdle technology is necessary to effectively reduce the number of *E. coli* on beef. Moreover, it confirms that the evaluation of pathogen intervention treatments used in the food industry should be based on a large numbers of strains that are taken from the food- processing environment.

The present study provides only indirect indication of the mechanism of the heat resistance. The induction of heat-shock proteins by sublethal thermal stress is an unlikely contributor because experiments in this study were carried out with stationary phase cells that were not exposed to sublethal heat prior to treatment. Constitutive expression of heat-shock proteins increases the pressure and heat resistance in *E. coli* LMM1030 (Hauben *et al.* 1997; Aertsen *et al.* 2004). However, this pressure and heat-resistant strain must be considered heat sensitive when compared to *E. coli* AW 1.7 (Table 3; Hauben *et al.* 1997). The σ^S-induced general stress response confers cross-resistance to acid, oxidative and heat stress, which argues against a role of σ^S in the heat resistance in *E. coli* AW 1.7 (Hengge-Aronis 2002). In addition to the general stress response or the heat-shock response of *E. coli*, past studies have identified several genes that confer increased resistance to heat. Amplification of a single master transcriptional regulator *evg*A improved survival at temperatures from 50 to 54°C (Christ and Chin 2008). Disruption of the *ubi*X gene, which is involved in ubiquinone biosynthesis, also increased heat resistance of *E. coli* (Park *et al.* 2005).

The heat resistance of slaughter plant isolates may be attributable to the selective pressure exerted by steam pasteurization in the processing environment, which eliminates all *E. coli* with the exception of few extremely heat-tolerant strains. Large beef-processing facilities can be estimated to process more than 10^{12} cells of *E. coli* per day, indicating that heat-tolerant strains will accumulate and persist even if they constitute only a minute fraction of the *E. coli* population entering the facility with live animals.

The heat-resistant strain *E. coli* AW 1.7 was not fully eliminated when hamburger patties are grilled to an internal temperature of 71°C according to CFIA guidelines (Anonymous, 2009). With the exception of the centre portion of the patties where the temperature was measured, the major portion of the hamburger patty was exposed to a temperature higher than 71°C for several minutes. The high D_{70} value (18 s) of *E. coli* AW 1.7 also questions its elimination by current pasteurization regimen applied in food production. Our work and literature data indicate that heat-resistant strains of *E. coli* constitute only a very minute fraction of organisms. However, these strains will have implications for food safety in a scenario that not only allows for their selection among the vast majority of heat-sensitive strains, as apparently is the case in beef processing, but also support re-growth of the heat-resistant strains during subsequent storage and distribution.

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