The locus of heat resistance (LHR) mediates heat resistance in *Salmonella enterica,* *Escherichia coli* and *Enterobacter cloacae*

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Abstract

Enterobacteriaceae comprise food spoilage organisms as well as food-borne pathogens including *Escherichia coli*. Heat resistance in *E. coli* was attributed to a genomic island called the locus of heat resistance (LHR). This genomic island is also present in several other genera of *Enterobacteriaceae*, but its function in the enteric pathogens *Salmonella enterica* and *Enterobacter cloacae* is unknown. This study aimed to determine the frequency of the LHR in food isolates of *E. coli*, and its influence on heat resistance in *S. enterica* and *Enterobacter* spp. Cell counts of LHR-positive strains of *E. coli*, *S. enterica* and *E. cloacae* were reduced by less than 1, 1, and 4 log (cfu/mL), respectively, after exposure to 60°C for 5 min, while cell counts of LHR-negative strains of the same species were reduced by more than 7 log (cfu/mL). Introducing an exogenous copy of the LHR into heat-sensitive enteropathogenic *E. coli* and *S. enterica* increased heat resistance to a level that was comparable to LHR-positive wild type strains. Cell counts of LHR-positive *S. enterica* were reduced by less than 1 log(cfu/mL) after heating to 60°C for 5 min. Survival of LHR-positive strains was improved by increasing the NaCl concentration from 0 to 4%. Cell counts of LHR-positive strains of *E. coli* and *S. enterica* were reduced by less than 2 log (cfu/g) in ground beef patties cooked to an internal core temperature of 71°C. This study indicates that LHR-positive *Enterobacteriaceae* pose a risk to food safety.

Keywords

Heat resistance; LHR; *Escherichia coli; Salmonella enterica* Senftenberg; food-borne pathogens; meat, undercooked burger

1. Introduction

Heat resistance of *Enterobacteriaceae* is highly variable. Heat resistance in species of *Enterobacteriaceae* results from the activity of alternative sigma factors (Dodd and Aldsworth, 2002; Noor, 2015), the presence of specialized heat shock chaperones and proteases (Arsène *et al*., 2000) and the accumulation of compatible solutes (Hengge-Aronis *et al*., 1991; Li and Gänzle, 2016; Pleitner *et al*., 2012). In *Escherichia coli*, elevated temperatures induce the expression of major heat shock proteins and molecular chaperones involved in protein folding, refolding and degradation (Noor, 2015). Additionally, the heat resistance of cultures increases upon entry to the stationary phase as a result of the activity of the alternative sigma factor σ^S (Dodd and Aldsworth, 2002). Similar mechanisms of heat resistance have been identified in related enteric species, including *Salmonella enterica* (Dodd *et al*., 2007). In addition to the inducible gene expression in response to heat shock or entry into the stationary phase of growth, *E. coli* stably adapts to growth at high temperature (Rudolph et al., 2010) and acquires increased resistance to lethal heat challenge through genomic adaptation (Vanlint et al., 2011).

E. coli AW1.7 is a food isolate with exceptional resistance to heat (Dlusskaya et al., 2011). The heat resistance of *E. coli* AW1.7 is not mediated by the σ^S regulon (Ruan *et al*., 2011) but was attributed to the 14-kb genomic island termed locus of heat resistance (LHR) (Mercer *et al*., 2015). The LHR encodes several putative heat shock proteins, proteases, and transport proteins, and is present in the genomes of 2% of all *E. coli* for which genome sequence data is available (Mercer *et al*., 2015). Fragments of the LHR were also linked to increased heat resistance in *Klebsiella pneumoniae* and *Cronobacter sakazakii* (Bojer *et al*., 2010; Gajdosova *et al*., 2011). Bioinformatic analyses also identified the LHR in *Yersinia enterocolitica*, *Citrobacter* sp. and *Enterobacter cloacae* (Mercer *et al*., 2015). LHR sequences from diverse

Enterobacteriaceae exhibit > 99% sequence identity. The high GC content and the presence of flanking mobile elements support the hypothesis that diverse species of the *Enterobacteriaceae* acquired this genomic island by horizontal gene transfer (Mercer *et al*., 2015).

Horizontal gene transfer of the LHR may allow transfer of the genomic island to pathogenic *Enterobacteriaceae* for which presence of the LHR has not yet been reported. Food-borne pathogens in the *Enterobacteriaceae* include *S. enterica* and Shiga-toxin producing *Escherichia coli* (STEC) (Scallan *et al*., 2011). In Canada, *Salmonella* and O157 STEC are estimated to be responsible for 30% of hospitalizations and 24% of deaths associated with food-borne illness, annually (Government of Canada, 2015). In food production and food preparation, enteric pathogens are controlled by pasteurization, steam or hot water intervention steps that are applied in production of meat, or domestic cooking of meat to an internal core temperature of 71°C (Health Canada, 2015; Minihan *et al*., 2003; Rajic *et al*., 2007; Yang et al., 2015). Strains of *E. coli* harbouring the LHR resist thermal interventions that are lethal to LHR-negative strains (Dlusskaya *et al*., 2011). The heat resistance of *E. coli* and *S. enterica* increases with increasing NaCl concentrations (Blackburn *et al*., 1997; Juneja *et al*., 2003; Pleitner et al., 2012). The effect of NaCl on heat resistance of the LHR positive *E. coli* AW1.7 was linked to increased accumulation of compatible solutes (Pleitner *et al*., 2012); however, the effect of NaCl on heat resistance of other LHR-positive *Enterobacteriaceae* has not been described.

The role of the LHR in heat resistance of *S. enterica*, *Enterobacter* spp. and pathogenic strains of *E. coli* has not been reported. Therefore, this this study aimed to investigate the effect of the LHR on heat resistance of several members of *Enterobacteriaceae*, the effect of NaCl on heat resistance of LHR-positive *Enterobacteriaceae*, and their survival after cooking in ground beef.

2. **Material and Methods**

2.1. Bacterial strains, plasmids and culture conditions

Strains of *E. coli*, *S. enterica,* and *Enterobacter* spp. used in this study are listed in Table 1. For this study, we selected the heat resistant *S. enterica* Senftenberg (Ng et al., 1969) and a heat-sensitive reference strain of *S. enterica*, and strains of *Enterobacter cloacae* from a collection of coliforms previously isolated from a beef processing plant (Aslam et al. 2004). Additionally, a total of 92 DNA samples of *E. coli* from a meat processing facility were screened for the LHR genotype. All four LHR-positive and four LHR-negative strains were obtained for further experimental analysis. Unless otherwise noted, strains were cultured at 37°C in Luria-Bertani (LB) media, which contains 1% NaCl (w/v). Media were supplemented with 15 µg/mL tetracycline-HCl when necessary for plasmid selection. For experiments determining the effect of NaCl on LHR-mediated resistance, LB media with addition of 0, 2 or 4% NaCl were also used. Plasmids and primers are listed in Table 2. The recombinant plasmids pRK767, pLHR and pLHR1-2 were transformed into wild type strains by electroporation and the transformed strains were plated on LB media containing 15 mg/L tetracycline-HCl (Mercer et al., 2015). The taxonomic position of *E. coli* strains was confirmed by PCR targeting the β-glucuronidase gene for *E. coli* (Yang et al, 2011; Table 2). The identity of other species was confirmed by PCR amplification and Sanger sequencing of genes coding for 16S rRNA by service of Macrogen (Rockville, MD), followed by sequence analysis using the ribosomal database project release 11 (http://rdp.cme.msu.edu/).

2.2. PCR screening to determine the presence of the LHR in *Enterobacteriaceae*

To identify LHR-positive strains, 3 target regions of the LHR were amplified by PCR as previously described (Mercer *et al*., 2015). Primer pairs HR-F1/HS-R1, HR-F2.2/HR-R2 and HS-F1 and HR-R3 (Table 2) were used in PCR reactions with a recombinant Taq DNA polymerase (Invitrogen, Burlington, Ontario). Genomic DNA from 92 strains of *E. coli* was used as templates for screening. These strains were selected to represent the diversity of more than 400 isolates that were previously obtained from a beef-processing facility (Yang *et al.*, 2015). *E. coli* AW1.7 and *E. coli* AW1.7ΔpHR1 (Pleitner *et al*., 2012) were used as LHR-positive and -negative controls, respectively. Colony PCR with the same primers was used to confirm the LHR genotypes for strains of *S. enterica* and *E. cloacae*.

2.3. Heat inactivation in laboratory media

Heat inactivation was used to determine the level of resistance for each strain as previously described, using 60°C as challenge temperature that allows straightforward differentiation of heat resistant and heat sensitive strains (Dlusskaya *et al*. 2011; Mercer *et al*., 2015). Cultures were grown overnight in LB broth containing 0, 1, 2 or 4% NaCl at 37°C with 200 rpm agitation (Pleitner et al., 2012). Heat treatments were performed at 60°C for 5, 10 or 20 min. For each experiment, *E. coli* AW1.7 and *E. coli* AW1.7ΔpHR1 were used as LHR-positive and -negative controls, respectively. The reduction in cell counts was determined in three biological replicates. Statistically significant differences (p-value < 0.05) were determined by analysis of variance (ANOVA).

2.4. Heat inactivation in ground beef patties

Ground beef was aseptically prepared from beef rounds with 4% fat that were obtained from a federally inspected beef-processing facility. Cell counts of uninoculated beef patties were

135 determined by mixing 200 g of ground beef with 200 mL of 0.1% buffered peptone water
136 (composition per litre: 10 g peptone, 3.5 g Na_2HPO_2, 1.5 g KH_2PO_4, 5 g NaCl) in a stomacher
137 and plating 100 µL on plate count agar [PCA; BD Difco™, Mississauga, Ontario, Canada] and
138 violet-red bile agar (VRBA; BD Difco™), followed by incubation at 37°C for quantification of
139 total aerobic plate counts and coliform bacteria, respectively. Total aerobic plate counts and
140 counts of coliform bacteria were less than 3000 and 50 cfu/g, respectively.

141 Ground beef was inoculated with strains of *E. coli*, *E. cloacae*, or *S. enterica* by mixing
142 approximately 10 mL of overnight cultures in LB with 200 g of refrigerated ground beef, and
143 massaging in a sterile bag by hand for 2 minutes. To determine the initial count of each sample,
144 20 g were removed and diluted with 200 ml of buffered peptone water and stomached for 2 min
145 using a Seward Lab Blender 400 (Seward Worthing, UK). The resulting solution was serially
146 diluted in buffered peptone water, plated on LB agar and the plates were incubated overnight at
147 37°C. The initial cell count was about 10^7 cfu/g. The remaining 180 g of ground beef was formed
148 into a patty with a diameter of 11.5 cm using a Single Hamburger Press (Weston Brand
149 Pragotrade, Strongsville, OH, USA). The patty was cooked on a grill (Cuisinart, Woodbridge,
150 Ontario) that was preheated to medium heat for at least 20 min to a temperature of 130 - 140°C.
151 The temperature of the patty was monitored with a Barnant Type K thermocouple thermometer
152 (Barnant, Barrington, USA) that was inserted in the geometric centre of the patty. Once the
153 internal temperature reached 71°C, the patty was removed, placed in 200 mL of iced buffered
154 peptone water, and stomached for 2 min. The solution was serially diluted, plated on LB agar
155 and incubated overnight at 37°C. Cell counts of samples before and after treatment were
156 determined in three replicate experiments and each experiment was analysed in duplicate.
157 Statistically significant differences (p-value < 0.05) were determined by ANOVA.

158 3. **Results**

159 **3.1. The locus of heat resistance (LHR) provides heat resistance to diverse *E. coli***

160 To determine the frequency of LHR positive *E. coli* present in beef-processing
161 environments, 92 isolates of *E. coli* from beef were screened by PCR. The LHR was present in 4
162 of the 92 strains (4.3%). Heat resistance of the LHR-positive strains *E. coli* 62, 68, 79, and 85
163 was compared with four LHR-negative strains that were isolated at the same time from the same
164 processing facility (Table 1 and data not shown). Heat treatment at 60°C for 5 min reduced cell
165 counts by less than 1 log(cfu/mL) for all 4 LHR-positive strains of *E. coli* (Figure 1); this
166 reduction of cell counts is similar to reductions for other LHR-positive strains (Mercer *et al*.,
167 2015). In contrast, cell counts of LHR-negative strains of *E. coli* 40, 50 60 and 70 were reduced
168 by more than 7 log (cfu/mL). None of LHR positive *E. coli* possessed plasmids large enough to
169 carry the LHR (data not shown), therefore, the LHR likely exists as a chromosomally-integrated
170 genomic island in these strains.

171 To further confirm that the LHR confers heat resistance in *E. coli*, the plasmid pLHR
172 (Mercer *et al*., 2015) was transformed into the LHR-negative *E. coli* MG1655 and the
173 enteropathogenic *E. coli* (EPEC) E2348/69. The plasmid pRK767 without the LHR served as a
174 control. The phenotype of *E. coli* carrying pLHR was consistent for both pathogenic and non-
175 pathogenic strains of *E. coli* (Figure 1). The same strains carrying the empty vector control,
176 pRK767, were similar in heat resistance to other LHR-negative strains.

177 **3.2. Sequence and function of the LHR is conserved in *Enterobacteriaceae***

178 LHR sequences are present in diverse species of *Enterobacteriaceae* (Mercer *et al*., 2015);
179 however, to date the LHR was not identified in *S. enterica*. Searching the National Center for
180 Biotechnology Information (https://www.ncbi.nlm.nih.gov/) whole genome shotgun sequence

database retrieved short, non-contiguous fragments of the LHR belonging to *S. enterica* strain ATCC 43845. Assembly of these fragments revealed 88% coverage and >99.9% sequence identity to the LHR sequence in *E. coli* AW1.7. PCR analysis of *S. enterica* ATCC 43845 with the same primers that were used for *E. coli* demonstrated that the strain carries the full length locus (data not shown). The heat resistance of the LHR-positive *S. enterica* ATCC 43845 (Fig. 2) was comparable to *E. coli* AW1.7 (Fig. 1). Conversely, the resistance of the LHR-negative *S. enteria* ATCC 13311 was comparable to heat-sensitive and LHR-negative strains of *E. coli* (Figure 2). Introducing the LHR on a plasmid into the heat sensitive strain yielded *S. enterica* ATCC 13311 pLHR, which had significantly ($p < 0.05$) increased heat resistance when compared to the wild type (Figure 2).

The PCR screening for presence of the LHR in *Enterobacteriaceae* was extended to strains of *Enterobacter* that were isolated from a beef processing plant (Aslam et al., 2004); meat isolates were compared to isolates from dairy products (Jones, 1956), or the scat of grizzly bears (Schwab *et al*., 2009) (Table 1). PCR screening identified 5 strains of *Enterobacter cloacae* carrying the LHR. Cell counts of LHR-positive *E. cloacae* were reduced by 2.0 to 3.4 log(cfu/mL) after treatment at 60°C for 5 min. Cell counts of the LHR-negative strain *E. cloacae* FUA1067 were reduced by more than 7 log (cfu/mL) (Figure 2). Introducing pLHR into the heat sensitive *E. cloacae* FUA1067 increased heat resistance and cell counts were reduced by 5 log (cfu/mL) (Figure 2).

3.3. Salt affects LHR-mediated resistance in *Enterobacteriaceae*

NaCl increased heat resistance of *E. coli* and *S. enterica* (Blackburn *et al*., 1997; Goepfert *et al*., 1970; Mattick *et al*., 2001; Pleitner *et al*., 2012). We therefore tested the effects of NaCl on resistance of LHR-positive *E. coli* and *S. enterica* to treatment in LB broth at 60°C (Figure 3).

NaCl was added to the pre-culture and the NaCl concentration was maintained for the heat treatment. Cell counts of LHR-negative strains were reduced to cell counts below the detection limit after 5 min of treatment at any NaCl concentration; corresponding to a reduction of cell counts by more than 5 log(cfu/mL) (data not shown). After 5 min of treatment at 60°C, cell counts of LHR-positive strains were reduced by less than 2 log(cfu/mL) at any NaCl concentration. However, addition of 4% NaCl significantly ($p < 0.05$) increased the survival of LHR-positive strains after 20 min of treatment at 60°C. Their cell counts were reduced by more than 6 log (cfu/mL) after 20 min at 60°C and 0% NaCl, but cell counts were reduced by less than 4 log (cfu/mL) after 20 min at 60°C and 4% NaCl (Figure 3). The plasmid pLHR1-2, which contains the native promotor of the LHR and genes coding 10 of the 16 putative proteins, also conferred heat resistance to *E. coli* AW1.7ΔpHR1, but the resistance of *E. coli* AW1.7ΔpHR1(pLHR1-2) was lower when compared to the resistance of AW1.7ΔpHR1(pLHR) at all salt concentrations (Figure 3).

3.4. Survival of LHR-positive *E. coli* and *S. enterica* in beef patties cooked to a core temperature of 71°C.

To assess the effects of the LHR on survival of *Enterobacteriaceae* during cooking of meat, LHR-positive and -negative strains were inoculated into beef patties. Patties were cooked to an internal temperature of 71°C according to Health Canada's recommended safe cooking temperature (Health Canada, 2015). Cell counts of the LHR-positive *E. coli* AW1.7, AW1.7ΔpHR1 (pLHR), and *S. enterica* ATCC 43845 were reduced by 2, 2.7 and 1.7 log (cfu/g), respectively (Figure 4), confirming that the heat resistance conferred by LHR is equivalent in *E. coli* and *S. enterica*. In contrast, cell counts of the LHR-negative *S. enterica* ATCC 13311 were reduced by more than 5 log (cfu/g); cell counts of *E. coli* AW1.7ΔpHR1 were reduced below the

detection level after cooking. Remarkably, the heat resistance in beef patties of the LHR-positive *E. cloacae* FUA1140 and the LHR-negative *E. cloacae* FUA1067 did not differ and cell counts of both strains was reduced by more than 5 log (cfu/g) (Figure 4). The improved survival of LHR-positive strains was found to be consistent in ground beef patties for *E. coli* and *S. enterica*, but differed for *E. cloacae*.

4. **Discussion**

The results of this study demonstrated that LHR-positive strains of *E. coli*, *S. enterica* and *E. cloacae* exhibit higher resistance to heat than LHR-negative strains of the same species, extending previous results obtained with *K. pneumoniae* and *C. sakazakii* (Bojer *et al*., 2010; Gajdosova *et al*., 2011). Moreover, the level of protection provided by the LHR is genus specific. LHR-positive *E. coli* and *S. enterica* are more resistant to heat than LHR-positive strains of *E. cloacae* (this study), *K. pneumoniae* (Bojer *et al*., 2010), and *C. sakazakii* (Gajdosova *et al*., 2011).

The presence of *E. coli* in processing environments is used an indicator for the presence of pathogenic strains (Castillo *et al*., 1998). The frequency of LHR-positive and heat resistant isolates that were previously obtained from a beef processing plant was determined in this study as 4.3%. Bioinformatic and genetic analysis indicate that the LHR is transferred between *Enterobacteriaceae* by horizontal gene transfer (Mercer et al., 2015). This study is the first to report LHR-mediated heat resistance in *S. enterica*; and demonstrates that the LHR mediates heat resistance in pathogenic *E. coli*. EPEC O127:H6 strain E2348/69 is a prototype strain that has been widely used to study EPEC biology, genetics and virulence (Iguchi *et al*., 2009). The wild type strain does not possess the LHR and is heat sensitive; however, transformation of the strain with a plasmid-coded LHR conferred heat resistance that is comparable to LHR-positive wild

type strains of *E. coli*. All wild type and LHR-positive *E. coli* identified to date maintain the element as a chromosomally-integrated genomic island (Mercer *et al*., 2015) while strains of *Klebsiella pneumoniae* possess plasmid-borne copies of the LHR (Bojer *et al*., 2010). Regardless of the genetic position (plasmid or chromosome), source (food or clinical isolate) or pathogenic ability (*eae*$^{+}$ or K-12 strain), LHR-positive *E. coli* demonstrate a similar and exceptional resistance to heat.

Heat resistance of *S. enterica* is highly variable from strain to strain (Lianou and Koutsoumanis, 2013). The exceptional heat resistance of the LHR-positive strain used in this study has been documented previously (Lianou and Koutsoumanis, 2013; Mañas *et al*., 2003; Murphy *et al*., 1999; Ng *et al*., 1969). *S. enterica* Senftenberg ATCC 43845 was originally described as a H_2S-negative strain capable of surviving 60°C for 5 min in liquid egg (Winter *et al*., 1946). It was suggested that heat resistance of this strain is atypical and that it should therefore not be used when constructing strain cocktails for food safety research (Juneja *et al*., 2003; Lianou *et al*., 2013; Ng *et al*., 1969; van Asselt and Zwietering, 2006). Our results indicate that the exceptional heat resistance of *S. enterica* Senftenberg ATCC 43845 is conferred by the LHR. Accordingly, introduction of the LHR into the heat sensitive *S. enterica* Typhimurium ATCC 13311 resulted in heat resistance that was comparable to the LHR-positive *S. enterica* ATCC 43845 and LHR-positive strains of *E. coli*. This is the first evidence that the LHR is the genetic determinant of unusually heat resistant strains of *S. enterica*. Due to the potential horizontal acquisition of the genomic island, these strains should be considered in future thermal inactivation studies.

Strains of *E. cloacae* are regarded as opportunistic pathogens (Hart, 2006) primarily associated with nosocomial infections (Gaston, 1988) and frequently demonstrate resistance to

multiple β-lactam antibiotics (Fung-Tomc *et al*., 1996). However, *E. cloacae* have also been frequently isolated from cattle, processing environments and retail beef (Kim and Wei, 2007). Strains used in this study were isolated from a beef processing facility and previously misidentified as *E. coli* or contaminants of strains of *E. coli* (Aslam et al., 2004). The wild type LHR-positive strains of *E. cloacae* tested in this study were significantly more heat resistant than LHR-negative strains of the same species when they were heated in broth. LHR-positive wild type strain of *E. cloacae* appeared to exhibit a higher heat resistance than a LHR-negative strain of *E. cloacae* that was transformed with pLHR; this may be attributable to the copy number of pLHR in this species. However, the difference in heat resistance between LHR-positive and LHR-negative strains was not evident when they were heated in meat; probably, the severity of the challenge exceeded the heat resistance even of LHR-positive strains.

Accumulation of compatible solutes in response to hyperosmotic conditions has been described as 'passe partout' for resistance to diverse environmental insults (Pleitner *et al*., 2012; Sleator and Hill, 2010). An increase of heat resistance in response to increased NaCl concentrations has been documented for both *E. coli* and *S. enterica* (Blackburn *et al*., 1997; Jujena *et al*., 2003; Pleitner *et al*., 2012). Our data demonstrate that the effect of NaCl on heat resistance is also observed with LHR-positive strains of both species. The LHR encodes putative transporters and proteases (Mercer et al., 2015) that may function in response to NaCl to delay thermal inactivation during prolonged periods of heat stress. Transport proteins can play a role in the accumulation of compatible substrates, while proteolytic processing of misfolded proteins is an essential part of moderating heat stress (Rosen *et al*., 2002).

Pathogen intervention methods in beef processing facilities include thermal interventions and washing or spraying with solutions of antimicrobials (Yang et al., 2015). These interventions

296 strongly reduce transfer of *E. coli* from the hide of animals to the carcass; however, *E. coli*
297 nevertheless contaminate beef products, particularly ground beef (Yang et al., 2015). Moreover,
298 improper cooking of beef or poultry provides an inadequate reduction of cell counts of
299 pathogenic *E. coli* and *S. enterica* and may contribute to foodborne disease (Liu *et al*., 2015;
300 Roccato *et al*., 2015). We demonstrate that LHR-positive pathogenic *E. coli* and *Salmonella*
301 survived cooking of meat according to safe handling and cooking label instructions. Both *E. coli*
302 and *S. enterica* that carry the LHR survived in ground beef patties cooked to an internal
303 temperature of 71°C. This temperature is currently referred to as a safe internal temperature for
304 ground beef to eliminate food-borne pathogens (Health Canada, 2015). LHR-positive pathogens
305 therefore present an additional risk to food safety. Assessment of this risk, however, requires
306 additional data on the frequency of the LHR in *E. coli* and *Salmonella*. The synergistic effect of
307 genetics (LHR) and osmotic stress (NaCl concentration) in protecting cells from thermal
308 inactivation also constitutes a novel risk to food safety that remains to be quantified.

309 We previously reported that the sequence of the LHR is highly conserved in
310 *Enterobacteriaceae* (Mercer et al., 2015). The present study demonstrates that the function of the
311 LHR is also conserved in *Enterobacteriaceae*. The LHR is a clear indicator of increased heat
312 resistance in *Enterobacteriaceae* but the magnitude of the resistance is species-dependent. The
313 presence of the LHR allows for novel and heat resistant pathogenic strains arising from
314 horizontal gene transfer amongst related species of *Enterobacteriaceae*.

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442 **Table 1. Bacterial strains used in this study.**

Strains	Description	Reference
	E. coli	
DH5α	Sub-cloning strain	Invitrogen
AW1.7	LHR-positive wild type food isolate	Dlusskaya *et al*., 2011
AW1.7ΔpHR1	LHR-negative, heat sensitive derivative of AW1.7	Pleitner *et al*., 2012
AW1.7ΔpHR1(pLHR)	Transgenic LHR-positive derivative	Mercer et al., 2015
AW1.7ΔpHR1(pLHR1-2)	Transgenic derivative with ORFs 1 – 10 of the LHR	This study
MG1655	K-12 strain	ATCC
MG1655(pLHR)	Transgenic LHR-positive derivative of MG1655	Mercer et al., 2015
MG1655(pRK767)	Vector control for MG1655 carrying pLHR	Mercer et al., 2015
EPEC O127:H6 E2348/69	Enteropathogenic *E. coli*; LEE-positive	Iguchi *et al*., 2009
EPEC O127:H6 E2348/69(pLHR)	Transgenic LHR-positive derivative of EPEC	This study
EPEC O127:H6 E2348/69(pRK767)	Vector control for EPEC carrying pLHR	This study
40	LHR-negative beef carcass isolate	Yang *et al*., 2015; this study
50	LHR-negative beef carcass isolate	Yang *et al*., 2015; this study
60	LHR-negative beef carcass isolate	Yang *et al*., 2015; this study
62	LHR-positive beef carcass isolate	Yang *et al*., 2015; this study
68	LHR-positive beef carcass isolate	Yang *et al*., 2015; this study
70	LHR-negative beef carcass isolate	Yang *et al*., 2015; this study
79	LHR-positive beef carcass isolate	Yang *et al*., 2015; this study
85	LHR-positive beef carcass isolate	Yang *et al*., 2015; this study
	S. enterica	
ATCC13311	serovar Typhimurium; LHR-negative	ATCC
ATCC13311(pLHR)	Transgenic, LHR-positive derivative of ATCC13311	This study
ATCC43845	serovar Senftenberg; LHR-positive	ATCC
	E. cloacae	
FUA1140	Isolated from equipment of beef processing facility	Aslam *et al*., 2004; this study
FUA1141	Isolated from equipment of beef processing facility	Aslam *et al*., 2004; this study
FUA1144	Isolated from equipment of beef processing facility	Aslam *et al*., 2004; this study
FUA1145	Isolated from equipment of beef processing facility	Aslam *et al*., 2004; this study
NCDO612	Subsp. *cloacae*; Dairy isolate	NCIMB
FUA1067	Isolated from bear feces	Schwab *et al*., 2009
FUA1067(pLHR)	Transgenic LHR-positive derivative of FUA1067	This study

443

444

Table 2. Plasmids and primers used in this study

Name	Description or Sequence (5' → 3')	Reference
Plasmids		
pUC19	High copy plasmid	Sigma
pRK767	Low copy plasmid	Gill and Warren, 1988
pLHR	Entire LHR, including promoter and *orfs1-16*, cloned into pRK767	Mercer *et al*., 2015
pLHR1-2	Partial LHR, including promoter and *orfs1-10* cloned into pRK767	Mercer *et al*., 2015
Primers		
HR-F1	TTAGGTACCGCTGTCCATTGCCTGA	Mercer *et al*., 2015
HS-R1	AGACCAATCAGGAAATGCTCTGGACC	Mercer *et al*., 2015
HR-F2.1	AGGGTACCAGCGATATCCGTCAATTGACT	Mercer *et al*., 2015
HR-R2	TATCTAGAATGTCATTTCTATGGAGGCATGAATCG	Mercer *et al*., 2015
HS-F1	GCAATCCTTTGCCGCAGCTATT	Mercer *et al*., 2015
HR-R3	GTCAAGCTTCTAGGGCTCGTAGTTCG	Mercer *et al*., 2015
URL-301	TGTTACGTCCTGTAGAAAGCCC	Bej *et al*., 1991
URR-432	AAAACTGCCTGGCACAGCAATT	Bej *et al*., 1991

Figure Legends

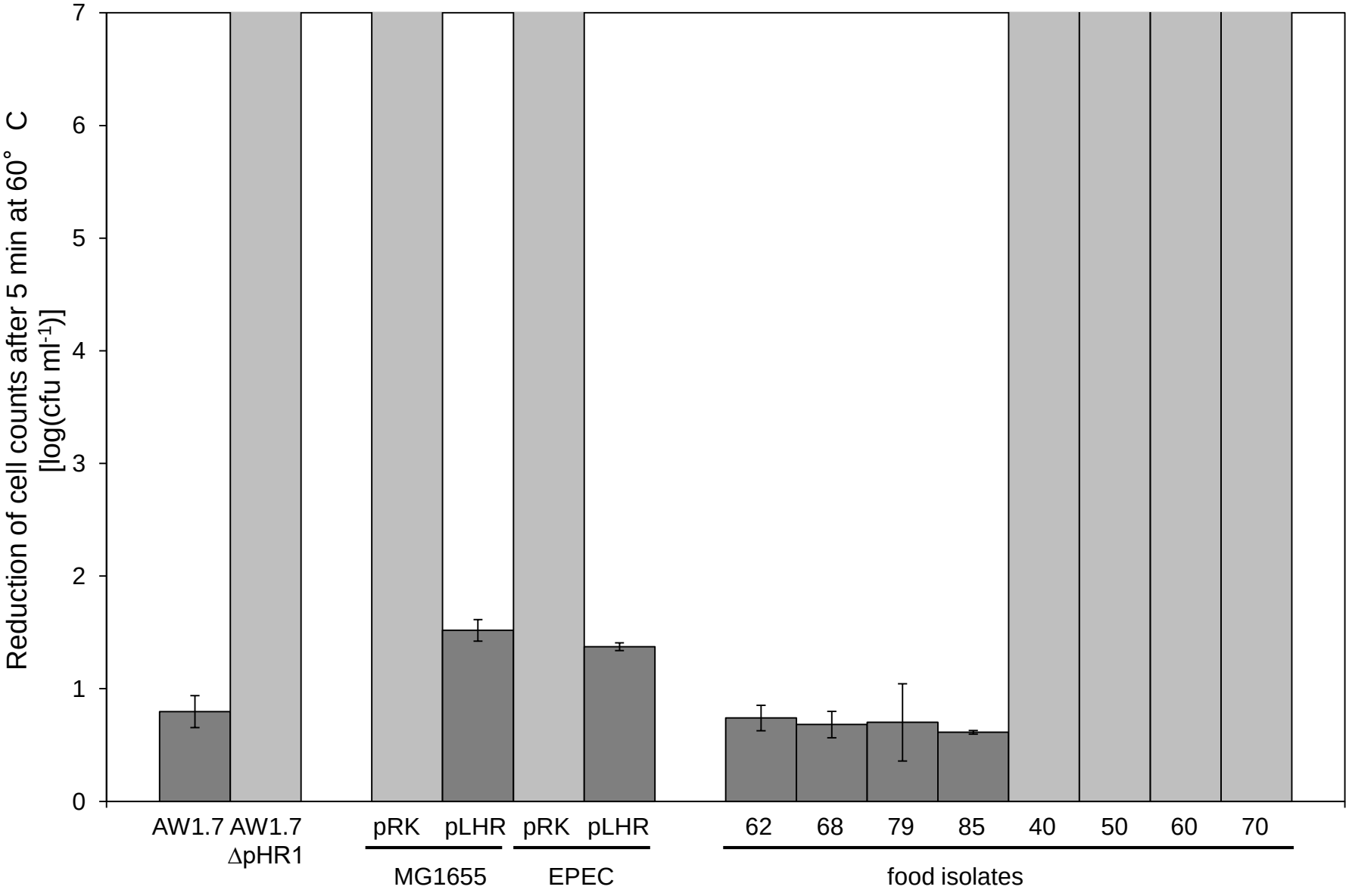
Figure 1. Reductions of cell counts of LHR-positive and LHR-negative *E. coli* heated to 60°C for 5 min in LB broth. Source (food), pathotype (K-12 or EPEC) and presence of recombinant plasmids (pRK= pRK767 or pLHR) are indicated for each strain. *E. coli* AW1.7 and AW1.7ΔpHR1 were used as LHR-positive and LHR-negative controls, respectively. Data are shown as means ± standard deviations of 3 replicate experiments. Bars representing values greater than 7 correspond to treatments that reduced cell counts to levels below the detection limit.

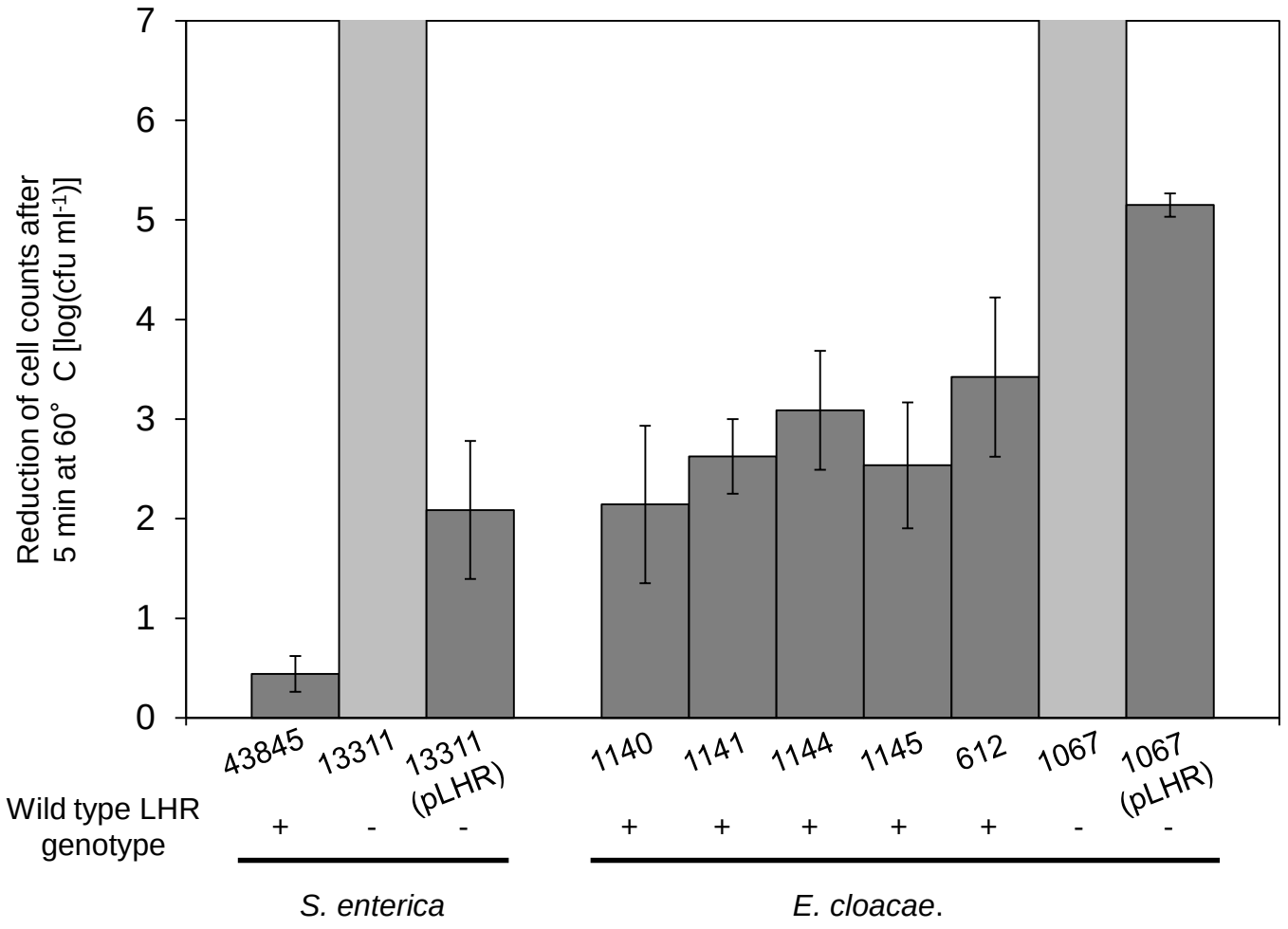
Figure 2. Reductions of cell counts of LHR-positive and LHR-negative *S. enterica* and *E. cloacae* heated to 60°C for 5 min in LB broth. The presence (+) or absence (-) of a wild type copy of the LHR, along with the presence of exogenous copies (pLHR) are indicated below each strain. Data are shown as means ± standard deviations of 3 replicate experiments. Bars representing values greater than 7 correspond to treatments that reduced cell counts to levels below the detection limit.

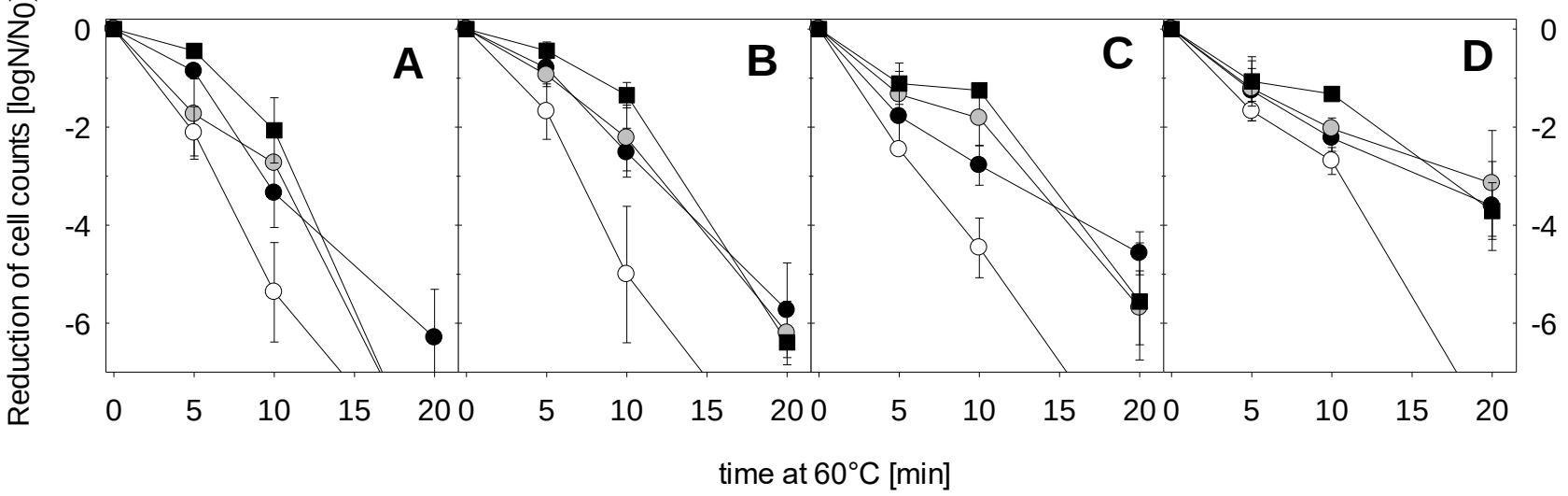
Figure 3. Reduction of cell counts of *E. coli* and *S. enterica* harbouring the complete or a partial LHR in response to NaCl concentrations. Samples of *E. coli* AW1.7 (●), *E. coli* AW1.7ΔpHR1 (pLHR) (●), *E. coli* AW1.7ΔpHR1 (pLHR1-2) (○) and *S. enterica* ATCC 43845 (■) were grown in LB media containing 0 (A), 1 (B), 2 (C) or 4 (D) % NaCl and treated at 60°C. Data are shown as means ± standard deviations of 3 replicate experiments.

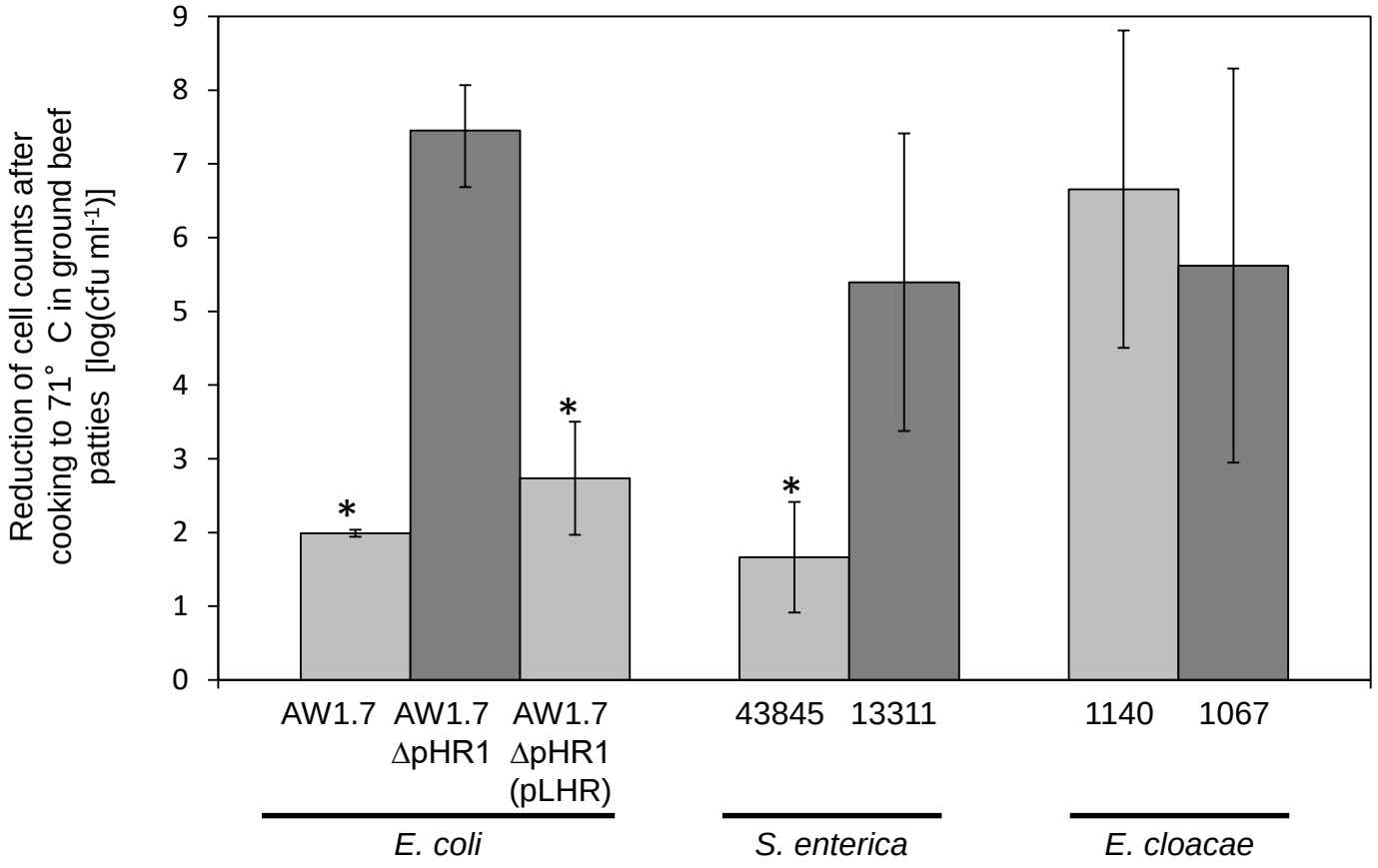
Figure 4. Thermal inactivation of *Enterobacteriaceae* in ground beef patties cooked to an internal temperature of 71°C. Reduction of cell counts for LHR-positive and LHR-negative *E. coli*, *S. enterica* and *E. cloacae* was determined with 3 biological replicates and means ±

standard deviations are shown. Statistically significant (p-value <0.05) differences between LHR-positive and LHR-negative strains of the same species are indicated by an asterisk (*).


Reduction of cell counts after 5 min at 60° C
[log(cfu ml-1)]
7
6
5
4
3
2
1
0
AW1.7
AW1.7 ΔpHR1
pRK
pLHR
pRK
pLHR
62
68
79
85
40
50
60
70
MG1655
EPEC
food isolates


Reduction of cell counts after 5 min at 60° C [log(cfu ml-1)]
7
6
5
4
3
2
1
0
43845
13311
13311 (pLHR)
1140
1141
1144
1145
612
1067
1067 (pLHR)
Wild type LHR genotype
+
-
-
+
+
+
+
+
-
-
S. enterica
E. cloacae.


Reduction of cell counts [logN/No)]
0
-2
-4
-6
A
B
C
D
0
5
10
15
20
time at 60°C [min]


Reduction of cell counts after cooking to 71° C in ground beef patties [log(cfu ml-1)]
9
8
7
6
5
4
3
2
1
0
*
*
*
AW1.7
AW1.7 ΔpHR1
AW1.7 ΔpHR1 (pLHR)
43845
13311
1140
1067
E. coli
S. enterica
E. cloacae