Food Microbiology 64 (2017) 96–103

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

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

Ryan G. Mercer [a], Brian D. Walker [a], Xianqin Yang [b], Lynn M. McMullen [a], Michael G. Gänzle [a, *]

[a] University of Alberta, Department of Agricultural, Food and Nutritional Science, Edmonton, Alberta, Canada
[b] Agriculture and Agri-Food Canada, Lacombe Research Centre, 6000 C&E Trail, Lacombe, Alberta, Canada

ARTICLE INFO

Article history:
Received 9 January 2016
Received in revised form
21 October 2016
Accepted 26 December 2016
Available online 29 December 2016

Keywords:
Heat resistance
LHR
Escherichia coli
Salmonella enterica Senftenberg
Food-borne pathogens
Meat
Undercooked burger

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.

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

* Corresponding author. University of Alberta, Dept. of Agricultural, Food and Nutritional Science, 4-10 Ag/For Centre, Edmonton, Alberta, T6G 2P5, Canada.
E-mail address: mgaenzle@ualberta.ca (M.G. Gänzle).

http://dx.doi.org/10.1016/j.fm.2016.12.018
0740-0020/

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

Ground beef was inoculated with strains of *E. coli*, *E. cloacae,* or *S. enterica* by mixing approximately 10 mL of overnight cultures in LB with 200 g of refrigerated ground beef, and massaging in a sterile bag by hand for 2 min. To determine the initial count of each sample, 20 g were removed and diluted with 200 ml of buffered peptone water and stomached for 2 min using a Seward Lab Blender 400 (Seward Worthing, UK). The resulting solution was serially diluted in buffered peptone water, plated on LB agar and the plates were incubated overnight at 37 °C. The initial cell count was about 10^7 cfu/g. The remaining 180 g of ground beef was formed into a patty with a diameter of 11.5 cm using a Single Hamburger Press (Weston Brand Pragotrade, Strongsville, OH, USA). The patty was cooked on a grill (Cuisinart, Woodbridge, Ontario) that was preheated to medium heat for at least 20 min to a temperature of 130–140 °C. The temperature of the patty was monitored with a Barnant Type K thermocouple thermometer (Barnant, Barrington, USA) that was inserted in the geometric centre of the patty. Once the internal temperature reached 71 °C, the patty was removed, placed in 200 mL of iced buffered peptone water, and stomached for 2 min. The solution was serially diluted, plated on LB agar and incubated overnight at 37 °C. Cell counts of samples before and