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THERMOTOLERANCE OF RIFAMPICIN-RESISTANT *ESCHERICHIA COLI* O157:H7 DERIVATIVES AND THEIR PARENTAL STRAINS IN A BROTH SYSTEM

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ABSTRACT

Rifampicin-resistant (Rif^R) strains have often been used in studies of *Escherichia coli* O157:H7 to assure more specific recovery. The present study compared the heat inactivation kinetics of rifampicin-resistant variants of pathogenic and nonpathogenic *E. coli* O157 strains with those of the wild-type parental strains. Spontaneous Rif^R derivatives of 11 pathogenic and six nonpathogenic *E. coli* O157 strains were selected. Stationary-phase cells of each strain in sterile tryptic soy broth were heated at 60C for 0, 30, 60, 90, 120, 150, 180 or 240 s, and aliquots were plated on tryptic soy agar supplemented with 0.1% pyruvate + 100 μg/mL rifampicin. *D* values of three pathogenic and two nonpathogenic Rif^R *E. coli* O157 strains were 35–70% lower ($P < 0.05$) than those of their wild-type counterparts. However, the heat resistance of most of the Rif^R *E. coli* O157:H7 strains (12 strains) was similar ($P \geq 0.05$) to that of their parental counterparts. Therefore, the findings of this study demonstrated that the majority of the rifampicin-resistant *E. coli* O157:H7 variants that were evaluated to assure more reliable recovery in microbial inactivation studies are suitable for use in various heat challenge studies.

PRACTICAL APPLICATIONS

More reliable media are needed for the isolation or recovery of *E. coli* O157:H7 in inoculated challenge studies. Prior to use of rifampicin-resistant *E. coli* O157:H7 strains in heat challenge studies, it is important that their thermal inactivation kinetics be compared with those of their wild-type parental strains. The results of this study showed that the thermotolerance at 60C of the majority of the tested rifampicin-resistant *E. coli* O157:H7 variants was not different than that of the corresponding wild-type strains. These rifampicin-resistant strains can, therefore, be used in heat challenge studies and can be recovered with non-selective culture media supplemented with rifampicin.

INTRODUCTION

Escherichia coli O157:H7 is a facultative gram-negative foodborne pathogen known to be the main cause of human hemorrhagic colitis (Padhye and Doyle 1992) and hemolytic-uremic syndrome (Loirat 2013). Most outbreaks of *E. coli* O157:H7 infection are associated with consumption of foods such as undercooked ground beef, raw milk, unpasteurized fruit juices and vegetables (Trevena *et al.* 1999; Yao *et al.* 2014). Rifampicin is an antibiotic that inhibits RNA synthesis by interacting with the β-subunit of DNA-dependent RNA polymerase (RpoB), encoded by the

rpoB gene (Jin and Gross 1988). Some *E. coli* strains are susceptible to rifampicin, whereas others are resistant to this antibiotic. Rifampicin resistance can be induced when the amount of rifampicin-resistant β-subunit is increased by introducing a multicopy plasmid that carries the mutated *rpoB* gene (Ogryzko and Nikiforov 1988).

A variety of selective media are available for the isolation of *E. coli* O157:H7, but their sensitivity and selectivity have not been completely reliable (Halkman *et al.* 1998; Duffy *et al.* 1999). In the United States Food and Drug Administration's Bacteriological Analytical Manual (Hitchins *et al.* 1998), sorbitol MacConkey agar supplemented with cefixime and potassium tellurite (CT-SMAC) is recommended as a selective medium for isolation of *E. coli* O157:H7. Nevertheless, CT-SMAC has some limitations such as low sensitivity, high detection limits (10^3 cfu/g) (Jordan and Maher 2006; Church *et al.* 2007) and masking of the *E. coli* O157:H7 phenotype by cohabitant flora (Dogan *et al.* 2003). Furthermore, non-O157:H7 *E. coli* colonies are not distinguishable from *E. coli* O157:H7 when the pour plate method is used (Jordan and Maher 2006). Some researchers claim that CT-SMAC is not suitable for the recovery of stressed or injured *E. coli* O157:H7 cells (McCarthy *et al.* 1998; Stephens *et al.* 1998). In general, most of the media currently used for detection of *E. coli* O157:H7 strains have low selectivity, especially in food matrices.

For such reasons, more reliable media are needed for the isolation or recovery of *E. coli* O157:H7 in inoculated challenge studies. Especially when cells are injured by treatments such as heating, the use of antibiotics for the inhibition of other strains is essential for more accurate detection of the pathogen. The use of rifampicin-resistant mutants may facilitate the recovery or isolation of the pathogen on media supplemented with rifampicin because the growth of contaminating strains originating from the test substrate or matrix can be inhibited. Therefore, rifampicin-resistant strains have often been used in studies of *E. coli* O157:H7 to assure more specific recovery (Shen *et al.* 2011; Adler *et al.* 2012).

For wider use of rifampicin-resistant mutants in various heat inactivation studies, it is necessary to confirm that there are no significant differences in heat resistance between rifampicin-resistant variants and their wild-type parental counterparts. However, few studies have compared the heat inactivation kinetics of rifampicin-resistant *E. coli* O157:H7 mutants with those of wild-type parental strains. The present study compared the heat inactivation kinetics of rifampicin-resistant variants of pathogenic and nonpathogenic *E. coli* O157:H7 or O157 strains with those of the wild-type parental strains to determine whether the use of rifampicin-resistant mutants is feasible in various heat challenge studies.

MATERIALS AND METHODS

Bacterial Strains and Selection of Rifampicin-Resistant *E. coli* O157:H7 or O157 Variants

In this study, 11 pathogenic and six nonpathogenic *E. coli* O157:H7 or O157 strains were used. The sources of these strains included hamburger meat implicated in a hemorrhagic colitis outbreak, clinical isolates, cattle feces and beef tissue (Table 1). The genotypes of the strains are presented in Table 1. Selection of the rifampicin-resistant variants was carried out by the method of Kaspar and Tamplin (1993). After culturing each pure strain in 10 mL tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD), the culture broth (0.1 mL, 10^9 cfu/mL) was spread plated on tryptic soy agar (TSA; Acumedia, Lansing, MI) supplemented with rifampicin (100 μg/mL, Sigma, St. Louis, MO; TSA_{100}). After incubation of the plates for 24 or 48 h, the colonies were isolated on TSA_{100} plates by streaking, and then it was incubated at 35C. A purified colony on the plate was inoculated into TSB supplemented with rifampicin (100 μg/mL) and then the culture broth was incubated under the same condition. After incubation, 100 μL of cultured TSB broth was inoculated to new TSB media including rifampicin (100 μg/mL). This step was repeated to ensure rifampicin resistance on consecutive days. Finally, the culture was streaked again on TSA_{100} and a rifampicin-resistant colony was obtained after incubation at 35C for 24 or 48 h.

Inoculum Preparation

The wild-type parental and rifampicin-resistant strains were cultured and subcultured for 22 h at 35C in 10 mL TSB or TSB + rifampicin (100 μg/mL), respectively. After subculturing, each culture (9 mL) was separately centrifuged (4629 × g, 15 min, 4C; Eppendorf, model 5810 R, Brinkmann Instruments Inc., Westbury, NY). The resulting cell pellets were resuspended in 0.9 mL of fresh TSB and the prepared inoculum solutions were used immediately for the heat challenge study.

Heat Challenge

Thermotolerance of the rifampicin-resistant derivatives at 60C was compared with that of the wild-type parental strains. Prior to the heat challenge, 29.7 mL of TSB in Oakridge tubes (Nalgene, Nalge Nunc, Rochester, NY) was equilibrated to 60C in a shaking water bath (80 strokes/min) for approximately 20 min. The temperature of the water in the water bath and the TSB in the tubes was continuously monitored with thermocouples (Pico Technology

TABLE 1. SOURCES AND GENOTYPE OF THE *ESCHERICHIA COLI* O157:H7 AND O157 STRAINS USED IN THE PRESENT STUDY

Strain	Source	Genotype*
Pathogenic *E. coli* O157:H7		
ATCC 43895	Raw hamburger meat implicated in a hemorrhagic colitis outbreak	Positive for *stx1* and *stx2*
ATCC 43895/ISEHGFP	Same as ATCC 43895 but with a green fluorescent protein marker†	Positive for *stx1* and *stx2*
ATCC 51658	Clinical isolate	–§
C1-010	Bovine feces‡	Positive for *stx2*, *rfb*, *fliC*$_{h7}$, *eae*
C1-072	Bovine feces	Positive for *stx1*, *stx2*, *rfb*, *fliC*$_{h7}$, *eae*
C1-094	Bovine feces	Positive for *stx1*, *stx2*, *rfb*, *fliC*$_{h7}$, *eae*
C1-097	Bovine feces	Positive for *stx1*, *stx2*, *rfb*, *fliC*$_{h7}$, *eae*
C1-109	Bovine feces	Positive for *stx1*, *stx2*, *rfb*, *fliC*$_{h7}$, *eae*
C1-146	Bovine feces	Positive for *stx1*, *stx2*, *rfb*, *fliC*$_{h7}$, *eae*
C1-154	Bovine feces	Positive for *stx2*, *rfb*, *fliC*$_{h7}$, *eae*
C1-158	Bovine feces	Positive for *stx1*, *rfb*, *fliC*$_{h7}$, *eae*
Nonpathogenic *E. coli* O157 and O157:H7		
O157 16785-1	Beef tissue¶ (has a plasmid-encoded GFP marker)	–
O157 6982	Beef tissue	–
C1-057	Bovine feces	Positive for *rfb*, *fliC*$_{h7}$, *eae*
C1-058	Bovine feces	Positive for *rfb*, *fliC*$_{h7}$, *eae*
ATCC 700728	–	–
ATCC 43888	Clinical isolate	Negative for *stx1*, *stx2*

* *stx1* encodes Shiga toxin 1, *stx2* encodes Shiga toxin 2, *rfb* encodes the O157 antigen, *fliC*$_{h7}$ encodes the H7 antigen, and *eae* encodes intimin.
† The strain was obtained from previous work by Noah *et al*. (2005).
‡ The strains isolated from bovine feces were obtained from previous work by Carlson *et al*. (2009).
§ No data or information.
¶ The strains isolated from beef tissue were provided by Dr. Mansour Samadpour (IEH Laboratories, Lake Forest Park, WA).

Ltd., Cambridge, U.K.) and recorded with real-time data recording software (PicoScope 6, Pico Technology Ltd.). The water level in the water bath was kept at about 2 cm above the level of TSB in the tubes. Aliquots (0.3 mL) of the prepared inoculum suspensions were dispensed into separate tubes containing 29.7 mL of preheated TSB; the initial inoculum levels were approximately 8 log cfu/mL. Aliquots (1 or 0.1 mL) were removed from the tubes after heating at 60C for 30, 60, 90, 120, 150, 180 or 240 s and placed in an ice-water slurry or immediately spiral-plated (Spiral Plater Model D, Spiral Biotech, Bethesda, MD) on TSA supplemented with 0.1% pyruvate (TSAP) for parental strains, and on TSAP and TSAP + rifampicin (100 μg/mL) for rifampicin-resistant strains. An aliquot of inoculated TSB that was not preheated was also plated and was considered as the time-zero sample (i.e., inoculation level prior to heat challenge). The plates were incubated at 35C for 48 h, survivors were enumerated (Colony Image Analysis CASBA 4 scanner and software system; Spiral Biotech), and bacterial counts were expressed as log cfu/mL. The detection limit of the analysis was 1.3 log cfu/mL. This test was performed in triplicate for each strain.

Data Analysis

Thermal inactivation kinetics of the strains were obtained using DMFit software (version 2.1) provided by Dr. J. Baranyi (Institute of Food Research, Reading, U.K.). The software was used to estimate the shoulder period, lower and upper asymptotes (Y_0 and Y_{end}, respectively), correlation coefficient (R^2) and death (inactivation) rates of the tested strains (Baranyi and Roberts 1994). Furthermore, the asymptotic D values representing 1 log reduction at 60C of each strain were determined by MATLAB software (version 7.6; http://www.mathworks.com/products/matlab/) using the equation developed by Juneja and Marks (2005). The D values and death rates obtained were statistically analyzed by the ANOVA test using the JMP program (version 7.0.2, SAS Institute, Cary, NC) to determine whether there were differences ($P < 0.05$) in heat resistance between the rifampicin-resistant variants and corresponding wild-type strains.

RESULTS AND DISCUSSION

Heat Resistance of Pathogenic and Nonpathogenic *E. coli* O157:H7 or O157 Strains

Thermal inactivation data are typically analyzed using linear regression. With nonlinear survival curves, linear regression may provide a poor fit and lead to inaccurate estimates of thermal resistance (Juneja *et al.* 1997). To more accurately assess the heat inactivation kinetics, this study utilized

DMFit and MATLAB software so that some curves were fitted to nonlinear thermal death curves, and asymptotic *D* values at 60C were estimated.

With respect to heat resistance of the pathogenic *E. coli* O157:H7 strains, the *D* values at 60C (D_{60C}) of the wild-type ATCC 43895, ATCC 43895/ISEHGFP and ATCC 51658 strains were 0.41, 0.29 and 1.05 min, respectively, while those of the corresponding rifampicin-resistant (Rif^R) derivatives were 0.36–0.40, 0.18–0.24 and 1.31–1.41 min, respectively (Table 2). The Y_{end} value (corresponding to the final bacterial count) of the Rif^R ATCC 43895 mutant was approximately 2 log lower than that of the corresponding parental strain. The ATCC 51658 parental and Rif^R strains showed a shoulder period in the survival curve and also had high thermotolerance (D_{60C} of 1.05–1.41 min), compared with the other strains, regardless of rifampicin resistance. However, overall, for ATCC 43895, ATCC 43895/ISEHGFP and ATCC 51658, no significant differences in *D* values and death rates at 60C were observed between the Rif^R mutants and their wild-type strains ($P \geq 0.05$).

TABLE 2. COMPARISON OF HEAT RESISTANCE OF PATHOGENIC, RIFAMPICIN-RESISTANT *E. COLI* O157:H7 STRAINS AND CORRESPONDING WILD-TYPE STRAINS IN TRYPTIC SOY BROTH DURING HEATING AT 60C ($N = 3$)

Strains	Type	Asymptotic *D* value (min)†	Shoulder (min)	Death rate (log cfu/mL/min) [x]	Y_0 (log cfu/mL) [y]	Y_{end} (log cfu/mL) [z]	R^2
ATCC 43895	Parental	0.41 ± 0.31[a]*	–‡	–2.62 ± 1.23[a]	8.11	3.63	0.974
	Rif^R-A§	0.40 ± 0.29[a]	–	–1.51 ± 0.57[a]	7.38	1.69	0.962
	Rif^R-B¶	0.36 ± 0.10[a]	–	–1.65 ± 0.59[a]	7.38	1.59	0.971
ATCC 43895/ISEHGFP	Parental	0.29 ± 0.17[a]	–	–2.26 ± 0.62[a]	7.82	3.49	0.957
	Rif^R-A	0.24 ± 0.17[a]	–	–1.54 ± 0.70[a]	7.13	2.43	0.835
	Rif^R-B	0.18 ± 0.11[a]	–	–2.37 ± 0.63[a]	7.54	2.98	0.894
ATCC 51658	Parental	1.05 ± 0.07[a]	0.40	–1.12 ± 0.21[a]	7.93	3.94	0.992
	Rif^R-A	1.31 ± 0.38[a]	0.40	–1.07 ± 0.17[a]	7.68	–	0.944
	Rif^R-B	1.41 ± 0.41[a]	0.41	–1.09 ± 0.37[a]	7.75	2.87	0.981
C1-010	Parental	0.97 ± 0.27[a]	0.20	–2.08 ± 0.24[a]	8.25	3.00	0.962
	Rif^R-A	0.53 ± 0.10[b]	–	–1.33 ± 1.17[a]	7.66	2.99	0.932
	Rif^R-B	0.34 ± 0.18[b]	–	–2.66 ± 0.13[a]	7.94	2.80	0.947
C1-072	Parental	1.30 ± 0.16[a]	0.88	–1.37 ± 0.27[a]	7.97	4.36	0.993
	Rif^R-A	0.88 ± 0.31[a]	0.61	–1.49 ± 0.29[a]	7.37	3.94	0.982
	Rif^R-B	0.82 ± 0.25[a]	0.19	–1.62 ± 0.30[a]	7.39	3.51	0.969
C1-094	Parental	0.88 ± 0.35[a]	0.41	–1.72 ± 0.40[a]	7.93	3.18	0.989
	Rif^R-A	0.58 ± 0.10[a]	–	–1.84 ± 0.31[a]	7.74	2.97	0.962
	Rif^R-B	0.50 ± 0.02[a]	0.10	–2.12 ± 0.30[a]	7.79	3.49	0.947
C1-097	Parental	0.80 ± 0.48[a]	0.65	–2.13 ± 0.39[a]	7.93	4.03	0.992
	Rif^R-A	0.64 ± 0.19[a]	0.42	–2.76 ± 0.28[a]	7.64	2.91	0.948
	Rif^R-B	0.61 ± 0.18[a]	–	–1.93 ± 0.67[a]	7.69	2.26	0.941
C1-109	Parental	1.09 ± 0.06[a]	0.73	–1.84 ± 0.38[a]	7.94	4.30	0.991
	Rif^R-A	1.63 ± 0.24[a]	0.57	–1.05 ± 0.39[a]	7.77	–	0.973
	Rif^R-B	1.46 ± 0.28[a]	0.54	–1.00 ± 0.22[a]	7.77	–	0.978
C1-146	Parental	0.66 ± 0.51[a]	0.14	–1.69 ± 0.24[a]	7.84	3.42	0.962
	Rif^R-A	0.92 ± 0.10[a]	0.34	–1.42 ± 0.20[a]	7.69	3.00	0.994
	Rif^R-B	0.87 ± 0.08[a]	0.22	–1.57 ± 0.03[a]	7.78	2.92	0.992
C1-154	Parental	1.33 ± 0.55[a]	0.80	–1.20 ± 0.06[a]	7.88	–	0.979
	Rif^R-A	0.53 ± 0.16[b]	–	–1.46 ± 0.16[a]	7.34	4.06	0.931
	Rif^R-B	0.61 ± 0.07[b]	0.08	–1.60 ± 0.58[a]	7.34	3.70	0.963
C1-158	Parental	0.97 ± 0.34[a]	0.71	–2.09 ± 0.10[a]	7.94	1.98	0.989
	Rif^R-A	0.49 ± 0.10[b]	0.21	–2.48 ± 0.32[a]	7.72	2.33	0.986
	Rif^R-B	0.43 ± 0.09[b]	0.02	–2.58 ± 0.64[a]	7.75	2.19	0.979

* Means within a column and within each strain that have a common letter are not significantly different ($P \geq 0.05$).

† Calculated using MATLAB software (version 7.6; http://www.mathworks.com/products/matlab/).

‡ No shoulder or no tail (i.e., no tail indicates Y_{end} was not reached).

[x] Inactivation (death) rate calculated using the Baranyi model.

[y] Upper asymptote; corresponds to the initial bacterial count.

[z] Lower asymptote; corresponds to the final bacterial count; shoulder, death rate, Y_0, Y_{end} and R^2 were obtained using DMFit software, version 2.1 (Baranyi and Roberts 1994).

§ Rif^R-A: rifampicin-resistant strain; inactivation kinetics calculated from counts obtained on TSA + 0.1% pyruvate (TSAP).

¶ Rif^R-B: rifampicin-resistant strain; inactivation kinetics calculated from counts obtained on TSAP + rifampicin (100 μg/mL).

The Rif^R C1-010 mutants had lower ($P < 0.05$) D values (0.34–0.53 min) than the wild-type strain (0.97 min), but the death rate of the Rif^R mutant was not significantly different from that of the parental strain ($P \geq 0.05$). The mean D_{60C} values of the parental C1-072, C1-094, C1-097 and C1-109 strains were 1.30, 0.88, 0.80 and 1.09 min, respectively, while those of the Rif^R counterparts were 0.82–0.88, 0.50–0.58, 0.61–0.64 and 1.46–1.63 min, respectively. The Rif^R C1-097 mutant had a lower Y_{end} value (1.1–1.7 log lower) than the corresponding parental strain. However, the D values and the death rates of the parental strains were not significantly different from those of the Rif^R derivatives ($P \geq 0.05$). The Rif^R derivative of C1-146 exhibited D values and death rates similar to those of the parental strain ($P \geq 0.05$); D_{60C} ranged from 0.66 to 0.92 min. The Rif^R derivatives of C1-154 and C1-158 had lower D values (0.53–0.61 min and 0.43–0.49 min, respectively) than the wild-type strains (1.33 and 0.97 min, respectively) ($P < 0.05$), but the death rates of the two Rif^R variants were not significantly ($P \geq 0.05$) different from those of the wild-type strains (Table 2). Overall, most of the pathogenic rifampicin-resistant *E. coli* O157:H7 derivatives (8/11) evaluated in the present study were not significantly different in thermal resistance from their parental controls. In conclusion, the tested pathogenic *E. coli* O157:H7 strains that had both *stx1* and *stx2* genes did not seem to be influenced by the mutation conferring rifampicin resistance.

With regards to the heat resistance of the tested nonpathogenic *E. coli* O157:H7 or O157 strains, the Rif^R variants of *E. coli* O157 16785-1 and 6982, isolated from beef tissue, exhibited lower D values (0.41–0.57 and 1.11–1.20 min, respectively) than their wild-type parents (1.08 and 1.69 min, respectively; $P < 0.05$). Furthermore, they showed higher death rates than their wild-type counterparts ($P < 0.05$) as shown in Table 3. Based on these results, we assumed that these Rif^R mutants will be inactivated more rapidly by heating at 60C than the wild-type strains. However, the D values and death rates of the Rif^R mutants of nonpathogenic *E. coli* O157:H7 C1-057, C1-058, ATCC 700728 and ATCC 43888 did not differ from those of their parental counterparts ($P \geq 0.05$) (Table 3). Like most of the pathogenic *E. coli* O157:H7 strains tested in the present study, the four nonpathogenic rifampicin-resistant *E. coli* O157:H7 mutants were not significantly different in thermal resistance from their parental controls. Although we assumed that exposure to rifampicin might result in

TABLE 3. COMPARISON OF HEAT RESISTANCE OF NONPATHOGENIC, RIFAMPICIN-RESISTANT *E. COLI* O157:H7 OR O157 STRAINS AND CORRESPONDING WILD-TYPE STRAINS IN TRYPTIC SOY BROTH DURING HEATING AT 60C ($N = 3$)

Strain	Type	Shoulder (min)	Asymptotic D value (min)†	Death rate (log cfu/mL/min) [x]	Y_0 (log cfu/mL) [y]	Y_{end} (log cfu/mL) [z]	R^2
O157 16785-1	Parental	1.15	1.08 ± 0.05[a]*	–1.23 ± 0.17[a]	7.88	4.63	0.961
	Rif^R-A§	–‡	0.41 ± 0.25[b]	–1.98 ± 0.19[b]	7.96	2.94	0.944
	Rif^R-B¶	–	0.57 ± 0.15[b]	–2.19 ± 0.20[b]	8.01	2.96	0.958
O157 6982	Parental	1.12	1.69 ± 0.55[a]	–0.80 ± 0.00[a]	7.81	–	0.938
	Rif^R-A	0.72	1.20 ± 0.22[b]	–1.26 ± 0.05[b]	8.06	–	0.983
	Rif^R-B	0.91	1.11 ± 0.15[b]	–1.54 ± 0.27[b]	8.09	3.56	0.987
C1-057	Parental	–	0.81 ± 0.20[a]	–1.49 ± 0.03[a]	8.13	3.06	0.949
	Rif^R-A	0.22	1.18 ± 0.34[a]	–0.99 ± 0.27[a]	7.63	2.56	0.992
	Rif^R-B	0.48	0.80 ± 0.68[a]	–1.17 ± 0.15[a]	7.71		0.974
C1-058	Parental	–	0.51 ± 0.14[a]	–1.24 ± 0.11[a]	8.10	3.83	0.984
	Rif^R-A	0.11	0.61 ± 0.10[a]	–1.71 ± 0.57[a]	7.15	2.48	0.966
	Rif^R-B	–	0.50 ± 0.11[a]	–1.87 ± 0.68[a]	7.33	2.53	0.968
ATCC 700728	Parental	–	0.09 ± 0.16[a]	–1.68 ± 0.66[a]	7.30	–	0.932
	Rif^R-A	–	0.12 ± 0.11[a]	–1.82 ± 0.08[a]	7.13	2.80	0.947
	Rif^R-B	–	0.10 ± 0.13[a]	–1.92 ± 0.23[a]	7.09	2.80	0.907
ATCC 43888	Parental	–	0.27 ± 0.07[a]	–1.95 ± 0.34[a]	7.83	2.09	0.951
	Rif^R-A	–	0.22 ± 0.26[a]	–1.86 ± 0.59[a]	7.57	3.62	0.928
	Rif^R-B	–	0.23 ± 0.20[a]	–1.91 ± 0.58[a]	7.60	3.43	0.934

* Means within a column and within each strain that have a common letter are not significantly different ($P \geq 0.05$).

† Calculated using MATLAB software (version 7.6; http://www.mathworks.com/products/matlab/).

‡ No shoulder or no tail (i.e., no tail indicates Y_{end} was not reached).

[x] Inactivation (death) rate calculated using the Baranyi model.

[y] Upper asymptote; corresponds to the initial bacterial count.

[z] Lower asymptote; corresponds to the final bacterial count; shoulder, death rate, Y_0, Y_{end} and R^2 were obtained using DMFit software, version 2.1 (Baranyi and Roberts 1994).

§ Rif^R-A: rifampicin-resistant strain; inactivation kinetics calculated from counts obtained on TSA + 0.1% pyruvate (TSAP).

¶ Rif^R-B: rifampicin-resistant strain; inactivation kinetics calculated from counts obtained on TSAP + rifampicin (100 μg/mL).

weak thermal resistance in some nonpathogenic *E. coli* O157:H7 strains, the heat tolerance of most of the nonpathogenic strains (4/6) in this study was not influenced by rifampicin exposure.

Kawai *et al.* (1976) indicated that the thermosensitivity of rifampicin-resistant mutants is due to the alteration of the β-subunit of RNA polymerase. Furthermore, Yura *et al.* (1970) reported that rifampicin-resistant *E. coli* strains isolated at low temperatures are susceptible to temperature and are unable to grow at high temperatures. In *E. coli*, there is a relationship between the temperature-sensitive (*Ts*) mutation and the mutation in the *rpoB* gene that confers rifampicin resistance; one mutation suppresses the other (Marshall and Gillespie 1972). On the other hand, Kashlev *et al.* (1989) suggested that elevated temperatures can promote rifampicin resistance in plasmid-containing cells by inducing production of heat shock proteins, which interfere with β-subunit aggregation. Heat shocking, a short-term exposure of bacterial cells to temperatures above their normal growth maximum, may be detrimental because it can induce unfavorable protein-folding interactions (Choi *et al.* 2013). The heat shock response in *E. coli* is regulated by the heat shock promoter-specific σ^{32} subunit of RNA polymerase, which is encoded by the *rpoH* gene (Lim *et al.* 2013), and the DnaK chaperone system and ftsH protease (Tomoyasu *et al.* 1998). In addition, the *rpoS* gene encodes the alternative sigma factor (σ_s), which can increase the resistance of stationary-phase cells to environmental stresses (Dodd and Aldsworth 2002). Furthermore, the *rpoS* gene is known to enhance the heat resistance of *E. coli* O157:H7 (Vanlint *et al.* 2013).

Rifampicin inhibits RNA synthesis by interacting with the β-subunit of DNA-dependent RNA polymerase, which is encoded by the *rpoB* gene (Jin and Gross 1988). However, the correlation between rifampicin exposure and the thermotolerance of microorganisms needs further investigation. Some researchers have reported that temperature-sensitive (*Ts*) mutations suppress genes conferring rifampicin resistance in *E. coli* (Lim *et al.* 2014). On the other hand, conditions that can induce heat shock protein synthesis (e.g., elevated temperature) make plasmid-containing cells more resistant to rifampicin (Zhou *et al.* 2013). Sörqvist (2003) reported that the *D* value at 60C of *E. coli* was 35–42 s, and Ahmed *et al.* (1995) showed that in pork sausage with 7, 10 and 30% fat, the *D* values at 60C of *E. coli* O157:H7 were 0.37, 0.46 and 0.55 min, respectively. Additionally, Smith *et al.* (2001) found that *E. coli* O157:H7 in 4.8% fat ground beef exhibited a *D* value at 61C of 0.32 min. On the other hand, Osaili *et al.* (2006) demonstrated that the *D* value at 60C of *E. coli* O157:H7 was 2.5 min in chicken-fried beef patties (14.6% fat). In the present study, the parental pathogenic *E. coli* O157:H7 strains ATCC 51658, C1-072, C1-109 and C1-154 and the parental nonpathogenic *E. coli* O157 strains 16785-1 and 6982 exhibited *D* values above 1.0 min at 60C. Our results differ somewhat from the *D* values reported by other researchers. This may be due to differences in the genetic properties and physiological characteristics of the *E. coli* O157:H7 strains tested, different methodology used for recovery of survivors, or experimental conditions including pH and chemical compounds.

The effects of rifampicin on the thermal resistance of pathogenic *E. coli* O157:H7 strains are assumed to be associated with their genotypes. This study found that three pathogenic rifampicin-resistant *E. coli* O157:H7 strains (C1-010, C1-154 and C1-158) and two nonpathogenic rifampicin-resistant *E. coli* O157 strains (16785-1 and 6982) had lower heat resistance than those of their wild-type counterparts ($P < 0.05$). However, overall, the heat tolerance of most of the pathogenic (8 of 11) and nonpathogenic (4 of 6) *E. coli* O157:H7 strains was not significantly different from their parental strains ($P \geq 0.05$). In addition, for pathogenic *E. coli* O157:H7 strains that had both the *stx1* and *stx2* genes, heat resistance did not seem to be affected by the mutation inducing rifampicin resistance. Therefore, this study demonstrated that the majority of the rifampicin-resistant *E. coli* O157:H7 variants evaluated are suitable for use in heat challenge studies.

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