DETERMINATION OF HEAT RESISTANCE OF 101 STRAINS OF VEROTOXIGENIC *ESCHERICHIA COLI*

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Abstract – This paper describes the screening of 101 strains of *E. coli* for heat resistance. In general, *E. coli* strains are sensitive to mild heat, but heat resistance is highly variable between strains. The presence of NaCl may play a role in increasing the heat resistance of certain strains.

Key Words – *E. coli* serotypes, heat treatment survival, NaCl

I. INTRODUCTION

Several *E. coli* pathotypes cause gastrointestinal infections owing to the presence of specific virulence factors [1]. Pathotypes causing foodborne illness include Verotoxigenic *E. coli* (VTEC), also known as Shigatoxigenic *E. coli* (STEC). VTEC are distinguished by the production of one or more verotoxins (shiga toxins) and may possess additional virulence factors, including the locus of enterocyte effacement [1]. The public health importance of VTEC is a consequence of the potentially severe health outcomes and low infectious dose of this pathogen [2]. Outbreaks VTEC illnesses have involved a wide range of foods, including fresh and processed meats (particularly beef), dairy products and vegetables [3, 4].

VTEC infection results in diarrhea, followed by hemorrhagic colitis (HC), which in a minority of cases develops into hemolytic uremic syndrome (HUS). HUS often results in long term health impacts, commonly as a consequence of kidney failure, and has a significant risk of death [2]. The development of HC and HUS result from the production of verotoxin in the victim's intestine and uptake of the toxin by a specific receptor on human kidney cells [2]. HUS is particularly life threatening in young children and elderly [2].

E. coli strains are serotyped on the basis of the antibodies for the O-antigen (lipopolysaccharide) and H-antigen (flagellin). VTEC of the serotype O157:H7 and nonmotile variant (O157:H- or NM) account for two thirds of reported VTEC illness in the USA [5]. A wide diversity of serotypes have been isolated are responsible for the remaining cases. However, certain serotypes predominate in cases of human illness, of 940 non O157 VTEC isolates submitted to the CDC between 1983 and 2002 70% of isolates belonged to 6 O-types (O26, O45, O103, O111, O121 and O145) [6]. A significant proportion of VTEC illness is attributed to isolates of these serotypes in other regions, and in some regions O157 isolates constitute a minority of reported cases [3].

The 6 serotypes (O26, O45, O103, O111, O121 and O145), were recently classified as adulterants by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS). It was announced in Sept, 2011 that these serotypes will be included together with O157:H7 in the routine sampling of beef products; and the presence of these microorganisms in raw ground beef or its precursors would lead to the prohibition of these products to enter commerce [7].

VTEC of unusual serotypes should not be ignored as major outbreaks are not limited to O157 and the top 6 US serotypes. In May of 2011 an outbreak of HC and HUS caused by *E. coli* O104:H4 initiated in Germany, resulted in 4075 cases of illness including 908 cases of HUS and 50 deaths across 16 countries [8, 9]. There was only one previous report of VTEC O104:H4 from Korea [10].

Heat treatment is a common intervention in reduce the numbers of vegetative cells on animal carcasses and as part of food preparation. In

E. coli, and specifically in *E. coli* O157:H7's, resistance to heat is highly variable between strains [11, 12]. The discovery of *E. coli* AW1.7, an extremely heat resistant strain, questioned the efficacy of current heat treatment interventions process [11, 13]. Moreover, heat resistant strains of *E. coli* are cross-resistant to high hydrostatic pressure [14].

It is the aim of this study to compare the heat resistance of a wide variety of VTEC strains. We hypothesize that VTEC are not significantly more heat resistant than non-pathogenic *E. coli.* In demonstrating that the heat resistance of *E. coli* AW1.7 is greater than that observed in the VTEC strains tested, we can confirm the suitability of AW1.7 as a surrogate for VTEC in the evaluation of the heat treatment of meats.

II. MATERIALS AND METHODS

Bacterial strains and growth conditions
E. coli AW1.7 and 87 VTEC strains and 14 VT negative *E. coli*, of 15 different serotypes, were used in this study. All strains were maintained frozen at -80°C and resuscitated before use by streaking onto Luria-Bertani agar plate (Difco, BD, Sparks, US) and incubated for 24 h at 37°C.

For experimental use single colonies of each strain to be tested were inoculated in 10 mL of LB media or LB with 2% NaCl. The broth was incubated in a shaking incubator at 200 rpm for 24 h at 37°C.

Screening for heat resistant strains
From the stationary phase *E. coli* culture 1 mL was withdrawn and diluted in 9 mL of 0.1% buffered Peptone Water. For each strain to be tested 100 µl of cell suspension was transferred to the wells of a Twin. Tec. PCR plate 96 microtiter plate (Eppendorf AG, Hamburg, DE). Four plates were prepared for each experiment; an untreated control, and for exposure to 60°C for 5, 15 or 30 min in an Eppendorf PCR thermal cycler (Eppendorf AG, Hamburg, DE). Following heat treatment, the microtiter plates, including the control, were incubated at 37°C for 48 h.

After incubation, the wells of the microtitre plate were examined for turbidity. Growth, indicating survival, was recorded if a plaque of cells formed at the bottom of the plate well. Screening experiments were conducted in duplicate for cells grown in LB or LB with 2% NaCl.

Enumeration of survivors following heat treatment
Strains of *E. coli* for which increased turbidity was observed following exposure to 60°C for 5 min. were selected for enumeration of survivors following heat treatment.

The cells were grown as described above in either LB or LB with 2% NaCl. Aliquots (100 µL) of culture were exposed to 60°C for 5 min as described above. Cells in heated and control samples were enumerated by plating onto agar medium with a Whitley Automatic Spiral Plater (Don Whitely Scientific, Shipely, UK). Cells grown in LB broth were diluted in 0.1% peptone water and plated onto LB agar. Cells grown in LB with 2% NaCl were diluted in 0.1% buffered peptone water with 0.85% NaCl and plated onto LB agar with 1% NaCl. Plates were incubated at 37°C for 48 h. The total colonies were counted and the reduction of cells during heat treatment was calculated in Log CFU/mL by comparing the treated with control samples. Enumeration experiments were conducted in triplicate.

Statistical analysis
Mean and standard deviation from at least three independent experiments was determined for colony count data. Welch's two-sample *t*-test was performed to determine whether the addition of NaCl in growth media affected the number of *E. coli* recovered following heat treatment.

III. RESULTS AND DISCUSSION

Screening for heat resistant strains
No increase in turbidity was observed following incubation for any of the *E. coli* strains tested when exposed to 60°C for 15 or 30 min, whether grown in LB or LB with 2% NaCl, with the exception of *E. coli* AW1.7. Of the VTEC tested, 25 strains showed visible turbidity following 5 min at 60°C (Table 1).

The results indicate that some of the *E. coli* strains tested are substantially more heat resistant than the majority of *E. coli* strains [11, 12]. All of VTEC tested, however, were less heat resistant than *E.*

coli AW1.7. Moreover, heat resistance is not dependent upon serotype.

Table 1. VTEC and other *E. coli* demonstrating resistance to 60 °C for 5 min

Strain ID	Serotype	Isolation	stx1	stx2	eae
1935	O157:H7	human	+	+	+
EC99	O157:H7	unknown	+	+	+
7236	O157:H7	human	+	+	+
7283	O157:H7	hamburger	+	+	+
C0283	O157:H7	cattle feces	+	+	+
E0122	O157:H7	cattle	-	+	+
E0139	O157:H7	deer jerky	-	+	+
CA 334	O145:H34	unknown	-	-	+
CA 728	O145:H34	unknown	-	+	-
03-6430	O145:NM	human	+	-	+
05-6544	O26:H11	human	+	-	+
99-4610	O26:H11	stool	+	-	+
00-4748	O111:NM	human	-	+	+
P 447	O111:NM	unknown	-	-	+
06-0434	O103:H2	human	+	-	+
P 444	O103:H2	unknown	-	-	-
05-6545	O45:H2	human	+	-	+
09-0525	O113:H4	unknown	+	+	-
92-0275	O117:H4	unknown	+	+	-
09-0523	O76:H19	unknown	+	+	-
03-2642	O121:H19	stool	-	+	+
03-4064	O121:NM	human	-	+	+
03-2832	O121:H19	human	-	+	+
96-0120	O121:H10	unknown	-	+	-
09-414	O104:H7	unknown	-	-	-

Enumeration of survivors following heat treatment
To determine the magnitude of heat resistance of the screened *E. coli*, cells were challenged at 60 °C for 5 min under growth conditions with or without NaCl. Of the 25 strains tested, only six strains had a lower than 5 log reduction (E0122, 03-6430, 05-6544, 03-2832, 09-0525) (Fig.1). *E. coli* AW1.7 was significantly more heat resistant than the *E. coli* strains tested ($P < 0.01$), with reductions of 1.08 and 0.34 log for cells grown in LB or LB with NaCl, respectively. By comparison reductions of 3 log or greater were observed for all *E. coli* strains. The observed sensitivity of the *E. coli* strains to 60°C was within the range previously reported in studies with *E. coli* [12, 15].

The addition of NaCl to the growth medium did not improve the survival of *E. coli* strains in the screening test. Comparison of *E. coli* recovered following heat treatment in LB with those in LB with NaCl did not indicate a significantly greater recovery ($P > 0.05$). However, the recovery of *E. coli* AW1.7 was significantly greater ($P < 0.01$) with the addition of NaCl to growth media. These results confirm the exceptional heat resistance of *E. coli* AW1.7 [11] and the observation that the heat resistance of *E. coli* AW1.7 was maximized between 2-4% NaCl [6].

The protective effective of NaCl on *E. coli* AW1.7 to heat stress likely is a consequence of accumulation of compatible solutes inside the cell by outer membrane transport protein NmpC and transport proteins in the cytoplasmic membrane [13, 16]. NmpC is expressed at higher levels in *E. coli* AW1.7 compared to that observed for heat sensitive *E. coli* strain [13].

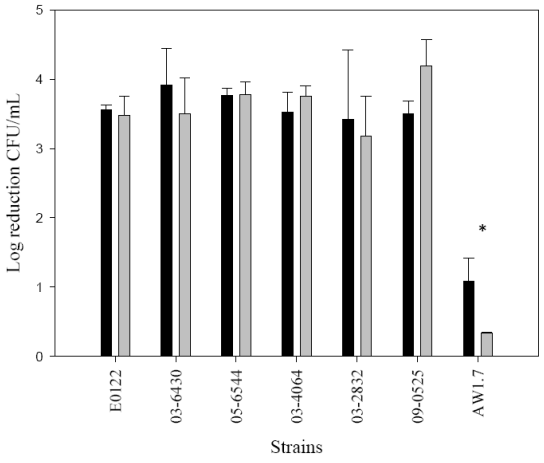

Figure 1. Survival of verotoxigenic *E. coli* after 60 °C 5 min heat treatment. Black bars indicate cells grown and enumerated on LB agar; grey bars indicate cells grown and enumerated on 1% NaCl LB. Error bars represent one standard deviation for triplicate experiments. * indicates statistically significant differences between cultures enumerated on LB or LB 1% NaCl ($P < 0.01$)

IV. CONCLUSION

With close to 87 strains of VTEC and 14 additional *E. coli* of common VTEC serotypes tested, this is the largest study on the heat resistance of VTEC. Though six VTEC strains were able to with stand 60 °C for 5 min with less than a 5 log reduction there is no indication that VTEC as group are significantly more heat resistant than other *E. coli*. None of the 101 strains tested demonstrated greater heat resistance than *E. coli* AW1.7. This indicates the suitability of *E. coli* AW1.7 as a surrogate of VTEC in thermal challenge studies and recovery of *E. coli* AW1.7 can be improved by the addition of NaCl to media. However, there was no evidence of a generalized protective effect of NaCl on *E. coli.*

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REFERENCES

1. Kaper, J. B., J. P. Nataro, and H. L. Mobley. 2004. Pathogenic *Escherichia coli*. Nature Reviews. Microbiology 2:123–140.
2. Karch, H., P. I. Tarr, and M. Bielaszewska. 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. International Journal of Medical Microbiology 295:405–418.
3. European Food Safety Authority. 2007. Scientific opinion of the panel on biological hazards on a request from EFSA on monitoring of verotoxigenic *Escherichia coli*. European Food Safety Authority Journal 579:1–61.
4. Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M. & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. Emerging Infectious Diseases 11:603-609.
5. Anonymous. 2010. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. Morbidity Mortality Weekly Reports 59:418–422.
6. Brooks, J. T., E. G. Sowers, J. G. Wells, K. D. Greene, P. M. Griffin, R. M. Hoekstra, and N. A. Strockbine. 2005. Non-O157 Shiga toxinproducing *Escherichia coli* infections in the United States, 1983–2002. Journal of Infectious Diseases. 192:1422–1429.
7. http://www.usda.gov/wps/portal/usda/usdamediafb?contentid=2011/09/0400.xml&printable=true&contentidonly=true. (04-09-2011).
8. http://www.euro.who.int/en/what-we-do/health-topics/emergencies/international-health-regulations/news/news/2011/07/outbreaks-of-e.-coli-o104h4-infection-update-30. (22-07-2011).
9. Frank, C., Werber, D., Cramer, J. P., Askar, M., Faber, M., an der Heiden, M., Bernard, H., Fruth, A., Prager, R., Spode, A., Wadl, M., Zoufaly, A., Jordan, S., Kemper, M., Follin, P., Müller, L., King, L. A., Rosner, B., Buchholz, U., Stark, K. & Krause, G. (2011). Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. New England Journal of Medicine 365:1771-1780.
10. Bae, W. K., Lee, Y.K., Cho, M. S., Ma, S. K., Kim, S. W., Kim, N. H. & Choi, K. C. (2006). A case of Hemolytic Uremic Syndrome caused by *Escherichia coli* O104:H4. Yonsei Medical Journal 47:437-439.
11. Dlusskaya, E. A., McMullen, L. M. & Gänzle, M. G. (2011). Characterisation of an extremely heat-resistant *Escherichia coli* obtained from a beef processing facility. Journal of Applied Microbiology 110:840-849.
12. Eblen, D. R., Annous, B. A. & Sapers, G. M. (2005) Studies to select appropriate nonpathogenic surrogate *Escherichia coli* strains for potential use in place of *Escherichia coli* O157:H7 and salmonella in pilot plant studies. Journal of Food Protection 68:282-291.
13. Ruan, L., Pleitner, A., Gänzle, M.G. & McMullen, L. M. (2011). Solute transport proteins and the outer membrane protein NmpC contribute to heat resistance of *Escherichia coli* AW1.7. Applied & Environmental Microbiology 77:2961-2967.
14. Liu, Y., M. Betti, and M. G. Gänzle. 2012. High pressure inactivation of Escherichia coli, Campylobacter jejuni, and spoilage microbiota on poultry meat. Journal of Food Protection 75: 497-503.
15. Benito, A., Venoura, G., Casadei, M., Robinson, T. & Mackey, B. (1999). Variation in resistance of natural isolates of *Escherichia coli* O157 to high hydrostatic pressure, mild heat, and other stresses. Applied & Environmental Microbiology 65:1564–1569.
16. Pleitner, A. 2011. Heat tolerance mechanisms of an exceptional strain of *Escherichia coli*. M.Sc. thesis, University of Alberta.