Variation among *Escherichia coli* O157:H7 strains relative to their growth, survival, thermal inactivation, and toxin production in broth

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Abstract

To estimate the potential outcomes of food processing on the fate of foodborne pathogens, variations in microbial parameters such as growth rate, survival time, thermal inactivation time, and toxin production must be known. Previous microbial studies using single strains or cocktails provide error estimates for the uncertainty of the experimental and statistical procedures, but not for variations among strains. In this study, the behavior of 17 strains of *Escherichia coli* O157:H7 were followed when placed in synthetic media that permitted growth, survival, or thermal inactivation. The parameter values were not rejected as being normal, lognormal, gamma, or Weibull distributions. The ratio of the standard deviation to mean (normal distribution) for the exponential growth rate was 0.16 and for the lag phase duration, it was 0.38. The ratios of times to achieve a $4 - \log_{10}$ reduction at two survival conditions were 0.39 and 0.46; ratios of thermal $D$ values at 55 and 60 °C were 0.42 and 0.33, respectively. The ratio of the negative log$_{10}$ of toxin production was 0.24. These distributions are larger than the coefficient of variations observed for experimental errors in single strain and cocktail experiments. This indicates the limitations in precision that predictions of future population numbers can have when the potential presence of all strains needs to be considered. This variation among strains is applicable whether predictions are made by traditional subjective and point estimates or by using models and risk assessments. Published by Elsevier Science B.V.

Keywords: Modeling; Distributions; Microbial pathogens; Risk assessments

1. Introduction

Management of microbial risks has increasingly relied on quantitative risk assessments for collecting microbial data and calculating estimates of the public health impact from consuming a food (FAO/WHO, 1991; ICMSF, 1998; ILSI, 1996). To make these calculations, risk assessments need models for growth, survival and thermal inactivation, surveys of food contamination and consumption, and epidemiological
data on illnesses (Buchanan et al., 2000; Buchanan and Whiting, 1998; Lammerding and Fazil, 2000; Whiting and Buchanan, 2001). These calculations require information on the distributions the parameter values, such as contamination levels, growth rates, and $D$ values, may have (Vose, 1998, 2000). A mean value by itself provides an incomplete, even misleading, impression of a parameter (Miller et al., 1993; Buchanan and Whiting, 1998; Marks and Coleman, 1998). The sources of this distribution are described as originating from uncertainty and variation. Uncertainty is from unknown factors and a lack of knowledge that affects the measurement of the parameter value. Variation represents real differences that exist for the parameter. In microbiology, uncertainty results from analytical errors, the limited number of replicates that can be made, and statistical errors in data analyses and modeling. The magnitude of the uncertainty can be reduced by improving the experimental procedure. A major source of variation is the differences among strains of the same species. Variation is an inherent property of the microorganisms, and when strains are identically treated under the conditions specified in the study, it cannot be reduced.

Most reports in the literature provide no or inadequate descriptions of the distributions that would be expected for the parameters measured. If a single strain is used, the reported parameters are from experimental and statistical errors. When strain cocktails are used, the measured data essentially derive from the fastest growing or the longest surviving strain(s). The experimental design does not acquire any information on strain variation. When an error term is calculated, a normal distribution is frequently assumed without close examination of the data set, and statistical procedures that require a normal distribution are applied. Often, an overall estimate of the error for the entire experimental design is provided without testing for uniformity of the variances for the different populations. Frequently, variation is presented only by letters or asterisks in the data tables.

A few microbiological studies have described sources of variation in the observed microbial parameters. Kelly and Rahn (1932) observed progeny from individual cells of *Bacillus cereus* and *Bacterium aerogenes* (now *Aeromonas*) under the microscope and recorded generation times. From their data of generation times for *B. aerogenes* in broth at 30 °C, the mean and standard deviation can be calculated to be 30.3 and 9.5 min, respectively, using a lognormal distribution. The generation times for *B. cereus* at 30 °C were best fitted to a Weibull distribution (Vose, 2000) and the mean and standard deviation were 48.7 and 23.8 min, respectively. Ratkowsky et al. (1996) explored the variation in generation times for bacteria at different temperatures and found an extended, right-handed tail of long generation times. Gamma or inverse Gaussian distributions were suggested for these data (Vose, 2000).

The statistical distribution of naturally occurring microorganisms in a food has usually been assumed to be lognormal, that is, the logarithm of the microbial counts are normally distributed (Kilsby and Pugh, 1981). Gill et al. (1996) reported the log counts for *Escherichia coli*, coliform, and aerobic counts on meat from beef carcasses in a commercial slaughterhouse destined for grinding were normally distributed. The average log$_{10}$ count and standard deviation for aerobic counts, for example, were 3.81 and 0.56 cfu/g, respectively.

There has been little examination of strain variation in the food microbiology literature, either in classical presentations or modeling papers, and no reporting of the characteristics of the distributions among strains. This has occurred because of the appreciable work required to obtain these data and because microbiologists likely considered the between-strain variation to be equal or smaller to the experimental and statistical variation and, thus, not necessary to be determined. Without providing numeric data, Mackey et al. (1990) reported that $D$ values for 27 *Listeria monocytogenes* strains isolated from beef, pork, and poultry processing plants had a fourfold difference in $D_{57}$ values between the least and most heat-resistant strains. Growth parameters for 28 or 39 strains of *L. monocytogenes* were determined at three temperatures in tryptic soy broth with added yeast extract by turbidity measurements (Barbosa et al., 1994). At 4 °C, for example, the lag phase duration was 150.9 ± 29.5 h and the exponential growth rate was 7.4 ± 1.8 AU/h. The growth parameters for 58 strains of *L. monocytogenes* from meat products and food plants were analyzed by cluster analyses by Begot et al. (1997). Differences among strains in lag phase duration were much greater than in the log phase. At 10 °C, pH 7, and $a_w$ 0.96, the lag phase durations for different strains ranged from 4 h to 4 days.
The \( D \) values in liquid whole eggs were determined for six serovars of *Salmonella* (excluding heat-resistant *S. senftenberg 775W*) and the range of the six \( D \) values at 140 °F was 0.40–2.2 min (Anellis et al., 1954). Shah et al. (1991) reported \( D \) values for 17 strains of *S. enteritidis* in liquid whole egg at 60 °C ranging from 11.8 to 31.3 s.

While some *E. coli* O157:H7 studies report individual data for four or fewer strains, only three studies presented sufficient data to indicate the magnitude of strain variability. Palumbo et al. (1995) reported that the time to achieve a \( 3 - \log_{10} \) growth, including the lag phase, at each of the 16 strains' minimum growth temperature (7.3–9.2 °C) ranged from 4.0 to 46.4 days. Buchanan and Edelson (1996) presented data for various combinations of acid adaptations and survival for six strains of EHEC and concluded that there were substantial differences among strains. Clavero et al. (1998) found that \( D \) values for 10 strains of *E. coli* O157:H7 heated at 54.4 °C in MSMA broth ranged from 17.4 to 35.4 min. However, none of these studies examined the shapes of the distributions or estimated the variation among the strains.

The above mentioned studies indicate that strain variation will be an important component in assessing the potential exposure of a consumer to a pathogen. Usually, a model is intended to represent all strains that are likely to be present in a food. A stochastic model incorporating the strain distribution may lead to a more complete analysis than a deterministic (point estimate) model of a pathogen’s parameter values or a calculation based on the “worst case” strain. This manuscript presents data on growth, survival, thermal inactivation, and toxin production by 17 strains of *E. coli* O157:H7 to estimate between-strain variations in a synthetic medium.

### 2. Materials and methods

#### 2.1. *E. coli* O157:H7

The 17 *E. coli* O157:H7 strains were archived in brain heart infusion (BHI; Difco Laboratories, Detroit, MI) broth supplemented with 10% glycerol at −70 °C in this laboratory’s culture collection. The strains were temporally and geographically unrelated and were isolated from both humans and foods. Strains ENT C9490, A9218-C1, 30-2C4, B6914, A9124-C1, 45753-32, A8959-C7, EDL-931, C984, B1409, 43890, C7927, and SEA 13B88 are clinical isolates originally obtained from the Centers for Disease Control and Prevention, Atlanta, GA. Strains 933, 45753-35, and 45750 were originally obtained from the USDA, Food Safety and Inspection Service, Washington, DC. Strain 932, a clinical isolate, was obtained from M.P. Doyle, University of Georgia, Griffin, GA. Stock cultures were grown overnight in BHI broth at 37 °C, and then 10% glycerol was added and 1.0-ml portions were stored at −70 °C. Working cultures were made by partially thawing each strain and adding the 1.0 into 10 ml of BHI broth and growing the culture at 37 °C overnight with shaking (150 rpm). These cultures were stored at 6 °C for up to 30 days. For each experiment, a 0.1-ml portion of the working culture (ca. 10^3 cfu/ml) was inoculated into 100 ml of BHI broth with 0.3% added dextrose (Difco) and incubated overnight (18–24 h) at 37 °C with shaking to give a stationary-phase culture. The metabolism of the added dextrose caused a reduction in the pH from 4.6–5.2 as the culture grew. Therefore, all strains were more acid resistant compared to strains cultured without added glucose (Buchanan and Edelson, 1996). Developing acid resistance would primarily affect a cell’s survival and thermal inactivation rates, there would be no effect after a culture resumes growth.

#### 2.2. Growth parameters

The BHI broth was adjusted to pH 5.3 with lactic acid and 1.5% NaCl was added before sterilization. The 100 ml of broth was pre-adjusted to 15 °C before 0.1 ml of each culture was added to a separate flask. This combination of pH, salt, and temperature was chosen to have three environmental factors affect the cells, while still allowing for a moderate growth rate. At appropriate times, a 100-μl sample was taken, diluted in peptone water (Difco), and plated on BHI agar using a Spiral Plater (Model D, Spiral Systems, Bethesda, MD). After incubating the plates for 24 h at 37 °C, the number of colonies was counted with a Model 500A Bacteria Colony Counter (Spiral Systems). Growth rates were determined in duplicate flasks with two replicates for each strain.

Data from each flask were fitted to the Gompertz equation (Whiting, 1995) using ABACUS, a Gauss–
Newton nonlinear fitting routine (Damert, 1994). The lag phase durations, generation times, and exponential growth rates were calculated from the Gompertz equation parameters (Whiting, 1995).

2.3. Survival parameters

Two broths were made, which did not allow growth, using factors from the aqueous phase of fermented and dried meat products that are important for microbial control. Salt, sodium nitrite, lactic acid, and hydrochloric acid were added to BHI broth to create the conditions of 5% NaCl, pH 4.8, 0.3% lactate, and 25 ppm nitrite (broth I) and 13% NaCl, pH 4.8, 0.8% lactate, and 25 ppm nitrite (broth II). Separate Erlenmeyer flasks with 19.4 ml of a test broth were individually inoculated with 0.6 ml of an E. coli O157:H7 strain to achieve about 108 cfu/ml and incubated at 12 °C. At appropriate times, a 100-μl sample was taken, diluted in peptone water, and plated on normal BHI agar using the Spiral Plater. After incubating the plates for 24 h at 37 °C, the number of colonies was counted with the Bacterial Colony Counter. Two replicates done in duplicate for each strain were modeled for each treatment.

For each flask, the declining number of survivors with storage time were fitted to a simple shoulder or lag time and linear decline model with ABACUS (Whiting, 1993). The shoulder time and D value were used to calculate the time for a 4 – log10-unit decline in pathogen numbers.

2.4. Thermal inactivation parameters

Stationary-phase cells of each of the 17 E. coli strains were separately diluted in normal BHI broth to 108 cfu/ml and a 10-ml sample was injected into a submerged-coil thermal inactivation apparatus (Tempette TE8D, Protrol Instruments, Surry, UK). At selected time intervals, 0.5-ml samples were expelled into vials which were immediately cooled on ice. The number of surviving cells was enumerated on BHI agar to include both fully viable and injured cells. The thermal inactivation data were fitted to a first-order inactivation model using ABACUS. Two flasks for each strain were tested at both 55 and 60 °C in replicate trials.

2.5. Toxin production

The 17 E. coli O157:H7 cultures were tested for verotoxin based on the procedures described by Riordan (1998). E. coli O157:H7 strains were individually grown in BHI broth for 24 h at 37 °C and 100 μl of serially diluted supernatant were added to microtiter plate wells containing Verocells (ATCC CCL 81). The plates were incubated for 72 h at 37 °C and visually examined under 10 × magnification (CK Olympus microscope, Tokyo, Japan) for toxin activity, as evidenced by the breakdown of the cell monolayer. The dilution at which 50% of the cells were detached from the base of the microwell (indicating cell death) was identified. The titer of toxin in a sample was expressed as the reciprocal of this end point dilution and is referred to as the CD50 (50% cell death) (Palumbo et al., 1995). As a control, sterile BHI (100 μl) was added to duplicate wells and serially diluted in the same manner as the cell-free supernatant. Each strain was separately grown three times and the toxin production was assayed in duplicate.

2.6. Distribution analyses

The mean value for each strain was used to determine the strain distributions. BestFit Version 2.0d (Palisade, Newfield, NY) determined the best distribution parameter values and tested the significance of the fit by Chi-squared, Kolmogorov–Smirnov and Anderson–Darling statistics (Palisade, 1997). The distributions tested for fits to each data set were the normal, lognormal, gamma, and Weibull and the fits were discounted if one of the three statistics failed at the 95% confidence level.

3. Results and discussion

The test conditions and results of the growth, survival, thermal death, and toxin production experiments for the 17 E. coli O157:H7 strains are summarized in Table 1. The lag phase durations varied from 13.7 to 55.6 h and the exponential growth rates varied from 0.055 to 0.106 log10/h. These data sets were not sufficiently large to show that one distribution was definitively better than another. None of the four distributions considered (gamma, normal, log-
normal, and Weibull) were rejected as possible fits by any of the three statistical tests. The means and standard deviations are presented for normal distributions.

To compare the strain variation with the confidence intervals representing experimental and statistical error from a large regression model, the USDA Pathogen Modeling Program Version 5.1 (PMP; USDA, 1997), based upon data presented by Buchanan and Bagi (1994) and Buchanan et al. (1993), was used to estimate the exponential growth rate for *E. coli* O157:H7 in the same environment. The PMP estimate of the growth rate was 0.073 h⁻¹, with lower and upper 95% confidence intervals (approximately ±2 standard deviations of the logarithm of the parameter value) of 0.063 and 0.086 h⁻¹, respectively. These values were converted from generation times to exponential growth rates for comparison (Table 2). The PMP model was based on a cocktail composed of three strains chosen from the 17 strains studied herein and includes the errors term from the laboratory procedures and the regression analyses. The strain data, presented in this study, found that the mean lag phase duration was 26.2 h and the range of two standard deviations was 14.4–37.9 h, a larger range than that estimated by the PMP. The ratios of standard deviation to mean (i.e., coefficient of variation) for the exponential growth rate and lag phase duration were 0.16 and 0.38, respectively. There was no correlation between the lag phase durations and exponential growth rates among the strains.

Survival models estimate the time a microorganism will remain viable in an environment that does not permit growth (Whiting, 1993). Many foods are preserved by conditions of low temperature, high salt, and other preservation methods.
and/or low pH that may allow survival of undesirable bacteria. Some survival data show initial shoulders where no declines in population occur. The shoulders are followed by linear decreases (log scale). The time required to achieve a 4 log₁₀-unit inactivation (T₄D) includes the shoulder and declining phases. In broth I, the mean T₄D for the 17 strains was 593 h with a standard deviation of 233 h (normal distribution). The range in observed data was from 142 (strain 932) to 998 h (strain C7927). The PMP estimated 649 h, with upper and lower 95% confidence intervals of 567 and 743 h, respectively. Changing to higher salt and lactate concentrations in broth II caused little change in the survival times compared to broth I. The mean T₄D was 564 h, with a standard deviation of 258 h. The PMP estimated a mean, lower, and upper 95% confidence intervals of 511, 457, and 572 h, respectively. As with the growth parameters, none of the four distributions (gamma, normal, lognormal, Weibull) were rejected as possible fits for the two survival environments. The coefficients of variation for the T₄D were 0.39 and 0.46 for broths I and II, respectively.

Thermal death times were determined at 55 and 60 °C. The D₅₅ ranged from 2.6 to 21.5 min and the D₆₀ ranged from 0.69 to 2.13 min. The two standard deviation ranges of these D values compare well to the 0.75-min D value reported for 60 °C in ground beef, for example (Doyle et al., 1997). As with the other parameters, none of the four distributions (gamma, normal, lognormal, Weibull) were rejected as possible fits for the two survival environments. The coefficients of variation for the D₄D were 0.39 and 0.46 for broths I and II, respectively.

Table 2
Comparison of between-strain variation vs. modeling uncertainty

<table>
<thead>
<tr>
<th></th>
<th>Between-strain variation</th>
<th>Uncertainty from Pathogen Modeling Program</th>
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<tbody>
<tr>
<td></td>
<td>95% LCLª</td>
<td>Mean</td>
</tr>
<tr>
<td>Exponential growth rate (log₁₀/h)</td>
<td>0.055</td>
<td>0.081</td>
</tr>
<tr>
<td>Lag phase duration (h)</td>
<td>14.4</td>
<td>26.2</td>
</tr>
<tr>
<td>Survival time broth I (h)</td>
<td>127</td>
<td>593</td>
</tr>
<tr>
<td>Survival time broth II (h)</td>
<td>48</td>
<td>564</td>
</tr>
</tbody>
</table>

ª LCL: lower confidence level.  
b UCL: upper confidence level.  
c Values calculated from the Pathogen Modeling Program generation times.

Because the dilutions in the toxin assay ranged from 1:16 to 1:4096, the negative log₁₀ fractions of original concentration were calculated, i.e., negative log (1/16) and negative log (1/4096), respectively (Table 1). Larger values indicate production of more toxins than lower values. The distributions of the calculated values were not rejected by any of the four distributions. The normal mean (log dilution⁻¹), standard deviation, and coefficient of variation were 2.71, 0.65, and 0.24, respectively (Table 1).

4. Conclusion

These data show the magnitude of the variation existing among strains of E. coli O157:H7. These variations are larger than the uncertainties which are frequently calculated from experimental procedures using a single strain or cocktail. Determining a single, best distribution would require testing a much larger collection of strains than what were used in this study. However, this study provides estimates of the magnitude of strain-to-strain variation for various E. coli O157:H7 parameters. When estimating the expected behavior of a species in a food, all strains likely to be present must be considered and the appropriate distribution for Monte Carlo simulations needs to include strain variation. This variation will limit the precision of any estimate provided by models and risk assessments. However, this reflects the diversity in the natural microbial world. Unless the identity and characteristics of a single strain are known, as when reconstructing a specific outbreak, interpretation of the potential hazard of a microbial pathogen in a food must consider this strain-to-strain variation.
References


