Research Note

Low Variability of Growth Parameters among Six O157:H7 and Non-O157:H7 Escherichia coli Strains

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ABSTRACT

Five Escherichia coli O157:H7 strains and one nonpathogenic E. coli strain were used. All strains were cultured in brain heart infusion broth and were inoculated in 16-well disposable module cassettes of a Bactometer impedance system. Two initial concentrations were obtained in the wells: 1.37 × 10^5 and 1.36 × 10^5 CFU/ml. The impedance measurements were monitored for 72 h at 5, 10, or 15°C, 48 h at 20°C, and 24 h at 25, 30, 35, 40, 45, 50, or 55°C. The lag time and the generation time of each culture were calculated from the detection time data. The coefficients of variation between the strains’ growth parameters were low (0.009 to 0.105 for generation time and 0.074 to 0.475 for lag time). An F test showed no significant differences between strains at 5 or 1% confidence levels.

Shiga toxin–producing Escherichia coli strains, including those with serotype O157:H7, are considered to be foodborne pathogens. This group is a major cause of gastroenteritis, which may be complicated by hemorrhagic colitis (HC) or by hemolytic uremic syndrome. Cattle are the principal reservoir of these microorganisms, and their transmission occurs through consumption of undercooked meat, unpasteurized dairy products, vegetables, or contaminated water (8).

Quantitative risk assessment forms the basis of the risk management decisions concerning foodborne microbial hazards (11). Predictive models and estimated model parameters (generation time [Tg] and lag time [λ]) are important in the assessment and description of the microbial responses (13). Although information on strain variability would also be helpful (16, 20), the absence of literature on strain variation (22) may lead to incomplete results in predictive models (16, 20). One reason for this lack of data is the appreciable work needed to obtain it with the use of classic viable count methods (13). Differences in the virulence of several E. coli O157:H7 (2) or non-O157:H7 (21) strains and high variability among O157:H7 strains under extreme growth conditions (4) have been reported.

The aim of the present work was to determine the variability of growth parameters (λ, and Tg) from different strains of E. coli (O157:H7 and non-O157:H7). The estimation uses detection times (DTs), which are the first data available when low concentrations of microorganisms are recorded with rapid methods.

MATERIALS AND METHODS

Strains and cultures. Five E. coli O157:H7 strains provided by the Spanish Type Culture Collection (CECT; Valencia, Spain) were used: CECT 4076 (E. coli O157:H7 isolated from a human with HC, Canada), CECT 4267 (E. coli O157:H7 VT1 VT2 isolated from a human with HC, Centers for Disease Control and Prevention [CDC], Atlanta, GA), CECT 4782 (E. coli O157:H7 VT1 VT2 exopolysaccharide + isolated from a human with HC, CDC), CECT 4783 (E. coli O157:H7 VT1 VT2 isolated from a human with HC, CDC), and CECT 4972 (nontoxigenic E. coli O157:H7). A non-O157:H7 and nonpathogenic strain was also used: CECT 516. All strains were cultured overnight in brain heart infusion broth (BHI; Difco, BD Diagnostics, Sparks, MD) at 37°C. Strains were stored in nutrient broth (Difco) plus 0.75% of nutrient agar (Difco) at room temperature.

Growth measurements. Aliquots (1 ml) from each cultured strain were transferred to tubes with 10 ml of BHI and were incubated at 37°C for 18 to 24 h. Populations were checked after they were plated onto Trypticase soy agar (TSA, Difco) plates and incubated at 37°C for 24 h. Final populations of 10^9 CFU/ml were obtained. Serial dilutions of cultures were made in sterile peptone water (Difco).

Impedance measurements were done as previously described (14). Briefly, aliquots of 0.9 ml of BHI broth were dispensed in each well of 16-well disposable module cassettes of a Bactometer impedance system (bioMerieux, Marcy-l’Étoile, France). The wells were inoculated with 0.1-ml portions from adequate dilutions of each strain. Concentrations ca. 1.37 × 10^5 and 1.36 × 10^5 CFU/ml were obtained in the wells, respectively. A control assay was done, with 0.1 ml of BHI broth inoculated into two wells per module. The cassettes were introduced into the Bactometer, and a capacitance test was monitored for 72 h at 5, 10, or 15°C, 48 h at 20°C, and 24 h at 25, 30, 35, 40, 45, 50, or 55°C. The assays were done in duplicate. Prior to the impedance tests, the population of

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each strain was determined by spreading 1-ml portions onto TSA (Difco) plates, followed by incubation at 37°C for 10 to 24 h.

**Growth parameters.** The DT value from each well (the time taken to reach a detectable population size from different initial levels) was collected and introduced in an Excel spreadsheet (Microsoft Inc., Redmond, WA). The differences in DT values between the 100-fold dilutions were recorded, and the mean Tg for each *E. coli* strain was calculated as follows (10):

\[
T_g = \frac{\log 2}{\mu} = \frac{\log 2}{(\log N_v - \log N_{\text{ini}})/(D_{\text{fin}} - D_{\text{ini}})}
\]

(1)

where \(\mu\) is the specific growth rate, \(N_v\) and \(N_{\text{ini}}\) are the counts of *E. coli* strains from initial inocula (ca. 10^5 and 10^3 CFU/ml, respectively), and \(D_{\text{fin}}\) and \(D_{\text{ini}}\) are the detection times obtained from each initial inoculum. The difference between the bacterial populations is twofold (\(\log N_v - \log N_{\text{ini}} = 2\); and \(\log 2 = 0.301\)). So, equation 1 could be redefined as:

\[
T_g = 0.151 \times (D_{\text{fin}} - D_{\text{ini}})
\]

(2)

The \(\lambda\) was estimated using the following expressions (15, 18):

\[
N_{(\text{GEN at DT})} = 3.32 \log \left( \frac{N_{\text{DT}}}{N_0} \right)
\]

(3)

\[
\lambda = DT - \left( T_g \times N_{(\text{GEN at DT})} \right)
\]

(4)

where \(N_{(\text{GEN at DT})}\) is the number of generations of the microorganism reached at \(D_T\). \(N_{\text{DT}}\) is the microorganism population at \(DT\) (10^5 CFU/ml, the threshold of detection of the Bactometer), and \(N_0\) is the microorganism population at \(t = 0\) (both initial populations were considered). An \(N_{(\text{GEN at DT})}\) value (equation 3) was obtained for each initial inoculum. DT (equation 4) was used as \(D_{\text{fin}}\) or \(D_{\text{ini}}\). Finally, two values of \(\lambda\) were calculated using equation 2: \(\lambda_{\text{ini}}\) or \(\lambda_{\text{fin}}\).

**Statistical analysis.** The means of the DT values for each initial inoculum, *E. coli* strain, and temperature were calculated. For \(\lambda\), the Pearson's correlation coefficients between the results of each inoculum at every temperature were calculated to see the linear correlation (dependence) between both sets of data. The mean, standard deviation, and variance of growth parameters (\(T_g\) and \(\lambda\)) were calculated. The coefficient of variation (CV) of the growth parameters was calculated (ratio between the standard deviation and the mean) to investigate the importance of variability among the temperatures or the strains. An \(F\) test was used to decide whether the difference between each *E. coli O157:H7* strain versus the non-O157:H7 strain was significant and also to explore differences between pathogenic and nonpathogenic strains. All statistical studies were performed with Excel software (Microsoft Inc.).

**RESULTS AND DISCUSSION.**

The average of duplicate DT values obtained from each strain inoculum (data not shown) was calculated and used in equation 2 for \(T_g\) calculation. Growth was not detected at 5 or 10°C for 72 h, or at 50 or 55°C for 24 h. The range of \(T_g\) values was between 2.4 and 2.7 h at 15°C, 1.1 and 1.4 h at 20°C, 0.7 and 0.8 h at 25°C, 0.35 and 0.4 h at 30°C, 0.3 and 0.4 h at 35°C, 0.2 and 0.3 h at 40°C, and 0.3 and 0.6 h at 45°C. The highest \(T_g\) values were found at 15°C (with a mean of 2.5 h), and the lowest at 40°C (a mean of 0.2 h). These results were compared with those obtained with the online Pathogen Modeling Program (PMP) (19). The model for aerobic growth in broth of *E. coli O157:H7* was selected with the following input conditions: temperature (15, 20, 25, 30, 35, 40, or 42°C; 45°C is not included in the model), pH 7.2, sodium chloride 0.5% (grams per deciliter), population initial level 4 log CFU/ml. The \(T_g\) values obtained at each temperature with the PMP were 2.26, 1.09, 0.61, 0.40, 0.30, 0.27, and 0.27, respectively. A good agreement between both sets of data is observed. At 15, 20, 25, and 45°C, the PMP results are in the lower limit of the \(T_g\) range of our study.

The \(\lambda\) values obtained from two initial concentrations of *E. coli O157:H7* and non-O157:H7 strains cultured in BHI at different temperatures were almost equal (data not shown). The Pearson's correlation coefficients between the \(\lambda\) values of each inoculum at every temperature were calculated to show the relationship between both sets of data. At all temperatures, the Pearson's correlation coefficient was 0.999, showing an almost totally positive correlation. When the inoculum is in the stationary phase, the \(\lambda\) is independent of the inoculum size (5). Means were found for both sets of \(\lambda\) values. Values of \(\lambda\) ranged between 20.9 and 26.2 h at 15°C, 6.9 and 12.1 h at 20°C, 3.7 and 4.9 h at 25°C, 3.0 and 3.8 h at 30°C, 2.2 and 2.7 h at 35°C, 1.4 and 1.9 h at 40°C, and 0.2 and 1.7 h at 45°C. The highest and lowest values were found at 15°C (a mean of 23.4 h) and 40°C (a mean of 1.6 h), respectively. The \(\lambda\) values obtained at every temperature with the PMP were 18.37, 6.97, 3.29, 1.93, 1.41, 1.28, and 1.31, respectively. The differences between the two sets of data are greater at 15 and 20°C. At 25, 30, and 35°C, the results from the PMP are in the lower limit of the range values obtained in our study.

The CVs of \(T_g\) and \(\lambda\) values are shown in Table 1. The higher CV for \(T_g\) was detected at 35°C (0.105 or 10.5%), and the lowest at 45°C (0.009, 0.9%). The variability among the strains was very small at all temperatures. The higher the CV, the greater the dispersion in the variable (1). All CV values for \(\lambda\) were between 0.074

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<th>Table 1. Coefficients of variation of the generation time and lag time obtained for Escherichia coli strainsa</th>
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*a CV, coefficients of variation; \(T_g\), generation time; \(\lambda\), lag time.*
and 0.187, except in the case of 45°C (0.475, 47.5%); an explanation could be that only when the inoculum is subjected to stress conditions might the inoculum size have an influence on the duration of the λ (5). Table 1 shows the CV for each E. coli strain, giving an indication of the variability associated with each strain at all temperatures studied. The results show similar values for all strains, ranging between 0.912 and 1.014 for Tg and between 1.144 and 1.342 for λ. These high CV values show the big differences among the temperatures and their direct influence on the growth of each strain.

Table 2 shows the F test statistic values calculated from the relationship between the variance of each E. coli O157:H7 strain and the variance of the nonpathogenic strain. The results show that, at 5 or 1% confidence levels, there is no significant difference between the strains (the critical values for the F test at 5 or 1% confidence levels are 3.79 and 6.99, respectively).

Conclusions on strain variability vary, reflecting the conditions, strains, or species used in the different studies. Duh and Schaffner (9) compared the growth rates and lag times of Listeria monocytogenes Scott A and Listeria innocua as a function of temperature (2 to 46°C) and found some differences between both species depending on the temperature selected. Begot et al. (3) found strain variation when investigating the growth response of L. monocytogenes and L. innocua strains under different growth conditions. The differences between the minimum and maximum parameter values among strains were greater for estimated λ values (25 times) than for estimated Tg values (3 times). De Jesus and Whiting (6) studied the variations in lag times and growth rates of L. monocytogenes from three different genotypic lineages. Some significant differences were observed between lineages, but extensive strain-to-strain variation was observed for all parameters tested (CV of 0.18).

Dengremont and Membré (7) identified three groups among five Staphylococcus aureus strains, using growth rates at different combinations of temperature, pH, and NaCl concentration. Lindqvist (13) characterized the variability in growth parameters of 34 S. aureus strains. The CV of growth parameters was up to six times larger among strains than within strains. Oscar (17) reported that the CVs for growth rates were similar among and within individual Salmonella strains growing in sterile ground chicken breast burgers at 25°C. The CV among strains was 9.4% for λ and 5.7% for μ.

Variation among strains of E. coli O157:H7 was reported by Whiting and Golden (22); the CVs of estimated growth parameters among 17 strains were larger than those assessed from experiments using single strains. Coleman et al. (4) found that the variability among nine E. coli O157:H7 strains studied was more apparent at the boundary conditions of growth (low pH [5.5] and low temperature [10°C]). Wenz et al. (21) found different results with E. coli strains from cattle mastitis. No predominant serotype was identified, and virulence genes were identified infrequently. Baker et al. (2) reported differences in the virulence of several E. coli O157:H7 strains isolated from humans and healthy cattle. Strains isolated from healthy cattle were less virulent than strains isolated from humans during disease outbreaks.

Strain variability has been observed by some of the authors cited above. The present study found no significant variability between the O157:H7 and non-O157:H7 strains, or the pathogenic and nonpathogenic strains. Recent results reported by Labhsetwar et al. (12) showed that gene expression can lead to phenotypic differences among cells even in isogenic populations growing under macroscopically identical conditions. However, a large proportion of the overall cell-to-cell variability in metabolic behavior could be attained by sampling only the 15 enzymes most likely to constrain growth. Furthermore, the CV of the growth rates was low (0.30). As a consequence, the metabolic changes of individual cells do not change the macroscopic appearance of the colonies and do not increase the CVs between the growth rates of species. Our results indicate that it could be possible to use non-O157:H7 and nonpathogenic strains for future studies on growth parameters. Further studies are in progress to evaluate the variability of E. coli strains.

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**REFERENCES**


