Strain effect on the heterogeneity of individual-cell growth kinetics of *Salmonella Typhimurium*

A. Lianou¹, G.-J.E. Nychas² and K.P. Koutsoumanis¹

¹Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece (alianou@agro.auth.gr, kkoutsou@agro.auth.gr)

²Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science, Technology and Human Nutrition, Agricultural University of Athens, Athens, Greece (gin@aua.gr)

In continuation to a previous study of ours, the findings of which demonstrated significant heterogeneity in the growth dynamics of microcolonies originating from single cells of *Salmonella enterica serotype Typhimurium*, the objective of the present work was the evaluation of the existence of a strain effect on such variability. For this purpose, the colonial growth of single cells of five *S. Typhimurium* strains was monitored by phase-contrast time-lapse microscopy, and the first, second and third division times (FDT, SDT and TDT, respectively) were determined. Furthermore, after cell counting, data were transformed to the respective growth curves (i.e., number of cells in each microcolony as a function of time), and the single-cell growth kinetic parameters (i.e., maximum specific growth rate and lag time) for each one of the tested strains were estimated. The results of the present study indicated that the heterogeneity of individual-cell division and growth dynamics may differ among strains of *S. Typhimurium*, and provide useful quantitative information for incorporating such variability in stochastic growth models and risk assessment studies.

**Abstract**

**Introduction**

Phenotypic variability among single bacterial cells has been reported (Konstantinou et al., 2004), attracting researchers working in the field of predictive microbiology. Given the contamination of foods with pathogens usually occurs at very low population levels, single-cell growth variability is of major importance in the context of quantitative microbial risk assessment (Voysey and Brown, 2000). Hence, recent studies have focused on the development of methods allowing for monitoring of the growth behavior (i.e., division times) of individual microbial cells (Ellweg et al., 2004; Wakamoto et al., 2005). In a recent study undertaken in our laboratory, a time-lapse microscopy method allowing for the evaluation of the growth dynamics of microcolonies originating from single cells was developed, and significant single-cell heterogeneity was demonstrated for *Salmonella enterica serotype Typhimurium* (Koutsoumanis and Lianou, 2013). Next research goals should include the determination of factors affecting single-cell growth behavior, and the development of effective stochastic models for microbial growth in foods taking into account such effects.

**Objective**

The objective of the present work was the characterization of the single-cell growth variability of different strains of *S. enterica serotype Typhimurium*, and the evaluation of the existence of a strain effect on such variability.

**Materials and methods**

**Bacterial strains and growth media**

The bacterial strains used in the study were *S. Typhimurium* FSL RB-085 (bovine isolate, Strain 1), FSL RB-075 (bovine isolate, Strain 2), FSD 05-250 (bovine isolate, Strain 3) and FSL 55-195 (bovine isolate, Strain 4), kindly provided by Dr. Martin Wedmann (Cornell University, Ithaca, New York), as well as an atypical (floor water) isolate (Strain 5) kindly provided by Dr. Consaldirion Geigenzorg (Aristotle University of Thessaloniki, Thessaloniki, Greece).

Stock cultures of the strains were stored frozen (-70°C) onto Microbank™ porous beads (Pro-Lab Diagnostics, Ontario, Canada). Working cultures were stored refrigerated (4°C) on tryptone soy agar (TSA; Lab M Limited) and incubating at 37°C for 24 h. Twenty microliters of the 24-h cultures of the strains, after two 10-fold serial dilutions in quarter-strength Ringer’s solution (Lab M Limited), were added to 500 µl of TSA solidified on glass slides, and the 20-µl volumes were left to dry in a biological safety cabinet for 5 min.

The inoculum size was approximately 10⁴ to 10⁵ CFU/ml.

**Time-lapse microscopy**

The colonial growth of single cells was monitored at 25°C by phase-contrast time-lapse microscopy using a motorized microscope (Olympus BX51, Tokyo, Japan) equipped with a ×100 objective (Olympus) and a high-resolution device camera (Olympus DP71). Working cultures were stored refrigerated (30°C) on tryptone soy agar (TSA; Lab M Limited, Lancashire, United Kingdom) slants.

Strains were activated by transferring a loopful from the respective TSA slant into 10 ml of tryptone soy broth (TSB; Lab M Limited) and incubating at 37°C for 24 h.

The first, second and third division times (FDT, SDT and TDT, respectively) were determined. The data collected in this study provide useful quantitative information for incorporating strain variability in individual-cell behavior in stochastic growth models and risk assessment studies.

**Data analysis and modelling**

The obtained growth curves were fitted to the primary model of Baranyi and Roberts (1994) for the estimation of lag time ($\lambda$) and maximum specific growth rate ($\mu_{max}$) for each one of the tested strains.

**Results**

- **Time-lapse microscopy images** of the behavior of a *S. Typhimurium* individual cell over time are shown below.

- **Results**

- **Dependent** on the tested strain, the mean FDT ranged from 1.69 to 2.75 h, while the corresponding ranges for the mean SDT and TDT were determined to be 0.79 to 1.03 and 0.39 to 0.65 h, respectively.

- The coefficient of variation (CV) of the division times of individual cells ranged from 33.8 to 43.3% for the FDT, from 43.9 to 62.4% for the SDT, and from 39.9 to 57.7% for the TDT.

- The analysis of variance showed significant (P<0.05) differences among the five strains for the means of all three division times.

- Using the Levene’s test and the Bonferroni confidence intervals for standard deviations, significant (P<0.05) differences were also found in the variance of individual-cell division times among the tested strains (Figures 1 and 2).

- The CV of $\lambda$ and $\mu_{max}$ ranged from 40.4 to 52.8% and from 13.7 to 21.2%, respectively (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean (h)</th>
<th>$\mu_{max}$ (h⁻¹)</th>
<th>CV %</th>
<th>Mean (%CV)</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.90</td>
<td>0.12</td>
<td>40.4</td>
<td>1.18</td>
<td>23.8</td>
</tr>
<tr>
<td>2</td>
<td>1.22</td>
<td>0.11</td>
<td>40.1</td>
<td>0.11</td>
<td>13.7</td>
</tr>
<tr>
<td>3</td>
<td>1.73</td>
<td>0.11</td>
<td>41.6</td>
<td>0.78</td>
<td>16.3</td>
</tr>
<tr>
<td>4</td>
<td>2.05</td>
<td>0.14</td>
<td>41.7</td>
<td>0.82</td>
<td>17.6</td>
</tr>
<tr>
<td>5</td>
<td>2.12</td>
<td>0.16</td>
<td>41.6</td>
<td>0.77</td>
<td>16.3</td>
</tr>
</tbody>
</table>

**Conclusions**

- The heterogeneity of individual-cell division and growth dynamics may differ among strains of *S. Typhimurium*.

- The data collected in this study provide useful quantitative information for incorporating strain variability in individual-cell behavior in stochastic growth models and risk assessment studies.

**Acknowledgements**

We acknowledge the action THALIS “Biological Investigation Of The Forces that Influence the Life of pathogens having as Mission to Survive in various Lifestyles, BIOFLMS”. The action falls under the Operational Programme (OP) “Education and Lifelong Learning (EUL)” and is co-financed by the European Social Fund (ESF) and National Resources.

**References**


Strain effect on the heterogeneity of individual-cell growth kinetics of *Salmonella* Typhimurium

Lianou A., Nychas G.-J.E. and Koutsoumanis K.P.

1 Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
2 Department of Food Science, Technology and Human Nutrition, Agricultural University of Athens, Athens 11855, Greece

OBJECTIVE

Phenotypic variability among single bacterial cells has been reported (Korobkova et al., 2004), attracting the attention and interest of researchers, particularly of those working in the field of predictive microbiology. Significant single-cell heterogeneity was demonstrated by the findings of a recent study of ours which, by utilizing a time-lapse microscopy method, allowed for the evaluation of the growth dynamics of microcolonies originating from single cells of *Salmonella enterica* (Koutsoumanis and Lianou, 2013). In continuation to the latter investigation, the objective of the present work was the characterization of the single-cell growth variability of different strains of *S. enterica* serotype Typhimurium, and the evaluation of the existence of a strain effect on such variability.

METHODS

Five *S. Typhimurium* strains were evaluated in the present study. Twenty microliters of 24-h cultures of the strains, after two 10-fold serial dilutions in Ringer’s solution, were added to 500 µl of tryptone soy agar solidified on a glass slide, and, after allowing for drying of the inoculum for 5 min, the inoculated agar was covered by a coverslip and sealed with silicone to avoid dehydration. The colonial growth of single cells was monitored at 25°C by phase-contrast time-lapse microscopy using a z-motorized microscope (Olympus BX61) equipped with a 100× objective (Olympus) and a high-resolution device camera (Olympus DP71) (Koutsoumanis and Lianou, 2013). The high quality of images allowed for monitoring the number of cells in each microcolony with time using the ImageProPlus image analysis software, and for the determination of the first, second and third division times (FDT, SDT and TDT, respectively). Furthermore, after cell counting, data were transformed to the respective growth curves (i.e., number of cells in each microcolony as a function of time), and the latter were fitted to the primary model of Baranyi and Roberts (1994) for the estimation of the single-cell growth kinetic parameters for each one of the tested strains.

RESULTS

Depending on the tested *S. Typhimurium* strain, the mean FDT ranged from 1.69 to 2.75 h, while the corresponding ranges for the mean SDT and TDT were determined to be 0.76 to 1.03 h and 0.39 to 0.65 h, respectively. The coefficient of variation of the division times of individual cells ranged from 33.8 to 43.3% for the FDT, from 43.9 to 62.4% for the SDT, and from 39.9 to 57.7% for the TDT. The analysis of variance showed significant (*P*<0.05) differences among the 5 strains for the means of all three division times. Using the Levene’s test and the Bonferroni confidence intervals for standard deviations, significant (*P*<0.05) differences were also found in the variance of individual-cell division times among strains. The observed distributions of the kinetic parameters (i.e., lag phase and maximum specific growth rate) of microcolonies originating from single cells were used to demonstrate the impact of variability on modelling growth of microbial populations of different strains using Monte Carlo simulations.

CONCLUSIONS AND IMPACT OF THE STUDY

The present study showed that the variability of individual-cell growth kinetics may differ among strains of *S. Typhimurium*. The results provide useful quantitative information for incorporating strain variability and heterogeneity in individual-cell behavior in stochastic growth models and risk assessment studies.
REFERENCES
