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

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Abstract

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.

Introduction

Phenotypic variability among single bacterial cells has been reported (Korobkova et al., 2004), attracting the interest of researchers working in the field of predictive microbiology. Given that 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 (Voyseya 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 (Elfwing 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 R6-065 (bovine isolate; Strain 1), FSL S5-370 (human isolate; Strain 2), FSL S5-520 (bovine isolate; Strain 3) and FSL S5-786 (bovine isolate; Strain 4), kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, New York), as well as an abattoir (floor water) isolate (Strain 5) kindly provided by Dr. Constantin Genigeorgis (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 (5°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.
- 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 10^6 to 10^7 CFU/ml.

Time-lapse microscopy

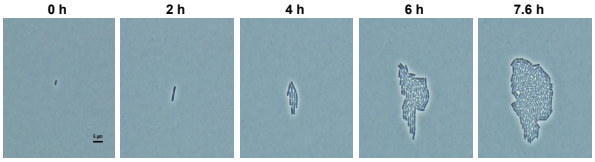
- The colonial growth of single cells was monitored at 25°C by phase-contrast time-lapse microscopy using a z-motorized microscope (Olympus BX61, Tokyo, Japan) equipped with a ×100 objective (Olympus) and a high-resolution device camera (Olympus DP71).
- Images of the field of view were acquired at 5-min intervals for 6 to 8 h after inoculation.
- The quality of the images was improved via an autofocus procedure with an extended depth of focus (EDF) system (Koutsoumanis and Lianou, 2013).
- The number of cells in each microcolony with time was monitored using the ImageProPlus image analysis software.
- The colonial growth of 15-25 single cells in the field of view to a final number of approximately 25-30 cells per colony were monitored in each experiment.

Data analysis and modelling

- The first, second and third division times (FDT, SDT and TDT, respectively) were determined.
- The means of FDT, SDT and TDT were compared using analysis of variance, while the Levene's test and the Bonferroni confidence intervals for standard deviations were used to evaluate the intra-species variance of the individual-cell division times.
- After cell counting, data were transformed to the respective growth curves showing the exact number of cells in each microcolony originating from a single cell over time.
- The obtained growth curves were fitted to the primary model of Baranyi and Roberts (1994) for the estimation of lag time (λ) and maximum specific growth rate (μ_{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.



- Depending 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 0.76 to 1.03 h 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**).
- With regard to the estimated individual-cell growth kinetic parameters, the mean λ values ranged from 1.22 to 2.12 h and the mean μ_{max} values ranged from 0.76 to 1.18 h^{-1} (**Table 1**).
- The CV of λ and μ_{max} ranged from 40.4 to 52.8% and from 13.7 to 21.2%, respectively (**Table 1**).

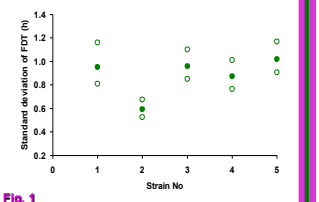


Fig. 1

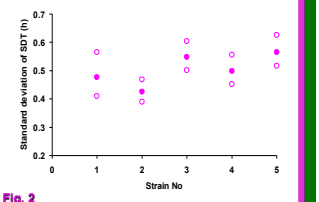


Fig. 2

Table 1

Mean, standard deviation (sd) and coefficient of variation (CV) of the growth kinetic parameters of the S. Typhimurium strains tested

Strain	λ (h)			μ_{max} (h^{-1})		
	Mean	sd	%CV	Mean	sd	%CV
1	1.90	0.77	40.4	1.18	0.23	19.8
2	1.22	0.57	47.1	0.81	0.11	13.7
3	1.73	0.91	52.8	0.76	0.16	20.7
4	2.05	0.85	41.7	0.82	0.14	17.6
5	2.12	0.88	41.6	0.77	0.16	21.2

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

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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.

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