



(P6.67) Insights into the behavioral heterogeneity of individual bacterial cells

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1 Introduction

Most studies concerning the behavior of microorganisms in food are conducted with large microbial populations (Aspridou & Koutsoumanis, 2015). These studies are based on deterministic approaches through which **point estimates** of microbial population levels are produced, without considering individual cells (Brehm-Stecher, 2007). However, **contamination** of food products occurs at **very low levels**, so deterministic models are not suitable for such situations due to the **variability in single cell behavior** (Aspridou & Koutsoumanis, 2020). In contrast, stochastic models which take into account the heterogeneity at the single cell level, are considered a proper tool for the **description of the high level of variability characterizing microbial dynamics** (Koutsoumanis & Lianou, 2013).

Objectives

- To **evaluate** and **describe** the **heterogeneity** in the behaviour of individual bacterial cells under growth conditions
- To **evaluate** population dynamics

2 Materials & Methods

- Escherichia coli*
- Tryptone Soy Agar w/o dextrose (TSA;** Lab M Limited, Lancashire, UK)
- 10⁶ to 10⁷ CFU/ml**

Data analysis

Kinetic model approach

- Cell counting** of each microcolony
- Fitting of obtained growth curves (up to **25-30 cells**) → **primary model of Baranyi and Roberts** (1994) → estimation of λ and μ_{max}

- Fitting of λ and μ_{max} to various **probability distributions** using @Risk 7.6 for Excel software (Palisade Corporation, Newfield, NY, USA)

- Phase-contrast time-lapse microscope** (Olympus BX61; Olympus, Tokyo, Japan)
- Monitoring of colonial growth of single cells (**30°C**)
- Image acquisition at **5-min intervals (6-8 h)**

Birth model approach

- Estimation of **1st-5th division time**
- Use of a **birth model** to predict the **growth of individual cells for various N₀** using Monte Carlo simulations

$$N_t = (N_0 - N_g) + \sum_{i=1}^{N_g} \begin{cases} 1 & \text{for } t \leq \lambda_i \\ e^{\mu_{max,i}(t-\lambda_i)} & \text{for } t > \lambda_i \end{cases}$$

N_t : total number of cells in a population at time t ,
 N_0 : initial number of cells in the population at $t = 0$,
 $N_g \sim \text{Binomial}(N_0, P_g)$: initial number of cells in the population at $t = 0$ that are able to grow and form a colony,
 P_g : mean probability of growth

References
 Aspridou, Z., & Koutsoumanis, K. (2020). Variability in microbial inactivation: From deterministic Bigelow model to probability distribution of single cell inactivation times. *Food Research International*, 137, 109579.
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 Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International journal of food microbiology*, 23(3-4), 277-294.
 Brehm-Stecher, B. F. (2007). New Technologies for Imaging and Analysis of Individual Microbial Cells. In *Imaging Cellular and Molecular Biological Functions* (pp. 313-343). Springer, Berlin, Heidelberg.
 Koutsoumanis, K. P., & Lianou, A. (2013). Stochasticity in colonial growth dynamics of individual bacterial cells. *Applied and Environmental Microbiology*, 79(7), 2294-2301.

3 Results

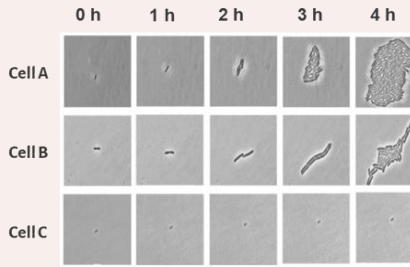


Figure 1. Time-lapse microscopy images of the behavior of representative *Escherichia coli* individual cells over time.

Kinetic model approach

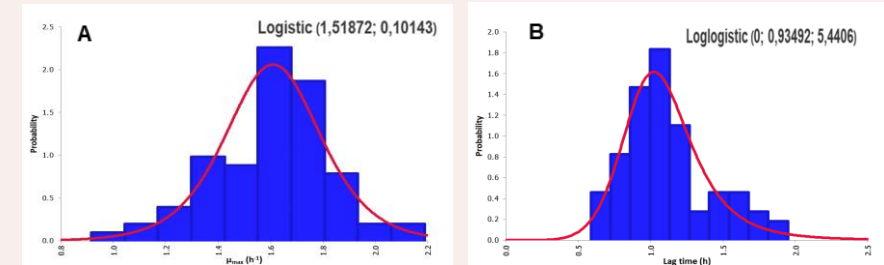


Figure 2. Variability in the kinetic parameters, λ and μ_{max} , for 80 colonies originating from an *Escherichia coli* individual cell. Probability histograms of (A) μ_{max} and (B) lag. Red lines represent the best-fitted distributions to the data.

Birth model approach

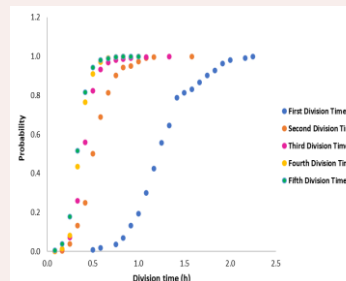


Figure 3. Cumulative distributions of 1st-5th division times of *Escherichia coli* individual cells.

Table 1. Best-fitted distribution for 1st-5th division times.

DT	Mean	Counts
1 st	1.29	113
2 nd	0.57	225
3 rd	0.45	448
4 th	0.40	891
5 th	0.38	1763

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Figure 4. Birth model development of *Escherichia coli* individual cells.

Kinetic model VS Birth model

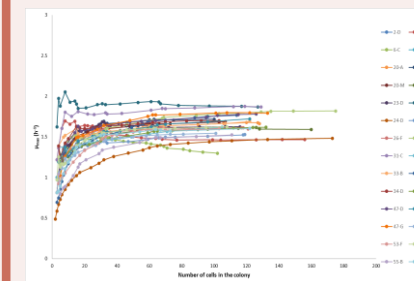


Figure 5. μ_{max} as affected by the increasing number of *Escherichia coli* cells in the microcolony over time.

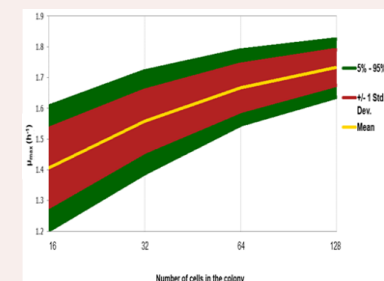


Figure 6. Relation between μ_{max} and the final number of *Escherichia coli* cells in a microcolony.

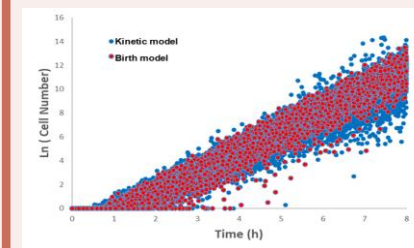


Figure 7. Simulation output of the stochastic model for the colonial growth of an *Escherichia coli* individual cell. Monte Carlo simulation with 10,000 iterations and a uniform distribution for time t ($t \sim \text{Uniform}(0, 8)$).

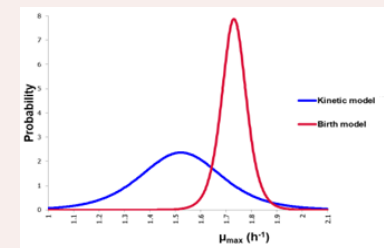


Figure 8. Probability distributions of stabilized μ_{max} values. Kinetic model approach vs Birth model approach.

4 Conclusions

- ✓ Variability in the growth dynamics of single cells i.e., in the kinetic parameters, μ_{max} & λ , and in division times

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- ✓ Kinetic model approach: μ_{max} stabilization when the final number of cells in the microcolony reaches 25-30
- ✓ Birth model approach: μ_{max} stabilization when the final number of cells in the microcolony exceeds 100 → high impact of 5th-6th division time distributions
- ✓ Variability in the growth dynamics is more notable following the kinetic model approach