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Develop mechanistic models of transition periods between lag/exponential and exponential/stationary phase

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Abstract

A continuing goal in predictive microbiology is models directly based on physiological behavior. Buchanan et al.[1] hypothesized that (1) the curvilinear lag/exponential transition represents the variability of cells in the adjustment (t_a) and metabolic (t_m) periods, and (2) the exponential/stationary transition is determined by limiting nutrient diffusion rates. Nutritional shift trials were conducted to estimate *E.coli* K-12 growth. Lactase production time suggest that lactase gene translation occurs after completion of lag phase. Agitation rates and inoculum sizes both influenced the shape of the exponential/stationary phase transition. Monte Carlo simulations allowed the generation of sigmoidal growth curves while considering physiological events.

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1. Introduction

Primary models are a set of time-dependent equations that can describe microbial population levels under certain environmental conditions[2]. Nowadays, primary models are widely accepted as a tool for quantitative food microbiology. They have been used in conjunction with curve-fitting software to evaluate food associated microbial growth. The three most commonly used models are Baranyi, Gompertzs, and three-phase linear models. The three-phase linear model was proposed by Buchanan et al.[1] in 1997. It simply divided the growth curve into three phases:

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- The lag phase: it is the time needed for the cells to adapt to the new environment and prepare for the first division. During this period, the specific growth rate $\mu=0$.
- The exponential phase: also known as log phase. The number of cells increase at a constant rate, $\mu=k$. If the bacterial counts are expressed in terms of logarithmic transformation, a straight line could be expected.
- The stationary phase: the growth rate balances out the death rate, so the cells reach the maximum population density that the medium could hold.

The three-phase linear model can be described as below:

$$\begin{aligned} &t < t_{lag}, \ \mathrm{Log}(N_t) = \mathrm{Log}(N_0); \\ &t_{lag} < t < t_{max}, \ \mathrm{Log}(N_t) = \mathrm{Log}(N_0) + \mu \cdot (t - t_{lag}); \\ &t > t_{max}, \ \mathrm{Log}(N_t) = \mathrm{Log}(N_{max}). \end{aligned} \quad (1)$$

Where: $\mathrm{Log}(N_0)$ [Log (CFU/ml)] is the initial population density, $\mathrm{Log}(N_t)$ [Log (CFU/ml)] is population density at time t (h), $\mathrm{Log}(N_{max})$ [Log (CFU/ml)] is the maximum population density at the stationary phase, μ [Log (CFU/ml)$\cdot h^{-1}$] is the specific growth rate and t_{lag} (h) is the lag phase duration. They hypothesized that when cells were grown to early stationary phase and then transferred to a medium that was identical in all attributes except the carbon source (nutritional-shift), the culture would experience a lag phase consisting of an adjustment period (t_a) when the cells alter their physiological state to adapt the new environment (eg. induction of a catabolic enzyme) and a metabolic period (t_m) when the cells synthesize energy and critical cellular component related to the first division[1]. Other than the widely accepted hypothesis that the specific growth rate increases gradually from zero to maximum[3,4], they further proposed that the curvilinear lag/exponential transition reflected the biological variance among the population.

The agitation rate during the culturing also plays an important role on microbial growth kinetics since it can substantially enlarge aeration and nutrient diffusion. When studying the transition period from exponential to stationary phase, another critical aspect to be considered is the inoculum size. A number of studies have reported that as the size of inoculum decreases, both the mean lag phase duration and the corresponding variation increase. However, the effect of inoculum size on late exponential and early stationary phase has not been fully investigated yet.

2. Materials and methods

2.1. Microorganisms and preculture conditions

E.coli K-12 (ATCC 23716) was kept frozen at -80ºC in brain heart infusion (BHI) broth supplemented with 20% glycerol for long-term preservation. A loopful of stock culture was streak on brain heart infusion agar (BHIA) and incubated at 37ºC for 24h. The plate serves as a short-term preservation and can keep active for up to one month if stored in 4ºC refrigerator. A single colony from the plate was picked and subcultured twice in sterile BHI broth for 24 h, with the first time incubated at 37ºC and the second time at temperatures same as the transferred environments. Cell cultivation under the defined conditions will yield early stationary cells at level of 10^8 - 10^9 CFU/ml.

2.2. Lag to exponential phase: nutritional-shift

BHI-grown *E. coli* K12 cells were transferred to diverse liquid medium: tryptic soy broth without dextrose (T-G) and TSB without dextrose but 0.5% lactose (T+L), and cultured at 35ºC, 30 rpm for designated time periods. Growth was measured both spectrophotometrically and culturally. GENESYS 20 viable spectrophotometer (Themo Fisher Scientific, model 4001/4, USA) was employed to measure the turbidity of the samples at 550 nm, using the uninoculated broth (T-G for T-G treatment, T+L for T+L treatment) as blanks. Serial dilutions (10^{-3} and 10^{-5}) were plated on TSA plates using Eddy Jet 2 spiral plater (NeuTec Group. Inc, Model 10001701/759, USA) and incubated at 35-37ºC for 24 hours. Enumeration was conducted by flash & go automatic plate counter (NeuTec Group. Inc, Model 10006021/182, USA). The cultures were assayed for lactase activity by the AOAC approved Ortho-

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