GROWTH OF *PSEUDOMONAS AERUGINOSA* SINGLE CELLS AND CELL POPULATIONS

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Abstract: In this article, a single cell growth image system was used to study the single cell growth of *Pseudomonas Aeruginosa*. A stochastic modelling was applied as a simulation process to connect the growth from *P. Aeruginosa* single cells to cell populations. Bacteria growth counts using different inoculum sizes were generated experimentally to validate the simulation process. Results demonstrated that the agreement between simulations and bacteria growth counts were good at both 25℃ and 35℃. This method made it possible to predict bacterial population growth with considering microbial single cells which useful for food safety control.

Ⅰ. INTRODUCTION

Traditional predictive microbiology uses deterministic models to describe the growth of large microbial populations without considering the microbial single cells. Since food contaminations caused by harmful microbes usually occur due to small amounts which consequently aggravate the effect of uncertainty and variability on the population growth of microorganisms.

Methods used to measure the growth of microbial individual cells could be traced back to the early 1930s when Kelly et al.[1] put a slice of inoculated solid agar under a microscope to study the growth rate of individual cells. For the last several decades, facilities have emerged in endlessly. Wakamoto et al.[2] suggested a method that could transfer a single bacterium from one well to another using microfluidic devices. Fritzsch et al.[3] invented an integrated microfluidic lab-on-a-chip system which allowed for isolation, contactless cultivation, and time-resolved analysis of single cells in a micro-flow. Elfwing et al.[4] designed a flow chamber to monitor the consecutive divisions of a single cell in which the cells attached to a solid surface and grew in a continuous flow environment.

Monitoring the growth of bacterial single cells plays an increasingly important role in predicting the growth of microbial populations, especially when the inoculum was small. How to establish the connection from microbial single cells to cell populations was also a fundamental issue. Inspired by the method suggested by Elfwing et al.[4], in this article, a flow chamber was improved as a whole system with time-lapse microscopic photography to study the single cell growth of *P. Aeruginosa*. Then a stochastic modeling process was applied as a simulation process to connect the growth from *P. Aeruginosa* single cells to cell populations.

Ⅱ. MATERIALS AND METHODS

One colony of *P. Aeruginosa* strain was picked from an agar plate and inoculated into a flask containing 100 mL sterile phosphate-buffer saline (PBS) (8g/L NaCl, 0.2g/L KCl, 0.24g/L KH_2PO_4, 1.44g/L Na_2HPO_4; pH 7.5). Another colony of *P. Aeruginosa* strain was picked and inoculated into a flask containing 300 mL of sterile Nutrient Broth (NB) (10g/L,

5g/L veal infusion, 10g/L NaCl; pH 7.2). This flask was shaken for 18h at 37°C until the culture reached its stationary phase.

The tubing system was first washed with 75% ethyl alcohol for at least 15 minutes and then washed by sterile PBS for another 15 minutes. 150μL of *P. Aeruginosa*-PBS suspension prepared before was inoculated onto a sterile slide surface and placed horizontally in the laminar flow cabinet for at least 30 minutes. Main parts of flow chamber were assembled orderly from "a" to "c" and tightly combined with pipe duct tape. Details were shown in Fig. 1.

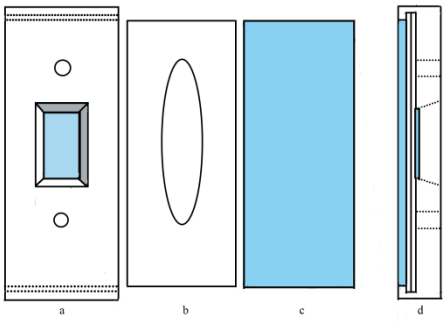


Fig.1 Main parts of flow chamber (a. Top block of flow chamber with cover slide in the middle and inlet and outlet pipes; b. polymer spacer made by qualitative filter paper; c. microscope slide; d. combination of flow chamber)

A single cell growth image system was established as showed in Fig. 2. Since *P. Aeruginosa* single cells were restrained in PBS, growth initiated when the valve changed from PBS to nutrient broth. The flow medium gave an average linear velocity of approximately 1.1cm/s. The driver (NIS-Elements 3.2) software installed in computer was used for taking pictures for every 3~5 minute intervals. Growth data of *P. Aeruginosa* single cells was expressed using pixels which acquired through image processing (Image-Pro Plus 6.0).

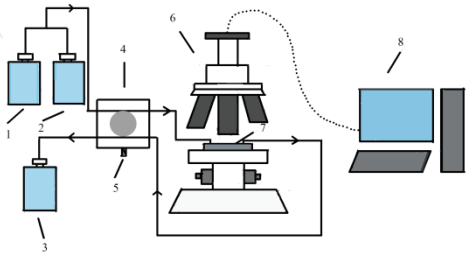


Fig.2 Single cell growth image system

(1. Nutrient broth; 2. phosphate buffer; 3. waste liquid; 4. peristaltic pump; 5. switch button; 6. microscope; 7. flow chamber; 8. computer)

The first *k*th generation times of 30 *P. Aeruginosa* single cells were recorded at both 25℃ and 35℃. Population growth simulation was carried out on the basis of binary fission multiplication and with an initial inoculum size of N_0 cells. The time for each generation was assigned accordingly to the distribution time acquired from single cell growth image system. After the *k*th division, the time for which beyond the *k*th generation was assigned by the *k*th generation time (The value of *k* was usually around 3 or 4)[5]. The iteration continued until the population reached a given number of cells (N_t), and then we could obtain a series of relevant time changing along with the multiplication of *P. Aeruginosa.* The simulation process was carried out by a created Java program and the result was dealt with by fitting the model of Gompertz as follows[6] :

$$\log N = N_0 + C * \exp\{-\exp[-B*(t-M)]\}$$

where log *N* was the decimal logarithm of

microbial counts [lg(CFU/mL)] at time t, N_0 was the asymptotic log count as time decreases indefinitely [lg(CFU/mL)], C was the log count increment as time increases indefinitely [lg(CFU/mL)], B was the relative maximum growth rate at time M (min^{-1}), M was the time required to reach the maximum growth rate (min).

Bacteria growth counts using different initial inoculum sizes [0 lg(CFU/mL), 1 lg(CFU/mL), 2 lg(CFU/mL)] were generated experimentally to validate the simulation process (Matlab®R2009b, Mathworks Company, USA).

Ⅲ. RESULTS AND DISCUSSION

Daughter cells with weaker adhesive abilities were flowed away by the shear force of nutrient broth. Pixels of a *P. Aaeruginosa* single cell were extracted from pictures obtained through the single cell growth image system. Fluctuated tendency of pixels as a function of time for a *P. Aaeruginosa* single cell were showed in Fig. 3. Each sudden drop represented an end of the cell division. Moreover, the time for each *P. Aaeruginosa* single cell began to divide was different. It suggested a stochastic growth property of *P. Aaeruginosa* single cells.

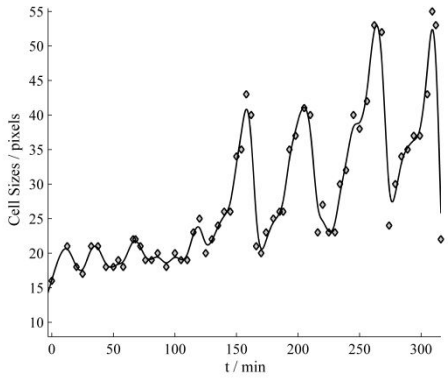
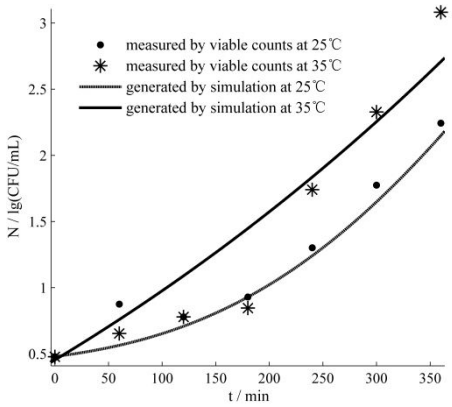
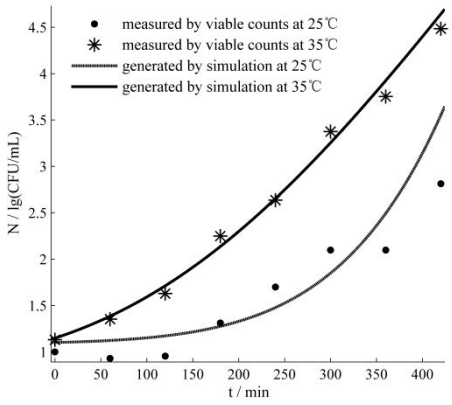


Fig.3 Changes of a *P. Aaeruginosa* single cell size

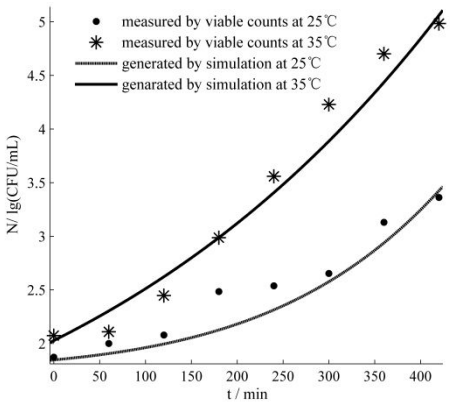
Growth of *P. Aaeruginosa* single cells in the flow chamber was obviously different from that under the optimal conditions. Because the flow liquid taking away some signaling molecules along with the daughter cells, it might weaken the effect of quorum sensing[7]. However, the results got from our experiment (Fig. 4) demonstrated an agreement result between bacterial growth counts and simulation curves at both 25℃ and 35℃. Results indicated that when the inoculum size was relatively small, the influence caused by quorum sensing might be ignored. In fact, it was more important to control food which contaminated with small harmful microorganisms instead of those already spoiled.



（A）



(B)



(C)

Fig.4 Simulation and validation of *P. Aaeruginosa* growth with different inoculum sizes (The initial inoculum size is about 0 lg(CFU/mL) (A), 1 lg(CFU/mL) (B), 2 lg(CFU/mL) (C))

Ⅳ. CONCLUSION

A stochastic growth property of *P. Aeruginosa* single cells was verified by using the single cell growth image system. The simulation process developed the relationship of the growth from *P. Aeruginosa* single cells to cell populations, and this method might provide a more accurate way to predict bacterial population growth.

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