Characteristics and Modeling of *Escherichia coli* Growth in Pouched Food

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Characteristics of the growth kinetics of *Escherichia coli* cells in pouched mashed potatoes under various conditions were studied with a mathematical model. Bacterial cells were inoculated in sterile mashed potatoes and then sealed in vinyl pouches, in which a very small amount of air was included. The growth curves of cells in the pouched mashed potatoes at constant temperature (12–34°C) were sigmoidal with time on a semi-logarithmic plot and were successfully described with a new logistic model recently developed by us. The rate constant of growth showed a highly linear relationship to the temperature with the square-root model, and the lag period was longer at lower temperatures. The growth curve in glass tubes containing a large volume of air was similar to that in pouches, showing that the rate of growth was not affected by the volume of the surrounding air. The growth curves in pouched mashed potatoes were very similar to those in nutrient broth or on the surface of nutrient agar, which we previously reported. These results suggested that the growth kinetics of the bacterial cells under various conditions of rich nutrition might be almost identical, and can be described with a simple growth model like ours.

**Key words:** modeling; logistic model; growth kinetics; pouched food

Introduction

Generally, both solid and liquid foods are consumed in our daily life. The microbial growth in liquids is planktonic. On solid foods, surface growth of microorganisms occurs, and con generate a biofilm, a unique microbial community. The inside of solid food is generally less well oxygenated than the surface. Commercial foods pouched in plastic bags characteristically contain a very small volume of air.

For the last two decades, a number of mathematical models have been developed to quantitatively describe microbial growth in culture media and food. This field is called predictive food microbiology. Most of the studies, however, have dealt with microbial growth in liquids and on surfaces. There seem to be few studies on the dependence of the growth kinetics on the volume of available air.

We recently developed a new growth model based on a logistic equation. The model successfully describes the growth curves of bacteria, including *E. coli*, *Salmonella* sp., and *Staphylococcus aureus*, in liquids such as broth, meat slurry, and milk. More recently, we found that the model could describe and predict the surface growth of *E. coli* on filtration membranes located on the surface of nutrient agar plates. However, no studies have been done on growth inside a food with the model.

In this study, therefore, we studied the kinetics of microbial growth in a location containing a very small volume of air, and examined the applicability of our model to such growth. The bacterial strain, *E. coli* 1952, studied here was one that we had previously used to assess growth in a liquid and on a surface. As a model food, we selected mashed potatoes, which are considered to be a homogenous food.

Materials and Methods

Cell preparation

Cell suspensions of *E. coli* 1952 were prepared by the method of Fujikawa et al. Briefly, bacterial cells were activated on a nutrient agar plate (Nissui Pharmaceuticals, Tokyo, Japan) at 35°C for 24 hr. Cells of several well-grown colonies on the plate were incubated in a nutrient broth (Nissui Pharmaceuticals) with shaking at 35°C for 24 hr. Cultured cells were washed twice with phosphate buffer, pH 7.0, by centrifugation. Cells were then suspended in the buffer and further diluted with sterile, purified water to make the final concentration of 10⁶ CFU/mL.

Mashed potatoes

Mashed potatoes (Ajinomoto Foods, Tokyo) (50 g) were mixed with nutrient broth powder (Nissui Pharmaceuticals) (7.5 g), and water (200 ml) and then steri-
lized at 121°C for 15 min. After having cooled to room temperature, the potatoes were thoroughly mixed with the cell suspension prepared above (50 mL). Portions (about eight grams) of the food sample were inserted into pouches (120 mm width, 80 mm height, and 1 mm depth), which were made with sterile stomaching bags and heat-sealed after visible air bubbles had been removed as thoroughly as possible. Portions (about two grams) of the sample were also inserted into sterile glass tubes (120-mL size) with tight plastic caps.

**Incubation**

The food samples in the pouches and glass tubes prepared above were incubated in duplicate in an incubator (PR-3G, Tabai Espec Co., Osaka) and a water bath unit (DH-12, Taitech, Tokyo), respectively, at constant temperature. The come-up time of the sample to reach the designated temperature was measured with a digital thermometer (AM-7002, Anritsu Meter Co., Tokyo). These times, which were 12 min for the pouches and 9.5 min for the glass tubes at the constant temperatures measured, were taken into consideration in this study.

**Cell counts**

Ten-percent homogenates of the food samples were prepared with buffered peptone water, pH 7.0 (Nissui Pharmaceuticals) in a sterile stomaching bag using a stomacher for 30 seconds. The number of viable cells in the homogenate was measured in duplicate using nutrient agar plates with the surface plating method. Incubation was done at 35°C for 24 hr. Average cell counts were logarithmically transformed (with base 10), the average of each point were then calculated.

**Growth model**

Recently we have developed a new logistic model of microbial growth\(^6\). The rate of growth of the model is expressed as follows:

\[
\frac{dN}{dt} = rN \left(1 - \frac{N}{N_{\text{max}}}\right) \left(1 - \frac{N_{\text{min}}}{N}\right) \quad (1)
\]

Here, \(N\) is the population (arithmetic number) of the organism at time \(t\), \(r\) is the rate constant of growth, \(N_{\text{max}}\) is the maximum population, and \(N_{\text{min}}\) corresponds to the inoculum size. \(n\) (\(\geq 0\)) is a parameter related to the curvature at the acceleration phase of growth. After slight modification\(^8\), our model was improved to more precisely describe microbial growth curves, as follows\(^9,10\):

\[
\frac{dN}{dt} = rN \left(1 - \frac{(N/N_{\text{max}})^m}{1 - (N_{\text{min}}/N)^n}\right) \quad (2)
\]

\(m\) is a curvature parameter (> 0); with a larger \(m\), the curvature of the deceleration phase of growth becomes smaller. Equation (2) might be a generic form of our logistic model\(^7\). Growth curves in this study were studied with Eq. (2).

**Mathematical and statistical procedures**

Equation (2) was solved numerically with the 4-order Runge-Kutta method, similar to that used with our previous models\(^6-10\). Parameter \(r\) was set to be a measured rate constant of growth during the exponential phase in an experimental growth curve. \(N_{\text{max}}\) and \(N_{\text{min}}\) in the model, which are both asymptotes, were estimated from experimental data. Parameters \(m\) and \(n\) were estimated with the least-squares method, minimizing the mean of the square error, \(MSE\), between log-transformed cell concentrations predicted with the model and those observed at the observation points. Namely, parameter \(n\) was first estimated using the experimental data from the initial time to that at the exponential phase, because the parameter strongly affects the duration of the lag period. Parameter \(m\) was then estimated using the whole data set and the estimated \(n\) value. With those optimal parameter values, growth curves were generated using Eq. (2).

The lag period, \(lag\), was estimated as the period between the initial point and the point where the regression line for the exponential phase intersects the horizontal line penetrating the initial point on the semi-logarithmic plot\(^6-10\). Statistical calculations were done with spreadsheet software, Microsoft Excel.

**Results and Discussion**

**Effect of nutrients supplemented in mashed potatoes**

Effect of the nutrients supplemented in mashed potatoes on the growth kinetics of \(E.\ coli\) cells in pouches was studied. Bacterial cells in the mashed potatoes and in the potatoes with the nutrients both showed sigmoidal growth curves, which could be successfully described with the new logistic model (Fig. 1). The supplement of nutrients in the potatoes caused the bacteria to grow faster; the value of \(r\) for the supplemented potatoes (1.4 L/hr) was larger than that of the potatoes only (0.90 L/hr) in Fig. 1. The value lag time of the supplemented potatoes (1.7 hr) was the same as that of the potatoes only (1.6 hr).

Effects of the nutrient level on the growth rate were also observed in \(E.\ coli\) on the surface of nutrient agar plates\(^10\). That is, the rate of growth on the surface increased with increasing nutrient concentration supplemented in the agar plates. We have studied the growth kinetics of \(E.\ coli\) under various conditions with

![Fig. 1. Growth curves of E. coli in pouch mashed potatoes with and without nutrients. Cells were incubated in mashed potatoes (■) and in mashed potatoes supplemented with nutrients (●) at 28°C. Lines are those predicted by the growth model.](image)
nutrient broth and nutrient agar plates. In this study, we used nutrients-supplemented mashed potatoes.

Growth at constant temperatures

Growth curves of E. coli cells in (nutrients-supplemented) mashed potatoes in pouches were sigmoidal at various constant temperatures (12–34°C). Some examples are shown in Fig. 2. The growth curves were successfully described with our model. The values of m and n were almost constant at the temperatures studied, with averages of 0.86±0.14 and 4.3±0.55, respectively. The values of N_max for the curves were also almost constant (about 9.6 log units/g).

The relationship of r to the temperature was highly linear with $R^2=0.993$ (Fig. 3). The linear regression line in the figure can be expressed as follows:

$$r = 0.047 \times T - 0.166$$

Here, T is the temperature (°C). In a preliminary study, r was also analyzed with the Arrhenius model. The linearity with the model was very high, but the fit was slightly less good ($R^2=0.985$) than that with the square root model.

The duration of lag was longer at lower temperature (Fig. 4). The relationship between lag and T could be experimentally expressed as an exponential equation, $\text{lag} = 2380 \times T^{-2.11}$ with a high $R^2$ value of 0.930.

Growth in glass tubes

The difference in the growth kinetics in mashed potatoes depending upon the surrounding atmospheric volume was then studied. The bacterial growth curve in pouches was compared with that in glass tubes containing a large volume of air. The growth curve in pouches was similar to that in the glass tubes at constant temperature (Fig. 5). In this figure, the values of r were 0.89 and 0.83 (L/hr) for the pouch and the tube, respectively, and the values of lag were 2.4 and 2.5 (hr). Similar results were obtained at other temperatures. These results showed that the volume of air surrounding the mashed potatoes did not affect the kinetics of cell growth. Zurera-Cosano et al. reported that there were no significant differences in k and lag between Staphylococcus aureus in a broth under aerobic and anaerobic conditions.

Comparison with growth in liquid and on a surface

The effect of the atmosphere surrounding bacterial cells on the growth kinetics was further studied. We have found that the growth curves on a surface at
The growth kinetics in pouches at a given temperature were very similar to those in broth (without shaking)\(^6\), \(^8\), \(^10\). In the present study, we compared the growth kinetics in pouches with that in liquid or on a surface. The nutrient supply was rich in all cases.

The growth curve in pouches at a given temperature was very similar to that in liquid or on a surface (Fig. 6). Only the growth curves described with the model are shown in Fig. 6, for the sake of clarity. The values of \( r \) for the pouch, the surface, and the broth were essentially identical, being 1.94, 2.05 and, 2.09 L/hr, respectively. The values of lag of the growth curve were also the same; 1.36, 1.65, and 1.24 hr, respectively. Similar results were observed at other temperatures. These results suggested that, regardless of the differences in physical (atmospheric) conditions, the growth kinetics of bacterial cells are identical if the supply of nutrient is good.

There is a possibility that some commercial, pouched foods might be contaminated with thermo-tolerant microorganisms such as \( \text{Bacillus} \) spp. or \( \text{Clostridium} \) spp. as spores. The growth kinetics of those aerobic and anaerobic microorganisms might be different from that of \( \text{E. coli} \) studied here, and thus, should be investigated.

In the present study, we clarified the growth kinetics of \( \text{E. coli} \) in pouched mashed potatoes and found that the growth kinetics tend to be similar in different physical surroundings, if the nutrient supply is good. We also confirmed the ability of the new logistic model to successfully describe bacterial growth curves in those surroundings. We have already shown that the growth model can describe microbial growth curves in a liquid and on a surface at various temperature \(^7\), \(^9\), \(^10\), and so the utility of the model has been extended.

**References**


**Fig. 6.** Growth curves of \( \text{E. coli} \) in pouches and broth and on the surface. Cells were grown at 34°C. Lines are those predicted by the growth model. Curves in broth and on a surface are taken from our previous papers\(^6\), \(^8\), \(^10\).