Single-Cell and Population Lag Times as a Function of Cell Age

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After inoculation, the times to the first divisions are longer and more widely distributed for those *Escherichia coli* single cells that spent more time in the stationary phase prior to inoculation. The second generation times are still longer than the typical generation times in the exponential phase, and this extended the apparent lag time of the cell population. The greater the variability of the single-cell interdivision intervals, the shorter are both the lag time and the doubling time of the population.

It has been shown experimentally (1, 15, 17) and theoretically (13) that the lag parameters of a bacterial population do not carry much information about the lag time of the individual cells. Single-cell measurements (6, 8, 10, 11, 14, 16–19) have made it possible to study the effect of the environment on the distribution of single-lag times, underlining the need for the quantification of growth parameters and their variability at the single-cell level.

We used the flow chamber technique of Elfwing et al. (5) to investigate how the age of the cells, quantified as the incubation time in the preinoculation culture, affects the distribution of the generation (i.e., interdivision) times of single *Escherichia coli* K-12 cells. LB media with 0.2% glucose was inoculated with ca. 10^3 cells/ml and incubated at 25°C. Stationary-phase cells after ca. 53, 77, 83, 144, 151, 193, 218, 360, and 602 h of incubation were removed from this culture and immediately used to inoculate the flow chamber. Thus, the age of the cells was defined as the time at which the cells were sampled from the primary culture. The generation times for single cells were calculated by observing the time intervals between two successive divisions after the first division occurred. The first division time (FDT) was considered to be the sum of the lag time and the first generation time.

Figure 1a shows the distributions of the natural logarithm of the FDTs of single cells of different ages. The older the cells, the greater are the averages of their FDTs. The FDTs continuously increase with the age of the cells (except for the unexpectedly high division times observed when inoculating the 144-h primary culture; this was attributed to experimental error). The standard deviations of the FDTs were not constant but increased with the average. However, the coefficient of variation (CV) (the ratio of the standard error to the mean) did not change with the age (Table 1).

Figure 1b shows that the age of the cells did not affect the distribution of the second generation time (the time interval between the first and second division). This indicates that the main effect of the age of the cells is on the lag period prior to the first division.

Pin and Baranyi (13) developed a stochastic model to simulate the growth of the population resulting from the single-cell division times. This approach proved to predict the growth of the population accurately. Simulations were carried out to study the following three main questions.

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What is the relationship between the single-cell division times measured in the flow chamber and the growth parameters of the population generated by those cells, for each inoculum age, based on 100 simulated growth curves?

Figure 2 compares the sum of the lag and the doubling times of the population with the average FDTs of the single cells within that population. The two were practically equal. Theoretically, the FDTs of single cells should be longer than the time at which the population doubles for the first time (2, 3). However, in these articles, it was assumed that, after the FDT, the cells were immediately in the exponential phase. Our observations disprove this hypothesis, as do other studies (12, 13). We found that the generation times decreased gradually after the first division. For example, for single cells inoculated on the slide after 53 h spent in the primary culture, the averages of the second, third, and fourth generation times were 0.74, 0.66, and 0.58, respectively. The fact that the second, third, and maybe even the fourth generation times are still longer than those in the exponential phase causes a further delay in the lag of the population. From a physiological point of view, this indicates that cells do divide before the adaptation process in the new environment is complete.

TABLE 1. Average FDTs of single cells measured in the flow chamber and growth parameters of populations simulated with the same single-cell measurements for inocula of various ages

<table>
<thead>
<tr>
<th>Inoculum age (h)</th>
<th>Single-cell measurement</th>
<th>Population simulation from 100 initial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.a</td>
<td>Avg FDT (h)</td>
</tr>
<tr>
<td>53</td>
<td>226</td>
<td>0.843</td>
</tr>
<tr>
<td>77</td>
<td>117</td>
<td>0.812</td>
</tr>
<tr>
<td>83</td>
<td>124</td>
<td>0.902</td>
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<tr>
<td>144</td>
<td>57</td>
<td>1.575</td>
</tr>
<tr>
<td>151</td>
<td>167</td>
<td>1.072</td>
</tr>
<tr>
<td>193</td>
<td>120</td>
<td>1.195</td>
</tr>
<tr>
<td>218</td>
<td>107</td>
<td>1.399</td>
</tr>
<tr>
<td>360</td>
<td>221</td>
<td>1.547</td>
</tr>
<tr>
<td>602</td>
<td>68</td>
<td>2.558</td>
</tr>
</tbody>
</table>

a Number of single cells observed or populations simulated.  
b CV of the FDT or the population lag time.  
c Sum of the lag time (L) and doubling time (Dt) of the population (to compare with the FDTs).  
d Lag, lag time of the population.  
e μmax, maximum specific growth rate of the population.

What effect did the variability of the single-cell division times have on the lag time observed at the population level? We generated, on computer, several series of single-cell first-division times according to the gamma distribution with 2.5 h as their mean (as measured for the cells incubated after 602 h in the primary culture) and standard deviations that varied from ca. 0.1 to 2 times their expected values. The initial number of cells in the population was 100. Each batch was simulated 100 times. Figure 3a shows that the population lag time is determined not only by the mean of the single-cell FDTs but also by their variability. The larger the variability of the single-cell FDTs, the greater the number of cells with short FDTs, which shortened the lag time of the population (Fig. 3a). According to our flow chamber measurements, the CV values observed for single-cell FDTs were between 0.3 and 0.4, independently of the age of the cells (Table 1). Similar values have

FIG. 2. Relationship between the average of the single-cell FDTs and the sum of the lag (L) and doubling times of the population (Dt), (L + Dt). The growth of a population with 100 initial cells was simulated for each inoculum age based on the distributions of the observed single-cell generation times as described in reference 13.

FIG. 3. Effect of the variability of the single-cell FDTs on the population lag time (a) and the relationship between the CV (coeff. variation) values of the population lag time and of the FDTs of the single cells (b).
been reported by Guillier and Augustin (7). Note that D’Arrigo et al. (4) reported greater CV values (0.76) for the lag time of single cells stressed previously. The CV values for single-cell FDTs were ca. 10 times larger than the respective parameters of the populations (Table 1). The simulations showed that the CV value of the population lag time increased as the CV values of the single-cell FDTs within that population increased (Fig. 3b), keeping a ratio that was ca. 10-fold greater, until a maximum value was reached.

**What effect did the variability of the generation time of single cells have on the exponential growth rate of the population?** A population in which all cells divide synchronously and exactly every 0.66 h was also simulated and compared to the situation when the generation times were distributed as described above, with an expected value of 0.66 h. Figure 4 shows that if all cells divide exactly at the same time (single-cell generation times with a standard deviation of zero), then the doubling time of the population is equal to the single-cell generation time, i.e., 0.66 h. As the variance of the single-cell generation times increases, the population doubling time becomes shorter, in agreement with reference 9. This is because the random appearance of cells with shorter generation times has an unexpectedly big effect on the overall population due to the exponential growth.

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**REFERENCES**