An analysis of the instability kinetics of plasmid pHSG415 during continuous culture of *Escherichia coli*

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The effect of dilution rate on the instability kinetics of *Escherichia coli* RV308(pHSG415) during glucose-limited continuous culture is examined. Two nonlinear models are fitted to the data, both of which characterize the plasmid-host system in terms of the rate parameters $R$ (for the plasmid segregation rate) and $d\mu$ (for the specific growth rate difference between plasmid-free and plasmid-bearing single cells). In the first model, both $R$ and $d\mu$ have constant values with respect to time. In the second, either $R$ or $d\mu$ is represented as a time-dependent function. Although both models fit the data equally well, it is demonstrated that the constant rate parameter model gives results which appear to be misleading. A comparison is also made among some of the many plasmid instability models (both mass-balance and segregated) which have appeared in the literature. It is found that all of these give identical trajectories and differ only in the definitions of the rate parameters used.

**Keywords:** plasmid instability, mathematical model

**INTRODUCTION**

The dynamics of non-conjugative bacterial plasmid maintenance depend upon the outcome of competition between the plasmid-bearing and plasmid-free cell fractions of the population. The overall growth rate of each species is determined by three principal phenomena: (i) the segregation rate (plasmid-bearing cells become plasmid-free due to unequal partition of plasmid molecules to each daughter at cell division), (ii) the specific growth rate of plasmid-bearing single cells and (iii) the specific growth rate of plasmid-free single cells. Two mathematical modelling approaches (mass-balance and segregated) have been used by various authors to express the proportion of plasmid-bearing cells in the population as a function of time. These expressions have been written in terms of two constant parameters: (i) a segregation rate or probability and (ii) a single-cell growth rate difference or quotient. Although all of these models are nonlinear, the rate parameters may be evaluated from biological data (obtained during batch or continuous culture) using linear regression to fit an exponential approximation describing part of the model solution (Cooper et al., 1987; Watson et al., 1988; San & Weber, 1989; Wei et al., 1989; Park et al., 1991; Mosrati et al., 1993). A more accurate evaluation is, however, obtained using the nonlinear methods employed by Lenski & Bouma (1987) and Davidson et al. (1990). Studies of plasmid instability have also been presented by Kim & Ryu (1984) and Sykora et al. (1989), although the curve-fitting procedures used by these authors were not described.

The condition that the rate parameter values are constant with respect to time means that it is impossible to fit these models to many of the data sets which have appeared in the literature. This is most often the result of large changes in plasmid copy number (Primrose et al., 1984; De Taxis Du Poët et al., 1987) or spontaneous mutations which arise in the host chromosome (Helling et al., 1981; De Taxis Du Poët et al., 1986; Sayadi et al., 1988) or plasmid (Godwin & Slater, 1979; Brownlie et al., 1990). In addition, it is likely that more subtle changes during some experiments mean that, although a reasonable fit is possible, the results of the analysis will be misleading. For
these reasons, the model used by Davidson et al. (1990) has been modified to describe either one of the two rate parameters as a time-dependent function. This paper compares the two models using previously published data for *Escherichia coli RV308(pHSG415).

**METHODS**

Numerical calculations were done on a DEC VAX 6420 using the NAG (Numerical Algorithms Group, Oxford, UK) Fortran routines (Anonymous, 1993) D02BBF (Runge–Kutta–Merson method to solve equation 12) and E04JBF (quasi-Newton method to fit equations 7 and 12). A key to the mathematical notation is given in Appendix 1.

**RESULTS AND DISCUSSION**

The mathematical model

The maintenance of bacterial plasmids during growth in a chemostat can be simulated using a mixed-culture, mass-balance approach to describe the change with time in the densities of the plasmid-bearing and plasmid-free cell fractions of the population. In this modelling strategy, each cellular species is viewed as a lumped biomass (which interacts as a whole with its environment) and such a system is termed non-segregated (Shuler, 1985). If $x_+$ and $x_-$ represent the respective concentrations (g l$^{-1}$) of plasmid-bearing and plasmid-free cells, then we may write the following system of first-order differential equations to describe the overall growth rate of each population:

\[
\begin{align*}
\frac{dx_+}{dt} &= \mu_+ x_+ - p_+ x_+ - D x_+ \quad (1a) \\
\frac{dx_-}{dt} &= \mu_- x_- + p_- x_+ - D x_- \quad (1b)
\end{align*}
\]

(Stewart & Levin, 1977) where $t$ is time (h), $\mu_+$ and $\mu_-$ are the single-cell specific growth rates (h$^{-1}$), $p$ is a dimensionless segregation parameter and $D$ is the environmental dilution rate (h$^{-1}$). Note that $pp_+$ is the segregation rate (h$^{-1}$) since segregants arise at cell division. The growth-limiting substrate concentration in the chemostat, $S$ (g l$^{-1}$), is described by

\[
\frac{dS}{dt} = D(S_i - S) - \frac{\mu_+ x_+}{y_+} - \frac{\mu_- x_-}{y_-} \quad (2)
\]

(see Pirt, 1975) where $y_+$ and $y_-$ are the yields of each cell type (g g$^{-1}$) and $S_i$ is the input nutrient concentration (g l$^{-1}$). If we assume that the hyperbolic function proposed by Monod (1949) gives an adequate approximation to the single-cell specific growth rates, then

\[
\mu_+ = \frac{\mu_m S}{S + K_+} \quad \text{and} \quad \mu_- = \frac{\mu_m S}{S + K_-}
\]

(3)

where $\mu_m$ is a maximum specific growth rate (h$^{-1}$) and $K_+$ is a half-saturation parameter (g l$^{-1}$). Note that the relative values $\mu_-/\mu_+$ and $K_+/K_-$ will depend on the metabolic activity associated with plasmid replication and gene expression. In experimental studies of plasmid instability, it is convenient to measure $x_+$ and $x_-$ as proportions of the total cell population density such that

\[
p_+ = \frac{x_+}{x_+ + x_-} \quad \text{and} \quad p_- = \frac{x_-}{x_+ + x_-}
\]

(4)

where $p_+ + p_- = 1$. A particular solution to the model is shown in Fig. 1. The parameter values used in this simulation may be regarded as being typical of *E. coli* (Pirt, 1975).

Equations 1 hold for $p_+$ and $p_-$ and can be rearranged to yield a single equation describing both batch and continuous culture:

\[
\frac{dp_+}{dn_+} = \frac{d\mu}{(d\mu + R)p_+} \quad (5)
\]

(Cooper et al., 1987), where $n_+$ is the number of generations of plasmid-bearing single cells, $R$ (gen$^{-1}$) is the segregation rate and $d\mu$ (gen$^{-1}$) is the specific growth rate difference between plasmid-free and plasmid-bearing single cells. That is,

\[
R = \mu_+ \tau_+ = \rho \ln 2 \quad (6a)
\]

and

\[
d\mu = (\mu_- - \mu_+)\tau_+ = \left(\frac{\mu_-}{\mu_+} - 1\right) \ln 2 \quad (6b)
\]

where $\tau_+ = \ln 2/\mu_+$ [the instantaneous generation time (h) of plasmid-bearing cells]. Thus we see that, if $\mu_- = 2\mu_+$, then $d\mu = 0.693$ gen$^{-1}$. If $\mu_+ = 2\mu_-$, then $d\mu = -0.347$ gen$^{-1}$.

**Constant rate parameter model.** Equation 5 can be solved analytically if the rate parameters ($R$ and $d\mu$) have constant values with respect to $n_+$. For the case $d\mu + R \neq 0$,

\[
p_+(n_+) = \frac{p_{+0}(d\mu + R)}{p_{+0}d\mu + (p_{-0}d\mu + R)e^{(d\mu + R)n_+}} \quad (7a)
\]

where $p_{+0} = p_+(0)$ and $p_{-0} = 1 - p_+(0)$. If $d\mu + R = 0$, then

\[
p_+(n_+) = \frac{p_{+0}}{1 + p_{+0}Rn_+} \quad (7b)
\]

(Davidson et al., 1990). As an alternative to this model, equation 12 of Imanaka & Aiba (1981) expresses $p_+(n_+)$ in terms of a single-cell specific growth rate ratio, $\alpha = \mu_-/\mu_+$, and a dimensionless segregation parameter, $p$ [incorrectly defined (Imanaka & Aiba, 1981) as a segregation probability]. From equations 6, we have

\[
p = \rho \quad \text{and} \quad \alpha = \frac{d\mu}{\ln 2} + 1 \quad (8)
\]

Similarly, it can be demonstrated that all of the mass-balance descriptions of $p_+(n_+)$ which have appeared in the
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In contrast with the mass-balance approach discussed above, Seo & Bailey (1985) derived an expression for $p_+(n_+)$ in terms of the single-cell specific growth rate ratio, $\alpha$, and the probability, $\theta$, that either one of the two daughters will be plasmid-free when a plasmid-bearing cell divides. It is necessary to consider the cellular age distributions in this case since newly formed plasmid-free cells will not divide until one generation after their initial appearance. However, if the contribution of plasmid-bearing single cells to the plasmid-free population by segregation is constant with respect to time, then both modelling strategies will give identical results (Seo & Bailey, 1985). To discover the relationship between $R$ and $\theta$, let both cell types grow at the same rate ($d\mu = 0$ or $n = 1$). Equation 38 of Seo & Bailey (1985) can then be simplified to yield

$$p_+(n_+) = p_{+o} \left(1 - \theta \frac{n_+}{2}\right)$$

(9a)

The equivalent solution to equation 5 is

$$p_+(n_+) = p_{+o} e^{-Rn_+}$$

(9b)

and hence

$$R = -\ln \left(1 - \theta \frac{n_+}{2}\right) \text{ or } \theta = 2(1 - e^{-R})$$

(10)

where $R$ and $\theta$ are constants. This relationship is depicted graphically in Fig. 2. It should be pointed out that it is common in the literature for $p$ or $R$ to be defined incorrectly as a probability. For examples, see Table 4 of Caulcott et al. (1987), equation 1 of Wittrup et al. (1990) or equations 1 and 2 of Mosrati et al. (1993).

The condition that the rate parameters values are constant with respect to $n_+$ leads to three problems when fitting all plasmid instability models to biological data. The first is that the independent variable in equations 7 is the number of generations of plasmid-bearing single cells (defined implicitly such that $dn_+ / dt = 1/n_+$). During the quasi-steady-state simulation depicted in Fig. 1, the substrate concentration in the chemostat decreases as the plasmid-bearing population is displaced by faster-growing plasmid-free cells. This behaviour occurs whenever $\mu_{m+} \neq \mu_{m-} \text{ or } K_{m+} \neq K_{m-}$. Consequently, when fitting the model to experimental data, $\tau_+$ is an unknown variable and it is therefore necessary to scale time using the approximation $n_+ \approx nD/ln2$. Since this model does not describe the data exactly (Fig. 1a), the parameter evaluation will be subject to a small error which is an increasing function of $|d\mu|$. For example, the values obtained by fitting $p_+(tD/ln2)$ (equations 7) to the data in Fig. 1(a) are $R = 0.0193$ gen$^{-1}$ and $d\mu = 0.144$ gen$^{-1}$. This compares with the true values of $R = 0.0173$ gen$^{-1}$ and $d\mu = 0.173$ gen$^{-1}$. The second difficulty becomes apparent when equations 3 are substituted into equation 6b, i.e.

$$d\mu = \left(\frac{\mu_{m+} S + K_{m+} - 1}{\mu_{m-} S + K_{m-} - 1}\right)ln2$$

(11)

This shows that $d\mu$ is constant with respect to $S$ (and
follows that \( R(n_+) = R_0 + R_-n_+ \) and \( dp(n_+) = dp_0 \). As Appendix 2 demonstrates, equation 12 can be solved analytically in this case only when \( R_1 \gg 0 \). It is therefore convenient to evaluate the model trajectory using a suitable numerical integration procedure (e.g. Runge–Kutta–Merson method).

**A measure of overall stability.** A widely used method of comparing the overall stability of different plasmids is to quote the proportion of plasmid-bearing cells remaining after a given period of time. On this basis, the two trajectories shown in Fig. 3 could be regarded as representing equally stable plasmids since \( p_+(80) = 0.2 \) in both cases. Clearly, this method is sensitive to the particular values of \( n_+ \) and \( p_+ \). In our opinion, a more appropriate measure is given by the area, \( \sigma(N_+) \), gen, under the curve \( p_+(n_+) \) in the interval \([0, N_+]\) (i.e. over the entire duration of the experiment). This can be obtained by integration of equations 7 between the limits \( n_+ = 0 \) and \( n_+ = N_+ \). For the case \( \mu_+ = 0 \) and \( \mu_+ + R = 0 \),

\[
\sigma(N_+) = \int_0^{N_+} p_+(n_+) \, dn_+ = \frac{1}{d\mu} \ln \left( \frac{d\mu + R}{d\mu + R - p_+d\mu(1 - e^{-(d\mu + R)N_+})} \right)
\]

(13a)

If \( d\mu = 0 \), then

\[
\sigma(N_+) = \frac{p_+(1 - e^{-RN_+})}{R}
\]

(13b)

and if \( d\mu + R = 0 \),

\[
\sigma(N_+) = \frac{1}{R} \ln(1 + p_+RN_+)
\]

(13c)

An alternative measure of overall stability is the average proportion of plasmid-bearing cells, \( \bar{p}_+(N_+) \), in the interval \([0, N_+]\). Thus, by the mean value theorem, we have

\[
\bar{p}_+(N_+) = \frac{1}{N_+} \int_0^{N_+} p_+(n_+) \, dn_+ = \frac{\sigma(N_+)}{N_+}
\]

(14)

When comparing data in this way (legend to Fig. 3), it is important to realize that the value of \( N_+ \) must be chosen to be the same in each of the cases considered. In the case of a completely stable plasmid \( [p_+(n_+) = p_+] \), the measures of stability are \( \sigma(N_+) = p_+N_+ \) and \( \bar{p}_+(N_+) = p_+ \).

**Instability kinetics of RV308(pHSG415)**

pHSG415 is a 7.1 kb plasmid which has a copy number of about 10 per average cell in *E. coli* (Hashimoto-Gotoh et al., 1981). It replicates via a 1.9 kb, temperature-sensitive replicon which was derived from pSC101 (Meacock & Cohen, 1980). The instability of *E. coli* RV308(pHSG415) during glucose-limited continuous culture at four dilution rates is shown in Fig. 4. These data, obtained at the
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However, one would expect an increase in copy number at the lower dilution rate. This may be the result of an unknown partition mechanism, in the sense that it does not support this hypothesis. Second, it seems improbable that this plasmid could increase the growth rate of its host \((du < 0)\) as indicated at the two highest dilution rates. It must therefore be concluded that these results are anomalous and are due to the incorrect assumption that \(R\) and \(du\) are time invariant.

**Time dependent parameter curve fit.** The best fit (quasi-Newton minimization) was obtained by representing the segregation rate as a first-degree polynomial \(R(n_+) = R_0 + R_1 p_{\alpha}\) and the single-cell specific growth rate difference as a constant value \(du(n_+) = du_0\). The fit of equation 12 (Fig. 4) is at least as good as the constant-parameter model described previously (i.e. the trajectories are virtually indistinguishable). In each case, this analysis indicates that the growth rate difference is negligible \((du \approx 0\) gen\(^{-1}\)) in comparison with the segregation rate (not shown). At the two lowest dilution rates, the segregation rate shows a marked increase in value with time (Fig. 6). The opposite is true at the higher dilution rates. It is interesting to note that the constant rate parameter model (Fig. 5) gave a positive value for \(du\) corresponding to increasing \(R(n_+)\), and a negative value corresponding to decreasing \(R(n_+)\).

**Plasmid biology.** The single-cell segregation probability, \(\theta\), is described by the zero class of the plasmid number distribution in the new-born cell fraction of the population (Nordström & Austin, 1989). For plasmids which are partitioned randomly at cell division, the mean and variance of this distribution depend upon two factors. First is the number of covalently linked multimers (concatamers and concatenanes) and non-covalent clusters of plasmid DNA, since these reduce the number of individual molecules which are free to be partitioned randomly to each daughter cell at division (Summers & Sherratt, 1984; Ryan & Parulekar, 1991). Second is the plasmid number distribution in the dividing cell fraction of the population. The mean and variance of this distribution are dictated by the mode of growth of single cells, the copy-control mechanism (Nordström et al., 1980; Seneta & Tavérè, 1983; Seo & Bailey, 1985) and also by the fact that replication initiation is stochastic in nature (Nordström & Aagaard-Hansen, 1984). This distribution is further widened by variation which occurs in the growth rates (Wittrup & Bailey, 1988; Wittrup et al., 1990) and in the division and birth volumes (Koch, 1987) of individual plasmid-bearing cells.

The parent from which the pHSG415 basic replicon was derived, pSC101, is stably maintained at a copy number of about 15 per dividing cell in *E. coli* (Tuckett et al., 1984). It encodes a function implicated in partition control (par) which comprises an 81 bp section of DNA having the potential to form a hairpin loop structure (Kalla & Gustafsson, 1984; Tucker et al., 1984). No gene products are thought to be expressed (Miller et al., 1983). The par locus is able to stabilize the unrelated replicon pACYC184 (Meacock & Cohen, 1980), but it does not promote stable inheritance when joined to minichromosomes (Hinchliffe et al., 1983). This suggests that pSC101 par is not an active partition mechanism, in the sense that it does not guarantee the inheritance of at least one plasmid molecule.

### Fig. 3. A comparison of two solutions to equations 7. ——, \(p_{\alpha} = 1\), \(R = 0.0001\) gen\(^{-1}\); \(du = 0.104\) gen\(^{-1}\) and hence \(p_{\alpha}(80) = 0.20\), \(\sigma(80) = 65\) gen and \(p_{\alpha}(80) = 0.81\); •••, \(p_{\alpha} = 1\), \(R = 0.0201\) gen\(^{-1}\); \(du = 0.0001\) gen\(^{-1}\) and hence \(p_{\alpha}(80) = 0.20\), \(\sigma(80) = 40\) gen and \(p_{\alpha}(80) = 0.50\).
Fig. 4. Instability of *E. coli* RV308(pHSG415) during glucose-limited continuous culture at various dilution rates (*D*) (data points from Caulcott *et al.*, 1987). Time is expressed as an approximation (ΔD/ln2) to the number of generations of plasmid-bearing single cells. Curves show the trajectories predicted by the variable-parameter model. □, *D* = 0·16 h⁻¹; ■, *D* = 0·30 h⁻¹; ○, *D* = 0·40 h⁻¹; ●, *D* = 0·50 h⁻¹.

![Graph showing instability of plasmids](image)

Fig. 5. Results of an analysis of the data in Fig. 4 using the constant-parameter method of Davidson *et al.* (1990). Error bars show the 90% confidence interval. □, *R*; ■, *dμ*.

![Graph showing parameter R](image)

**Table 1. Predicted stability of pHSG415 during 100 generations of continuous culture**

<table>
<thead>
<tr>
<th>Dilution rate (h⁻¹)</th>
<th>Stability (gen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0·16</td>
<td>34</td>
</tr>
<tr>
<td>0·30</td>
<td>33</td>
</tr>
<tr>
<td>0·40</td>
<td>61</td>
</tr>
<tr>
<td>0·50</td>
<td>59</td>
</tr>
</tbody>
</table>

by each daughter at cell division. In comparison with the parent plasmid, pHSG415 has a similar copy number of 10 per average cell (Hashimoto-Gotoh *et al.*, 1981) and hence approximately 10/ln2 = 14 per dividing cell (see Bremer & Denis, 1987). Although existing in the monomeric form and encoding approximately 40% of the *par* locus, pHSG415 (and derivatives containing the same basic replicon) exhibits a rate of segregational instability which is somewhat greater than that predicted by random partition at cell division (Meacock & Cohen, 1980; Tucker *et al.*, 1984; Caulcott *et al.*, 1987). This implies that *par* may be involved in resolving non-covalent clusters of plasmid DNA (Tucker *et al.*, 1984). More recent work demonstrates that the hairpin loop structure is a specific binding site for DNA gyrase (Wahle & Kornberg, 1988; Biek & Cohen, 1992), and so the abnormal inheritance characteristics of pHSG415 could be due to a defect in DNA topology. In the light of such evidence, Nordström & Austin (1989) suggest that pSC101 *par* may act in some way to assist the replication mechanism to narrow the distribution of copy number in dividing cells. We therefore conceive that the predicted time dependency in the segregation rate of pHSG415 (Fig. 6) is a consequence of a change in either: (i) the mean or variance of the plasmid number distribution in dividing cells or (ii) the number of non-covalent clusters of plasmid DNA.

**Conclusion**

The present results indicate the need for caution when fitting mathematical models of plasmid instability to experimental data. Although both the constant and time dependent parameter models fit the data equally well, very different conclusions are reached with respect to the nature of the plasmid instability. It is not possible to prove absolutely that the segregation rate is time dependent for the data set analysed here, although this is certainly the more sensible of the two results. Whether this phenomenon is unique to pHSG415 is not known. As it stands, this new method of analysis is restricted to situations in which either *R* or *dμ* can be regarded as being constant with respect to time. This limitation arises since it is not possible to obtain two unique time-dependent functions when only the proportion of plasmid-bearing cells (*p+*) is known. Given the growth-limiting substrate concentra-
tration ($S$) and the absolute densities of the two cell types ($x_+$ and $x_-$) in the chemostat, it is theoretically possible to resolve $p(t)$, $\mu_+(t)$ and $\mu_-(t)$ by fitting equations 1 and 2. However, considerable difficulty is encountered in measuring these quantities with sufficient accuracy and experimental studies rarely attempt to do so [although see Ryan & Parulekar (1991) for a notable exception to this rule].

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APPENDIX 1: Nomenclature

<table>
<thead>
<tr>
<th>Greek</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>Single-cell specific growth rate ratio $[\mu_/\mu_+]$</td>
</tr>
<tr>
<td>$D$</td>
<td>Chemostat dilution rate (h$^{-1}$)</td>
</tr>
<tr>
<td>$d\mu$</td>
<td>Single-cell specific growth rate difference $[\mu_- - \mu_+]$ (gen$^{-1}$)</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Single-cell segregation probability</td>
</tr>
<tr>
<td>$K_{p+}$, $K_{p-}$</td>
<td>Half-saturation parameters (g l$^{-1}$)</td>
</tr>
<tr>
<td>$\mu_+$, $\mu_-$</td>
<td>Single-cell specific growth rates (h$^{-1}$)</td>
</tr>
<tr>
<td>$\mu_{m+}$, $\mu_{m-}$</td>
<td>Maximum specific growth rates (h$^{-1}$)</td>
</tr>
<tr>
<td>$n_+$</td>
<td>Number of generations of plasmid-bearing single cells</td>
</tr>
<tr>
<td>$p_+$, $p_-$</td>
<td>Proportion of population comprising plasmid-bearing and plasmid-free cells</td>
</tr>
<tr>
<td>$p_{+0}$, $p_{-0}$</td>
<td>Values of $p_+$ and $p_-$ at $n_+ = 0$</td>
</tr>
<tr>
<td>$\tilde{p}<em>+(N</em>+)$</td>
<td>Mean proportion of plasmid-bearing cells in the interval $[0, N_+]$</td>
</tr>
<tr>
<td>$R$</td>
<td>Segregation rate (gen$^{-1}$)</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Dimensionless segregation parameter</td>
</tr>
<tr>
<td>$\rho_+\rho_-$</td>
<td>Segregation rate (h$^{-1}$)</td>
</tr>
<tr>
<td>$S$, $S_t$</td>
<td>Substrate concentrations in chemostat and in feed reservoir (g l$^{-1}$)</td>
</tr>
<tr>
<td>$\sigma(N_+)$</td>
<td>Overall plasmid stability in the interval $[0, N_+]$ (gen)</td>
</tr>
<tr>
<td>$t$</td>
<td>Absolute time (h)</td>
</tr>
<tr>
<td>$\tau_+$</td>
<td>Instantaneous generation time $[\ln2/\mu_+]$ of plasmid-bearing cells (h)</td>
</tr>
<tr>
<td>$x_+$, $x_-$</td>
<td>Cellular concentrations (g l$^{-1}$)</td>
</tr>
<tr>
<td>$y_+$, $y_-$</td>
<td>Cellular yields (g g$^{-1}$)</td>
</tr>
</tbody>
</table>

Subscripts

+ Plasmid-bearing species

- Plasmid-free species

APPENDIX 2: The solution to equation 12

Let $R(n_+) = R_0 + R_1n_+$ and $d\mu(n_+) = d\mu_0$. Equation 12 then has the explicit solution

$$p_+(n_+) = \frac{p_{+0}}{1 - p_{+0}d\mu_0(n_+)e^{(d\mu_0 + R_0)n_+e^{R_1n_+}}}$$

where

$$f(n_+) = \begin{cases} \frac{A}{\sqrt{2\pi}} \left[ \text{erf}(b) - \text{erf}(a) \right]; R_1 > 0 \\ 1 - e^{-(d\mu_0 + R_0)n_+}; R_1 = 0 \\ \frac{A}{\sqrt{-R_1}} \int_a^b e^{u^2} du; R_1 < 0 \end{cases}$$

with $A = \frac{(d\mu_0 + R_0)^2}{\sqrt{2\pi}R_1}$ and $b = a + t_{e^{-R_1}}$. The error function (case $R_1 > 0$) is defined such that $\text{erf}(x) = (2/\sqrt{\pi}) \int_0^x e^{-u^2} du$ and $\text{erf}(-x) = -\text{erf}(x)$. This is classed as an analytic solution since the error function is available in tabulated form (e.g. NAG routine S15AEF). The definite integral in case $R_1 < 0$, however, must be evaluated numerically using a suitable quadrature formula (e.g. Patterson method: NAG routine D01AHF). Finally, case $R_1 = 0$ is equivalent to Equation 7a.

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