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

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Abbreviation: gen, generation(s).

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:

$$\frac{dx_+}{dt} = \mu_+ x_+ - \rho \mu_+ x_+ - D x_+ \quad (1a)$$

$$\frac{dx_-}{dt} = \mu_- x_- + \rho \mu_+ x_+ - D x_- \quad (1b)$$

(Stewart & Levin, 1977) where t is time (h), μ_+ and μ_- are the single-cell specific growth rates (h^{-1}), ρ is a dimensionless segregation parameter and D is the environmental dilution rate (h^{-1}). Note that $\rho \mu_+$ 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

$$\mu_+ = \frac{\mu_{m+} S}{S + K_{s+}} \quad \text{and} \quad \mu_- = \frac{\mu_{m-} S}{S + K_{s-}} \quad (3)$$

where μ_m is a maximum specific growth rate (h^{-1}) and K_s is a half-saturation parameter ($g\ l^{-1}$). Note that the relative values μ_{m-}/μ_{m+} and K_{s-}/K_{s+} 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

$$p_+ = \frac{x_+}{x_+ + x_-} \quad \text{and} \quad p_- = \frac{x_-}{x_+ + x_-} \quad (4)$$

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:

$$\frac{dp_+}{dn_+} = d\mu p_+^2 - (d\mu + R)p_+ \quad (5)$$

(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,

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

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

Fig. 1. A quasi-steady-state solution to equations 1-4. The model parameters are: $S_i = 4 \text{ g l}^{-1}$, $D = 0.5 \text{ h}^{-1}$, $\mu_m = 1 \text{ h}^{-1}$, $\mu_{m+} = 0.8 \text{ h}^{-1}$, $K_{s-} = K_{s+} = 4 \text{ mg l}^{-1}$, $y_- = y_+ = 0.5 \text{ g g}^{-1}$ and $\rho = 0.025$. (a) $\square$, p_+ (equation 4); —, $p_+(tD/\ln 2)$ (i.e. the fit of equations 7). (b) —, μ_+ ; ---, μ_- . The horizontal line represents the chemostat dilution rate. (c) —, S ; ---, $x_+ + x_-$.

literature give identical trajectories and differ only in the definitions of the rate parameters used.

In contrast with the mass-balance approach discussed above, Seo & Bailey (1985) described plasmid instability using a segregated, population-balance framework (Shuler, 1985; Metz & Diekmann, 1986). Segregated models recognize explicitly the distribution of properties among individual members of the population. They have the advantage of direct incorporation of basic information on the mechanisms of plasmid replication and partition at the single-cell level. By integrating the ideal age distribu-

tions (Powell, 1956) of the two species over all cell ages, Seo & Bailey (1985) derived an expression for $p_+(n_+)$ in terms of the single-cell specific growth rate ratio, α , and the probability, θ , 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 θ , let both cell types grow at the same rate ($d\mu = 0$ or $\alpha = 1$). Equation 38 of Seo & Bailey (1985) can then be simplified to yield

$$p_+(n_+) = p_{+0} \left(1 - \frac{\theta}{2}\right)^{n_+} \quad (9a)$$

The equivalent solution to equation 5 is

$$p_+(n_+) = p_{+0} e^{-Rn_+} \quad (9b)$$

and hence

$$R = -\ln\left(1 - \frac{\theta}{2}\right) \text{ or } \theta = 2(1 - e^{-R}) \quad (10)$$

where R and θ are constants. This relationship is depicted graphically in Fig. 2. It should be pointed out that it is common in the literature for ρ 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 $dn_+/dt = 1/\tau_+$). 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 $\mu_{m+} \neq \mu_{m-}$ or $K_{s+} \neq K_{s-}$. Consequently, when fitting the model to experimental data, τ_+ is an unknown variable and it is therefore necessary to scale time using the approximation $n_+ \approx tD/\ln 2$. 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 $p_+(tD/\ln 2)$ (equations 7) to the data in Fig. 1(a) are $R = 0.0193 \text{ gen}^{-1}$ and $d\mu = 0.144 \text{ gen}^{-1}$. This compares with the true values of $R = 0.0173 \text{ gen}^{-1}$ and $d\mu = 0.173 \text{ gen}^{-1}$. The second difficulty becomes apparent when equations 3 are substituted into equation 6b, i.e.

$$d\mu = \left(\frac{\mu_{m-}S + K_{s+}}{\mu_{m+}S + K_{s-}} - 1\right) \ln 2 \quad (11)$$

This shows that $d\mu$ is constant with respect to S (and

Fig. 2. Relationship between the segregation rate (R) and segregation probability (θ). Since a dividing plasmid-bearing cell must contain at least one copy of the plasmid, it therefore follows that $0 \leq \theta \leq 1$ and thus $0 \leq R \leq \ln 2$.

therefore with respect to n_+ only when $K_{s+} = K_{s-}$. If this is not the case, then the fitted value of $d\mu$ will represent an approximation to the arithmetic mean growth rate difference. Note that it is not established whether plasmid replication and gene expression do have any influence on the value of K_{s+} . However, caution must be exercised when drawing conclusions from equation 11 since the Monod model (equations 3) is inaccurate at the very low growth-limiting substrate concentrations (Monod, 1949) present during continuous culture. In particular, it may therefore be incorrect to assume that $\mu_{m-}/\mu_{m+} = \mu_-/\mu_+$ when $K_{s+} = K_{s-}$. Theoretically, neither of the above caveats applies to (batch) serial subculture experiments if it can be guaranteed that $\mu_+ = \mu_{m+}$. In this case, it is more appropriate to use absolute time (h) as the independent variable in equations 7 because τ_+ is an unknown constant. Thus the equivalent rate parameters would be $\rho\mu_+$ and $(\mu_- - \mu_+)$. Since μ_{m-} can be measured directly (and assuming that $\mu_- = \mu_{m-}$), this will then allow τ_+ and hence R and $d\mu$ to be evaluated (equations 6). The third (and possibly the most important) problem is that μ_m , K_s and ρ are not necessarily constants. As indicated in the Introduction, this may mean that it is impossible to fit equations 7 to the data. In other cases, more subtle changes in these values could produce misleading results.

Time dependent parameter model. If the segregation and growth rate difference parameters are functions of time, then we write the model as

$$\frac{dp_+}{dn_+} = d\mu(n_+)p_+^2 - (d\mu(n_+) + R(n_+))p_+ \quad (12)$$

where $R(n_+)$ and $d\mu(n_+)$ may be represented by polynomials. It can be shown that either one of these two polynomials must be of degree zero (i.e. a constant value) in order for a unique set of polynomial coefficients to be produced by the fitting procedure. The degree of the second can then be chosen to give the best fit to the particular set of data. For the purpose of this paper, let

$R(n_+) = R_0 + R_1 n_+$ and $d\mu(n_+) = d\mu_0$. As Appendix 2 demonstrates, equation 12 can be solved analytically in this case only when $R_1 \geq 0$. 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_{+0} . 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 $d\mu \neq 0$ and $d\mu + R \neq 0$,

$$\begin{aligned} \sigma(N_+) &= \int_0^{N_+} p_+(n_+) dn_+ \\ &= \frac{1}{d\mu} \ln \left(\frac{d\mu + R}{d\mu + R - p_{+0} d\mu (1 - e^{-(d\mu + R)N_+})} \right) \end{aligned} \quad (13a)$$

If $d\mu = 0$, then

$$\sigma(N_+) = \frac{p_{+0}}{R} (1 - e^{-RN_+}) \quad (13b)$$

and if $d\mu + R = 0$,

$$\sigma(N_+) = \frac{1}{R} \ln(1 + p_{+0}RN_+) \quad (13c)$$

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

$$\bar{p}_+(N_+) = \frac{1}{N_+} \int_0^{N_+} p_+(n_+) dn_+ = \frac{\sigma(N_+)}{N_+} \quad (14)$$

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 [$p_+(n_+) = p_{+0}$], the measures of stability are $\sigma(N_+) = p_{+0}N_+$ and $\bar{p}_+(N_+) = p_{+0}$.

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

Fig. 3. A comparison of two solutions to equations 7. —, $p_{+0} = 1$, $R = 0.0001 \text{ gen}^{-1}$, $d\mu = 0.104 \text{ gen}^{-1}$ and hence $p_{+}(80) = 0.20$, $\sigma(80) = 65 \text{ gen}$ and $\bar{p}_{+}(80) = 0.81$; ---, $p_{+0} = 1$, $R = 0.0201 \text{ gen}^{-1}$, $d\mu = 0 \text{ gen}^{-1}$ and hence $p_{+}(80) = 0.20$, $\sigma(80) = 40 \text{ gen}$ and $\bar{p}_{+}(80) = 0.50$.

permissive temperature for replication and in the absence of selective pressure, were originally presented by Caulcott *et al.* (1987). We begin by presenting a mathematical analysis of the instability data. The results of this analysis are then discussed in context with the biology of the plasmid.

Constant-rate parameter curve fit. Fig. 5 shows the results obtained by fitting equations 7 (quasi-Newton minimization) to the data in Fig. 4. This analysis indicates that the plasmid segregation rate (R , gen^{-1}) remains almost constant as the chemostat dilution rate increases, whereas the cellular growth rate difference ($d\mu$, gen^{-1}) decreases in value and becomes negative at the two highest dilution rates. A negative value of $d\mu$ means that plasmid-bearing single cells have a growth rate advantage compared with plasmid-free single cells. Although a relationship is demonstrated between the magnitudes of R and μ and the chemostat dilution rate, it is not clear under which condition the plasmid is most or least stable. This is also true of Fig. 4 because each of the trajectories starts at a different value. Table 1 shows the overall stability, $\sigma(100)$ gen, of pHSG415 as a function of the chemostat dilution rate. These are the values expected if, starting with a population of 100% plasmid-bearing cells ($p_{+0} = 1$), each experiment were run for 100 generations of growth. For a completely stable plasmid, $\sigma(100) = 100$ gen in this case. Clearly, despite the wide-ranging rate parameter values depicted in Fig. 5, two distinct levels of overall stability are exhibited at the dilution rates shown in Table 1.

These results are surprising for two reasons. First, it is unclear why the predicted value of $d\mu$ should change with respect to dilution rate. This may be the result of an increase in the copy number of the plasmid at the lower dilution rates (not measured) which would tend to reduce the growth rate of the plasmid-bearing strain ($d\mu > 0$). However, one would expect an increase in copy number to be associated with a reduction in the rate of segregation (Novick *et al.*, 1975). The values of R given in Fig. 5 do

not support this hypothesis. Second, it seems improbable that this plasmid could increase the growth rate of its host ($d\mu < 0$) 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 $d\mu$ 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 [$R(n_{+}) = R_0 + R_1 n_{+}$] and the single-cell specific growth rate difference as a constant value [$d\mu(n_{+}) = d\mu_0$]. 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 ($d\mu \approx 0 \text{ gen}^{-1}$) 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 $d\mu$ corresponding to increasing $R(n_{+})$, and a negative value corresponding to decreasing $R(n_{+})$.

Plasmid biology. The single-cell segregation probability, θ , 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 & Tavaré, 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* (Tucker *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. 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 ($tD/\ln 2$) to the number of generations of plasmid-bearing single cells. Curves show the trajectories predicted by the variable-parameter model. $\square$, $D = 0.16 \text{ h}^{-1}$; $\blacksquare$, $D = 0.30 \text{ h}^{-1}$; $\circ$, $D = 0.40 \text{ h}^{-1}$; $\bullet$, $D = 0.50 \text{ h}^{-1}$.

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. $\square$, R ; $\blacksquare$, $d\mu$.

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

Dilution rate (h^{-1})	Stability (gen)
0.16	34
0.30	33
0.40	61
0.50	59

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 $10/\ln 2 = 14$ per dividing cell (see Bremer

Fig. 6. Time histories of parameter R determined using the new analytical method. In all cases investigated, $d\mu \approx 0 \text{ gen}^{-1}$ (not shown). —, $D = 0.16 \text{ h}^{-1}$; ---, $D = 0.30 \text{ h}^{-1}$; ···, $D = 0.40 \text{ h}^{-1}$; -·-, $D = 0.50 \text{ h}^{-1}$.

& 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 $d\mu$ 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 concen-

tration (S) and the absolute densities of the two cell types (x_+ and x_-) in the chemostat, it is theoretically possible to resolve $\rho(t)$, $\mu_+(t)$ 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

α	Single-cell specific growth rate ratio [μ_-/μ_+]
D	Chemostat dilution rate (h^{-1})
$d\mu$	Single-cell specific growth rate difference [$(\mu_- - \mu_+)\tau_+$] (gen^{-1})
θ	Single-cell segregation probability
K_{s+}, K_{s-}	Half-saturation parameters (g l^{-1})
μ_+, μ_-	Single-cell specific growth rates (h^{-1})
μ_{m+}, μ_{m-}	Maximum specific growth rates (h^{-1})
n_+	Number of generations of plasmid-bearing single cells
p_+, p_-	Proportion of population comprising plasmid-bearing and plasmid-free cells
p_{+0}, p_{-0}	Values of p_+ and p_- at $n_+ = 0$
$\bar{p}_+(N_+)$	Mean proportion of plasmid-bearing cells in the interval $[0, N_+]$
R	Segregation rate (gen^{-1})
ρ	Dimensionless segregation parameter
$\rho\mu_+$	Segregation rate (h^{-1})
S, S_i	Substrate concentrations in chemostat and in feed reservoir (g l^{-1})
$\sigma(N_+)$	Overall plasmid stability in the interval $[0, N_+]$ (gen)
t	Absolute time (h)
τ_+	Instantaneous generation time [$\ln 2/\mu_+$] of plasmid-bearing cells (h)
x_+, x_-	Cellular concentrations (g l^{-1})
y_+, y_-	Cellular yields (g g^{-1})
Subscripts	
+	Plasmid-bearing species
-	Plasmid-free species

APPENDIX 2: The solution to equation 12

Let $R(n_+) = R_0 + R_1 n_+$ and $d\mu(n_+) = d\mu_0$. Equation 12 then has the explicit solution

$$p_+(n_+) = \frac{p_{+0}}{[1 - p_{+0}d\mu_0 f(n_+)]e^{(d\mu_0 + R_0)n_+}e^{\frac{1}{2}R_1 n_+^2}}$$

where

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

with $A = e^{(d\mu_0 + R_0)^2/2R_1}$, $a = (d\mu_0 + R_0)/\sqrt{2|R_1|}$ and $b = a + n_+ \sqrt{\frac{1}{2}|R_1|}$. The error function (case $R_1 > 0$) is defined such that $\text{erf}(z) = (2/\sqrt{\pi}) \int_0^z e^{-u^2} du$ and $\text{erf}(-z) = -\text{erf}(z)$. 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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