Acta Physica Academiae Scientiarum Hungaricae, 53 (1—2), pp. 255—262 (1982)

MATHEMATICAL MODELLING OF SEGREGATION PROCESSES IN MICROBIAL POPULATIONS CONTAINING A SINGLE PLASMID SPECIES*

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This paper presents two mathematical models for plasmid segregation processes of bacteria containing a single plasmid species with various copies. A Markov chain model allows to study the general properties of these processes for stable inheritance of the plasmid and for plasmid loss during continuous culture. The other, more specialized model is based upon the following assumptions: (1) random replication of the plasmid copies concerning the replicated copy number, (2) equal number partitioning of the copies after replication into the daughter cell.

Introduction

The stability of inheritance of a multi-copy-plasmid in a chemostat culture depends on the type of plasmid, its host cell and the culture conditions (Adams et al [1], Godwin and Slater [2], Roth et al [3], Jones et al [4], Noack et al [5]). There are two possible alternatives: either stable inheritance of the plasmid for a long time of cultivation or the loss of the plasmid. The kinetics of plasmid loss in a chemostat and the conditions for their stable maintenance were studied in several mathematical models (Baumberg [6], Stewart and Levin [7], Levin et al [8], Levin and Stewart [9], Levin and Rice [10]). These models describe the dynamic behaviour of the whole population without consideration of the various mechanisms of the segregation process. Other authors discussed the problems of plasmid segregation in relation to these mechanisms (Novick et al [11], [12], [13], Uhlin and Nordström [14], Cullum and Broda [15], Molin and Nordström [16], Nordström et al [17] I, II, Hashimoto-Gotoh and Timmis [18]).

In addition to the experimental studies stochastic models allow a quantitative comparison between theoretical and experimental data and their interpretation.

Such models were based on various assumptions for the basic mechanisms of segregation in the process of inheritance of the plasmid copies to the daughter cells at

* Dedicated to Prof. I. Tarján on his 70th birthday.

cell division. Mathematical models of the mechanisms of replication (rep), replication control (cop) and partitioning (par) of plasmid copies at cell division were discussed quantitatively only in connection with problems of plasmid incompatibility (Ishii et al [19], Novick and Hoppensteadt [20], Cullum and Broda [15], Nordström et al [17] I, II). All discussed mathematical models for plasmid incompatibility have in common the dissection of segregation process of two different plasmid types into two processes: replication of the plasmid copies and their partitioning into the daughter cells, as well as they are common in the assumption of a constant whole copy number of both plasmid types in all generations and in each cell after replication. The assumption of a $N \to 2N \to N$ cycle in the course of replication and partitioning allows to calculate the alteration of relative frequencies of mixed plasmid states and the averaged segregation probability. Conclusions can be drawn on the mode of replication and partitioning of the plasmid.

In this paper we present two stochastic models describing the loss of a single multi-copy-plasmid during continuous culture. In both models we assume a random replication of the plasmid copies and a random partitioning, for instance a binomial partitioning, or an equal number partitioning, of the replicated plasmid copies. The mathematical description of the plasmid segregation in agreement with genetic results is a difficult task, because the average number of plasmid copies per cell of the whole population decreases in time in contrast to the mentioned models of plasmid incompatibility. We have solved this problem in two ways. In the first model the basic properties of the segregation process of a single multi-copy-plasmid are discussed. By means of special matrices R and Q for the replication and partition the second mathematical model describes in detail the segregation rate of the plasmid and the proportion of plasmid-free and plasmid-containing cells in the whole population.

A general segregation model

In the first model we study the basic properties of the segregation process described as a Markov chain. Let τ be the number of generations ($\tau = 0, 1, 2, 3, \ldots$) and ξ_τ a random variable which describes the copy number per cell in the generation τ. ξ_τ has possible values $k = 0, 1, 2, \ldots, N$. We call

$$p(\xi_\tau = k) = p_\tau(k)$$

the probability that $\xi_\tau = k$. The vector $(p_0(k))$ $k = 0, 1, 2, \ldots N$ is the initial distribution vector of the plasmid copy numbers in the population. A Markov chain with discrete time is defined by the conditional probability:

$$\pi(\xi_{\tau+1} = k | \xi_0 = j_0, \xi_1 = j_1, \ldots, \xi_\tau = j_\tau) = \pi(\xi_{\tau+1} = k | \xi_\tau = j_\tau) = \pi_{jk}\,.$$

π_{jk} is the transfer probability of the j-th state to the k-th state after one generation. We assume that π_{jk} is independent of the number of generations. Therefore, the Markov

chain is homogeneous and we have the transfer matrix (π_{jk}) $j, k=0, 1, \ldots, N$. This is a stochastic matrix with the properties:

$$\sum_{k=0}^{N} \pi_{jk} = 1\,, \qquad j=0, 1, \ldots, N$$

and

$$\pi_{jk} \geqq 0\,, \qquad j, k=0, 1, \ldots, N\,.$$

Writing $p_\tau(k)$, $\tau=0, 1, 2, \ldots$ as a line vector P_τ and (π_{jk}) as the square matrix Π, we have, for example,

$$P_\tau = P_{\tau-1} \cdot \Pi\,. \tag{1}$$

This recursive formula allows to calculate the distribution vector of plasmids in the generation τ

$$P_\tau = P_0 \cdot \Pi^\tau\,. \tag{2}$$

Π^n is the n-fold matrix product. In our model we consider the segregation process from one generation to the next, described by the stochastic matrix Π, as a result of two processes, replication and partition of the plasmid copies. We represent the processes with two matrices R and Q which have the form:

$$R = \begin{bmatrix} 1 & 0 & \cdot & \cdot & \cdot & \cdot & 0 \\ 0 & R_{11} & \cdot & \cdot & \cdot & \cdot & R_{1N} \\ 0 & 0 & & R_{22} & & & \cdot \\ \cdot & & & & & & \cdot \\ \cdot & & & & & & \cdot \\ \cdot & & & & & & \cdot \\ 0 & \cdot & \cdot & \cdot & \cdot & 0 & R_{NN} \end{bmatrix}$$

and

$$Q = \begin{bmatrix} 1 & 0 & \cdot & \cdot & \cdot & 0 \\ P_{10} & P_{11} & & & & 0 \\ \cdot & & P_{22} & & & \cdot \\ \cdot & & & & & \cdot \\ \cdot & & & & & \cdot \\ \cdot & & & & & 0 \\ P_{N0} & & & & & P_{NN} \end{bmatrix}.$$

Then the transfer matrix Π may be written

$$\Pi = R \cdot Q = \begin{bmatrix} 1 & 0 & . & . & . & . & . & . & 0 \\ \pi_{10} & \pi_{11} & . & . & . & . & . & . & \pi_{1N} \\ \pi_{20} & \pi_{21} & \pi_{22} & . & . & . & . & . & \pi_{2N} \\ . & & & & & & & & . \\ . & & & & & & & & . \\ . & & & & & & & & . \\ \pi_{N0} & . & . & . & . & . & . & . & \pi_{NN} \end{bmatrix} . \tag{3}$$

a) Non-segregation case

The case of stable inheritance of the plasmid during continuous culture is described by the stochastic matrix Π with the condition for the first column

$$\pi_{i0} = \begin{cases} 1, & i=0 \\ 0, & i=1, 2, \ldots, N \end{cases}$$

(no plasmid containing cells change over into the plasmid free state).

Eq. (2) shows that in this case the first element of the initial distribution vector P_0 does not change during the process of cultivation. From the matrix Π we can obtain the submatrix Π' without the first column and first line of Π. The properties of Π' usually satisfy the conditions of the ergodic theorem for Markov chains and therefore there exists in each case a stationary limit distribution P'_∞ independent of the initial probability distribution P'_0:

$$\lim_{n \to \infty} P'_0 \Pi'^n = P'_\infty .$$

b) Segregation case

The case of continuously decreasing proportion of plasmid containing cells in the population is characterized by the special form of matrix Π:

$$\pi_{00} = 1$$

and

$$\pi_{i0} > 0 \text{ at least for one of } i .$$

The study of the asymptotic behaviour of these homogeneous Markov chains is in direct connection with the existence of eigenvalues of the matrix Π'. With the known notation we get the matrix equation:

$$\lambda P'_\tau = P'_\tau \Pi' \tag{4}$$

with λ as an eigenvalue of Π' and P'_τ a corresponding eigenvector. If $\lambda = 1$, the corresponding eigenvector P'^*_τ in the equation $P'^*_\tau = P'^*_\tau \Pi'$ is the stationary limit distribution of case a) independent of P'_0.

From the standard Perron—Frobenius theory for positive matrices (Canning [21]) and from the detailed classification of the various possibilities of Markov chains and their asymptotic behaviour (Bartlett [22], Feller [23]) we can conlude for the segregation case that eigenvalues $\lambda < 1$ of the reduced matrix equation (4) will appear.

From this result and Eq. (1) it can be concluded that the plasmid copy distribution functions of the plasmid containing cell population are not changed in the course of long term cultivation. For instance the mean value of copies per cell of the plasmid containing cell population is constant in the process of segregation.

Specialized segregation model

In the first model the processes of replication and partitioning are not specified, in the second one, however, explicit calculations will be carried out. We assume a random replication process in the following sense (not in the sense of genetic replication models) and describe this process with the replication factor $\varkappa$, $0 \leqq \varkappa \leqq 1$.

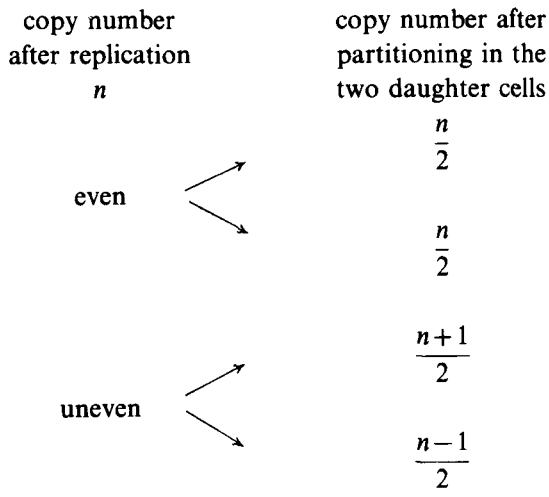
generation	copy number before replication	mean value of copy number after replication
τ	z_τ	$z_\tau + \varkappa z_\tau$

Assumption: after the process of replication the copy number per cell undergoes a binomial distribution. Let P_{ks} be the transfer probabilities that k copies result in s copies after replication. Then

$$p_{ks} = \binom{k}{s-k} \varkappa^{s-k}(k) \, [1 - \varkappa(k)]^{2k-s} \tag{5}$$

with $k = 1, 2, \ldots, N$ and $s = k, \ldots, 2k$. The dependence $\varkappa = \varkappa(k)$ on the copy number makes a more general formulation possible.

The initial distribution vector P_0 can be chosen arbitrarily. We could show that the asymptotic behaviour of the model parameters are independent of P_0. The partitioning of the replicated copies is assumed to be equally distributed and is illustrated in the following scheme:



The equal number assortment model is in agreement with the experimental results of Hashimoto—Gotoh and Sekiguchi [24] for a low copy number plasmid (pSC 101, with 10—14 copies per cell). Novick et al [12] also interpreted experimental results with a high copy number plasmid (about 32 per cell) as supporting equal number partitioning. In contrast to these cases a lack of partitioning (par$^-$) leads to a random distribution of the plasmid molecules between the daughter cells at all divisions (binomial distribution of plasmid copy number in the process of assortment, Nordström et al [17] Part I).

With these assumptions we can calculate the series of distribution vectors of the copy numbers in each generation

$$\{P_\tau\}_{\tau=1,2,\ldots}$$

Further it is possible to calculate the segregation rate δ_τ for each generation as the transfer probability of cells to the plasmid free state:

$$\delta_\tau = \frac{1-\varkappa(1)}{2-(1-\varkappa(1))\sum_{i=0}^{\tau-1} B_i} \cdot B_\tau . \tag{6}$$

The terms B_i, $i=0, 1, \ldots, \tau$ are multiple sums, dependent on the initial distribution P_0, the replication factors $\varkappa(n)$, $n=1, 2, \ldots$ and the transfer probabilities p_{ks}. The experimental measurable proportion of plasmid free and plasmid containing cells can be determined as follows:

$$P(z_\tau=0) = \frac{1-\varkappa(1)}{2} \sum_{i=1}^{\tau} B_i , \tag{7}$$

$$P(z_\tau>0) = 1-P(z_\tau=0) , \qquad (z_\tau \leqq N) .$$

The segregation process can be described with the replication factor $0 \leqq \varkappa < 1$. The limiting value of segregation rate has been calculated. If the number of generations $\tau \to \infty$, then the segregation rate $\delta_\tau \to \frac{1-\varkappa(1)}{2}$. The function δ_τ depends on τ, the replication factor $\varkappa$ and the maximum copy number N. Our calculations have shown that δ_τ rapidly converges to the limit $\frac{1-\varkappa(1)}{2}$.

Discussion

A favourable assumption previously made in the mathematical descriptions of segregation processes concerning plasmid incompatibility was the constant whole number of plasmid copies per cell after replication. These models described the change in relative frequencies of mixed plasmid states and studied in detail the kinetics of the segregation process. With the aid of the first mathematical model proposed in this paper we analysed the more general properties of the structure of models for the segregation of a single plasmid type if in the segregation case the whole copy number decreases. The two important results are that in the non-segregation case the segregation models must have the property that the plasmid copy number has a limit distribution which is independent of the initial distribution and that the proportion of plasmid free cells is constant during cultivation. These results are in agreement with experimental results and are not trivial.

On the other hand, theoretical results of the real segregation process show that the proportion of plasmid containing cells decreases within the population during cultivation, but the plasmid distribution functions of this part of population remain unchanged.

We considered these general conditions of segregation models in the detailed calculations of the specialized segregation model in the second part of the paper. We have chosen special mechanisms for replication and partitioning, but the dependence of the replication factor $\varkappa$ on the appropriate copy number in the cell gives much more possibilities for use. If one assumes a decreasing function $\varkappa(k)$ as the copy number increases it is possible to describe the loss of the plasmid during continuous culture as well as to replace the failing assumption of a constant copy number used in models for incompatibility.

Acknowledgements

The authors would like to thank Dr. M. Roth for his valuable discussions and for useful comments on the manuscript.

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