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Global analysis of a model of plasmid-bearing, plasmid-free competition in a chemostat

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Abstract. A model of competition between plasmid-bearing and plasmid-free organisms in a chemostat was proposed in a paper of Stephanopoulis and Lapidus. The model was in the form of a system of nonlinear ordinary differential equations. Such models are relevant to commercial production by genetically altered organisms in continuous culture. The analysis there was local (using index arguments). This paper provides a mathematically rigorous analysis of the global asymptotic behavior of the governing equations in the case of uninhibited specific growth rate.

Key words: Competition – Plasmid – Chemostat

1 Introduction

Genetically altered organisms are frequently used to produce foreign products. The alteration is accomplished by the introduction of DNA into the cell in the form of a plasmid. The load imposed by the production can result in the genetically altered (the plasmid-bearing) organism being a less able competitor than the plasmid free (or "wild" type) organism. Unfortunately, the plasmid can be lost in the reproductive process. Since commercial production can take place on a scale of many generations, it is possible for the plasmid-free organism to take over the culture.

The chemostat is a common model in microbial ecology. (It is also known as a "continuous culture" or as a "continuously stirred tank reactor".) It is used as an ecological model of a simple lake, as a model of waste-treatment, and as a model for

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commercial production of fermentation processes. It is important in ecology because the parameters are readily measurable, and thus, the mathematical results are readily testable.

The following model of competition between plasmid-bearing and plasmid-free organisms in a chemostat based on the mass balances of the organisms was proposed by Ryder and DiBiasio [RD].

$$\begin{aligned} S' &= (S^{(0)} - S)D - x_1\sigma_1(S) - x_2\sigma_2(S) \\ x_1' &= x_1(f_1(S)(1-q) - D) \\ x_2' &= x_2(f_2(S) - D) + qx_1f_1(S) \\ S(0) &\geqq 0, \qquad x_i(0) \geqq 0, \quad i = 1, 2\,. \end{aligned} \tag{1.1}$$

$S(t)$ is the nutrient concentration at time t, $x_1(t)$ is the concentration of plasmid-bearing organisms at time t, and $x_2(t)$ is the concentration of plasmid-free organisms at time t. The consumption rates and the specific growth rates of plasmid-bearing and plasmid-free organisms are σ_1, σ_2, f_1 and f_2, respectively. The probability that a plasmid is lost in reproduction is represented by q, and hence

$$0 < q < 1\,.$$

The operating parameters are $S^{(0)}$, the input concentration of the nutrient and D, the washout rate of the chemostat.

Assuming that $\sigma_1(S) = \sigma_2(S)$, Ryder and DiBiasio [RD] presented a local stability analysis of the rest points for very general growth kinetics. Based on this analysis they suggest an operational strategy involving feedback control to enhance plasmid stability in chemostat systems.

Instead of assuming that $\sigma_1(S) = \sigma_2(S)$, we consider the model proposed by Stephanopoulis and Lapidus [SL], who assume that

$$\sigma_i(S) = \frac{f_i(S)}{\gamma} \quad \text{for } i = 1, 2\,,$$

where γ is the yield constant (assumed to be the same for both populations). They used very clever index theory arguments to determine the steady state portraits based on the shape and mutual disposition of the specific growth rate curves. They do an exhaustive analysis for the two most common growth models, the Monod model (also referred to as Michaelis–Menten kinetics or Holling type II) for uninhibited growth,

$$\frac{\mu_{\max} S}{K_S + S} \tag{1.2}$$

and the Andrews model for inhibited growth,

$$\frac{\mu_{\max} S}{K_S + S + \dfrac{S^2}{K_I}}\,. \tag{1.3}$$

We restrict our attention in this paper to arbitrary response functions that are uninhibited within the range of interest. That is, we assume only that the functions

$f_i(S)$ satisfy:

$$f_i(S) \text{ is continuously differentiable}, \tag{1.4}$$

$$f_i(0) = 0, \tag{1.5}$$

$$f_i'(S) > 0 \quad \text{for all } 0 < S \leqq S^0. \tag{1.6}$$

Besides the Monod model (1.2), another important model of uninhibited growth has an S-shaped or sigmoidal form and is often referred to as Holling Type III (see for example [JDFT]),

$$\frac{\mu_{\max} S^2}{K_S + S + \dfrac{S^2}{K_I}}. \tag{1.7}$$

Ivlev's functional response,

$$\mu_{\max}(1 - e^{-\lambda s}), \tag{1.8}$$

also an example of uninhibited growth, is the most common functional response used to describe zooplankton grazing.

In fact, inhibited growth is also covered in this analysis provided (1.6) holds. In particular, the Andrews model (1.3) for inhibited growth would be covered provided that $\sqrt{K_S K_I} > S^0$.

The arguments given by Stephanopoulis and Lapidus do not exclude the possibility of nontrivial periodic solutions and hence they cannot make assertions about the eventual outcome that are global in the sense that they are independent of the initial conditions. (See [BWb] and [HW] for examples of other similar chemostat models that do have limit cycles.) Although we do not find any new asymptotic behavior patterns under the above conditions, we prove rigorously that there are no nontrivial periodic solutions and hence provide a mathematically rigorous determination of the global asymptotic behavior of solutions of (1.1). We thus predict the outcome of competition and show how the outcome depends on the parameters of the system, but is independent of the initial concentrations in the inoculant. Given the unavoidable competition within cultures of genetically altered organisms, it seems important to have the problem rigorously understood from the mathematical viewpoint. Further comments may be found in Sect. 5.

By measuring concentrations of nutrient in units of $S^{(0)}$, time in units of $1/D$, and x_i in units of $\gamma S^{(0)}$, the number of parameters can be reduced and the equations take the form

$$\begin{aligned} S' &= 1 - S - x_1 f_1(S) - x_2 f_2(S) \\ x_1' &= x_1(f_1(S)(1-q) - 1) \\ x_2' &= x_2(f_2(S) - 1) + q x_1 f_1(S) \\ S(0) &\geqq 0, \qquad x_i(0) \geqq 0, \quad i = 1, 2, \end{aligned} \tag{1.9}$$

where we abuse notation, since each $f_i(S)$ in (1.9) is defined to be $f_i(S^{(0)} S)/D)$ in (1.1). Therefore, these scaled response functions $f_i(S)$ satisfy (1.4), and (1.5)–(1.6) for all $0 < S < 1$.

The operating parameters have been scaled out, or, from another point of view, such parameters as $\mu_{\max}$, K_S and K_I are measured relative to the operating environments. (These parameters have changed their biological meaning.) This is mathematically convenient although to be useful, the results must be returned to biologically meaningful units. We do this in the discussion in Sect. 5 where we

interpret our results in terms of the operating parameters and thus provide a strategy to maintain mixed cultures in the chemostat.

The reader is referred to Levin and Stewart [LS] and Macken, Levin and Waldstätter [MLW] and the references included in these papers for other approaches to modeling competition between plasmid-bearing and plasmid-free organisms.

2 Preliminaries

Let $\Sigma(t)=1-S(t)-x_1(t)-x_2(t)$. The system (1.9) may be written equivalently as

$$\begin{aligned}
\Sigma' &= -\Sigma \\
x_1' &= x_1(f_1(1-\Sigma-x_1-x_2)(1-q)-1) \\
x_2' &= x_2(f_2(1-\Sigma-x_1-x_2)-1)+qx_1f_1(1-\Sigma-x_1-x_2), \\
1-\Sigma(0)-x_1(0)-x_2(0) &\geqq 0, \qquad x_i(0)\geqq 0, \quad i=1,2.
\end{aligned}$$

Clearly $\lim_{t\to\infty}\Sigma(t)=0$, and so the omega limit set of any solution of (1.9) is contained in the set

$$\Omega^3=\{(S,x_1,x_2)\,|\,S\geqq 0,\ x_1\geqq 0,\ x_2\geqq 0,\ \Sigma=0\}. \tag{2.1}$$

The limiting system, obtained by restricting the initial conditions to the set Ω^3, is

$$\begin{aligned}
x_1' &= x_1(f_1(1-x_1-x_2)(1-q)-1) \\
x_2' &= x_2(f_2(1-x_1-x_2)-1)+qx_1f_1(1-x_1-x_2).
\end{aligned} \tag{2.2}$$

These equations, of course, are restricted to the region

$$\Omega=\{(x_1,x_2)\,|\,x_1\geqq 0,\ x_2\geqq 0,\ x_1+x_2\leqq 1\}.$$

The boundary of Ω satisfies the following properties.

$$(x_1+x_2)(\tau)=1, \quad \text{for some } \tau\geqq 0\Rightarrow x_i'(\tau)=-x_i(\tau)\leqq 0, \quad i=1,2. \tag{2.3}$$

$$x_1(\tau)=0 \quad \text{for some } \tau\geqq 0\Rightarrow x_1'(\tau)=0. \tag{2.4}$$

$$x_2(\tau)=0 \quad \text{for some } \tau\geqq 0\Rightarrow x_2'(\tau)=qx_1(\tau)f_1(1-x_1(\tau))\geqq 0. \tag{2.5}$$

Therefore, Ω is a positively invariant region (where uniqueness of initial value problems is applied in case (2.4)). Similar arguments show that Ω^3 defined in (2.1) is positively invariant.

We use the following notation for the relevant rest points of system (1.9). We say that a rest point of (1.9) does not exist if any one of its components is negative. Since $\lim_{t\to\infty}\Sigma(t)=0$, any rest point $\bar{E}=(\bar{S},\bar{x}_1,\bar{x}_2)$ of (1.9) must satisfy

$$1-\bar{S}-\bar{x}_1-\bar{x}_2=0.$$

The washout rest point is denoted $E_1^3=(1,0,0)$. There is only one possible rest point involving plasmid-free organisms but no plasmid-bearing organisms, denoted $E_2^3=(\lambda_2,0,1-\lambda_2)$ where λ_2 is defined as the unique value of S where

$$f_2(\lambda_2)=1 \tag{2.6}$$

(if one exists). The mixed culture rest point is denoted $E_c^3=(\lambda^*, x_1^*, x_2^*)$, where λ^* is defined as the unique value of S where

$$f_1(\lambda^*)=\frac{1}{(1-q)} \tag{2.7}$$

(if it exists) and we assume $x_i^*>0$ for $i=1$ and 2. When E_c^3 exists (this will be discussed in Sect. 3.2),

$$x_1^*=\frac{(1-\lambda^*)\,(1-f_2(\lambda^*))}{f_1(\lambda^*)-f_2(\lambda^*)}\,, \tag{2.8}$$

$$x_2^*=(1-\lambda^*-x_1^*)\,. \tag{2.9}$$

It can easily be seen that no rest point can exist where there are plasmid-bearing organisms but no plasmid-free organisms.

The corresponding rest points of (2.2) are simply the projections on (x_1-x_2)-space and are denoted:

$$E_1=(0,0), \quad E_2=(0,1-\lambda_2), \quad E_c=(x_1^*, x_2^*)\,.$$

We assume that these do not exist if either component is negative or if the sum of the components exceeds 1, since then it would be outside Ω. (This would force the S component in the corresponding rest point in (1.9) to be negative.)

3 Analysis on Ω

We shall proceed by first determining the dynamics on the two dimensional globally attracting set Ω. To justify our conclusions for arbitrary initial conditions for the full three dimensional system (1.9), and hence prove the main result of the paper, Theorem 4.1, we will use the theory of asymptotically autonomous systems (see Thieme [T]).

The analysis of the two dimensional system (2.2) breaks conveniently into four cases. These are summarized in Table 1. Note that strict inequalities are made to avoid non-hyperbolic cases (cases where a rest point has a variational matrix with an eigenvalue with zero real part). For example, due to the strict inequalities, we make the implicit assumption that when both λ_2 and λ^* exist, $\lambda_2 \neq \lambda^*$. In each case,

Table 1.

Case	Criteria for existence of rest points and global stability of boxed rest point			Rest points
I	$f_1(1)(1-q)<1$	$f_2(1)<1$		$\{\boxed{E_1}\}$
II	$f_1(1)(1-q)>1$	$f_2(1)<1$		$\{E_1, \boxed{E_c}\}$
III	$f_1(1)(1-q)<1$	$f_2(1)>1$		$\{E_1, \boxed{E_2}\}$
IVa	$f_1(1)(1-q)>1$	$f_2(1)>1$	$f_1(\lambda_2)(1-q)<1$	$\{E_1, \boxed{E_2}\}$
IVb			$f_1(\lambda_2)(1-q)>1$	$\{E_1, E_2, \boxed{E_c}\}$

we also state which rest points exist. The rest point that will be proven to be the global attractor in each case is enclosed in a box.

The main result in this section can now be stated.

Theorem 3.1
i) In case I, E_1 is a global attractor of Ω.
ii) In cases III and IVa, E_2 is a global attractor of any solution initiating at a point in Ω with $x_1(0)+x_2(0)>0$.
iii) In cases II and IVb, E_c is a global attractor of any solution initiating at a point in Ω with $x_1(0)>0$.

In order to prove this we require some preliminary work.

3.1 Elimination of nontrivial periodic solutions on Ω

Lemma 3.1. *System (2.2) admits no nontrivial periodic solutions.*

Proof. We apply the Dulac criterion (see [ALGM]) with the auxiliary function

$$B(x_1, x_2) = \frac{1}{x_1 x_2}$$

to the vector field given by (2.2). An easy computation yields

$$\frac{\partial B(x_1, x_2) x_1'}{\partial x_1} + \frac{\partial B(x_1, x_2) x_2'}{\partial x_2} =$$
$$-\left(\frac{f_1'(1-x_1-x_2)}{x_2} + \frac{f_2'(1-x_1-x_2)}{x_1} + \frac{q f_1(1-x_1-x_2)}{x_2^2}\right) < 0 .$$

Hence there are no nontrivial periodic solutions. □

3.2 Rest points of Ω and their local stability

First we discuss when the rest points exist. The washout rest point $E_1=(0, 0)$ always exists. Since f_2 is monotone for $0<S<1$ with $f_2(0)=0$,

$$\lambda_2 \text{ exists, satisfying } 0<\lambda_2<1 \quad \text{and} \quad f_2(\lambda_2) = 1 \Leftrightarrow f_2(1)>1 . \tag{3.1}$$

In this case there is a plasmid-free rest point E_2, where $E_2=(0, 1-\lambda_2)$. Otherwise, no such rest point exists.

Next consider the mixed-culture (interior) rest point $E_c=(x_1^*, x_2^*)$. Since f_1 is monotone with $f_1(0)=0$,

$$\lambda^* \text{ exists, satisfying } 0<\lambda^*<1 \quad \text{and} \quad f_1(\lambda^*) = \frac{1}{1-q} \Leftrightarrow f_1(1) > \frac{1}{1-q} . \tag{3.2}$$

In addition, for E_c to exist, both x_1^* and x_2^* must be positive. By (2.8)–(2.9),

$$\begin{aligned} x_2^* &= 1-\lambda^*-x_1^* \\ &= 1-\lambda^*-\frac{(1-\lambda^*)(1-f_2(\lambda^*))}{f_1(\lambda^*)-f_2(\lambda^*)} , \\ &= \frac{(1-\lambda^*)(f_1(\lambda^*)-1)}{f_1(\lambda^*)-f_2(\lambda^*)} . \end{aligned}$$

Assuming $f_1(1) > \dfrac{1}{1-q}$, it follows that the numerator of x_2^* is always positive, since $f_1(\lambda^*) - 1 > 0$. Therefore, for $x_2^* > 0$ to hold, the denominator must also be positive. But x_1^* has the same denominator (see (2.8)), and so the numerator of x_1^* must also be positive. This is true if and only if either no λ_2 exists (i.e. $f_2(1)) < 1$) or $\lambda_2 > \lambda^*$. Since $f_1(\lambda^*) > 1$, if the numerator of x_1^* is positive, then so is the denominator. Note that

$$\lambda_2 > \lambda^* \Leftrightarrow f_1(\lambda_2) > f_1(\lambda^*) = \frac{1}{1-q}. \tag{3.3}$$

Thus E_c exists if and only if

$$f_1(1)(1-q) > 1 \quad \text{and} \quad \text{either } f_2(1) < 1 \text{ or } f_1(\lambda_2) > \frac{1}{1-q}. \tag{3.4}$$

Therefore, E_2 exists in cases III and IV and E_c exists in cases II and IVb. This is indicated in Table 1.

Next we investigate the local stability of these rest points by finding the eigenvalues of the associated variational matrices.

The variational matrix J of (2.2) takes the form

$$J = \begin{bmatrix} j_{11} & j_{12} \\ j_{21} & j_{22} \end{bmatrix}$$

where

$$\begin{aligned}
j_{11} &= f_1(1-x_1-x_2)\,(1-q) - 1 - x_1 f_1'(1-x_1-x_2)\,(1-q)\,, \\
j_{12} &= -x_1 f_1'(1-x_1-x_2)(1-q)\,, \\
j_{21} &= -x_2 f_2'(1-x_1-x_2) + q f_1(1-x_1-x_2) - q x_1 f_1'(1-x_1-x_2)\,, \\
j_{22} &= f_2(1-x_1-x_2) - 1 - x_2 f_2'(1-x_1-x_2) - q x_1 f_1'(1-x_1-x_2)\,.
\end{aligned}$$

At E_1,

$$J(E_1) = \begin{bmatrix} f_1(1)(1-q) - 1 & 0 \\ q f_1(1) & f_2(1) - 1 \end{bmatrix}. \tag{3.5}$$

The eigenvalues lie on the diagonal.

When E_2 exists (cases III and IV), the variational matrix at E_2 is

$$J(E_2) = \begin{bmatrix} f_1(\lambda_2)(1-q) - 1 & 0 \\ -(1-\lambda_2) f_2'(\lambda_2) + q f_1(\lambda_2) & -(1-\lambda_2) f_2'(\lambda_2) \end{bmatrix}. \tag{3.6}$$

Again the eigenvalues lie on the diagonal.

When E_c exists (cases II and IVb), the variational matrix at E_c takes the form

$$J(E_c) = \begin{bmatrix} -x_1^* f_1'(\lambda^*)(1-q) & -x_1^* f_1'(\lambda^*)(1-q) \\ -x_2^* f_2'(\lambda^*) + q f_1(\lambda^*) - q x_1^* f_1'(\lambda^*) & f_2(\lambda^*) - 1 - x_2^* f_2'(\lambda^*) - q x_1^* f_1'(\lambda^*) \end{bmatrix} \tag{3.7}$$

If we perform an elementary column operation on $J(E_c)$: *replace column* 2 *of* $J(E_c)$ *by column* 2 *minus column* 1, *to obtain* J_P, the matrix J_P has the same determinants as $J(E_c)$.

$$J_P = \begin{bmatrix} -x_1^* f_1'(\lambda^*)(1-q) & 0 \\ -x_2^* f_2'(\lambda^*) + q f_1(\lambda^*) - q x_1^* f_1'(\lambda^*) & f_2(\lambda^*) - 1 - q f_1(\lambda^*) \end{bmatrix}. \tag{3.8}$$

Whenever E_c exists, by (2.6)–(2.7) and (3.2)–(3.4), $f_2(\lambda^*) < 1$ and so the two diagonal elements of J_P are always negative, and so the determinant of $J(E_c)$ is positive. Similarly, the trace of E_c, given by

$$\text{trace}(E_c) = f_2(\lambda^*) - 1 - x_1^* f_1'(\lambda^*) - x_2^* f_2'(\lambda^*) .$$

is always negative and so E_c is always locally asymptotically stable.

We are now in a position to prove the main (global) results of this section.

3.3 Proof of Theorem 3.1

In all cases Ω is bounded and positively invariant (see (2.3)–(2.5)). Also, by Lemma 3.1 there are never any nontrivial periodic solutions in Ω.

i) From (3.5), E_1 is locally asymptotically stable if

$$f_1(1)(1-q) - 1 < 0 \quad \text{and} \quad f_2(1) < 1 . \tag{3.9}$$

This is case I. Note that the second inequality precludes the existence of E_2 and the first, precludes the existence of E_c (see (3.1) and (3.2), respectively). Hence in case I, E_1 is the only rest point as claimed in Table 1. Moreover, since Ω is bounded and positively invariant, the result follows directly from the Poincaré–Bendixson Theorem.

ii) First consider case III. By (3.2), in this case, E_c does not exist, and so there is no interior rest point. By (3.5), E_1 has one positive and one negative eigenvalue and so is unstable. Since $f_2(1) > 1$ and f_2 is continuous, for any $t \geqq 0$, provided that $(x_1(t), x_2(t))$ satisfies $(x_1 + x_2)(t) > 0$ and is sufficiently close to E_1, then $x_2'(t) > 0$. Hence, the stable manifold of E_1 cannot intersect $\Omega \setminus \{E_1\}$. By (3.6), both eigenvalues of E_2 are negative, and hence E_2 is locally asymptotically stable. Again, the result follows by the Poincaré–Bendixson Theorem.

Next consider case IVa. We already showed that E_c does not exist in this case and so there is no interior rest point. By (3.5), E_1 is a repellor and by (3.6), E_2 is locally asymptotically stable. Again the result follows by the Poincaré–Bendixson Theorem.

iii) First consider case II. By (3.4), E_c exists, and from (3.8) it is locally asymptotically stable. E_2 does not exist by (3.1). From (3.5), E_1 has one positive and one negative eigenvalue. In this case, the stable manifold of E_1 is one dimensional and lies along the x_2-axis. Again the result follows by the Poincaré–Bendixson Theorem.

Finally, consider case IVb. In this case all three rest points exist. E_c is locally asymptotically stable. E_1 is a repellor. E_2 has a one dimensional stable manifold that lies along the x_2-axis. Again the result follows by the Poincaré–Bendixson Theorem. □

4 Global analysis of (1.9)

We now use the results of asymptotically autonomous systems to show the connection between the dynamics for system (2.2) and system (1.9).

Theorem 4.1
i) In case I, E_1^3 is a global attractor for solutions of (1.9).
ii) In cases III and IVa, E_2^3 is a global attractor of any solution of (1.9) *initiating at a point where* $x_1(0)+x_2(0)>0$.
iii) In cases II and IVb, E_c^3 is a global attractor of any solution of (1.9) *initiating at a point where* $x_1(0)>0$.

Proof. Note that because all of the inequalities in Table 1 are strict, in all cases I–IV, all rest points for both systems (2.2) and (1.9) are hyperbolic and hence isolated. From the proof of Theorem 3.1, it is clear that every solution of (2.2) converges to a rest point and that there are no homoclinic orbits or chains of rest points for (2.2). It follows by Corollary 4.3 of [T], that every solution of (1.9) converges to one of the rest points of (1.9), either E_1^3, E_2^3, or E_c^3. Since an unstable rest point cannot be the only point in the omega limit set of a solution unless that solution lies in its stable manifold it remains only to consider the stable manifolds of E_1^3 and E_2^3, when they are unstable. Since Ω^3 is globally attracting, except for an additional negative eigenvalue, the eigenvalues of E_1^3, E_2^3, and E_c^3 are identical to those of E_1, E_2 and E_c, respectively.

i) No rest point is unstable in this case and so the result follows.
ii) In these two cases the only unstable rest point is E_1^3.

In case III, the stable manifold is two dimensional, containing the S-axis but not interesecting $\Omega^3\setminus\{E_1^3\}$, where Ω^3 was defined in (2.1) (see the proof of Theorem 3.1 *ii*). Therefore, the only solutions that E_1^3 can attract in this case are solutions initiating on the S-axis.

In case IVa, E_1^3 is a repellor with respect to Ω^3 (see the proof of Theorem 3.1*ii*)). Its stable manifold is the one dimensional S-axis.

Hence in cases III and IVa, E_2^3 attracts all solutions with $x_1(0)+x_2(0)>0$.
iii) In case II the only unstable rest point is E_1^3 and its stable manifold is the same as in Case III (see the proof of Theorem 3.1*iii*)).

In case IVb, E_1^3 and E_2^3 exist and are both unstable. The stable manifold of E_1^3 is as in case IVa (see the proof of Theorem 3.1*iii*)). The two dimensional stable manifold of E_2^3 is the entire $(S-x_2)$-face where $x_1\equiv 0$.

Hence, in cases II and IVb E_2^3 attracts any solution where $x_1(0)>0$. □

The above theorem could also be proved directly, using the Butler–McGehee Lemma (see Appendix 1 of [FW]) as in the proof of Theorem 3.3 of Butler and Wolkowicz [BWa].

5 Discussion

The foregoing sections gave a global analysis of the asymptotic behavior of the model studied by Stephanopoulis and Lapidus [SL], assuming uninhibited growth, i.e. monotone response functions, as well as a very restrictive case of inhibited growth. The analysis of [SL] dealt rigorously only with existence and stability of

rest points. For example, no account was taken there of the possibility of nontrivial periodic solutions. Since we are able to prove that in all cases there is a globally attracting rest point, the outcome of the competition between the plasmid-free and the plasmid-bearing organisms, depends only on the relative values of the parameters in the model and is always independent of the initial concentrations, provided that initially there is at least some of the appropriate organism present.

There are a number of models for plasmid loss referenced in [SL]. There is also a nice paper of Simonsen [S], surveying a number of experiments and giving an estimate of q as 10^{-3}–10^{-5} HR^{-1}. Since commercial production of products manufactured by genetically altered organisms is a reality, understanding these models in a mathematically rigorous fashion seems important. For example, the existence (and resulting global stability) of E_c^3 represents a loss of the (desired) efficiency of the biological reactor. On the other hand, the global stability of E_2^3 represents no production at all. In this case, the plasmid-free organism has taken over the chemostat and excluded the plasmid-bearing organism.

In order to easily see how the operating parameters D and $S^{(0)}$ effect whether a mixed culture can be achieved, we give a bifurcation analysis for model (1.1) in terms of the biological meaningful parameters. The rest points in terms of the original parameters are:

$$\mathscr{E}_1 = (S^{(0)}, 0, 0)$$

$$\mathscr{E}_2 = (\lambda_2, 0, \gamma(S^{(0)} - \lambda_2)) \quad \text{where } f_2(\lambda_2) = D\,.$$

$$\mathscr{E}_c = \left(\lambda^*, \frac{\gamma(S^{(0)} - \lambda^*)(D - f_2(\lambda^*))}{f_1(\lambda^*) - f_2(\lambda^*)}, \frac{\gamma(S^{(0)} - \lambda^*)(f_1(\lambda^*) - D)}{f_1(\lambda^*) - f_2(\lambda^*)}\right)$$

$$\text{where } f_1(\lambda^*)(1-q) = D$$

Table 2 is just the rescaling of Table 1 to obtain criteria in terms of the biologically meaningful parameters.

If one fixes $S^{(0)}$ one can in theory always select the dilution rate D, large enough so that case I of Table 2 holds and both organisms washout. If one assumes that the functions $(1-q)f_1(S)$ and $f_2(S)$ intersect at most once for $S > 0$, as in the case that the specific growth rate functions are either both Monod (see (1.2)) or both Ivlev (see (1.8)) in form, then either:

1. $(1-q)f_1(S) > f_2(S)$ for all $S > 0$, or
2. $(1-q)f_1(S) < f_2(S)$ for all $0 < S < \hat{S}$ and $(1-q)f_1(S) > f_2(S)$ for all $S > \hat{S}$, or
3. $(1-q)f_1(S) < f_2(S)$ for all $S > 0$, or
4. $(1-q)f_1(S) > f_2(S)$ for all $0 < S < \hat{S}$ and $(1-q)f_1(S) < f_2(S)$ for all $S > \hat{S}$.

For the sake of genericity, assume $S^{(0)} \neq \hat{S}$. Without loss of generality, assume also that $S^{(0)} > \hat{S}$. (If not, cases 2. and 4. would be covered by cases 3. and 1., respectively.)

If case 1 holds, then as D is gradually decreased, eventually there is a bifurcation and case II of Table 2 holds. In this case the coexistence rest point becomes the global attractor. Decreasing D further, since λ_2 must initially be very close to $S^{(0)}$, the next bifurcation is to case IVb and so the coexistence rest point remains the global attractor. Since, in this case, λ_2 remains larger than λ^* no matter how much more D is decreased, there is not another case change. Thus, in this case, the sequence of bifurcations as D decreases, results in the global stability passing from $\mathscr{E}_1$ to $\mathscr{E}_c$ and remaining there.

Table 2.

Case	Criteria for existence of rest points and global stability of boxed rest point			Rest points
I	$f_1(S^0)(1-q) < D$	$f_2(S^0) < D$		$\{\boxed{\mathscr{E}_1}\}$
II	$f_1(S^0)(1-q) > D$	$f_2(S^0) < D$		$\{\mathscr{E}_1, \boxed{\mathscr{E}_c}\}$
III	$f_1(S^0)(1-q) < D$	$f_2(S^0) > D$		$\{\mathscr{E}_1, \boxed{\mathscr{E}_2}\}$
IVa	$f_1(S^0)(1-q) > D$	$f_2(S^0) > D$	$f_1(\lambda_2)(1-q) < D$	$\{\mathscr{E}_1, \boxed{\mathscr{E}_2}\}$
IVb			$f_1(\lambda_2)(1-q) > D$	$\{\mathscr{E}_1, \mathscr{E}_2, \boxed{\mathscr{E}_c}\}$

If case 2 holds, then as D is decreased, the same initial sequence of bifurcations occurs as in case 1, but there is an additional bifurcation as D is decreased further so that one passes from case IVb to case IVa and one obtains a plamid-free culture. Thus the bifurcation sequence has the global stability passing from $\mathscr{E}_1$ to $\mathscr{E}_c$ to $\mathscr{E}_2$ and then remaining there. In this case, the experimenter must be very careful to operate the chemostat at low enough dilution rate, but not too low, if the aim is to maintain a mixed culture.

Similarly, in case 3, one can show that the bifurcation sequence is from case I to case III to case IVa and hence global stability passes from $\mathscr{E}_1$ to $\mathscr{E}_2$ and so it is not possible to obtain a mixed culture in this case. In case 4, the bifurcation sequence is from I to III to IVa to IVb and hence global stability passes from $\mathscr{E}_1$ to $\mathscr{E}_2$ to $\mathscr{E}_c$ and remains there. Thus in this case, it is only necessary to operate the chemostat at low enough dilution rate to obtain a mixed culture.

If one wanted to allow one or both of the specific growth rate functions to be sigmoidal using for example the form (1.7), one would have to consider two more cases, since it is then possible to have two intersections of $(1-q)f_1(S)$ and $f_2(S)$ for $S>0$:

5. $(1-q)f_1(S) < f_2(S)$ for all $0<S<\hat{S}_1$,
   $(1-q)f_1(S) > f_2(S)$ for all $\hat{S}_1<S<\hat{S}_2$,
   $(1-q)f_1(S) < f_2(S)$ for all $\hat{S}_2 < S$.
6. $(1-q)f_1(S) > f_2(S)$ for all $0<S<\hat{S}_1$,
   $(1-q)f_1(S) < f_2(S)$ for all $\hat{S}_1<S<\hat{S}_2$,
   $(1-q)f_1(S) > f_2(S)$ for all $\hat{S}_2<S$.

Case 5 is initially the same as case 4, but there is an additional bifurcation resulting in a final case change from case IVb to case IVa. Thus the sequence of bifurcations is from $\mathscr{E}_1$ to $\mathscr{E}_2$ to $\mathscr{E}_c$ and then back to $\mathscr{E}_2$. Case 6 is initially the same as case 2, but there is an additional bifurcation resulting in a final case change from case IVa to case IVb. Thus the sequence of bifurcations is from $\mathscr{E}_1$ to $\mathscr{E}_c$ to $\mathscr{E}_2$ and then back to $\mathscr{E}_c$.

In a similar manner it is easy to see that effect of varying $S^{(0)}$. The principal question is the relative values of $S^{(0)}$ and $\hat{S}$. Although q is not usually under the investigators control, one could also consider the effect of q. The determining factor is how varying q effects the relative values of $(1-q)f_1(S)$ and $f_2(S)$ and whether or not they intersect.

It is not possible to achieve total efficiency (exclusion of plasmid-free organisms) since there is no attracting solution with $x_2(t)=0$ and $x_1(t)>0$. The best that can be done is to optimize the location of $\mathscr{E}_c$, that is, to operate the chemostat so as to maximize the concentration of plasmid-bearing organism in $\mathscr{E}_c$ within, of course, the many other practical limitations on the operating parameters.

A common way to alter the parameters intrinsic to the organisms, so as to alter the competitive advantage of the plasmid-free type is to add an inhibitor to the chemostat along with the nutrient. This was done in the experiments of Hansen and Hubbell [HH] who use Naladixic acid to modify the value of m_i. The chemostat with an inhibitor has been analyzed in [HW]. It would be interesting to investigate the effect of an inhibitor in the present context.

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