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A MODEL OF THE EFFECT OF ANTI-COMPETITOR TOXINS ON PLASMID-BEARING, PLASMID-FREE COMPETITION*

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Abstract. The usual models of the chemostat assume that the competition is purely exploitative, the competition is only through the consumption of the nutrient. However, it is known that microorganisms can produce toxins against its competitors. In this work, we consider a model of competition in the chemostat between plasmid-bearing, plasmid-free organisms for a single nutrient where plasmid-bearing organism can produce a toxin (allelopathic agent) against the plasmid-free organism at some cost to its reproductive abilities. We give a characterization of the outcome of this competition in terms of the relevant parameters in hyperbolic cases. The global asymptotic behavior of the solutions is proved by using the perturbation of a globally stable steady state for a sufficiently small plasmid loss rate.

1. Introduction

Genetically altered organisms are frequently used to manufacture products. The alteration is accomplished by the introduction of DNA into the cell in the form of a plasmid. The metabolic load imposed by this 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. One approach is for the plasmid to code for resistance to an antibiotic which is then added to the medium. Our model assumes that the plasmid codes for

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the production of and resistance to an allelopathic agent (hereafter referred to as a "toxin" or as an inhibitor.)

The basic chemostat is a standard example of an open system with purely exploitative competition. It consists, essentially, of three vessels. The first contains the nutrient which is pumped at a constant rate into the second vessel, the culture vessel. This vessel is charged with micro-organisms which compete, in a purely exploitative manner, for the nutrient. The contents of the second vessel is pumped, at a constant rate, into the third or overflow vessel. The key assumptions are that the culture vessel is well stirred, that temperature, pH, etc., are kept constant and that the turnover of the vessel is sufficiently fast that no wall growth occurs and that there is no buildup of metabolic products. In ecology the chemostat is a model of a simple lake but in chemical engineering it also serves as a laboratory model of a bio-reactor used to manufacture products with genetically altered organisms. In more complicated situations, it is often the starting point for construction of models in waste water treatment, Schuler and Kargi [15], or of the mammalian large intestine, Freter [2]. Early analyses can be found in the articles of Levin and Stewart, [14], and Hsu, Hubbell and Waltman, [10]. The recent monograph of Smith and Waltman, [17], provides a mathematical description of the chemostat and its properties.

In a paper of Stephanopoulis and Lapius [20], a model of competition between plasmid-bearing and plasmid-free organisms in a chemostat was proposed. The global analysis of the model in case of uninhibited specific growth rate was provided in the paper of Hsu, Waltman and Wolkowicz, [11].

The above models assume that no agents are produced by one organism to inhibit the other thus making for purely exploitative competition. However, in nature it is known that micro-organisms produce inhibitors against their rivals. In a fundamental paper, Chao and Levin [1], provided basic experiments on anti-bacterial toxins. In [9], Hsu and Waltman proposed a model of competition in the chemostat of two competitors for a single nutrient where one of the competitors can produce a toxin against its opponent at some cost to its reproductive abilities. In this paper we combine the models in [8] and [9] to consider a new model of competition in the chemostat of plasmid-bearing, plasmid-free organism for a single nutrient where plasmid-bearing organism can produce an allelopathic agent (hereafter called a toxin) against the plasmid-free organism at some cost to its reproductive abilities. The loss of the plasmid renders the organsim free from the metabolic burden it was designed to carry but also makes it susceptible to the toxin. The goal of the paper is to describe the asymptotic behavior of the model in terms of the system parameters (the operating parameters of the chemostat and the parameters of the organisms.) The utility of this information will be illustrated in the discussion section. To put this into perspective, we comment on some other models of inhibitors in the chemostat.

Lenski and Hattingh [12] produced a model of the chemostat with an external inhibitor and provided numerical experiments to illustrate the behavior of solutions. The introduction of an inhibitor produces a selective medium. The model of Lenski and Hattingh is appropriate for detoxification problems in that the external inhibitor interferes with the growth of one competitor while being taken up without ill effect by the other. The model proposed by Lenski and Hattingh was analyzed by Hsu and Waltman [7], where the possible outcomes were classified in terms of the parameters of the system and the global asymptotic behavior of the system determined. See also Hsu and Luo [6] for another approach. This is important in bio-reactors because inhibitors are used to suppress the competitors of the organism manufacturing a product. If a competitor produces the inhibitor (the toxin) it also produces a selective medium in the same sense as the external inhibitor only "naturally". We investigate here whether a substance that inhibits the growth of a cell produces different qualitative behavior than one that is lethal to it.

A model for toxins in the chemostat was given by Levin [13]. He provided numerical evidence of the presence of bi-stable attrators. See, in particular, Figure 1 of the above cited paper. In this case, the winner of the competition is determined by the initial conditions.

A mathematical analysis of the chemostat with an internally produced selective medium can be found in Hsu and Waltman [8]. In this approach, the inhibitor reduces the growth of the competitor rather than being lethal. The models there focused on the effect of plasmid loss to create the competitor. In the models of Lenski and Hattingh. [12], Hsu and Waltman, [7], the inhibitor affected the nutrient uptake-and consequently the growth-of the sensitive cell.

In section 2 we present the model and the preliminary stability analysis of "washout" state E_0 and "plasmid-free" state E_2. In Section 3 we study the existence and stability of the coexistence states E_{c1} and E_c. In Section 4, we apply the technique of perturbation of a globally stable steady state [17] to show that the global stability of E_{c1} and E_2 respectively for sufficiently small plasmid loss rate $q, 0 < q \ll 1$. We note that biologically $q \approx 10^{-3} \sim 10^{-5}/hr$ [16], and it is appropriate to assume that q is small.

2. The Model

Let $S(t)$ denote the concentration of nutrient in the vessel, let $x(t)$ and $y(t)$ denote the concentration of the plasmid-bearing and plasmid-free organisms at time t, respectively, and let $P(t)$ denote the concentration of toxin present. The model takes the form:

$$
\begin{aligned}
S' &= (S^{(0)} - S)D - \frac{m_1 S}{a_1 + S}\frac{x}{r_1} - \frac{m_2 S}{a_2 + S}\frac{y}{r_2} \\
x' &= x\left[(1 - q - k)\frac{m_1 S}{a_1 + S} - D\right] \\
y' &= y\left[\frac{m_2 S}{a_2 + S} - D - rP\right] + q\frac{m_1 S}{a_1 + S}x \\
P' &= k\frac{m_1 S}{a_1 + S}x - DP
\end{aligned}
\tag{2.1}
$$

where $S^{(0)}$ is the input concentration of nutrient, D is the washout rate, m_i, the maximal growth rates, a_i, the Michaelis-Menten constant and r_i, the yield constant, $i = 1, 2$. q, $0 < q < 1$ is the fraction of plasmid-bearing organisms that loose the plasmid in reproduction and so convert into plasmid-free organisms; $k > 0$ is the fraction of growth that the plasmid-bearing population sacrifices for producing toxin, $0 < k < 1 - q$. We perform the usual scaling for the chemostat. Specifically, let

$$
\begin{aligned}
\bar{S} &= \frac{S}{S^{(0)}},\ \bar{x} = \frac{x}{r_1 S^0},\ \bar{y} = \frac{y}{r_2 S^{(0)}},\ \bar{P} = \frac{P}{r_2 S^0} \\
\tau &= Dt,\ \bar{m}_i = \frac{m_i}{D},\ \bar{a}_i = \frac{a_i}{S^{(0)}},\ \bar{r} = \frac{r_2 r S^{(0)}}{D}.
\end{aligned}
$$

Then (2.1) becomes

$$
\begin{aligned}
\frac{d}{d\tau}\bar{S} &= (1 - \bar{S}) - \frac{\bar{m}_i \bar{S}}{\bar{a}_1 + \bar{S}}\bar{x} - \frac{\bar{m}_2 \bar{S}}{\bar{a}_2 + \bar{S}}\bar{y} \\
\frac{d}{d\tau}\bar{x} &= \bar{x}\left[(1 - q - k)\frac{\bar{m}_1 \bar{S}}{\bar{a}_1 + \bar{S}} - 1\right] \\
\frac{d}{d\tau}\bar{y} &= \bar{y}\left[\frac{\bar{m}_2 \bar{S}}{\bar{a}_2 + \bar{S}} - 1 - \bar{r}\bar{P}\right] + q\frac{\bar{m}_1 \bar{S}}{\bar{a}_1 + \bar{S}}\frac{r_1}{r_2}\bar{x} \\
\frac{d}{d\tau}\bar{P} &= k\frac{\bar{m}_1 \bar{S}}{\bar{a}_1 + \bar{S}}\frac{r_1}{r_2}\bar{x} - \bar{P}
\end{aligned}
$$

If we drop the bars, and assume two plasmid populations have the same yield constants, i.e., $r_1 = r_2$: the system becomes

$$
\begin{aligned}
S' &= (1 - S) - \frac{m_1 S}{a_1 + S}x - \frac{m_2 S}{a_2 + S}y \\
x' &= x\left[(1 - q - k)\frac{m_1 S}{a_1 + S} - 1\right] \\
y' &= y\left[\frac{m_2 S}{a_2 + S} - 1 - rP\right] + q\frac{m_1 S}{a_1 + S}x \\
P' &= k\frac{m_1 S}{a_1 + S}x - P.
\end{aligned}
\tag{2.2}
$$

It is easy, from the form of the equations, to show that the solutions of (2.2) are positive if $x(0) > 0$, $y(0) > 0$, $S(0) \geq 0$, $P(0) \geq 0$. Let $\Sigma(t) = S + x + y + P$. Then

$$\Sigma'(t) = 1 - (S + x + y + P) - rPy \leq 1 - \Sigma(t);$$

or,

$$\limsup_{t \to \infty} \Sigma(t) \leq 1.$$

The solution, $(S(t), x(t), y(t), P(t))$, is bounded for $t \geq 0$ since each component is non-negative. The system (2.2) is dissipative and, thus, has a compact, global attractor. To simplify (2.2), let

$$z = P - \frac{k}{1 - k - q}x.$$

This change of variables yields the system

$$
\begin{aligned}
z' &= -z \\
S' &= (1 - S) - \frac{m_1 S}{a_1 + S}x - \frac{m_2 S}{a_2 + S}y \\
x' &= x\left[(1 - q - k)\frac{m_1 S}{a_1 + S} - 1\right] \\
y' &= y\left[\frac{m_2 S}{a_2 + S} - 1 - r\left(z + \frac{k}{1 - k - q}x\right)\right] + q\frac{m_1 S}{a_1 + S}x.
\end{aligned}
\tag{2.3}
$$

Clearly, $z(t) \to 0$, so the system (2.3) is an asymptotically autonomous system with the following limiting system:

$$
\begin{aligned}
S' &= (1 - S) - \frac{m_1 S}{a_1 + S}x - \frac{m_2 S}{a_2 + S}y \\
x' &= x\left[(1 - q - k)\frac{m_1 S}{a_1 + S} - 1\right] \\
y' &= y\left[\frac{m_2 S}{a_2 + S} - 1 - \frac{rk}{1 - k - q}x\right] + q\frac{m_1 S}{a_1 + S}x
\end{aligned}
\tag{2.4}
$$

We shall study the behavior of solutions of (2.4), and from the work of Thieme, [21], we obtain the asymptotic behavior of solutions of (2.3).

Let $f_i(s) = \frac{m_i S}{a_i + S}$, $i = 1, 2$ and define λ_1, λ_2 to satisfy

$$
f_1(\lambda_1) = \frac{1}{1 - q - k}; \tag{2.5}
$$

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

The equilibrium point $E_0 = (1, 0, 0)$ always exists. If $\lambda_2 < 1$, there is an equilibrium of (2.4) in the form $E_2 = (\lambda_2, 0, 1 - \lambda_2)$. Notice that with $q > 0$, there is no equilibrium of (2.4) in the form $E_1 = (a, b, 0)$ with $a > 0, b > 0$.

In the following we discuss the local stability of the equilibria by evaluating the variational matrix of system (2.4) at each equilibrium.

Lemma 2.1. (1) *If* $\lambda_1 > 1$, *and* $\lambda_2 > 1$, *then* E_0 *is local asymptotically stable*; *it is unstable if either inequality is reversed*. (2) *If* E_2 *exists and* $\lambda_2 < \lambda_1$, *then* E_2 *is local asymptotically stable*; *it is unstable if either inequality is reversed.*

Proof. The variational matrix of system (2.4) takes the form

$$
J = \begin{bmatrix}
-1 - xf_1'(S) - yf_2'(S) & -f_1(S) & -f_2(S) \\
x(1 - q - k)f_1'(S) & (1 - q - k)f_1(S) - 1 & 0 \\
yf_2'(S) + qxf_1'(S) & -\frac{rk}{1-q-k}y + qf_1(S) & f_2(S) - 1 - \frac{rk}{1-q-k}x
\end{bmatrix}.
$$

At $E_0 = (1, 0, 0)$,

$$
J_0 = \begin{bmatrix}
-1 & -f_1(1) & -f_2(1) \\
0 & (1 - q - k)f_1(1) - 1 & 0 \\
0 & qf_1(1) & f_2(1) - 1
\end{bmatrix}.
$$

The eigenvalues of J_0 are on the diagonal and E_0 is local asymptotically stable if

$$f_1(1) < \frac{1}{1 - q - k}$$

and

$$f_2(1) < 1, \quad \text{or if}, \quad \lambda_1 > 1 \ \text{ and } \ \lambda_2 > 1.$$

At $E_2 = (\lambda_2, 0, 1 - \lambda_2)$,

$$J_2 = \begin{bmatrix} m_{11} & m_{12} & m_{13} \\ 0 & m_{22} & 0 \\ m_{31} & m_{32} & 0 \end{bmatrix}$$

where

$$m_{11} = -1 - f_2'(\lambda_2)(1 - \lambda_2); \ m_{12} = -f_1(\lambda_2); \ m_{13} = -1$$

$$m_{22} = (1 - q - k)f_1(\lambda_2) - 1; \ m_{31} = (1 - \lambda_2)f_2'(\lambda_2)$$

$$m_{32} = -\frac{rk}{1 - q - k}(1 - \lambda_2) + qf_1(\lambda_2)$$

The characteristic polynomial of J_2 is

$$\varphi(\lambda) = \det(\lambda I - J) = (\lambda - m_{22})(\lambda^2 - m_{11}\lambda - m_{13}m_{31})$$

By the Routh-Hurwitz criterion, E_2 is local asymptotically stable if $m_{22} < 0$, $m_{11} < 0$, and $m_{13}m_{31} < 0$. Clearly, $m_{11} < 0$ and $m_{13}m_{31} < 0$. Moreover,

$$m_{22} < 0$$

if and only if

$$f_1(\lambda_2) < \frac{1}{1 - q - k},$$

that is, $\lambda_2 < \lambda_1$ This completes the proof of Lemma 2.1.

Before proving the following global results for the washout state E_0 and the plasmid-free state E_2, we state two lemmas.

Lemma 2.2. [4] *Let* $f : R^+ \longrightarrow R$ *be differentiable. If*

$$\liminf_{t \to \infty} f(t) < \limsup_{t \to \infty} f(t),$$

there are sequences $t_m \nearrow \infty$ *and* $s_m \nearrow \infty$, *such that for all* m

$$\lim_{m \to \infty} f(t_m) = \limsup_{t \to \infty} f(t), \ f'(t_m) = 0$$

and

$$\lim_{m\to\infty} f(s_m) = \liminf_{t\to\infty} f(t), \quad f'(s_m) = 0.$$

Lemma 2.3. [3] *Let* $a \in \mathbb{R}$ *and* $f : [a, \infty) \to \mathbb{R}$ *be differentiable. If*

$$\lim_{t\to\infty} f(t)$$

exists and the derivative $f'(t)$ *is uniformly continuous on* (a, ∞), *then*

$$\lim_{t\to\infty} f'(t) = 0.$$

Next the basic global results for E_0 and E_2 are established.

Lemma 2.4. (1) *If* $\lambda_1 > 1$, *then*

$$\lim_{t\to\infty} x(t) = 0.$$

Moreover, if, in addition, $\lambda_2 < 1$, *then* E_2 *is global asymptotically stable.*

(2) *If* E_0 *is local asymptotically stable, then* E_0 *is global asymptotically stable.*

Proof. (1) First, we prove that $\lim_{t\to\infty} x(t)$ exist by contradiction. If $x(t)$ does not tend to a limit, then the lim sup and the lim inf are different, i.e.,

$$0 \le \liminf_{t\to\infty} x(t) < \limsup_{t\to\infty} x(t) = \delta.$$

Then, from Lemma 2.2, we can choose a sequence $t_m \nearrow \infty$ such that $x'(t_m) = 0$, for all m, and

$$\lim_{m\to\infty} x(t_m) = \limsup_{t\to\infty} x(t) = \delta > 0.$$

It follows that

$$\lim_{m\to\infty} x(t_m)\left((1-q-k)\frac{m_1 S(t_m)}{a_1 + S(t_m)} - 1\right) = \lim_{m\to\infty} x'(t_m) = 0.$$

Thus,

$$\lim_{m\to\infty}\left((1-q-k)\frac{m_1 S(t_m)}{a_1 + S(t_m)} - 1\right) = 0.$$

This implies $\lim_{m\to\infty} S(t_m) = \lambda_1 > 1$, and this a contradiction to the fact that $\limsup_{t\to\infty} \Sigma(t) \le 1$.

Next, we prove $\lim_{t\to\infty} x(t) = 0$. If not, then $\lim_{t\to\infty} x(t)$ exists and $\lim_{t\to\infty} x(t) > 0$. From Lemma 2.3, we obtain

$$\lim_{t\to\infty} x'(t) = 0.$$

Hence,

$$\lim_{t\to\infty} x(t)\left[(1-q-k)\frac{m_1 S(t)}{a_1+S(t)}-1\right]=\lim_{t\to\infty} x'(t)=0.$$

This leads to a contradiction, $\lim_{t\to\infty} S(t)=\lambda_1>1$.

Consider the flow in the invariant set, $x=0$. For $x=0$, system (2.4) is a two dimensional system:

$$\begin{aligned} S' &= (1-S)-\frac{m_2 S}{a_2+S}y \equiv F(S,y)\\ y' &= y\left[\frac{m_2 S}{a_2+S}-1\right]\equiv G(S,y). \end{aligned}$$

From [5], it follow that $\lim_{t\to\infty} S(t)=\lambda_2$, and $\lim_{t\to\infty} y(t)=1-\lambda_2$. Using Thieme [21], it follows that

$$\lim_{t\to\infty}(S(t),x(t),y(t))=(\lambda_2,0,1-\lambda_2).$$

Thus the proof of (1) is complete.

(2) Since E_0 is local asymptotically stable, i.e., $\lambda_1>1$ and $\lambda_2>1$, similar arguments as in the proof of (1) establishes (2). We omit the details.

3. The Existence and Stability of Interior Equilibria

In this section, we consider the existence and stability of the interior equilibrium $E_c=(S_c,x_c,y_c)$. From the equation for x in (2.4), one has directly that $S_c=\lambda_1$. From the equation for S it follow that

$$1-\lambda_1-f_1(\lambda_1)x_c-f_2(\lambda_1)y_c=0,$$

or that

$$y_c=\frac{1}{f_2(\lambda_1)}(1-\lambda_1-f_1(\lambda_1)x_c). \tag{3.1}$$

From the equation for y, it follows that

$$y_c\left(f_2(\lambda_1)-1-\frac{rk}{1-k-q}x_c\right)+qf_1(\lambda_1)x_c=0. \tag{3.2}$$

From (3.1) and (3.2) one has

$$\frac{1}{f_2(\lambda_1)}(1-\lambda_1-f_1(\lambda_1)x_c)\left(f_2(\lambda_1)-1-\frac{rk}{1-k-q}x_c\right)+qf_1(\lambda_1)x_c=0.$$

Define the function

$$H(x) = \frac{1}{f_2(\lambda_1)}\Big(1-\lambda_1 - f_1(\lambda_1)x\Big)\Big(f_2(\lambda_1) - 1 - \frac{rk}{1-k-q}x\Big) + q f_1(\lambda_1)x.$$

First consider the case $0 < \lambda_1 < \lambda_2 < 1$. It will be shown that the equation $H(x) = 0$ has a unique root x_c which lies between 0 and 1. As a consequence, one has that

$$y_c = \frac{1}{f_2(\lambda_1)}(1-\lambda_1 - f_1(\lambda_1)x_c) > 0,$$

or that

$$0 < x_c < \frac{1-\lambda_1}{f_1(\lambda_1)}.$$

In this case there is a unique interior equilibrium $E_c = (S_c, x_c, y_c)$.

Obviously,

$$H(0) = \frac{1}{f_2(\lambda_1)}(1-\lambda_1)\left(\frac{m_2\lambda_1}{a_2+\lambda_1} - 1\right) < 0, \text{ if } \lambda_1 < \lambda_2 \text{ and } \lambda_1 < 1$$

and

$$H\left(\frac{1-\lambda_1}{f_1(\lambda_1)}\right) = q(1-\lambda_1) > 0, \text{ if } \lambda_1 < 1$$

Note that $0 < \frac{1-\lambda_1}{f_1(\lambda_1)} = (1-\lambda_1)(1-k-q) < 1$. From the Intermediate-Value theorem, there is a point x_c between 0 and $\frac{1-\lambda_1}{f_1(\lambda_1)}$ such that $H(x_c) = 0$. Next, we show that the x_c is unique and $y_c > 0$. The graph of $y = H(x)$ is a parabola with a positive coefficient of the x^2 term. Since $H(0) < 0$ and $H(\frac{1-\lambda_1}{f_1(\lambda_1)}) > 0$, $y = H(x)$ intersects the x-axis in exactly one point. From the discussion above, the following lemma holds.

Lemma 3.1. *Suppose that* $0 < \lambda_1 < 1$. *If* $\lambda_1 < \lambda_2 < 1$, (i.e., E_2 *is unstable*), *then the interior equilibrium* $E_c = (\lambda_1, x_c, y_c)$ *exists and is unique.*

Remark 3.1. *Write* $E_c = E_c(q) = (S_c(q), x_c(q), y_c(q))$. *If* $q = 0$ *then* $S_c(0) = \lambda_1(0)$ *where* $f_1(\lambda_1(0)) = \frac{1}{1-k}$, $x_c(0) = \frac{1-\lambda_1(0)}{f_1(\lambda_1(0))}$ *and* $y_c(0) = 0$. *Hence*

$$E_c(q) \longrightarrow E_1$$

as $q \to 0$, *where* $E_1 = (\lambda_1(0), x_c(0), 0)$.

Because of Remark 3.1, we denote the interior equilibrium by E_{c1} as a reminder that $\lim_{q\to 0} E_{c1}(q) = E_1$.

To determine the stability of E_{c1}, we investigate the eigenvalues of the Jacobian matrix,

$$J_c = \begin{bmatrix} m_{11} & m_{12} & m_{13} \\ m_{21} & 0 & 0 \\ m_{31} & m_{32} & m_{33} \end{bmatrix}$$

where

$$\begin{aligned}
m_{11} &= -1 - f_1'(\lambda_1)x_c - f_2'(\lambda_1)y_c < 0, \\
m_{21} &= (1-q-k)f_1'(\lambda_1)x_c > 0, \\
m_{31} &= f_2'(\lambda_1)y_c + qf_1'(\lambda_1)x_c > 0, \\
m_{12} &= -f_1(\lambda_1) < 0, \\
m_{32} &= -\frac{rk}{1-q-k}y_c + qf_1(\lambda_1) = f_1(\lambda_1)(q - rky_c), \\
m_{13} &= -f_2(\lambda_1) < 0, \\
m_{33} &= f_2(\lambda_1) - 1 - \frac{rk}{1-q-k}x_c < 0 \quad \text{if } \lambda_1 < \lambda_2.
\end{aligned}$$

Since

$$H(x_c) = \frac{1}{f_2(\lambda_1)}(1-\lambda_1 - f_1(\lambda_1)x_c)(f_2(\lambda_1) - 1 - f_1(\lambda_1)rkx_c) + qf_1(\lambda_1)x_c = 0,$$

it follows that

$$\frac{1}{f_2(\lambda_1)}(1-\lambda_1 - f_1(\lambda_1 x_c) = \frac{qf_1(\lambda_1)x_c}{rkf_1(\lambda_1)x_c + 1 - f_2(\lambda_1)},$$

and

$$y_c = \frac{qf_1(\lambda_1)x_c}{rkf_1(\lambda_1)x_c + 1 - f_2(\lambda_1)}.$$

Hence,

$$m_{32} = f_1(\lambda_1)(q - rky_c) = qf_1(\lambda_1)\left(\frac{f_2(\lambda_2) - f_2(\lambda_1)}{rkf_1(\lambda_1)x_c + (f_2(\lambda_2) - f_2(\lambda_1))}\right) > 0$$

if $\lambda_1 < \lambda_2$.

Lemma 3.2. *If* $0 < \lambda_1 < \lambda_2 < 1$, *then* E_{c1} *exist and is local asymptotically stable.*

Proof. By the Routh-Hurwitz criterion, E_{c1} is local asymptotically stable if and only if

(3.3) $$A_1 > 0, A_3 > 0$$

$$A_1A_2 > A_3 \tag{3.4}$$

where the characteristic polynomial of J_c is

$$f(\lambda) = \lambda^3 + A_1\lambda^2 + A_2\lambda + A_3$$

with

$$\begin{aligned}
A_1 &= -(m_{11}m_{33}); A_2 = m_{11}m_{33} - m_{13}m_{31} - m_{12}m_{21}\\
A_3 &= m_{21}(m_{12}m_{33} - m_{13}m_{32})\\
A_1A_2 - A_3 &= m_{11}m_{12}m_{21} - m_{11}^2m_{33} + m_{11}m_{31}m_{33} - m_{11}m_{33}^2\\
&\quad +m_{31}m_{13}m_{33} + m_{21}m_{13}m_{32}.
\end{aligned}$$

Clearly, (3.3) holds. Since $\lambda_1 < \lambda_2$, and since

$$\begin{aligned}
m_{11}m_{12} + m_{13}m_{32} &= f_1(\lambda_1)[1 + f_1'(\lambda_1)x_c + f_2'(\lambda_1)y_c]\\
&\quad + f_2(\lambda_1)f_1(\lambda_1)(rky_c - q)\\
&= f_1(\lambda_1)[1 + f_1'(\lambda_1)x_c + f_2'(\lambda_1)y_c + f_2(\lambda_1)rky_c\\
&\quad - f_2(\lambda_1)q]\\
&> f_1(\lambda_1)[1 - f_2(\lambda_1)q]\\
&= f_1(\lambda_1)[f_2(\lambda_2) - f_2(\lambda_1)q]\\
&> f_1(\lambda_1)[f_2(\lambda_2) - f_2(\lambda_1)] > 0.
\end{aligned}$$

it follows that

$$m_{11}m_{12}m_{21} + m_{21}m_{13}m_{32} = m_{21}(m_{11}m_{12} + m_{13}m_{32}) > 0.$$

Since the remainder of the terms in $A_1A_2 - A_3$ are positive, it follows that (3.4) holds which completes the proof of Lemma 3.2.

Next, for the case $0 < \lambda_2 < \lambda_1 < 1$, we consider the existence of interior equilibria and their stability properties. As above for the case $0 < \lambda_2 < \lambda_1 < 1$, the interior equilibrium $E_c = (S_c, x_c, y_c)$ satisfies $S_c = \lambda_1$, $H(x_c) = 0$, $0 < x_c < \frac{1-\lambda_1}{f_1(\lambda_1)}$. Obviously if $0 < \lambda_1 < \lambda_2 < 1$ then $H(0) > 0$ and $H(\frac{1-\lambda_1}{f_1(\lambda_1)}) > 0$. Since $H(x)$ is a quadratic polynomial, the minimum of $H(x)$ is attained at $x^* = x^*(q) = \frac{(f_2(\lambda_1)-1)+rk(1-\lambda_1)-qf_2(\lambda_1)}{2rkf_1(\lambda_1)}$. From $H(0) > 0$ and $H(\frac{1-\lambda_1}{f_1(\lambda_1)}) > 0$, if $0 < x^* < \frac{1-\lambda_1}{f_1(\lambda_1)}$ and $H(x^*) < 0$, then there are two roots x_c, x_{1c} of $H(x) = 0$ satisfying $0 < x_c < x^* < x_{1c} < \frac{1-\lambda_1}{f_1(\lambda_1)}$. Equivalently, there are two interior equilibria which we denote by $E_c = (\lambda_1, x_c, y_c)$, and $E_{c1} = (\lambda_1, x_{c1}, y_{c1})$. We note that $0 < x^* < \frac{1-\lambda_1}{f_1(\lambda_1)}$ is equivalent to

$$(f_2(\lambda_1) - 1) + (1 - \lambda_1) > qf_2(\lambda_1) > (f_2(\lambda_1) - 1) - rk(1 - \lambda_1) \tag{3.5}$$

and $H(x^*) < 0$ is equivalent to

$$\begin{aligned} &rk(1-\lambda_1) - (f_2(\lambda_1) - 1) + qf_2(\lambda_1))((f_2(\lambda_1) - 1) \\ &- rk(1-\lambda_1) + qf_2(\lambda_2)) + 2q((f_2(\lambda_1) - 1) + rk(1-\lambda_1) \\ &- qf_2(\lambda_1)) < 0 \end{aligned} \tag{3.6}$$

If one of (3.5) and (3.6) is violated, then there are no interior equilibria. We note that when $q = 0$, (3.6) is automatically satisfied and condition (3.5) is reduced to the condition $\lambda_1 < \hat{\lambda}$ in [8] where $\hat{\lambda}$ is unique root of $g(x) = f_2(x) - 1 - rk(1 - x) = 0$.

For $q > 0$ sufficiently small, (3.6) holds. Thus (3.5) is violated if and only if

$$f_2(\lambda_1) - 1 - rk(1 - \lambda_1) > 0 \tag{3.7}$$

Lemma 3.3. *If $\lambda_2 < \lambda_1$ then*

(i) *If* (3.5) *and* (3.6) *hold then these exist two interior equilibria $E_{c1} = (\lambda_1, x_{1c}, y_{1c})$ and $E_c = (\lambda_1, x_c, y_c)$ where $0 < x_{1c} < x^* < x_c < \frac{1-\lambda_1}{f_1(\lambda_1)}$. The equilibrium $E_2 = (\lambda_2, 0, 1 - \lambda_2)$ is locally asymptotically stable.*

(ii) *If one of* (3.5), (3.6) *is violated, then no interior equilibrium exists. E_2 is the only equilibrium which is locally asymptotically stable.*

Remark 3.2. We conjecture that E_{c1} is locally stable and E_c is unstable with a two dimensional stable manifold. The conjecture is true for the case $q = 0$ [8].

4. Global Convergence

The previous sections all provide local results, the existence and local stability of rest points. The more interesting question is that of global behavior. Unfortunately, for three dimensional systems that is a major difficulty. We turn instead to a perturbation in the parameter q which we have already observed is small. We outline the basic approach.

Consider

$$x' = f(x, \lambda) \tag{4.1}$$

where $f : U \times \Lambda \to R^n$ is continuous and where $U \subset R^n$ and $\Lambda \subset R^k$ and $D_x f(x, \lambda)$ is continuous on $U \times \Lambda$. Suppose that solutions of initial value problems are unique and remain in U for all $t > 0$ and $\lambda \in \Lambda$. Denote the solution of (4.1) by $x(t; z; \lambda)$ where $x(0) = z$. The following is Corollary 2.3 of [18].

Theorem 4.1. *Assume that* $(x_0, \lambda_0) \in U \times \Lambda$; $x_0 \in \mathrm{Int}\, U$; $f(x_0, \lambda_0) = 0$; *all eigenvalues of* $D_x f(x_0, \lambda_0)$ *have negative real part*; *and* x_0 *is globally attracting for solutions of* (4.1) *with* $\lambda = \lambda_0$. *If*

(H1) *there exists a compact set* $D \subset U$ *such that for each* $\lambda \in \Lambda$ *and each* $z \in U$, $x(t; z; \lambda) \in D$ *for all large* t;

then there exists $\epsilon > 0$ *and a unique point* $\hat{x}(\lambda) \in U$ *for* $\lambda \in B_\Lambda(\lambda_0, \epsilon)$ *such that* $f(\hat{x}(\lambda), \lambda) = 0$ *and* $x(t; z; \lambda) \to \hat{x}(\lambda)$ *as* $t \to \infty$ *for all* $z \in U$.

While the statement of the theorem seems complex, in our case it is fairly straightforward. The role of λ in the theorem is played by q and $\lambda_0 = 0$. We will apply it in the case that we have a globally stable rest point, either E_{c1} or E_2 for $q = 0$, The ϵ restricts q so that the rest point continues to exist (with perhaps different coordinates) and – the important conclusion in the last phrase of the statement – that the rest point retains its global stability. The difficulty is to satisfy (H1) which we will do using known results on persistence. Since the definition of λ_1 given in (2.5) depended on q we will write it as $\lambda_1(q)$ but retain λ_1 instead of $\lambda_1(0)$.

Theorem 4.2. *For* $q > 0$ *sufficiently small*

(i) *If* $\lambda_1(q) < \lambda_2$ *then* E_{c1} *is global asymptotically stable.*

(ii) *If* $\lambda_2 < \lambda_1(q)$ *and either one of* (3.5), (3.6) *does not hold then* E_2 *is global asymptotically stable.*

Before beginning the proof, we make a few comments. We have already noted that all trajectories with initial conditions in the non-negative orthant eventually lie in the compact set $Q = \{(S, x, y) | S \geq 0, x \geq 0, y \geq 0, S + x + y \leq 1\}$. Constructing the compact set required in Theorem 4.1 will be a question of uniform persistence uniformly in the parameter q. Finally, in order not to interrupt the flow of the proof, we note that the standard comparison theorem allows one to compare

$$y' \leq y\left(\frac{m(1 - \epsilon_1 - y)}{a + 1 - \epsilon_1 - y} - 1\right) + \epsilon_2$$

with solutions of the equality

$$y' = y\left(\frac{m(1 - \epsilon_1 - y)}{a + 1 - \epsilon_1 - y} - 1\right) + \epsilon_2. \tag{4.3}$$

For ϵ_1 and ϵ_2 small, the form of the equation (4.3) yields a unique interior rest point and the linearization shows it to be stable. Global stability follows from natural monotonicity since it is first order. Smoothness in the parameters shows that the rest point tends to that of

$$y' = y\left(\frac{m(1-y)}{a+1-y} - 1\right)$$

as the ϵ's end to zero. The limiting rest point is given by $1 - \frac{a}{m-1}$. A similar result applies to the reversed inequality with the signs in front of the ϵ's reversed.

Proof of Theorem 4.2. For part (i), we note that from Theorem 3.2 in [9], if $q = 0$, then the equilibrium $E_1 = (\lambda_1, x^*, 0)$ is global asymptotically stable. We have already noted that $E_{c1}(q) \to E_1$ as $q \to 0$. To apply the conclusion of Theorem 4.1, it suffices to show that there exists $\eta > 0$ and $q_0 > 0$ such that if $0 < q < q_0$, then $\liminf_{t\to\infty} x(t) > \eta$. Equivalently, the system (2.4) is uniformly persistent, uniformly in q near 0. Theorem 5 in [19] shows that one can prove $\liminf_{t\to\infty} x(t) > \eta$, provided that one can prove $\limsup_{t\to\infty} x(t) > \epsilon$ for some $\epsilon > 0$, uniformly in q. Suppose on the contrary, there exists $q_n \to 0$, $q_n > 0$ such that the corresponding solutions, (S_n, x_n, y_n), of (2.4) with $q = q_n$ satisfy $\lim_{n\to\infty} \limsup_{t\to\infty} x_n(t) = 0$. We may assume, after shifting the start time forward if necessary and adding a $\frac{1}{n}$, that $\lim_{n\to\infty} \sup_{t\geq 0} x_n(t) = 0$.

From (2.4), it follows that

$$\begin{aligned} \frac{dS_n}{dt} &= 1 - S_n - \frac{m_2 S_n}{a_2 + S_n} y_n - \eta_n(t) \\ \frac{dy_n}{dt} &= y_n\left[\frac{m_2 S_n}{a_2 + S_n} - 1\right] + \xi_n(t) \end{aligned} \tag{4.4}$$

where

$$\begin{aligned} \eta_n(t) &= \frac{m_1 S_n(t)}{a_1 + S_n(t)} x_n(t) \\ \xi_n(t) &= q_n \frac{m_1 S_n(t) x_n(t)}{a_1 + S_n(t)} - \frac{rk}{1-k-q_n} x_n(t) y_n(t). \end{aligned}$$

For $\epsilon > 0$ and n sufficiently large,

$$\eta_n(t) > 0, \ \limsup_{t\to\infty} \eta_n(t) < m_1 \limsup_{t\to\infty} x_n(t) < \epsilon/2 \tag{4.5}$$

and

$$\begin{aligned} \xi_n(t) &< \epsilon - \frac{rk}{1-k-q_n} x_n(t) y_n(t) < \epsilon, \\ \xi_n(t) &> -\frac{rk}{1-k-q} x_n(t) y_n(t) > -C x_n(t) \\ &> -\epsilon/2. \end{aligned} \tag{4.6}$$

From (4.4), (4.5) and (4.6), we have that for $n \ge N_0$, N_0 large,

$$
\begin{aligned}
(S_n + y_n)' &= 1 - (S_n + y_n) + \eta_n(t) - \xi_n(t) \\
&\le 1 - (S_n + y_n) + \epsilon
\end{aligned}
\tag{4.7}
$$

and that

$$
(S_n + y_n)' \ge 1 - (S_n + y_n) - \epsilon \tag{4.8}
$$

By the differential inequalities (4.7) and (4.8), for ϵ, $\epsilon_1 > 0$ and N_0 as above, there exists $T = T(\epsilon; \epsilon_1; N_0)$ such that

$$
1 - \epsilon - \epsilon_1 \le S_n(t) + y_n(t) \le 1 + \epsilon + \epsilon_1; \quad t \ge T. \tag{4.9}
$$

Using the second equation in (4.4), and (4.9) for $t \ge T$, one has

$$
\frac{dy_n}{dt} \le y_n \left[\frac{m_2(1 + \epsilon + \epsilon_1 - y_n(t))}{a_2 + (1 + \epsilon + \epsilon_1 - y_n(t))} - 1 \right] + \epsilon \tag{4.10}
$$

$$
\frac{dy_n}{dt} \ge y_n \left[\frac{m_2(1 - \epsilon - \epsilon_1 - y_n(t))}{a_2 + (1 - \epsilon - \epsilon_1 - y_n(t))} - 1 \right] - \epsilon. \tag{4.11}
$$

By the differential inequalities (4.10), (4.11) and the standard comparison theorem, we have

$$
\underline{y}(t) \le y_n(t) \le \overline{y}_n(t); \quad t \ge T_{\epsilon_1}; \tag{4.12}
$$

and

$$
\lim_{t \to \infty} \underline{y}_n(t) = \hat{\underline{y}}_n; \quad \lim_{t \to \infty} \overline{y}_n(t) = \hat{\overline{y}}_n.
$$

$\underline{y}_n(t)$ and $\overline{y}_n$ are solutions of the corresponding equalities and, as noted above, these solutions have limits. For n sufficiently large, the limits $\hat{\underline{y}}_n$ and $\hat{\overline{y}}_n$ are close to $y^* = 1 - \lambda_2$. From (4.12) and (4.9), $S_n(t)$ is close to λ_2. However the assumption $\lambda_1(q) < \lambda_2$ and the second equation of (2.4) imply that, in this case, $x_n(t) \to \infty$ as $t \to \infty$; contradicting boundedness of $x_n(t)$.

To prove part (ii), we note that, from Theorem 3.4 in [9], if $q = 0$ and if $\lambda_1 > \hat{\lambda}$ (which exactly violates of (3.5) when $q = 0$), then E_2 is globally asymptotically stable. We begin by restricting q by $0 \le 1 - k$. Under the conditions in (ii), there are exactly two equilibria $E_0 = (1; 0; 0)$ and $E_2 = (\lambda_2; 0; y^*)$ if $q > 0$. To find the compact set needed to apply Theorem 4.1, it suffices to show that there exists $\eta > 0$ and $q > 0$ such that if $0 < q < q_0$ then

$$
\liminf_{t \to \infty} y(t) > \eta.
$$

As we have done in part (i), it suffices to show that

$$\limsup_{t\to\infty} y(t) > \varepsilon$$

for some $\varepsilon > 0$ uniformly in q, that is, for some q_0, $0 < q < q_0$. Suppose on the contrary, there exists $q_n \to 0$, $q_n > 0$ and the corresponding solutions $(S_n; x_n; y_n)$ of (2.4) with $q = q_n$ such that

$$\lim_{n\to\infty} \limsup_{t\to\infty} y_n(t) = 0.$$

Assume, without loss of generality,

$$\lim_{n\to\infty} \sup_{t\geq 0} y_n(t) = 0.$$

From (2.4) it follows that

$$\begin{aligned} \frac{dS_n}{dt} &= 1 - S_n - \frac{m_1 S_n}{a_1 + S_n} x_n - \delta_n(t) \\ \frac{dx_n}{dt} &= x_n \left[(1 - q_n - k)\frac{m_1 S_n}{a_1 + S_n} - 1 \right] \end{aligned} \tag{4.13}$$

where

$$\delta_n(t) = \frac{m_2 S_n(t)}{a_2 + S_n(t)} y_n(t) > 0$$

satisfies that, given $\varepsilon > 0$, there is an N_0 such that for $n > N_0$

$$\limsup_{t\to\infty} \delta_n(t) < m_2 \limsup_{t\to\infty} y_n(t) < \varepsilon \tag{4.14}$$

Let

$$\tilde{x}_n(t) = \frac{1}{1 - q_n - k} x_n(t).$$

Then (4.13) is converted into the following equations

$$\begin{aligned} \frac{dS_n}{dt} &= 1 - S_n - \frac{M_1 S_n}{a_1 + S_n} \tilde{x}_n - \eta_n(t) \\ \frac{d\tilde{x}_n}{dt} &= \tilde{x}_n \left[\frac{M_1 S_n}{a_1 + S_n} - 1 \right] \end{aligned} \tag{4.15}$$

where $M_1 = m_1(1 - q_n - k)$. From (4.13), (4.15), we have that

$$\begin{aligned} (S_n' + \tilde{x}_n)' &= 1 - (S_n + \tilde{x}_n) - \delta_n(t) \\ &\leq 1 - (S_n + \tilde{x}_n) \end{aligned} \tag{4.16}$$

and

$$(S_n + \tilde{x}_n)' \geq 1 - (S_n + \tilde{x}_n) - \epsilon. \tag{4.17}$$

Given $\epsilon_1 > 0$, and small, using the differential inequalities (4.16) and (4.17), there exists $T = T(\epsilon, \epsilon_1, N_0)$ such that

$$1 - \epsilon - \epsilon_1 \leq S_n(t) + \tilde{x}_n(t) \leq 1 + \epsilon_1, \quad t \geq T_{\epsilon_1}. \tag{4.18}$$

Consider the second equation in (4.15); from (4.18), for $t \geq T$, one has that

$$\frac{d\tilde{x}_n}{dt} \leq \tilde{x}_n \left[\frac{M_1(1 + \epsilon_1 - \tilde{x}_n(t))}{a_1 + (1 + \epsilon - \tilde{x}_n(t))} - 1 \right] \tag{4.19}$$

$$\frac{d\tilde{x}_n}{dt} \geq \tilde{x}_n \left[\frac{M_1(1 - \epsilon - \epsilon_1 - \tilde{x}_n(t))}{a_1 + (1 - \epsilon_1 - \epsilon_1 - \tilde{x}_n(t))} - 1 \right]. \tag{4.20}$$

By the differential inequalities (4.19) and (4.20) we have

$$\underline{\psi}_n(t) \leq \tilde{x}_n(t) \leq \bar{\psi}_n(t), \quad t \geq T \tag{4.21}$$

and

$$\lim_{t \to \infty} \underline{\psi}_n(t) = \underline{x}_n, \quad \lim_{t \to \infty} \bar{\psi}_n(t) = \bar{x}_n.$$

For n sufficiently large, the limits $\underline{x}_n$ and $\bar{x}_n$ are close to $x^* = 1 - \frac{a_1}{M_1 - 1} = 1 - \lambda_2$. From (4.18), (4.21), $S_n(t)$ is close to λ_1.

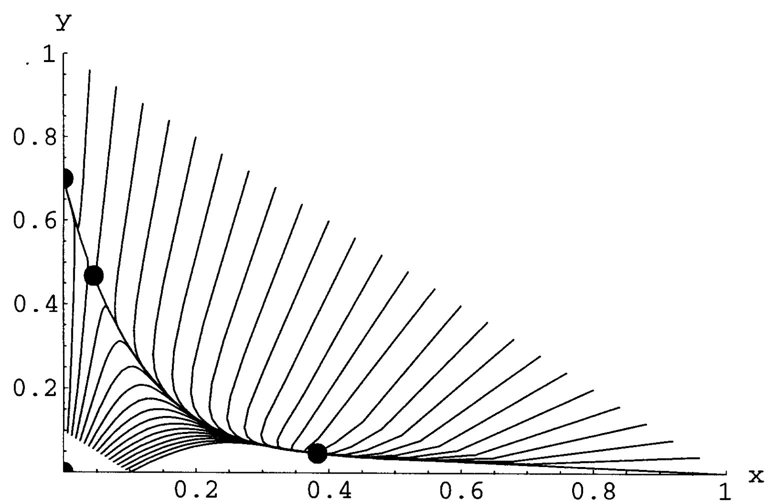

Figure 4.1. Bistable Attractors: Projection on the $x - y$ plane.

Given $\epsilon > 0$, define the neighborhood $\Omega = \{(S, x, y) : |S - \lambda_1| < \epsilon,\ |x - x_1^*| < \epsilon,\ 0 < y < \epsilon\}$. There exists N_0 such that for $n \geq N_0$, the corresponding trajectory $(S_n(t), x_n(t), y_n(t))$ stays in Ω for t sufficiently large. Then from the third equation of (2.4), it follows that

$$y_n' \geq \left[\left(\frac{m_2 \lambda_1}{a_2 + \lambda_1} - 1 - \frac{rk}{1 - k} x^*\right) - \epsilon'\right] y_n \tag{4.22}$$

$$= [(f_2(\lambda_1) - 1 - rk(1 - \lambda_1)) - \epsilon'] y_n \tag{4.23}$$

Since (3.7) holds, one may choose $\epsilon' > 0$ sufficiently small such that $f_2(\lambda_1) - 1 - rk(1 - \lambda_1) - \epsilon' > 0$. Since Ω contains no rest points, the trajectory cannot remain in Ω. This contradiction establishes the theorem.

Of course, it can happen that $\lambda_2 < \lambda_1$ and both (3.5) and (3.6) hold so that there are two interior rest points, one locally stable and one locally unstable, and, in addition, E_2 is locally stable. This is the case of bistable attractors and the outcome depends on the initial conditions. The usual case of bistable attractors is such that extinction of one population results; however, since one of the attractors is an interior rest point, it represents a co-existence state. Inuitively, the stable manifold of the unstable interior rest point divides E^3 into two region where the trajectories tend to the respective rest points. We are unable to prove this. However, the choice $m_1 = 2.5$, $m_2 = .2$, $a_1 = a_2 = .3$, $k = .2$, $q = .1$, $r = 12$, produces such a case. Figure 4.1 is a projection onto the $x - y$ plane of the flow in E^3 with these parameters.

5. Discussion

We have considered competition in the chemostat between plasmid-bearing and plasmid-free organisms where the plasmid codes for the production of a toxin (an allelopathic agent) against the plasmid-free organism. We have given a rigorous mathematical description, sometimes with the assumption that q is small, of all of the outcomes in terms of the parameters of the system except that we cannot prove the extent of convergence in the case of bistable attractors. The bistable case here is different from other chemostat systems in that one of the attractors is an interior rest point and so does not represent an extinction state.

The results should be of interest in biotechnology. Since plasmids are used to code for the manufacture of a product, the loss of the plasmid results in an organism that is a better competitor. To guard against such, the results here suggest that coding for an anti-competitor toxin in addition is a viable strategy. The can never be a steady state consisting of only the plasmid-bearing organism since the loss of the plasmid creates its competitor, so the best the engineer can hope for is a coexistence state where the plasmid-bearing organism dominates.

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