Allelopathy of plasmid-bearing and plasmid-free organisms competing for two complementary resources in a chemostat

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(Received 6 August 2011; final version received 14 February 2012)

We consider a model of competition between plasmid-bearing and plasmid-free organisms for two complementary nutrients in a chemostat. We assume that the plasmid-bearing organism produces an allelopathic agent at the cost of its reproductive abilities which is lethal to plasmid-free organism. Our analysis leads to different thresholds in terms of the model parameters acting as conditions under which the organisms associated with the system cannot thrive even in the absence of competition. Local stability of the system is obtained in the absence of one or both the organisms. Also, global stability of the system is obtained in the presence of both the organisms. Computer simulations have been carried out to illustrate various analytical results.

Keywords: chemostat; allelopathy; plasmid-bearing; plasmid-free; complementary nutrients

AMS Subject Classifications: 92B05; 92D25

1. Introduction

Genetic alteration of organisms is accomplished by introducing DNA into the cell in the form of a plasmid. Plasmids contain DNA sequence that serves as an origin of replication which enables the plasmid DNA to be duplicated independently from the chromosomal DNA [12]. This genetic material is reproduced when the cell divides. The plasmid-bearing organism is likely to be a lesser competitor than the plasmid-free organism because of the added load on its metabolic machinery. During replication, the plasmid can be lost by not being passed to the daughter cells, producing a plasmid-free organism [15]. 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. To inhibit the growth of the plasmid-free organism, the plasmid is coded for resistance to an allelopathic agent and is added to the medium. A plausible interpretation is that the lethal release of toxins kills non-producing competitors, promoting the spread of the remaining clone mates that carry the plasmid encoding immunity to the toxin [9].
Allelopathy is observed in bacteria where the agent of the allelopathic interaction is ‘bacteriocin’. Some bacteria can produce toxic bacteriocins that kill or inhibit the competing bacteria of different genotypes [11]. In general, bacteria that are capable of producing such chemicals are immune to their own action. Colicin, the most extensively studied class of bacteriocins, is produced by *Escherichia coli* (*E. coli*) and Enterobacteriaceae. *E. coli* contains a plasmid that produces colicin and an immune protein to protect the cell from colicin, while other species without this plasmid do not produce colicin and are sensitive to it. In the absence of colicin in the environment, it is observed that the colicin-sensitive strain has a faster growth rate than the colicin-producing one. The lethal release of colicin kills colicin-sensitive competitors, promoting the spread of *E. coli* that carry the plasmid [2].

At an elementary level, the nutritional requirements of *E. coli* are revealed by the cell’s elemental composition, which consists of carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorous, potassium, magnesium, iron, calcium, manganese, and traces of zinc, cobalt, copper, and molybdenum. These elements are found in the form of water, inorganic ions, small molecules, and macromolecules which serve either a structural or functional role in the cells. In order to grow a bacterium in the laboratory, we must have an energy source, a source of carbon and other required nutrients, and a permissive range of physical conditions. In this paper, we consider the competition between plasmid-bearing and plasmid-free organisms with allelopathy growing in a chemostat with two essential nutrients which are metabolically independent requirements for their growth [7]. The main emphasis of this paper will be to put into studying the asymptotic behaviour of the system in the absence of one or both the organisms and in the presence of both the organisms. We have studied the model analytically as well as numerically with the proofs that are all presented in the appendix.

### 2. The basic model

We take the model of a food chain [7,8] in which there are constant supplies of two nutrients having concentrations $S(t)$ and $R(t)$ at time $t$. Plasmid-bearing and plasmid-free bacteria are growing on these two complementary nutrients with concentrations $x(t)$ and $y(t)$, respectively, at time $t$. The concentration of colicin, generated by $x(t)$, is $p(t)$ which is lethal to $y(t)$.

The basic equations with all the parameters are as follows:

\[
\begin{align*}
\frac{dS}{dt} & = (S^0 - S)D - \frac{xf_1(S, R)}{\gamma_1} - \frac{yf_2(S, R)}{\gamma_2}, \\
\frac{dR}{dt} & = (R^0 - R)D - \frac{xf_1(S, R)}{\gamma_1} - \frac{yf_2(S, R)}{\gamma_2}, \\
\frac{dx}{dt} & = x[1 - q - k]f_1(S, R) - D], \\
\frac{dy}{dt} & = y[f_2(S, R) - D - \gamma p] + qxf_1(S, R), \\
\frac{dp}{dt} & = kxf_1(S, R) - pD, \quad (1)
\end{align*}
\]

$S(0) = S_0 \geq 0$, $R(0) = R_0 \geq 0$, $x(0) = x_0 \geq 0$, $y(0) = y_0 \geq 0$, and $p(0) = p_0 \geq 0$, where $f_1(S, R) = \min\{m_{11}S/(a_{11} + S), m_{12}R/(a_{12} + R)\}$ and $f_2(S, R) = \min\{m_{21}S/(a_{21} + S), m_{22}R/(a_{22} + R)\}$.

Here, $S^0$ and $R^0$ are the input concentrations of the two complementary nutrients, $D$ is the dilution (‘washout’) rate of the chemostat, $k$ is the fraction of the consumption of plasmid-bearing
organisms devoted to produce the inhibitor, \( q \) is the fraction of plasmid-bearing organisms that lose the plasmid in reproduction and so convert into plasmid-free organisms \((0 < k, q < 1)\) and \( \gamma \) is the toxin coefficient. Also, \( m_{is} \) and \( m_{ir} \) are the maximal growth (‘birth’) rates of the \( i \)th organism with respect to the nutrients \( S \) and \( R \), \( a_{is} \) and \( a_{ir} \) are the Michaelis–Menten or ‘half-saturation’ constants with respect to the nutrients \( S \) and \( R \), which are the nutrient concentrations at which the functional response of the \( i \)th organism is half maximal, and \( \gamma_{is} \) and \( \gamma_{ir} \) are yield constants, representing the conversion of nutrient to organism \((i = 1, 2)\); all of these are positive quantities \([5,14,16]\). \( S^0, R^0, \) and \( D \) are environmental variables while \( m_{is}, m_{ir}, a_{is}, a_{ir}, \gamma_{is}, \gamma_{ir}, k, q, \) and \( \gamma \) are properties of the organisms \([14]\). Also, similar to the approach as considered in \([7]\), we assume that \( \gamma_{is} = \gamma_1 = \gamma_{is} \) and \( \gamma_{ir} = \gamma_2 = \gamma_{ir} \).

### 3. Simplification of the basic model

To simplify Equation (1), let us apply \( z = p - kx/(1 - q - k) \).

Then, \( \lim_{t \to \infty} z(t) = 0 \) and so the limiting system becomes

\[
\frac{dS}{dt} = (S^0 - S)D - \frac{x f_1(S, R)}{\gamma_1} - \frac{y f_2(S, R)}{\gamma_1},
\]
\[
\frac{dR}{dt} = (R^0 - R)D - \frac{x f_1(S, R)}{\gamma_2} - \frac{y f_2(S, R)}{\gamma_2},
\]
\[
\frac{dx}{dt} = x ((1 - q - k)f_1(S, R) - D),
\]
\[
\frac{dy}{dt} = y \left\{ f_2(S, R) - D - \frac{\gamma kx}{1 - q - k} \right\} + q f_1(S, R),
\]

\( S(0) = S_0 \geq 0, \ R(0) = R_0 \geq 0, \ x(0) = x_0 \geq 0, \) and \( y(0) = y_0 \geq 0. \)

Obviously, the right-hand sides of system (2) are continuous smooth functions on \( \mathbb{R}^4_+ = \{(S, R, x, y) : S, R, x, y \geq 0\} \). Indeed, they are Lipschitzian on \( \mathbb{R}^4_+ \) and so the solution of the system (2) exists and is unique. Therefore, the interior of the positive octant of \( \mathbb{R}^4 \) is an invariant region.

### 4. Boundedness of the system

**Theorem 4.1** All the solutions of Equation (2) enter into the set \( \{(S, R, x, y) \in \mathbb{R}^4 : S(t) + R(t) + x(t) + y(t) < S^0 + R^0 + \epsilon, \epsilon > 0\} \).

Let us define \( \lambda_{is} = a_{is}D/((1 - q - k)m_{is} - D), \ \lambda_{ir} = a_{ir}D/((1 - q - k)m_{ir} - D), \ \lambda_{2i} = a_{2i}D/(m_{2i} - D), \ \lambda_{2r} = a_{2r}D/(m_{2r} - D), C = \gamma_1/\gamma_2, \) and \( T_i = (R^0 - \lambda_{ir}/(S^0 - \lambda_{is}))(i = 1, 2) \), where \( (1 - q - k)m_{is}, (1 - q - k)m_{ir}, m_{2i}, m_{2r} > D \).

Then, \( \lambda_{is} \) and \( \lambda_{ir} \) represent the concentrations of the nutrients \( S \) and \( R \), respectively, needed for zero-net growth of both the organisms; \( \lambda_{2i} \) and \( \lambda_{2r} \) represent the concentrations of the nutrients \( S \) and \( R \), respectively, needed for zero-net growth of plasmid-free organisms in the absence of plasmid-bearing organisms.

Also, \( C \) is the ratio of units of \( R \) consumed and units of \( S \) consumed, \( T_i \) is the ratio of units of \( R \) regenerated and units of \( S \) regenerated \((i = 1, 2)\).

Therefore, in the absence of plasmid-bearing organism, \( T_2 > C \) implies that \( S \) is regenerating at a slower rate than \( R \) with respect to the required consumption ratio for \( y \) so that the growth rate...
of the plasmid-free organism is $S$-limited and $T_2 < C$ implies that the growth rate of plasmid-free organism is $R$-limited [7].

Similarly, $T_1 > C$ implies that the growth rate of the plasmid-bearing organism is $S$-limited and $T_1 < C$ implies that the growth rate of plasmid-bearing organism is $R$-limited.

5. Equilibria and their stability

The system (2) possesses the following equilibria:

(i) organism-free equilibrium $E_0 = (S^0, R^0, 0, 0)$;
(ii) plasmid-bearing organism-free equilibrium: 
(a) $E_{1s} = (\lambda_{2s}, R_s, 0, y_s)$, when the growth rate of plasmid-free organism is $S$-limited, where $R_s = R^0 - (\gamma_1/\gamma_2)(S^0 - \lambda_{2s})$ and $y_s = \gamma_2(S^0 - \lambda_{2s})$;
(b) $E_{1r} = (S_r, \lambda_{2r}, 0, y_r)$, when the growth rate of the plasmid-free organism is $R$-limited, where $S_r = S^0 - (\gamma_2/\gamma_1)(R^0 - \lambda_{2r})$ and $y_r = \gamma_2(R^0 - \lambda_{2r})$;
(iii) the equilibrium of coexistence:
(a) $E_s^* = (\lambda_{1s}, R_s^*, x_s^*, y_s^*)$, when the growth rate of plasmid-bearing organism is $S$-limited, where $R_s^* = R^0 - (\gamma_1/\gamma_2)(S^0 - \lambda_{1s}) > \lambda_{1s}$, and $x_s^*$ is a positive root of the algebraic equation $(S^0 - \lambda_{1s})D - xD/\gamma_1(1 - q - k) - (\theta_s(x)/\gamma_1)f_2(\lambda_{1s}, R_s^*) = 0$, with $y_s^* = \theta_s(x_s^*) = qx_s^*D/(\gamma kx_s^* - (1 - q - k)f_2(\lambda_{1s}, R_s^* - D))$;
(b) $E_r^* = (S_r^*, \lambda_{1r}, x_r^*, y_r^*)$, when the growth rate of the plasmid-bearing organism is $R$-limited, where $S_r^* = S^0 - (\gamma_2/\gamma_1)(R^0 - \lambda_{1r}) > \lambda_{1s}$ and $x_r^*$ is a positive root of the algebraic equation $(R^0 - \lambda_{1r})D - xD/\gamma_2(1 - q - k) - (\theta_r(x)/\gamma_2)f_2(S_r^*, \lambda_{1r}) = 0$, with $y_r^* = \theta_r(x_r^*) = qx_r^*D/(\gamma kx_r^* - (1 - q - k)f_2(S_r^*, \lambda_{1r} - D))$.

The following lemma states the condition under which the organisms cannot survive even in the absence of competition.

**Lemma 5.1**

(i) If $(1 - q - k)f_1(S^0, R^0) < D$ and $f_2(S^0, R^0) < D$, then $\lim_{t \to \infty} x(t) = 0 = \lim_{t \to \infty} y(t)$.
(ii) If $(1 - q - k)f_1(S^0, R^0) < D$, $f_2(S^0, R^0) > D$, and $T_2 > C$, then $\lim_{t \to \infty} x(t) = 0$ and the growth rate of $y$ is $S$-limited.
(iii) If $(1 - q - k)f_1(S^0, R^0) < D$, $f_2(S^0, R^0) > D$, and $T_2 < C$, then $\lim_{t \to \infty} x(t) = 0$ and the growth rate of $y$ is $R$-limited.

We analyse the local stability of the system (2) by using eigenvalue analysis of the Jacobian matrix evaluated at the appropriate equilibrium. The detailed calculations are given in the appendix.

**Lemma 5.2** The critical point $E_0 = (S^0, R^0, 0, 0)$ of the system (2) is locally asymptotically stable if $(1 - q - k)f_1(S^0, R^0) < D$ and $f_2(S^0, R^0) < D$.

Local stability at $E_0$ implies the non-existence of the equilibria $E_{1s}, E_{1r}$, and $E^*$.

**Lemma 5.3** The critical point $E_{1s} = (\lambda_{2s}, R_s, 0, y_s)$ of the system (2) is locally asymptotically stable if $\lambda_{2s} < \lambda_{1s}$ or $R_s < \lambda_{1s}$.

**Lemma 5.4** The critical point $E_{1r} = (S_r, \lambda_{2r}, 0, y_r)$ of the system (2) is locally asymptotically stable if $S_r < \lambda_{1s}$ or $\lambda_{2r} < \lambda_{1r}$.
Both the organisms in the system will persist if \( \lim_{t \to \infty} x(t) > 0 \) and \( \lim_{t \to \infty} y(t) > 0 \) [10]. The condition given in the following lemma rules out the possibility of extinction of any organism in the system.

**Lemma 5.5** If \((1 - q - k)f_1(S^0, R^0) > D, \lambda_{1r} \leq \min\{S^*, \lambda_{2r}\} \) and \( \lambda_{1r} \leq \min\{R^*, \lambda_{2r}\} \) hold, then both the organisms will persist in the system, where \( E^* = (S^*, R^*, x^*, y^*) \) is one of \( E_x^* \) and \( E_r^* \) according to S-limiting or R-limiting equilibrium.

Having established the existence of the positive steady-state \( E^* \), we now turn our attention to its local stability.

**Lemma 5.6** The positive equilibrium \( E^* \) of the system (2) is locally asymptotically stable if \( AB > C \), where

\[
A = 2D - f_2|E^*| + \frac{\gamma_k x^*}{1 - q - k} + x^*\left(\frac{f_1R|E^*|}{y_2} + \frac{f_1S|E^*|}{y_1}\right) + y^*\left(\frac{f_2R|E^*|}{y_2} + \frac{f_2S|E^*|}{y_1}\right),
\]

\[
B = D\left(D - f_2|E^*| + \frac{\gamma_k x^*}{1 - q - k}\right) + x^*\left(D - (1 - q)f_2|E^*| + \frac{\gamma_k x^*}{1 - q - k}\right)
+ (1 - q - k)f_1|E^*| + \gamma k y^*\left(\frac{f_1R|E^*|}{y_2} + \frac{f_1S|E^*|}{y_1}\right) + y^*\left(D + \frac{\gamma_k x^*}{1 - q - k}\right)\left(\frac{f_2R|E^*|}{y_2} + \frac{f_2S|E^*|}{y_1}\right),
\]

and

\[
C = x^*\left[f_1|E^*|\left(D + \frac{\gamma_k x^*}{1 - q - k}\right) - f_2|E^*|\left((1 - q)f_1|E^*| + \frac{\gamma k y^*}{1 - q - k}\right)\right]\left(\frac{f_1R|E^*|}{y_2} + \frac{f_1S|E^*|}{y_1}\right).
\]

### 6. Global stability at interior equilibrium

Now, we will study the global behaviour of the system at the interior equilibria. For this we consider a Lyapunov function as

\[
V(S,R,x,y) = \left(S - S^0 + \frac{x}{y_1} + \frac{y}{y_1}\right)^2 + \left(R - R^0 + \frac{x}{y_2} + \frac{y}{y_2}\right)^2.
\]

Then,

\[
\frac{dV}{dt} = -2\left(S - S^0 + \frac{x}{y_1} + \frac{y}{y_1}\right)\left(S - S^0 + \frac{x}{y_1} + \frac{y}{y_1}\right)D
+ \frac{xk}{y_1}\left((1 - q - k)f_1(S,R) + \gamma y\right)
- 2\left(R - R^0 + \frac{x}{y_2} + \frac{y}{y_2}\right)\left(R - R^0 + \frac{x}{y_2} + \frac{y}{y_2}\right)D
+ \frac{xk}{y_2}\left((1 - q - k)f_1(S,R) + \gamma y\right)
\leq -2D\left(S - S^0 + \frac{x}{y_1} + \frac{y}{y_1}\right)^2 + \left(R - R^0 + \frac{x}{y_2} + \frac{y}{y_2}\right)^2 \leq 0.
\]
By the LaSalle lemma, it follows that all the trajectories approach the set
\[
\Delta = \left\{ (S, R, x, y) : \frac{dV}{dt} = 0 \right\} = \left\{ (S, R, x, y) : S^0 = S + \frac{x}{\gamma_1} + \frac{y}{\gamma_1}, R^0 = R + \frac{x}{\gamma_2} + \frac{y}{\gamma_2}, S, R, x, y \geq 0 \right\}.
\]
Therefore, the \( \omega \)-limit set of the trajectory of the system (2) lies in \( \Delta \), and it is sufficient to study the behaviour of the solutions of the following two-dimensional system:
\[
\frac{dx}{dt} = xg_1(x, y) = G_1(x, y),
\]
\[
\frac{dy}{dt} = yg_2(x, y) + \frac{xq}{1 - q - k} \{ g_1(x, y) + D \} = G_2(x, y),
\]
where \( g_1(x, y) = (1 - q - k) f_1(S^0 - x/\gamma_1 - y/\gamma_1, R^0 - x/\gamma_2 - y/\gamma_2) - D \) and \( g_2(x, y) = f_2(S^0 - x/\gamma_1 - y/\gamma_1, R^0 - x/\gamma_2 - y/\gamma_2) - D - \gamma kx/(1 - q - k) \).

**Lemma 6.1** The system (3) has no periodic solution.

Since there is no periodic solution for the system (3) in \( \Delta \), by Poincare–Bendixon theorem, it follows that every trajectory of the system (2) approaches asymptotically to an equilibrium point of the system (2). This leads to the following result:

**Lemma 6.2** If the system (2) is locally asymptotically stable at the positive equilibrium, then it is globally asymptotically stable at that point.

7. **Numerical simulations**

In this section, we investigate numerically, as demonstrated in [1,3,4,6,13], the effect of the various parameters on the qualitative behaviour of the system using parameter values given in Table 1 throughout, unless otherwise stated.

We first observe that under the given set of parameter values, the system becomes stable at \( E_r^* = (0.0595, 0.8357, 0.5309, 0.006) \). Also we obtain \( R_s^* = 0.8357 \) and \( \lambda_{1r} = 0.0595 \) so that \( R_s^* > \lambda_{1r} \), satisfying the existence criterion for the equilibrium of coexistence with \( S \)-limited growth of \( x \) (cf. Figure 1).

We will now verify the feasibility of the stability criterion in Section 5.

**Example 1** For \( R^0 = 1.2 \) and all other parameters as in Table 1, it is observed that the system is stable at \( E_r^* = (0.992, 0.0595, 0.5189, 0.0063) \). Also we obtain \( S_r^* = 0.992 \) and \( \lambda_{1s} = 0.0595 \) so that \( S_r^* > \lambda_{1s} \), satisfying the existence criterion for the equilibrium of coexistence with \( R \)-limited growth of \( x \) (cf. Figure 2).

**Example 2** For \( S^0 = 0.05 \) and all other parameters as in Table 1, it is observed that the system is stable at \( E_{1s} = (0.0179, 1.9807, 0, 0.0096) \). Also we obtain \( (1 - q - k)f_1(S^0, R^0) = 0.0855 < D_1f_2(S^0, R^0) = 0.25 > D, T_2 = 61.667, \) and \( C = 0.6 \), satisfying the existence criterion for the equilibrium with \( S \)-limited growth of \( y \) in the absence of \( x \). Also we obtain \( \lambda_{2s} = 0.0179 \) and \( \lambda_{1s} = 0.0593 \), so that the condition for stability as given in Lemma 5.3 is satisfied (cf. Figure 3).
Table 1. A default set of parameter values.

| Parameters | Description of Parameters | Default value | Dimension |
|------------|---------------------------|---------------|-----------|
| $S^0$      | Constant input nutrient concentration | 2             | Mass/volume |
| $R^0$      | Constant input nutrient concentration | 2             | Mass/volume |
| $m_{IS}$   | Maximal growth rate of $x$ on $S$ | 1             | 1/time    |
| $m_{IR}$   | Maximal growth rate of $x$ on $R$ | 1             | 1/time    |
| $m_{2S}$   | Maximal growth rate of $y$ on $S$ | 1.5           | 1/time    |
| $m_{2R}$   | Maximal growth rate of $y$ on $R$ | 1.5           | 1/time    |
| $a_{1S}$   | Half-saturation constant for uptake of $S$ by $x$ | 0.5           | Mass/volume |
| $a_{1R}$   | Half-saturation constant for uptake of $R$ by $x$ | 0.5           | Mass/volume |
| $a_{2S}$   | Half-saturation constant for uptake of $S$ by $y$ | 0.25          | Mass/volume |
| $a_{2R}$   | Half-saturation constant for uptake of $R$ by $y$ | 0.25          | Mass/volume |
| $\gamma_1$| Yield constant of $x$ and $y$ on $S$ | 0.3           | –          |
| $\gamma_2$| Yield constant of $x$ and $y$ on $R$ | 0.5           | –          |
| $D$        | Dilution rate of the chemostat | 0.1           | 1/time    |
| $q$        | Fraction of $x$ that loose the plasmid in reproduction | 0.01          | –          |
| $k$        | Fraction of consumption of $x$ that produce toxin | 0.05          | –          |
| $\gamma$  | Toxin coefficient | 10            | –          |

Figure 1. Time series analysis of the system for the parameters given in Table 1, the system is stable at $E^*_1$.

**Example 3** For $R^0 = 0.05$ and all other parameters as in Table 1, it is observed that the system is stable at $E_{1r} = (1.9464, 0.0179, 0, 0.0161)$. Also we obtain $(1 - q - k)f_1(S^0, R^0) = 0.0855 < D$, $f_2(S^0, R^0) = 0.25 > D$, $T_2 = 0.0162$, and $C = 0.6$, satisfying the existence criterion for the equilibrium with $R$-limited growth of $y$ in the absence of $x$. Also we obtain $\lambda_{2r} = 0.0179$ and $\lambda_{1r} = 0.0595$, so that the condition for stability as given in Lemma 5.4 is satisfied (cf. Figure 4).

**Example 4** For $S^0 = 0.01$, $R^0 = 0.01$, and all other parameters as in Table 1, it is observed that the system is stable at $E_0 = (0.01, 0.01, 0, 0)$. Also we obtain $(1 - q - k)f_1(S^0, R^0) = 0.0184 < D$ and $f_2(S^0, R^0) = 0.0577 < D$, satisfying the criterion of stability of $E_0$ as given in Lemma 5.2 (cf. Figure 5).
Figure 2. Time series analysis of the system for $R_0 = 1.2$ and other parameters as given in Table 1, the system is stable at $E_r^*$. 

Figure 3. Time series analysis of the system for $S_0 = 0.05$ and other parameters as given in Table 1, the system is stable at $E_1^s$.

7.1. Combined effects of input nutrient concentrations and dilution rate of the chemostat

We have observed that under the set of parameter values as given in Table 1, the system is stable at $E_r^*$ with $S$-limited growth of the plasmid-bearing organism in the chemostat. If the dilution rate of the chemostat is increased (viz., $D = 0.2$), the system becomes stable at $E_1^s$ with $S$-limited growth of plasmid-free organism. Further increase of the dilution rate (viz., $D = 1.5$) stabilizes the system at the organism-free equilibrium (cf. Figure 6).

Also, we have observed that for $R_0 = 1.2$ and all other parameter values as given in Table 1, the system is stable at $E_r^*$ with $R$-limited growth of plasmid-bearing organism in the chemostat. If the dilution rate is increased (viz., $D = 1$), the system becomes stable at $E_{1r}$ with $R$-limited growth.
of the plasmid-free organism. Further increase of the dilution rate (viz., $D = 1.5$) stabilizes the system at the organism-free equilibrium (cf. Figure 7).

### 7.2. Effect of toxin

It is observed that under the set of parameter values as given in Table 1, the system becomes stable at $E^*_{s}$ in the presence of an allelopathic agent produced by $x$. When the plasmid-bearing organism stops producing this toxin ($k = 0, \gamma = 0$), with all other parameter values as in Table 1, the system becomes stable at $E_{1s}$ (Figure 8).
Figure 6. Phase plane diagram of the system projected on $xy$-plane for the parameters as given in Table 1 with initial value $I_1 = (0.6, 0.4)$. The system is stable at $E_s^*$ (in solid blue). For $D = 0.2$, other parameters as given in Table 1, the system is stable at $E_{1s}$ (in dotted blue). For $D = 1.5$, other parameters as given in Table 1, the system is stable at $E_0$ (in dotted black).

Figure 7. Phase plane diagram of the system projected on $xy$-plane for $R_0 = 1.2$ and other parameters as given in Table 1 with an initial value $I_1$. The system is stable at $E_r^*$ (in solid blue). For $D = 1$, other parameters as given in Table 1, the system is stable at $E_{1r}$ (in dotted blue). For $D = 1.5$, other parameters as given in Table 1, the system is stable at $E_0$ (in dotted black).

8. Discussion

We have considered a chemostat in which plasmid-bearing and plasmid-free organisms are growing in the presence of two complementary nutrients where the plasmid-bearing organism is producing a toxin which is lethal to the plasmid-free organism. The threshold values for the existence and stability of various steady states of the system are worked out. We have also provided numerical simulations to substantiate our analytic results. Furthermore, numerical simulations
Figure 8. Phase plane diagram of the system projected on the \(xy\)-plane for \(k = 0.05\), \(\gamma = 10\), and other parameters as given in Table 1 with initial value \(I_2 = (0.7, 0.8)\). The system is stable at \(E_1^*\) (in solid blue). For \(k = 0\), \(\gamma = 0\), and other parameters as given in Table 1, the system is stable at \(E_{1b}\) (in dotted blue).

demonstrate the following conclusions:

(i) If the concentration of nutrients are very low, both the organisms in the chemostat would go to extinction.

(ii) Increase of the dilution rate leads to the extinction of the plasmid-bearing organism in the chemostat.

(iii) If the plasmid-bearing organism stops producing a toxin, the plasmid-free organism will take over the culture leading to the extinction of plasmid-bearing organism.

Throughout the article (analytical and numerical), an attempt is made to search for a suitable way to control the growth of plasmid-bearing and plasmid-free organisms and maintain stable coexistence of both the species. A numerical attempt is made to analyse the situation of extinction of some of the species in the system by changing the values of the parameters.

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Appendix

Proof of boundedness of the system (Theorem 4.1) Let \( \Sigma(t) = S(t) + R(t) + x(t) + y(t) \). Then \( \frac{d}{dt}(\Sigma(t)) \leq (S^0 + R^0)D - (\Sigma(t))D, (0 < y_1, y_2 < 1) \). Let \( u(t) \) be the solution of \( \frac{du}{dt} + uD = (S^0 + R^0)D \) satisfying \( u(0) = \Sigma(0) \). Then \( u(t) = S^0 + R^0 + (\Sigma(0) - (S^0 + R^0))e^{-D} = S^0 + R^0 \) as \( t \to \infty \). By comparison, it follows that \( \lim_{t \to \infty} \sup[S(t) + R(t) + x(t) + y(t)] \leq (S^0 + R^0) \), proving the theorem.

Proof of Lemma 5.1 (i) Since \( dS/dt \leq (S^0 - S)D \) and \( dR/dt \leq (R^0 - R)D \), it follows that \( S(t) \leq S^0 \) and \( R(t) \leq R^0 \) as \( t \to \infty \). Thus, corresponding to \( \epsilon > 0 \), there exists \( T_e > 0 \) such that \( S(t) \leq S^0 + \epsilon \) and \( R(t) \leq R^0 + \epsilon \) for all \( t \geq T_e \). For all \( t \geq T_e \), if \( (1 - q - f_1 S^0 + \epsilon, R^0 + \epsilon) < D \), then

\[
\frac{dx}{dt} \leq x[(1 - q - f_1 S^0 + \epsilon, R^0 + \epsilon) - D] < 0
\]

and so

\[
\int_{x(T_e)}^{x(t)} \frac{dx}{x} \leq (1 - q - f_1 S^0 + \epsilon, R^0 + \epsilon) - D) (t - T_e) \Rightarrow x(t) \leq x(T_e) e^{(1 - q - f_1 S^0 + \epsilon, R^0 + \epsilon) - D) (t - T_e) \to 0 \text{ as } t \to \infty.
\]

Therefore, if \( (1 - q - f_1 S^0, R^0) < D \), then \( x(t) \to 0 \) as \( t \to \infty \). Also, if \( (1 - q - f_1 S^0, R^0) < D \) then \( \lim_{t \to \infty} x(t) = 0 \) and so \( \frac{dy}{dt} \leq y[f_2(S, R) - D] \) as \( t \to \infty \) (since \( f_1(S, R) \) is bounded). Therefore, for all \( t \geq T_e \), if \( (1 - q - f_1 S^0 + \epsilon, R^0 + \epsilon) < D \) and \( f_2(S^0 + \epsilon, R^0 + \epsilon) < D \) hold, then

\[
\frac{dy}{dt} \leq y[f_2(S^0 + \epsilon, R^0 + \epsilon) - D] \Rightarrow \int_{y(T_e)}^{y(t)} \frac{dy}{y} \leq [f_2(S^0 + \epsilon, R^0 + \epsilon) - D)(t - T_e) \Rightarrow y(t) \leq y(T_e) e^{[f_2(S^0 + \epsilon, R^0 + \epsilon) - D)(t - T_e) \to 0 \text{ as } t \to \infty.}
\]

Hence, if \( (1 - q - f_1 S^0, R^0) < D \) and \( f_2(S^0, R^0) < D \), then \( \lim_{t \to \infty} x(t) = \lim_{t \to \infty} y(t) = 0 \).

(ii) For \( (1 - q - f_1 S^0, R^0) < D \), we have \( \lim_{t \to \infty} x(t) = 0 \). Since for \( \epsilon > 0 \), \( f_2(S^0 + \epsilon, R^0 + \epsilon) > D \), it follows that for all \( t \geq T_e \), \( dy/dt \geq y[f_2(S^0 + \epsilon, R^0 + \epsilon) - D] > 0 \) and so \( 0 < \lim_{t \to \infty} y(t) < \infty \) (since the system is bounded). Let \( \Sigma(t) = S(t) + S(t) = (1/\gamma_1) y(t) \) and \( \Sigma_2(t) = R(t) - R(t) = (1/\gamma_2) y(t) \). Then \( d\Sigma_1/dt = -D\Sigma_1 \) and \( d\Sigma_2/dt = -D\Sigma_2, \) and so \( \lim_{t \to \infty} \Sigma_1 = 0 \) (for \( i = 1, 2 \)). As \( t \to \infty \), the positive components of plasmid-free equilibria, \( S, \bar{R} \), and \( y, \) must satisfy \( y_1 \bar{S} + \bar{y} = y_1 S^0 + y_2 \bar{R} + \bar{y} = y_2 R^0 \). This gives \( \bar{R} = (y_1/y_2) (\bar{S} - S^0) + R^0 \) and \( \bar{S} = (y_2/y_1) (\bar{R} - R^0) + S^0 \). If \( T_2 > C \), then for \( \delta = \lambda_2 \), we obtain \( R^0 - \lambda_2 > (y_1/y_2) (S^0 - \lambda_2) = R^0 - \bar{R} \Rightarrow \bar{R} > \lambda_2 \), and so the growth rate of \( y \) is \( \bar{S} \)-limited in the absence of \( x \).

(iii) If \( T_2 < C \), then for \( \bar{R} = \lambda_2 \), we obtain \( R^0 - \lambda_2 > (y_2/y_1) (R^0 - \lambda_2) = S^0 - \bar{S} \Rightarrow \bar{S} > \lambda_2 \), and so the growth rate of \( y \) is \( \bar{R} \)-limited in the absence of \( x \).
Proof of Lemma 5.2  At $E_0$ the variational matrix is
\[
V(E_0) = \begin{bmatrix}
-D & 0 & -f_1(S^0, R^0) / \gamma_1 & -f_2(S^0, R^0) / \gamma_2 \\
0 & -D & -f_1(S^0, R^0) / \gamma_1 & -f_2(S^0, R^0) / \gamma_2 \\
0 & 0 & (1 - q - k) f_1(S^0, R^0) - D & 0 \\
0 & 0 & q f_1(S^0, R^0) & f_2(S^0, R^0) - D
\end{bmatrix}.
\]  
(A1)

The eigenvalues are $-D, -D, (1 - q - k) f_1(S^0, R^0) - D$ and $f_2(S^0, R^0) - D$. Therefore, the system is stable at $E_0$ if $(1 - q - k) f_1(S^0, R^0) < D$ and $f_2(S^0, R^0) < D$.

Proof of Lemma 5.3  At plasmid-bearing organism-free equilibria $\hat{E}_1 = (\hat{S}, \hat{R}, 0, \hat{y})$, the characteristic polynomial of the variational matrix $V(\hat{E}_1)$ is
\[
\begin{vmatrix}
-D & -\gamma_1 (1 - q - k) f_1(\hat{S}, \hat{R}) & -f_1(\hat{S}, \hat{R}) & -D \\
-\gamma_2 (1 - q - k) f_1(\hat{S}, \hat{R}) & -D & -f_1(\hat{S}, \hat{R}) & -D \\
0 & -\gamma_2 (1 - q - k) f_1(\hat{S}, \hat{R}) & -f_1(\hat{S}, \hat{R}) & -D \\
\gamma_2 f_2(\hat{S}, \hat{R}) & \gamma_2 f_2(\hat{S}, \hat{R}) & \gamma_2 f_2(\hat{S}, \hat{R}) & \gamma_2 f_2(\hat{S}, \hat{R}) & -D
\end{vmatrix}.
\]  
(A2)

where $\hat{E}_1$ is one of $E_{1s}$ and $E_{1r}$ according to $S$-limiting or $R$-limiting equilibrium, $f_{2s} = \partial f_2 / \partial S$, and $f_{2r} = \partial f_2 / \partial S$.

Hence, we get
\[
\begin{vmatrix}
-D & -\gamma_1 (1 - q - k) f_1(\hat{S}, \hat{R}) & -f_1(\hat{S}, \hat{R}) & -D \\
-\gamma_2 (1 - q - k) f_1(\hat{S}, \hat{R}) & -D & -f_1(\hat{S}, \hat{R}) & -D \\
0 & -\gamma_2 (1 - q - k) f_1(\hat{S}, \hat{R}) & -f_1(\hat{S}, \hat{R}) & -D \\
\gamma_2 f_2(\hat{S}, \hat{R}) & \gamma_2 f_2(\hat{S}, \hat{R}) & \gamma_2 f_2(\hat{S}, \hat{R}) & \gamma_2 f_2(\hat{S}, \hat{R}) & -D
\end{vmatrix}.
\]  
(A3)

When $R_1' = R_1 + (1/\gamma_1) R_3$ and $R_2' = R_2 + (1/\gamma_2) R_3$, we get
\[
\begin{vmatrix}
-D & 0 & -f_1(\hat{S}, \hat{R}) / \gamma_1 & -D \\
0 & -D & -f_1(\hat{S}, \hat{R}) / \gamma_1 & -D \\
\gamma_1 f_2(\hat{S}, \hat{R}) & \gamma_1 f_2(\hat{S}, \hat{R}) & \gamma_1 f_2(\hat{S}, \hat{R}) & -\mu
\end{vmatrix} = (D + \mu)^2 \begin{vmatrix}
1 & (1 - q - k) f_1(\hat{S}, \hat{R}) - D - D \\
0 & -D - \mu & -f_1(\hat{S}, \hat{R}) / \gamma_1 \\
\gamma_1 f_2(\hat{S}, \hat{R}) & \gamma_1 f_2(\hat{S}, \hat{R}) & \gamma_1 f_2(\hat{S}, \hat{R}) & -\mu
\end{vmatrix}.  
\]  
(A4)

The eigenvalues are $-D, -D, (1 - q - k) f_1(\hat{S}, \hat{R}) - D$ and $-\gamma_2 f_2(\hat{S}, \hat{R}) / \gamma_1$. Thus, $E_{1s} = (\lambda_{1s}, R_s, 0, y_s)$ is stable if $(1 - q - k) f_1(\lambda_{1s}, R_s) < D$, where $R_s = R^0 - (\gamma_1 / \gamma_2)(S^0 - \lambda_{2s})$ and $y_s = \gamma_1 (S^0 - R_s - \lambda_{1s})$. If $f_1 = m_{1s} S / (a_{1s} + S)$, then $(1 - q - k) f_1(\lambda_{1s}, R_s) < D \Rightarrow \lambda_{2s} < \lambda_{1s}$. If $f_1 = m_{1s} R / (a_{1r} + R)$, then $(1 - q - k) f_1(\lambda_{1s}, R_s) < D \Rightarrow R_s < \lambda_{1r}$. Therefore, $E_{1s}$ is stable if $\lambda_{2s} < \lambda_{1s}$ or $R_s < \lambda_{1s}$.

Proof of Lemma 5.4  $E_{1r} = (S_r, \lambda_{2s}, 0, y_r)$ is stable if $(1 - q - k) f_1(S_r, \lambda_{2s}) < D$, where $S_r = S^0 - (\gamma_2 / \gamma_1)(R^0 - \lambda_{2s})$ and $y_r = \gamma_2 (R^0 - \lambda_{1s})$. If $f_1 = m_{1s} S / (a_{1s} + S)$, then $(1 - q - k) f_1(S_r, \lambda_{2s}) < D \Rightarrow S_r < \lambda_{1r}$. If $f_1 = m_{1s} R / (a_{1r} + R)$, then $(1 - q - k) f_1(S_r, \lambda_{2s}) < D \Rightarrow \lambda_{2s} < \lambda_{1r}$. Therefore, $E_{1r}$ is stable if $S_r < \lambda_{1r}$ or $\lambda_{2s} < \lambda_{1r}$.

Proof of Lemma 5.5  In order to prove the persistence of the system, we shall show that all the boundary equilibria of the system are repellers. It is observed that if $(1 - q - k) f_1(S^0, R^0) \geq D$, then $E_0$ is unstable. If $\lambda_{2s} \geq \lambda_{1s}$ and $R^0 \geq \lambda_{1r}$, then $E_{1s}$ is unstable. If $S^0 \geq \lambda_{1s}$ and $\lambda_{2s} \geq \lambda_{1r}$, then $E_{1r}$ is unstable. Combining all the cases, we can conclude that all the boundary equilibria of the system are unstable if $(1 - q - k) f_1(S^0, R^0) \geq D$, $\lambda_{1r} \leq \min\{S^0, \lambda_{2s}\}$ and $\lambda_{1s} \leq \min\{R^0, \lambda_{2r}\}$. Also, by Theorem 4.1, it follows that the system (2) is bounded. Therefore, the system is persistent under the above conditions.
Proof of Lemma 5.6  At interior equilibria $E^* = (S^*, R^*, x^*, y^*)$, the characteristic polynomial of the variational matrix $V(E^*)$ is

$$
\begin{align*}
-D - \frac{x f_{1S}(E^*)}{1 - q - k} &- \frac{x f_{2S}(E^*)}{1 - q - k} - \mu \\
-\frac{x f_{1R}(E^*)}{1 - q - k} &- \frac{x f_{2R}(E^*)}{1 - q - k} - \mu \\
&- \frac{-f_1(E^*)}{1 - q - k} - \frac{-f_2(E^*)}{1 - q - k} \\
&- \frac{-f_1(E^*)}{1 - q - k} - \frac{-f_2(E^*)}{1 - q - k} \\
&- \frac{-y f_{2S}(E^*)}{1 - q - k} - \frac{q f_1(E^*)}{1 - q - k} \\
&- \frac{-y f_{2R}(E^*)}{1 - q - k} - \frac{q f_1(E^*)}{1 - q - k} \\
&- \frac{-y k x^*}{1 - q - k} - \frac{-y k x^*}{1 - q - k} \\
&- \frac{-D - \mu}{1 - q - k} - \frac{-D - \mu}{1 - q - k},
\end{align*}
$$

where $E^*$ is one of $E_1^*$ and $E_2^*$ according to $S$-limiting or $R$-limiting equilibrium, $f_{1S} = \partial f_1/\partial S$ and $f_{1R} = \partial f_1/\partial R$. When $R_1^* = R_1 - (\gamma_2/\gamma_1) R_2$, we get

$$
\begin{align*}
\begin{vmatrix}
-1 & \frac{\gamma_2}{\gamma_1} \\
-x f_{1S}(E^*) & -y f_{2S}(E^*) & -\gamma_2(D + \mu) - x f_{1R}(E^*) & -y f_{2R}(E^*) & 0 & 0 \\
 x f_{1S}(E^*) & x f_{1R}(E^*) & -\mu & 0 \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k}
\end{vmatrix},
\end{align*}
$$

where $f(\mu) = (1 - q - k)(D + \mu)/\gamma_2$.

When $R_1^* = R_1 - q R_2$, we get

$$
\begin{align*}
\begin{vmatrix}
-1 & \frac{\gamma_2}{\gamma_1} \\
x f_{1S}(E^*) & -y f_{2S}(E^*) & -\gamma_2(D + \mu) - x f_{1R}(E^*) & -y f_{2R}(E^*) & 0 & 0 \\
x f_{1S}(E^*) & x f_{1R}(E^*) & -\mu & 0 \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k}
\end{vmatrix},
\end{align*}
$$

When $R_2^* = R_2^* + R_3^* + R_4^*$, we get

$$
\begin{align*}
\begin{vmatrix}
-1 & \frac{\gamma_2}{\gamma_1} \\
0 & -\gamma_2(D + \mu) & (q - 1) \left( f_1(E^*) + \frac{\mu}{1 - q - k} \right) - \frac{-y k x^*}{1 - q - k} & (D + \mu) - \frac{y k x^*}{1 - q - k} & 0 \\
x f_{1S}(E^*) & x f_{1R}(E^*) & -\mu & 0 \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k} \\
y f_{2S}(E^*) & y f_{2R}(E^*) & -\gamma k x^* + \frac{\mu q}{1 - q - k} & + q f_1(E^*) & f_1(E^*) & - \frac{y k x^*}{1 - q - k}
\end{vmatrix},
\end{align*}
$$

where

$$
A = 2D - f_2(E^*) + \frac{\gamma k x^*}{1 - q - k} + x^* \left( f_1(E^*) + \frac{f_1(E^*)}{\gamma_2} + \frac{f_1(E^*)}{\gamma_1} \right) + y^* \left( f_2(E^*) + \frac{f_2(E^*)}{\gamma_1} \right),
$$

$$
B = D \left( D - f_2(E^*) + \frac{\gamma k x^*}{1 - q - k} \right)
+ x^* \left( D - (1 - q) f_2(E^*) + \frac{\gamma k x^*}{1 - q - k} + (1 - q - k) f_1(E^*) + \gamma k x^* \right) \left( f_1(E^*) + \frac{f_1(E^*)}{\gamma_2} + \frac{f_1(E^*)}{\gamma_1} \right),
$$

$$
y^* \left( D - \frac{\gamma k x^*}{1 - q - k} \right) \left( f_2(E^*) + \frac{f_2(E^*)}{\gamma_1} \right),
$$

and

$$
C = x^* \left[ f_1(E^*) \left( D + \frac{\gamma k x^*}{1 - q - k} \right) - f_2(E^*) \left( (1 - q) f_1(E^*) + \frac{\gamma k x^*}{1 - q - k} \right) \right] \left( f_1(E^*) + \frac{f_1(E^*)}{\gamma_1} \right).
$$

Therefore, the interior equilibrium is locally asymptotically stable if $AB > C$.  \[\square\]
Proof of Lemma 6.1 Let $p_i(S) = m_{ij}S/(a_{ij} + S)$ and $q_i(R) = m_{ij}R/(a_{ij} + R)$ $(i = 1, 2)$. Then, $x + y = \gamma_1(S^0 - S) = \gamma_2(R^0 - R)$ and so $R = R^0 + \gamma_1/\gamma_2(S - S^0) \equiv h(S)$. Let $Q_i(S) = q_i(h(S)) = q_i(R)$ $(i = 1, 2)$. Also, $p_i(S) - Q_i(S) = 0$ if $m_{ij}a_{ij}S - m_{ij}a_{ij}R + (m_{ij} - m_{ij})RS = 0$.

We consider the following cases:

Case I: Let $p_1(S) - Q_1(S) = 0$ has no root in $0 < S < S^0$ $(i = 1, 2)$. For $p_1(S) < Q_1(S)$, $p_2(S) > Q_2(S)$ in $0 < S < S^0$, the system becomes

$$\frac{dx}{dt} = x(1 - q - k)p_1(S) - D \equiv G_1,$$

$$\frac{dy}{dt} = y \left[ Q_2(S) - D - \frac{\gamma kx}{1 - q - k} \right] + qxp_1(S) \equiv G_2,$$

where $S = S^0 - \frac{1}{\gamma_1}(x + y)$.

Considering the auxiliary function $U(x, y) = 1/xy$, we get

$$\frac{\partial}{\partial x} (UG_1) + \frac{\partial}{\partial y} (UG_2) = -\frac{1}{y\gamma_1}(1 - k)p_1(S) - \frac{q}{y^2}p_1(S) - \frac{1}{xy\gamma_1}'Q_2(S) < 0.$$

For $p_1(S) < Q_1(S)$, $p_2(S) < Q_2(S)$ in $0 < S < S^0$, the system becomes

$$\frac{dx}{dt} = x(1 - q - k)p_1(S) - D \equiv G_1,$$

$$\frac{dy}{dt} = y \left[ p_2(S) - D - \frac{\gamma kx}{1 - q - k} \right] + qxp_1(S) \equiv G_2,$$

where $S = S^0 - \frac{1}{\gamma_1}(x + y)$.

$$\frac{\partial}{\partial x} (UG_1) + \frac{\partial}{\partial y} (UG_2) = -\frac{1}{y\gamma_1}(1 - k)p_1(S) - \frac{q}{y^2}p_1(S) - \frac{1}{xy\gamma_1}'p_2(S) < 0.$$

For $p_1(S) > Q_1(S)$, $p_2(S) < Q_2(S)$ in $0 < S < S^0$, the system becomes

$$\frac{dx}{dt} = x(1 - q - k)Q_1(S) - D \equiv G_1,$$

$$\frac{dy}{dt} = y \left[ p_2(S) - D - \frac{\gamma kx}{1 - q - k} \right] + qxQ_1(S) \equiv G_2,$$

where $S = S^0 - \frac{1}{\gamma_1}(x + y)$.

$$\frac{\partial}{\partial x} (UG_1) + \frac{\partial}{\partial y} (UG_2) = -\frac{1}{y\gamma_1}(1 - k)Q_1(S) - \frac{q}{y^2}Q_1(S) - \frac{1}{xy\gamma_1}'p_2(S) < 0.$$

Therefore, there is no periodic solution.

Case II: We assume that for $i = 1, 2$, $p_i(S)$ and $Q_i(S)$ intersect at a point $S_i \in (0, S^0)$. We have $h(0) = R^0 - (\gamma_1/\gamma_2)S^0$. If $q_i(h(0)) > 0$, then $p_i(S) < Q_i(S)$ for $0 < S < S_i$ and $p_i(S) > Q_i(S)$ for $S > S_i$, otherwise, $p_i(S) > Q_i(S)$ for $0 < S < S_i$ and $p_i(S) < Q_i(S)$ for $S > S_i$ $(i = 1, 2)$. Without any loss of generality, we assume that $S_1 < S_2$ and $q_i(h(0)) > 0$ $(i = 1, 2)$. If possible, let there exists a periodic orbit $C$ in the first quadrant of the $xy$-plane. Then $Q_i(S) > p_i(S)$ for $S < S_1 < S_2$. If $S_1 < S < S_2$, then $p_1(S) > Q_1(S)$ and $p_2(S) < Q_2(S)$. If $S > S_2$, then $p_1(S) > Q_1(S)$ and $p_2(S) > Q_2(S)$. In the $xy$-plane, $S < S_1 \Rightarrow x + y > \gamma_1(S^0 - S_1)$, $S_1 < S < S_2 \Rightarrow \gamma_1(S^0 - S) < x + y < \gamma_1(S^0 - S_1)$ and $S > S_2 \Rightarrow x + y < \gamma_1(S^0 - S_2)$. We define the following regions:

$$C_1 = \{(x, y) : x, y \geq 0, x + y > \gamma_1(S^0 - S_1)\},$$

$$C_2 = \{(x, y) : x, y \geq 0, \gamma_1(S^0 - S_2) < x + y < \gamma_1(S^0 - S_1)\},$$

$$C_3 = \{(x, y) : x, y \geq 0, x + y < \gamma_1(S^0 - S_2)\}.$$

On the region $C_1$

$$\frac{dx}{dt} = x(1 - q - k)p_1(S) - D \equiv G_1,$$

$$\frac{dy}{dt} = y \left[ p_2(S) - D - \frac{\gamma kx}{1 - q - k} \right] + qxp_1(S) \equiv G_2.$$
On the region $C_2$

\[
\frac{dx}{dt} = x[(1 - q - k)Q_1(S) - D] \equiv G_1,
\]

\[
\frac{dy}{dt} = y \left[ p_2(S) - D - \frac{y'kx}{1 - q - k} \right] + qxQ_1(S) \equiv G_2.
\]

On the region $C_3$

\[
\frac{dx}{dt} = x[(1 - q - k)Q_1(S) - D] \equiv G_1,
\]

\[
\frac{dy}{dt} = y \left[ Q_2(S) - D - \frac{y'kx}{1 - q - k} \right] + qxQ_1(S) \equiv G_2.
\]

Therefore,

\[
\frac{\partial}{\partial x} (UG_1) + \frac{\partial}{\partial y} (UG_2) = \begin{cases} 
-\frac{1}{y'\gamma_1} (1 - k)p_1'(S) - \frac{q}{y'\gamma_1} p_1(S) - \frac{1}{x'\gamma_1} p_2'(S) < 0, & \text{if } C \subset C_1, \\
-\frac{1}{y'\gamma_1} (1 - k)Q_1'(S) - \frac{q}{y'\gamma_1} Q_1(S) - \frac{1}{x'\gamma_1} p_2'(S) < 0, & \text{if } C \subset C_2, \\
-\frac{1}{y'\gamma_1} (1 - k)Q_2'(S) - \frac{q}{y'\gamma_1} Q_2(S) - \frac{1}{x'\gamma_1} Q_2'(S) < 0, & \text{if } C \subset C_3 
\end{cases}
\]

(A9)

contradicting our assumption. Therefore, no periodic orbit exists. Next, we assume that $C \subset C_1 \cup C_2$ and $\epsilon > 0$ (Figure A1). Let us consider the straight lines

\[L_1 : x + y = \gamma_1(S^0 - S_1)\]

\[L_1^\epsilon : x + y = \gamma_1(S^0 - S_1 - \epsilon)\]

\[L_1^{-\epsilon} : x + y = \gamma_1(S^0 - S_1 + \epsilon)\]

(cf. Figure A1).

Also, let $W = U(G_1 \ dy - G_2 \ dx)$. Then

\[
\oint_C W = \int_{N_1EM_1} W + \int_{M_1M_2} W + \int_{M_2F_2N_2} W + \int_{N_2N_1} W
\]

\[
= \left[ \int_{N_1EM_1} W + \int_{M_1N_1} W \right] + \left[ \int_{M_2F_2N_2} W + \int_{N_2M_2} W \right] + \int_{M_1M_2} W + \int_{N_2N_1} W + \left[ - \int_{M_1N_1} W + \int_{M_2N_2} W \right].
\]
Also,
\[
\lim_{\epsilon \to 0} \left[ \int_{N_1 E M_1} W + \int_{M_1 N_1} W \right] = \lim_{\epsilon \to 0} \int_{I_{1,\epsilon}} \left\{ -\frac{1}{y \gamma_1} (1 - k)p'_1(S) - \frac{q}{y^2} p_1(S) - \frac{1}{x \gamma_1} p'_2(S) \right\} \, dx \, dy < 0
\]
and
\[
\lim_{\epsilon \to 0} \left[ \int_{M_2 FN_2} W + \int_{N_2 M_2} W \right] = \lim_{\epsilon \to 0} \int_{I_{2,\epsilon}} \left\{ -\frac{1}{y \gamma_1} (1 - k)Q'_1(S) - \frac{q}{y^2} Q_1(S) - \frac{1}{x \gamma_1} p'_2(S) \right\} \, dx \, dy < 0,
\]
where \( I_{1,\epsilon} \) and \( I_{2,\epsilon} \) are the regions bounded by the closed curves \( \overline{BEA} \) and \( \overline{AFB} \), respectively. Also, \( \lim_{\epsilon \to 0} \int_{M_1 N_1} W = 0 = \lim_{\epsilon \to 0} \int_{N_2 M_2} W \).

\[
\int_{M_1 N_1} W = -\int_{m_1} U(G_1 + G_2) \, dx
\]
\[
= -\int_{m_1} \left\{ x(1 - k)p_1(S_1 - \epsilon) + yp_2(S_1 - \epsilon) - \frac{\gamma k x}{1 - q - k} - (S^0 - S + \epsilon) \gamma_1 D \right\} \, dx,
\]
\[
\int_{M_2 N_2} W = -\int_{m_2} \left\{ x(1 - k)Q_1(S_1 + \epsilon) + yp_2(S_1 + \epsilon) - \frac{\gamma k x}{1 - q - k} - (S^0 - S - \epsilon) \gamma_1 D \right\} \, dx.
\]
So
\[
\lim_{\epsilon \to 0} \left[ -\int_{M_1 N_1} W + \int_{M_2 N_2} W \right] = 0.
\]
Therefore, as \( \lim_{\epsilon \to 0} \), we have \( f_C W < 0 \), contradicting the fact that \( f_C W = 0 \). Finally, we assume that the periodic orbit \( C \) intersects each region \( C_i \) \( (i = 1, 2, 3) \). Let us consider the straight lines:

\[
L_1 : x + y = \gamma_1 (S^0 - S_1),
\]
\[
L'_1 : x + y = \gamma_1 (S^0 - S_1 - \epsilon),
\]
\[
L^-_1 : x + y = \gamma_1 (S^0 - S_1 + \epsilon),
\]
\[
L_2 : x + y = \gamma_1 (S^0 - S_2 - \epsilon),
\]
\[
L^-_1 : x + y = \gamma_1 (S^0 - S_2 + \epsilon).
\]
Then, we have
\[
\int_C W = \int_{D_1 \cup D_2} \left\{ -\frac{1}{y \gamma_1} (1 - k)p'_1(S) - \frac{q}{y^2} p_1(S) - \frac{1}{x \gamma_1} p'_2(S) \right\} \, dx \, dy
\]
\[
+ \int_{D_3 \cup D_4} \left\{ -\frac{1}{y \gamma_1} (1 - k)Q'_1(S) - \frac{q}{y^2} Q_1(S) - \frac{1}{x \gamma_1} p'_2(S) \right\} \, dx \, dy
\]
\[
+ \int_{D_5 \cup D_6} \left\{ -\frac{1}{y \gamma_1} (1 - k)Q'_1(S) - \frac{q}{y^2} Q_1(S) - \frac{1}{x \gamma_1} Q'_2(S) \right\} \, dx \, dy
\]
\[
< 0,
\]
where \( D_1 \) and \( D_2 \) are the regions bounded by \( C \) and \( L_1 \); \( D_3 \) and \( D_4 \) are the regions bounded by \( C, L_1 \), and \( L_2 \); \( D_5 \) and \( D_6 \) are the regions bounded by \( C \) and \( L_2 \). This leads to a contradiction. Hence, the system has no periodic solution around the interior point.