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A Mathematical Model of Gene Transfer in a Biofilm

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Summary. Based on our previous work, a model of plasmid transfer between micro-organisms in a heterogeneous environment consisting of a biofilm immersed in a fluid medium is constructed. A review of previous modeling of gene transfer is provided in order to place our work in context. The key question is whether the plasmid can persist in the bacterial population. We answer this question by constructing a basic reproductive number which takes into account the advantages conferred by the plasmid and its costs to the bacterial host.

6.1 Introduction

Plasmids, small circular strands of DNA separate from the main genome of the organism, are common in natural bacterial populations such as soils, lakes and stream and in the gut of mammals. They often carry genes for such beneficial factors as resistence to antibiotics and heavy metals, the ability to ferment sugars, or to produce toxins. Some carry genes for pili production and mating pair formation that allow the infectious transfer of the plasmid to other bacteria – a process called conjugation. However, many plasmids have no known function in bacteria and may simply be parasitic. Vertical transmission of plasmids occurs during cell division when the plasmids in the cell are duplicated and partitioned among the daughter cells; rarely, however, one daughter cell may end up without plasmid while the other daughter cell receives multiple copies. This loss of plasmid is referred to a segregative loss. Furthermore, there is some cost to an organism carrying plasmids since the cell may produce plasmid gene products and must duplicate it during cell division which leads to a reduced reproductive rate. See Simonsen (1991) for a readable review, particularly of modeling aspects, and Summers (1996) for a general review. Because the benefits of plasmid carriage, if any, depend on ever-changing environmental conditions while the costs are always present, a longstanding focus of theoretical studies has been to determine conditions under which plasmids can be maintained in bacterial populations. See Stewart

and Levin (1977), Levin and Rice (1980) and Bergstrom, Lipsitch, and Levin (2000) for modeling results related to this problem.

According to Angles and Goodman (2000):

> Biofilms are environments of high microbial density where cell-cell contact is likely. Such conditions create a favorable niche for the spread of self-transmissible as well as mobilisable plasmids among members of the bacterial communities. Studies have demonstrated plasmid transfer among bacteria in a wide range of biofilm habitats, including the surface of stones in a river, the air-water interface, surfaces in soil and water microcosms, plant surfaces and insect as well as animal intestinal surfaces.

In a recent paper Ghigo (Ghigo 2001) established that several natural conjugative plasmids express factors that induce some bacteria to form biofilms. Experimental studies showed that a strain of *E. Coli* bearing a certain plasmid formed a thick biofilm within one day while those not carrying the plasmid produced no macroscopically observable biofilm. Interestingly, Ghigo's results suggest that the pili responsible for the horizontal transfer of the plasmid, may also act as an adhesion factor for cell-to-surface contact. See also Pratt and Kolter (1998) and O'Toole and Kolter (1998). Ghigo points out the many beneficial aspects for bacteria in biofilms relative to the fluid environment and speculates that such factors "may provide a rationale for the unexplained vertical maintenance of the numerous uninfectious cryptic plasmids found in natural populations". He also observes that by inducing bacteria to form the denser communities characteristic of biofilms the plasmid increases the likelihood of its own horizontal transfer via conjugation.

In this chapter, we explore the suggested link between plasmid maintenance and biofilms by modifying slightly the mathematical model proposed by us in (Imran et al. preprint) of a bacterial population consisting of plasmid-bearing and plasmid-free organisms in a continuous culture with a surface on which a biofilm may form. The question we address is under what circumstances can the plasmid be maintained in a population. Heuristically, the advantageous genes carried by the plasmid together with the ability of the plasmid-bearing organism to pass the plasmid to other organisms must compensate for the energetic cost of bearing the plasmid and the occasional segregative loss of the plasmid during cell division. We seek to quantify this trade-off. Our model builds on the plasmid model of Stephanopoulus and Lapidus (1988) and Ryder and DiBiasio (1984), includes conjugation terms used by Stewart and Levin (1977), and models the biofilm following the model of Pilyugin and Waltman (1999). Consequently, we briefly review these models in order that the foundation of our model is made more clear.

Two cases are considered: (1) the plasmid is parasitic, conferring no advantage on its host, and (2) the plasmid codes for enhanced biofilm forming ability in its bacterial host which in its absence can form only a macroscopically unobservable biofilm. In the first case, the question is under what

circumstances can a parasitic plasmid can be maintained. In the second case, the question is under what circumstances can the ability to form a robust biofilm community in which conjugative transfer of the plasmid may occur be sufficiently advantageous for the plasmid-bearing organism to compensate for the energetic cost of bearing the plasmid and the segregative loss of the plasmid. In each case, we provide a quantitative expression of a potential mechanism which may be significant in plasmid maintenance.

Our work corroborates the conjectures of Ghigo. The availability of colonalizable surfaces that provide a selective advantage for an organism carrying a plasmid containing a biofilm-enhancing gene may contribute to the maintenance of such plasmids in natural bacterial populations.

The same models developed in this paper could also be used to study the important phenomena of horizontal spread of antibiotic resistance in the gut. Rather than assuming the plasmid codes for enhanced biofilm forming ability one would assume that it codes for antibiotic resistance. Selection for the resistant strain could, of course, be arranged by adding antibiotic. Ingestion of bacteria containing plasmid coding for antibiotic resistance could lead to the spread of resistance to the gut microflora. This phenomena may play a significant role in the proliferation of antibiotic resistant pathogens (Summers 1996).

6.2 A model of plasmid transfer with wall growth

We consider a population of bacteria in a continuous culture which colonize both the fluid environment and a portion of a surface immersed in the

Table 6.1. Model parameters for the chemostat: t =time, m = mass, l =length

Symbol	Description	Dimension
u, u_+	biomass concentration of planktonic bacteria.	ml^{-3}
w, w_+	areal biomass density of adherent bacteria.	ml^{-2}
β, β_+	sloughing rate.	t^{-1}
α, α_+	rate constant of adhesion.	t^{-1}
S	concentration of limiting substrate.	ml^{-3}
S^0	concentration of the substrate in the feed.	ml^{-3}
γ	yield constant.	–
a	half saturation constant.	ml^{-3}
m	maximum growth rate of plasmid-free organism.	t^{-1}
c	fractional energetic cost of plasmid carriage, $0 < c < 1$.	–
q	fractional segregation loss factor, $0 < q < 1$.	–
D	dilution rate.	t^{-1}
μ	biofilm conjugational transfer parameter.	$l^2(mt)^{-1}$
$\bar{\mu}$	planktonic conjugational transfer parameter.	$l^3(mt)^{-1}$

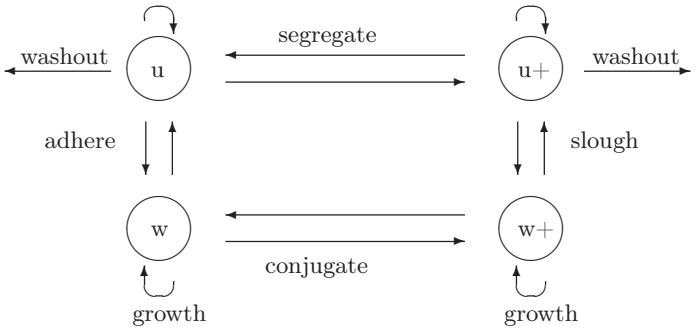

Fig. 6.1. Flow chart of biomass flow between model compartments, u, u_+, w, w_+

fluid. Bacteria are labelled according to their location (fluid or wall: the former called planktonic cells, the latter called adherent cells) and according to whether or not they possess the plasmid of interest (subscript '+' means they have plasmid). Let u (u_+) denote the density of planktonic plasmid-free (plasmid-bearing) organism and w (w_+) denote the areal density of wall-adherent plasmid-free (plasmid-bearing) organism. These populations are supported by the substrate S in continuous culture.

Model parameters are described in the Table 6.1 and a schematic diagram of the model is depicted below it. Bacterial variables and parameters without the "+" sign refer to plasmid-free cells while those with subscript "+" refer to plasmid-bearing cells.

The model equations in the setting of a continuous culture of volume V, colonizable surface area A and flow rate Φ takes the form ($D = \Phi/V$, $\delta = A/V$):

$$\begin{aligned}
S' &= D(S^0 - S) - \gamma^{-1}\left[f_u(S)u + f_u(S)(1-c)u_+\right] \\
&\quad - \gamma^{-1}\left[\,\delta f_w(S)w + \delta f_w(S)(1-c)w_+\right] \\
u' &= (f_u(S) - D)u + qf_u(S)(1-c)u_+ - \alpha u + \beta\delta w - \bar{\mu}uu_+ \\
w' &= f_w(S)w + qf_w(S)(1-c)w_+ + \alpha\delta^{-1}u - \beta w - \mu w w_+ \\
u'_+ &= [f_u(S)(1-c)(1-q) - D]u_+ - \alpha_+ u_+ + \beta_+\delta w_+ + \bar{\mu}uu_+ \\
w'_+ &= f_w(S)(1-c)(1-q)w_+ + \alpha_+\delta^{-1}u_+ - \beta_+ w_+ + \mu w w_+
\end{aligned} \tag{1}$$

These equations represent a modification of the model first proposed in (Imran et al. preprint). See the discussion section for a description of this modification. Because the model is an amalgamation of models in the literature we do not give a detailed description of it here. Instead, we indicate below how it was constructed from earlier models and, in subsequent sections, review these earlier and simpler models. In this way, the basic features of the model may be discussed in a simpler setting without all the distractions present in the full model. After doing this, we return to the full model.

In the absence of the plasmid-bearing organism ($u_+ = w_+ = 0$), the system reduces to the wall growth model of Pilyugin and Waltman(1999):

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1}\left[f_u(S)u + \delta f_w(S)w\right] \\ u' &= (f_u(S) - D)u - \alpha u + \beta\delta w \\ w' &= f_w(S)w + \alpha\delta^{-1}u - \beta w \end{aligned} \tag{2}$$

This system is described in detail in the next section.

Neglecting the wall-attached population ($w = w_+ = 0$ and $\alpha = 0$) in (1) we obtain a model of plasmid transfer in the fluid environment of the chemostat:

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1}[f_u(S)u + f_{u_+}(S)u_+] \\ u' &= (f_u(S) - D)u + qf_{u_+}(S)u_+ - \bar{\mu}uu_+ \\ u'_+ &= [f_{u_+}(S)(1-q) - D]u_+ + \bar{\mu}uu_+ \\ f_{u_+}(S) &:= (1-c)f_u(S) \end{aligned} \tag{3}$$

This model is similar to the classic model of Stewart and Levin (1977). We consider and compare both of these in Sect. 6.4.2.

Ignoring the wall population and plasmid transfer (conjugation), but not segregation, the system reduces to a special case of the model of Stephanopoulus and Lapidus (1988) for (non-infectious) plasmid-bearing organisms in the chemostat:

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1}[f_u(S)u + f_{u_+}(S)u_+] \\ u' &= (f_u(S) - D)u + qf_{u_+}(S)u_+ \\ u'_+ &= [f_{u_+}(S)(1-q) - D]u_+ \\ f_{u_+}(S) &:= (1-c)f_u(S) \end{aligned} \tag{4}$$

Stephanopoulus and Lapidus borrowed ideas from the earlier work of Ryder and DiBiasio (1984). System (4) and similar models are especially relevant to issues in biotechnology involving the production of biologically useful compounds by genetically altered organisms. See Hsu et. al. (1994, 1997, 2004).

As (1) is inspired by these earlier models, it is also perhaps best understood once one is familiar with them. We review them in the sections immediately following before returning to analyze (1).

6.3 Pilyugin–Waltman model

Pilyugin–Waltman (1999) proposed a simple chemostat model with wall growth in the form of three nonlinear differential equations. The key difference between their model and the standard chemostat model (see e. g. Smith and Waltman 1995) is that the population growing on the wall does

not wash out of the chemostat. Due to this modification the basic conservation principle of the chemostat is lost so the system is no longer reducible to a planar system. With some change in notation, their model is given by

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1} f_u(S)u - \gamma^{-1}\delta f_w(S)w \\ u' &= (f_u(S) - D)u - \alpha u + \beta\delta w \\ w' &= f_w(S)w + \alpha\delta^{-1}u - \beta w \end{aligned} \tag{5}$$

where u denotes the volume density of the organism in the fluid (planktonic cells) and w denotes the areal density of the organism on the wall (adherent cells). Planktonic cells adhere to the wall at rate α and adherent cells slough off the wall at rate β. D is the dilution rate, γ is the yield coefficients expressing the proportionality between the uptake rate and growth rate. The nutrient uptake rate $f_u(S)$ should satisfy

$$f_u(0) = 0\,, \quad f_u'(S) > 0\,.$$

In practice, they are often taken to be of Michaelis–Menten form

$$f_u(S) = \frac{mS}{a+S}\,, \quad m, a > 0\,. \tag{6}$$

The same conditions hold for f_w, which may be distinct from f_u. Parameter δ is the ratio of the colonizable area A to the volume V of the chemostat. It can be scaled out of the system by replacing w by δw in the model which we routinely do in computing Jacobian matrices below.

A key parameter in the model is the mean residence time (MRT) of a bacterial cell in the chemostat. From the Appendix, we have

$$\text{MRT} = \left[\frac{2}{D + \alpha + \beta - \sqrt{(D+\alpha+\beta)^2 - 4\beta D}}\right] \tag{7}$$

Because α and β are assumed to be positive, there are only two possible types of steady states, the washout steady state $(S^0, 0, 0)$ and possibly one or more survival steady states having the form $(\bar{S}, \bar{u}, \bar{w})$ with all components positive. The stability of the washout steady state can be determined by the eigenvalues of the variational matrix at $(S^0, 0, 0)$

$$J := \begin{pmatrix} -D & -\gamma^{-1} f_u(S^0) & \gamma^{-1} f_u(S^0) \\ 0 & f_u(S^0) - D - \alpha & \beta \\ 0 & \alpha & f_w(S^0) - \beta \end{pmatrix}$$

We denote by A the lower right two-by-two sub-matrix of J and by $s(A)$ its stability modulus, the maximum of its two real eigenvalues. Evidently, the washout steady state $(S^0, 0, 0)$ is hyperbolically stable if $s(A) < 0$ and unstable if $s(A) > 0$.

The survival steady state can be described most efficiently by introducing the quasi-positive irreducible matrix function of S given by

$$B(S) := \begin{pmatrix} f_u(S) - D - \alpha & \beta \\ \alpha & f_w(S) - \beta \end{pmatrix} \tag{8}$$

In order that $(\bar{S}, \bar{u}, \bar{w})$ be a positive steady state, $(\bar{u}, \delta\bar{w})$ must be a positive eigenvector corresponding to the zero eigenvalue of $B(\bar{S})$. As $S \to B(S)$ is increasing (along the diagonal), Perron–Frobenius theory (Berman and Plemmons 1979) implies that $S \to s(B(S))$ is strictly increasing so there can be at most one value of S at which $s(B(S)) = 0$. Since $A = B(S^0)$ and $s(B(0)) < 0$, we see that if $s(A) > 0$, there is a unique $\bar{S} \in (0, S^0)$ such that $s(B(\bar{S})) = 0$. Then, $(\bar{u}, \delta\bar{w})$ is uniquely determined up to a positive multiple, p, as the positive eigenvector of $B(\bar{S})$. This scalar multiple p is uniquely determined by the steady state equation $S' = 0$ when $\bar{S} < S^0$. If $s(A) \leq 0$, then there may be no $\bar{S}$ for which $s(B(\bar{S})) = 0$ and even if there is one, $\bar{S} \geq S^0$ so no survival steady state exists.

The main result is the following:

Theorem 6.3.1 /Pilyugin & Waltman/*The following hold for (5):*

(a) The washout state is globally attracting when it is locally asymptotically stable in the linear approximation, i. e., when $s(A) < 0$.

(b) there is a positive "survival" steady state if and only if the washout state is unstable in the linear approximation. When it exists, it is unique and asymptotically stable in the linear approximation.

(c) If the washout steady is unstable, then the bacterial population persists. More precisely, there exists $\epsilon > 0$, *independent of initial data, such that for all solutions of (5) satisfying* $u(0) + \delta w(0) > 0$, *there is* $T > 0$ *such that*

$$u(t) + \delta w(t) > \epsilon\,, \quad t > T\,.$$

(d) If $f_u = f_w$, *then the washout state is stable if* $R_0 := MRT \cdot f_u(S^0) < 1$ *and unstable when* $R_0 > 1$. *In the latter case, the survival steady state* $(\bar{S}, \bar{u}, \bar{w})$ *attracts all solutions with* $u(0) + \delta w(0) > 0$.

In part (d), R_0 represents the number of progeny produced by a single cell introduced into the washout steady state.

Pilyugin and Waltman establish the global stability assertion in part (d) by passing to new variables $z = u + \delta w$ and $v = u/z$ and noting that one can reduce the dimension by one since v converges. An interesting open problem is to show that the global stability assertion in (d) holds more generally.

Part (a) is not contained in the results of Pilyugin and Waltman 1999 so we give the argument here. If $s(A) < 0$ then $s(B(S^0 + \epsilon)) < 0$ for sufficiently small $\epsilon > 0$ by continuity of the stability modulus. The first of Eqs. (5) implies that $S' \leq D(S^0 - S)$ so there exists $T > 0$ such that $S(t) < S^0 + \epsilon$ for $t \geq T$.

Consequently, for $t \geq T$,

$$\begin{aligned} u' &\leq (f_u(S^0 + \epsilon) - D)u - \alpha u + \beta\delta w \\ w' &\leq f_w(S^0 + \epsilon)w + \alpha\delta^{-1}u - \beta w \end{aligned} \tag{9}$$

By a well-known comparison theorem (see Theorem B.1 in (Smith and Waltman 1995)), it follows that

$$(u(t), w(t)) \leq (U(t), W(t)) \,, \quad t \geq T$$

where $(U(t), W(t))$ satisfies the linear system obtained by replacing the inequalities by equalities in (9) and the initial conditions $(U(T), W(T)) = (u(T), w(T))$. Because $s(B(S^0 + \epsilon)) < 0$, we conclude that $(U(t), W(t)) \to (0, 0)$ as $t \to \infty$ so the same holds for $(u(t), w(t))$, completing the argument.

6.4 Models of plasmid transfer without wall growth

6.4.1 Stewart and Levin model

Stewart and Levin (1977) presented a model that describes the dynamics of conjugationally transmitted plasmids in bacterial populations. They also analyzed the steady state properties of the model. With some change in notation, their model is

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1} f_u(S)u - \gamma_+^{-1} f_{u_+}(S)u_+ \\ u' &= (f_u(S) - D)u + qu_+ - \bar{\mu}uu_+ \\ u'_+ &= [f_{u_+}(S) - D - q]u_+ + \bar{\mu}uu_+ \end{aligned} \tag{10}$$

where u and u_+ are plasmid-free and plasmid-bearing bacterial population densities and S is the concentration of substrate on which they grow. These populations reproduce at rates $f_u(S)$ and $f_{u_+}(S)$ respectively, with properties as in the previous section. Parameters γ^{-1} and γ_+^{-1} are yield coefficients.

Stewart and Levin model conjugation as a mass action type infectious process for the reaction $u + u_+ \to 2u_+$ with infectious rate constant $\bar{\mu}$. This mass action model of conjugation, similar to that used in epidemiological modeling (Diekmann and Heesterbeek 2000), will be used throughout this paper. Segregation is modelled as if a plasmid-bearing cell has per unit time probability q of losing its plasmid and reverting to a plasmid-free organism.

We briefly summarize the results of Stewart and Levin. The washout steady state $(S^0, 0, 0)$ is locally asymptotically stable if $f_u(S^0) < D$ and unstable if the reverse inequality holds. A unique plasmid-free steady state, $(\lambda, \bar{u}, 0)$ where $\bar{u} = \gamma(S^0 - \lambda)$ and $f_u(\lambda) = D$, exists only when the washout steady state is unstable, i. e. when $f_u(S^0) > D$. The plasmid-free steady state is stable if $\bar{\mu}\bar{u} < D + q - f_{u_+}(\lambda)$. They found that a unique coexistence steady

state (S^*, u^*, u_+^*) will exist if:

$$\bar{\mu}\bar{u} > D + q - f_{u_+}(\lambda)$$

which can be rewritten as

$$\bar{\mu}\bar{u} > \chi D + q$$

where $\chi = 1 - \frac{f_{u_+}(\lambda)}{f_u(\lambda)}$. In the usual case that $\chi > 0$ when the plasmid-bearing population is at a growth disadvantage, the plasmid is maintained and the coexistence steady state exists if the density of plasmid-free organisms is sufficiently large relative to the cost of carrying the plasmid (χ) and the miss-segregation rate q. If

$$f_{u_+}(S) = f_u(S)(1 - c) \tag{11}$$

where c is the fractional energetic cost for plasmid carriage with $0 < c < 1$, the condition for coexistence becomes $\bar{\mu}u > cD + q$.

The following result can be proved in a similar manner as those to follow.

Theorem 6.4.1 *Assume that (11) holds and $\gamma = \gamma_+$. Then the following hold for (10):*

(a) The washout state is globally stable whenever it is locally stable, which holds when $f_u(S^0) < D$.
(b) When $f_u(S^0) > D$, the plasmid-free steady state exists and it is asymptotically stable in the linear approximation if and only if $\bar{\mu}\bar{u} < cD + q$. In this case, it attracts all solutions with $u(0) + u_+(0) > 0$.
(c) When $\bar{\mu}\bar{u} > cD + q$ the unique coexistence steady state exists and attracts all solutions with $u_+(0) > 0$.

6.4.2 Stephanopoulus–Lapidus competition model

Stephanopoulus–Lapidus (1988) proposed a chemostat model of competition between plasmid-free and plasmid-bearing organisms which takes the form (4). It is based on earlier work of Ryder–DiBiasio (1984) who modeled segregation in a much different way than Stewart and Levin. They proposed that a fraction q of the daughter cells of the plasmid-bearing population produced in the time interval $[t, t + \mathrm{d}t]$, given by $f_{u_+}(S)u_+ \,\mathrm{d}t$, acquire no plasmid during cell division, and therefore contribute to the plasmid-free population, while the fraction $1 - q$ acquire one or more plasmid and thus contribute to the plasmid-bearing population. More precisely, of the daughter cells $f_{u_+}(S)u_+ \,\mathrm{d}t$, $qf_{u_+}(S)u_+ \,\mathrm{d}t$ are plasmid-free cells while $(1 - q)f_{u_+}(S)u_+ \,\mathrm{d}t$ are plasmid-bearing cells. This treatment of segregation seems to us more faithful to the biology since miss-segregation is associated with cell division. Cells don't lose plasmid, they just may not get one from the mother cell.

See also Hsu and Waltman (1997, 2004) for a similar approach in a different application.

The model of Stephanopoulus–Lapidus is given by

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1}[f_u(S)u + f_{u_+}(S)u_+] \\ u' &= (f_u(S) - D)u + qf_{u_+}(S)u_+ \\ u'_+ &= [f_{u_+}(S)(1-q) - D]u_+ \end{aligned} \tag{12}$$

It has little to do with gene transfer so we include it here only because we adopt their approach to the modeling of segregation. The model is important in biotechnology where u_+ has been genetically engineered to produce some useful protein but miss-segregation implies that it must compete with the "wild-type" organism u. See the review article of Hsu and Waltman (2004) for more on models of this sort.

6.4.3 A model of gene transfer without wall growth

In this section we consider a model that is similar to the Stewart Levin model except that we employ the modeling of segregation introduced in (Ryder and DiBiasio 1984). Using (11), we are lead to consider the system

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1} f_u(S)[u + (1-c)u_+] \\ u' &= (f_u(S) - D)u + qf_u(S)(1-c)u_+ - \bar{\mu}uu_+ \\ u'_+ &= [f_u(S)(1-c)(1-q) - D]u_+ + \bar{\mu}uu_+ \end{aligned} \tag{13}$$

where u and u_+ are the biomass concentrations of plasmid-free and plasmid-bearing organisms. As all the terms in (13) carry over from previous sections, no further motivation is needed. Observe that the plasmid bearing organism is assumed to have no advantage over the plasmid-free organism.

Adding all three equations in the above model and using the new variable $\Sigma = \gamma(S^0 - S) - u - u_+$ in place of S gives

$$\begin{aligned} \Sigma' &= -D\Sigma \\ u' &= (f_u(S) - D)u + qf_u(S)(1-c)u_+ - \bar{\mu}uu_+ \\ u'_+ &= [f_u(S)(1-c)(1-q) - D]u_+ + \bar{\mu}uu_+ \\ S &= S^0 - \gamma^{-1}(\Sigma + u + u_+) \end{aligned} \tag{14}$$

It follows at once that $\Sigma(t) \to 0$, so the limiting system given by:

$$\begin{aligned} u' &= (f_u(S) - D)u + qf_u(S)(1-c)u_+ - \bar{\mu}uu_+ \\ u'_+ &= [f_u(S)(1-c)(1-q) - D]u_+ + \bar{\mu}uu_+ \\ S &= S^0 - \gamma^{-1}(u + u_+) \end{aligned} \tag{15}$$

is the key to understanding the global dynamics of (13). We observe that in both (14) and (15), there are additional restrictions on the initial data aside from nonnegativity.

It is a routine exercise to show that solutions remain nonnegative. The ultimate boundedness of solutions of (13) is obvious from the fact that $\Sigma \to 0$.

Exactly as for the Stewart and Levin model, the steady states of (13) consist of a washout steady state $(S^0, 0, 0)$, a plasmid-free steady state $(\lambda, \bar{u}, 0)$ where $\bar{u} = \gamma(S^0 - \lambda)$ and a coexistence steady state denoted by (S^*, u^*, u^*_+).

We summarize our main results for (13).

Theorem 6.4.2 *The following hold:*

(a) the washout steady state is globally asymptotically stable whenever it is locally asymptotically stable and this occurs if and only if $f_u(S^0) < D$.
(b) When $f_u(S^0) > D$, *the plasmid-free steady state exists and it is asymptotically stable in the linear approximation if and only if* $f_u(\lambda)(1-c)(1-q) + \bar{\mu}\bar{u} < D$. *It attracts all solutions with* $u(0) + u_+(0) > 0$.
(c) When $f_u(\lambda)(1-c)(1-q) + \bar{\mu}\bar{u} > D$ *then a unique coexistence equilibrium exists and attracts all solutions with* $u_+(0) > 0$.

The proof of this theorem follows from Poincare–Bendixson Theorem and the following lemmas.

The stability of washout steady state of (13) can be determined by the eigenvalues of the variational matrix at $(S^0, 0, 0)$

$$J_1 := \begin{pmatrix} -D & -\gamma^{-1} f_u(S^0) & -\gamma^{-1} f_u(S^0)(1-c) \\ 0 & f_u(S^0) - D & q f_u(S^0)(1-c) \\ 0 & 0 & f_u(S^0)(1-c)(1-q) - D \end{pmatrix}$$

This leads immediately to the following result.

Lemma 6.4.3 *The washout steady state* $(S^0, 0, 0)$ *of (13) is locally asymptotically stable if and only if* $f_u(S^0) < D$.

Lemma 6.4.4 *If* $f_u(S^0) < D$ *then* $u, u_+ \to 0$ *as* $t \to \infty$.

Proof : Since $f_u(S^0) < D$, we can choose $\epsilon > 0$ small enough so that $f_u(S^0) + \epsilon < D$. From the first equation of (13)

$$S' \leq D(S^0 - S)$$

from which we conclude that $\limsup_{t\to\infty} S(t) \leq S^0$. Monotonicity of f_u implies that, for large enough t, $f_u(S(t)) \leq f_u(S^0) + \epsilon/2$, where ϵ is chosen above. Adding the last two equations of (13) and taking $v = u + u_+$ we have for large t,

$$\begin{aligned} v' &= (f_u(S) - D)u + f_u(S)(1-c)u_+ - Du_+ \\ &\leq 2(f_u(S) - D)v \\ &\leq 2(f_u(S^0) + \epsilon/2 - D)v \\ &\leq -\epsilon v\,. \end{aligned}$$

Since $u \geq 0$ and $u_+ \geq 0$, the result follows immediately. □

The criterion for stability of the plasmid-free steady state $(\lambda, \bar{u}, 0)$ of (13) is related to the variational matrix of (14) at the corresponding steady state $(0, \bar{u}, 0)$:

$$J_2 := \begin{pmatrix} -D & 0 & 0 \\ z & z & z + qD(1-c) - \bar{\mu}\bar{u} \\ 0 & 0 & D(1-c)(1-q) - D + \bar{\mu}\bar{u} \end{pmatrix}$$

where $z := -\gamma^{-1} f_u'(\lambda)\bar{u}$ and we have used that $f_u(\lambda) = D$.

Lemma 6.4.5 *The plasmid-free steady state $(\lambda, \bar{u}, 0)$ of (13) is stable if and only if $\bar{\mu}\bar{u} < D[1 - (1-c)(1-q)]$.*

Proof: The variational matrix J_2 of (14) has eigenvalues $-D$ and the eigenvalues of the lower right two-by-two sub-matrix. The eigenvalues of J_2 have negative real parts when the above stated condition is satisfied. □

The plasmid-free steady state $(\lambda, \bar{u}, 0)$ is unstable if

$$\frac{\bar{\mu}\bar{u}}{D} + \frac{f_u(\lambda)(1-c)(1-q)}{D} > 1\,. \tag{16}$$

The first term on the left gives the number of infections produced by a single plasmid-bearing cell in the environment determined by the plasmid-free steady state before being washed out. The second term gives the number of plasmid-bearing daughter cells of a single plasmid-bearing cell before washing out. Of course, the factor $f_u(\lambda)/D = 1$ but we leave it in for interpretations sake. Thus, the sum gives the number of horizontal and vertical transmissions of the plasmid before washout. That number must exceed one for plasmid persistence.

Lemma 6.4.6 *There exists a unique coexistence steady state (S^*, u^*, u_+^*) where $S^0 > S^* > \lambda$ when the plasmid-free steady state is unstable. There can be no coexistence steady state when it is stable.*

Proof: Adding the three steady state equations for (13) gives

$$\gamma(S - S^0) = u + u_+ \tag{17}$$

Solving the second and third equation for u and u_+, we get

$$u = \frac{D - f_u(S)(1-c)(1-q)}{\bar{\mu}}$$

$$u_+ = \frac{[D - f_u(S)]}{f_u(S)(1-c) - D} \frac{D - f_u(S)(1-c)(1-q)}{\bar{\mu}}$$

Substituting these into (17) and a little algebra leads to a single equation for S:

$$\bar{\mu}\gamma(S^0 - S) = cf_u(S)[1 + \frac{qf_u(S)(1-c)}{D - f_u(S)(1-c)}]$$

Positivity of u, u_+ implies that we must have $f_u(S) > D$ and $f_u(S)(1-c) < D$; clearly, $0 < S < S^0$. Let $F(S)$ denote the left hand side and $G(S)$ denote the right hand side of the equality. F is obviously decreasing. The term in square brackets in G is a monotonically increasing function of S and is positive when $D - f_u(S)(1-c) > 0$. Thus $G(S)$ is a monotonically increasing function of S so long as $D - f_u(S)(1-c) > 0$ and it satisfies $G(0) = 0$, $G(\lambda) = D[1-(1-c)(1-q)]$ since $f_u(\lambda) = D$. Thus, there is at most one value of S where $F(S) = G(S)$ on the interval where $D - f_u(S)(1-c) > 0$. Note that $F(\lambda) = \bar{\mu}\bar{u}$. If the plasmid-free state is hyperbolically stable then $F(\lambda) < G(\lambda)$ so the intermediate value theorem gives the unique value $S^* \in (0, \lambda)$ where $F = G$. But $f_u(S^*) < f_u(\lambda) = D$ implying that u and u_+ are not both positive. There exists no coexistence steady state when the plasmid-free state is hyperbolically stable. Similarly, when $\bar{\mu}\bar{u} = D[1-(1-c)(1-q)]$ we get the same contradiction. When $\bar{\mu}\bar{u} > D[1-(1-c)(1-q)]$, that is, when the plasmid-free steady state is unstable, then $F(\lambda) > G(\lambda)$ so $S^* > \lambda$ if it exists. There are two cases depending on whether $D - f_u(S^0)(1-c) > 0$ or $D - f_u(S^0)(1-c) \leq 0$. In the first case, $F(S^0) = 0 < G(S^0)$ so $S^* \in (\lambda, S^0)$ exists by the intermediate value theorem. In the second case, G has a vertical asymptote at some $\tilde{S} \leq S^0$ and in this case too, the intermediate value theorem implies the existence of $S^* \in (\lambda, \tilde{S})$. Since $D - f_u(S^*) < D - f_u(\lambda) = 0$, the values of u and u_+ above are positive. □

The local stability of the coexistence steady state (S^*, u^*, u_+^*) of (13) can be determined from the eigenvalues of the variational matrix of (14) at its corresponding steady state $(0, u^*, u_+^*)$

$$J_3 := \begin{pmatrix} -D & 0 & 0 \\ \cdot & j_{22} & j_{23} \\ \cdot & j_{32} & j_{33} \end{pmatrix}$$

where

$$\begin{aligned}
j_{22} &= f_u(S^*) - D - \gamma^{-1}u^*f_u'(S^*) - q\gamma^{-1}u_+^*f_u'(S^*)(1-c) - \bar{\mu}u_+^* \\
j_{23} &= -\gamma^{-1}u^*f_u'(S^*) + qf_u(S^*)(1-c) - \gamma^{-1}qu_+^*f_u'(S^*)(1-c) - \bar{\mu}u^* \\
j_{32} &= -\gamma^{-1}u_+^*f_u'(S^*)(1-c)(1-q) + \bar{\mu}u_+^* \\
j_{33} &= -\gamma^{-1}u_+^*f_u'(S^*)(1-c)(1-q)
\end{aligned}$$

Note that the entries denoted "$\cdot$" play no role in the stability of coexistence steady state. In the remainder of the proof, we use the notation

$$d = 1 - c\,, \quad p = 1 - q$$

in order to shorten lengthy formulae. The coexistence steady state of (13) is stable if the eigenvalues of the matrix $E = (j_{jk})_{j,k\in\{2,3\}}$ have negative real parts i. e. trace$(E) < 0$ and det$(E) > 0$. Since $\bar{\mu}u_+^* > f_u(S^*) - D$, $j_{22} < 0$ and since $j_{33} < 0$, trace$(E) < 0$. In order to show that det$(E) > 0$ we simplify j_{23} as follows:

$$\begin{aligned} j_{23} &= -\gamma^{-1}u^* f_u'(S^*) + qf_u(S^*)d - q\gamma^{-1}u_+^* f_u'(S^*)d - \bar{\mu}u^* \\ &= -\gamma^{-1}u^* f_u'(S^*) + qf_u(S^*)d - q\gamma^{-1}u_+^* f_u'(S^*)d \\ &\quad - D + f_u(S^*)dp \\ &= -\gamma^{-1}u^* f_u'(S^*) - q\gamma^{-1}u_+^* f_u'(S^*)d - D + f_u(S^*)d \\ &< 0\,. \end{aligned}$$

because the sum of the last two terms is negative. If $\bar{\mu} \geq \gamma^{-1}f_u'(S^*)dp$ then $j_{32} \geq 0$ so $det(E) > 0$ while if $\bar{\mu} < \gamma^{-1}f_u'(S^*)dp$ then

$$\begin{aligned} \det(E) &= -\gamma^{-1}u_+^* f_u'(S^*)dpf_u(S^*) + \gamma^{-1}u_+^* f_u'(S^*)dpD \\ &\quad + \gamma^{-1}u_+^* f_u'(S^*)dpqf_u(S^*)d + \bar{\mu}\gamma^{-1}(u_+^*)^2 f_u'(S^*)dp \\ &\quad - \bar{\mu}u^*\gamma^{-1}u_+^* f_u'(S^*)dp + \bar{\mu}u_+^*\gamma^{-1}u^* f_u'(S^*) \\ &\quad - \bar{\mu}u_+^* qf_u(S^*)d + \bar{\mu}u_+^*\gamma^{-1}u_+^* qf_u'(S^*)d + \bar{\mu}u^*\bar{\mu}u_+^* \\ &= \gamma^{-1}f_u'(S^*)dpu_+^*[\bar{\mu}u_+^* + D - f_u(S^*)] \\ &\quad + qf_u(S^*)du_+^*[\gamma^{-1}f_u'(S^*)dp - \bar{\mu}] \\ &\quad + \gamma^{-1}f_u'(S^*)\bar{\mu}u^*u_+^*[1 - dp] + \gamma^{-1}u_+^* qf_u'(S^*)d + \bar{\mu}u^*\bar{\mu}u_+^* \\ &> 0\,. \end{aligned}$$

The above inequality is true because all three terms inside the square brackets are positive. Thus we have, trace$(E) < 0$ and det$(E) > 0$ and so (S^*, u^*, u_+^*) is locally asymptotically stable.

Lemma 6.4.7 *System (15) has no periodic solutions.*

Proof of Lemma 6.4.7: We apply the Dulac criterion with the auxiliary function

$$g(u, u_+) = \frac{1}{uu_+}$$

to the system (15) and find that

$$\begin{aligned} &\frac{\partial}{\partial u}[g(u,u_+)u'] + \frac{\partial}{\partial u_+}[g(u,u_+)u'_+] \\ &= -\frac{\gamma^{-1}f_u'(S)}{u_+} - q(1-c)\frac{\gamma^{-1}uf_u'(S) + f_u(S)}{u^2} - \frac{\gamma^{-1}f_u'(S)(1-c)(1-q)}{u} < 0\,. \end{aligned}$$

Hence, the Dulac criterion implies that the above system does not have any periodic solution. □

Proof of Theorem 6.4.2: Part (a) follows from Lemma 6.4.4. For parts (b) and (c), we first consider the planer system (15). If $f_u(S^0) > D$ and $f_u(\lambda)(1-c)(1-q) + \bar{\mu}\bar{u} < D$, there are two steady states: the washout state $(0,0)$ is unstable and the plasmid-free state $(\bar{u},0)$ is asymptotically stable and it is attracts all orbits with $u(0) > 0$ by the Poincare–Bendixson theorem and Lemma 6.4.7. If If $f_u(S^0) > D$ and $f_u(\lambda)(1-c)(1-q)+\bar{\mu}\bar{u} > D$, both the washout and the plasmid-free states are unstable and the coexistence state (u^*, u_+^*) is stable. Again, by the Poincare–Bendixson theorem and Lemma 6.4.7, the coexistence state attracts all orbits with initial condition $u_+(0) > 0$.

Now we consider the system (14). For case (b) and (c), all steady states are hyperbolic under our hypotheses so hypotheses $(H1) - (H4)$ of theorem (F.1) of [17] are satisfied. There are no cycles of equilibria, so $(H5)$ is also satisfied. Theorem (F.1) of (Smith and Waltman 1995) implies that those trajectory identified in cases (b) and (c) tend to the locally asymptotically stable steady state. □

Figure 6.2 depicts the invasion of the plasmid-free state by a tiny inoculum of plasmid-bearing organisms. We use (6) for growth and uptake. The output has been scaled by S/a, $u/(a\gamma)$ and $u_+/(a\gamma)$. Parameter values are chosen as in Freter (1983), as used in Jones et al. (2002). In particular, $\gamma = 0.5$,

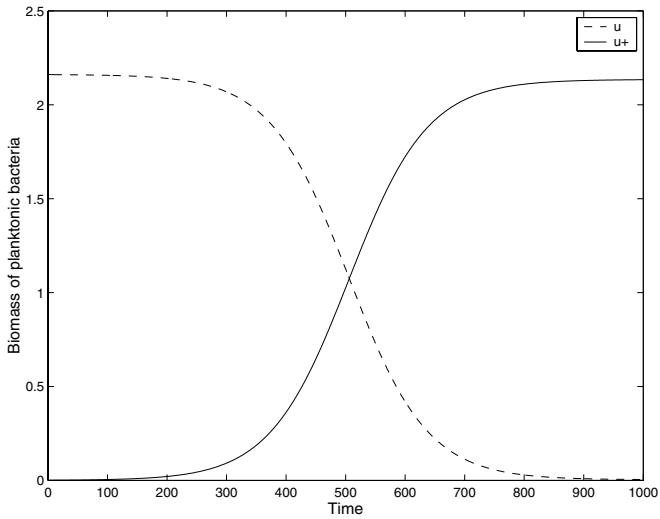

Fig. 6.2. Time series of the invasion of the plasmid-free steady state by an inoculum of plasmid-bearing organisms with $\bar{\mu} = .0018 \times 10^7$

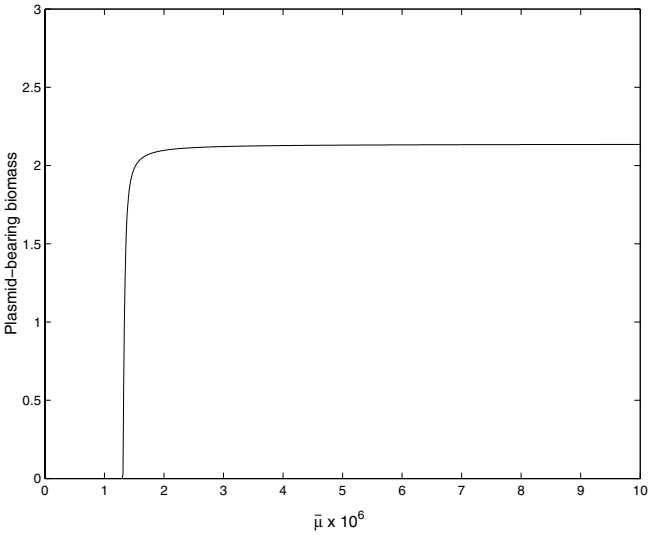

Fig. 6.3. Bifurcation diagram depicting the coexistence steady state value of u_+ versus $\bar{\mu}$

$a = 9\times10^{-7}\,\mathrm{g/ml}$, $m = 1.66\,\mathrm{hr}^{-1}$, $S^0 = 2.09\times10^{-6}\,\mathrm{g/ml}$, $D = 0.23\,\mathrm{hr}^{-1}$, $V = 1\,\mathrm{cm}^3$, $A = 6\,\mathrm{cm}^2$. Simonsen (1991) suggests $c = 0.01$ and $q = 0.0001$. He also points out that the value of $\bar{\mu}$ ($a\gamma\bar{\mu}$ with current scaling) is highly uncertain. We take $\bar{\mu} = .0018\times10^7$, a factor of 10^7 larger than biologically reasonable, in order to satisfy condition (c) of Theorem 6.4.2.

Initial data are chosen to be near the plasmid-free steady state $(\lambda, \bar{u}) = (0.16084, 2.1614)$ with S, u exactly at steady state and $u_+ = 0.001$.

Figure 6.3 plots the coexistence value of u_+ versus the conjugational transfer parameter $\bar{\mu}$. A very large value of $\bar{\mu}$ is required for the persistence of the plasmid-bearing organism, reflecting our assumption that the plasmid confers no advantage on its host.

6.5 A model of gene transfer in biofilms

In this section we obtain our main results concerning (1), restated below for the convenience of the reader.

$$\begin{aligned}
S' &= D(S^0 - S) - \gamma^{-1} f_u(S)\,[u + (1-c)u_+] - \gamma^{-1}\delta f_w(S)\,[w + (1-c)w_+]\\
u' &= (f_u(S) - D)u + q f_u(S)(1-c)u_+ - \alpha u + \beta\delta w - \bar{\mu}uu_+\\
w' &= f_w(S)w + q f_w(S)(1-c)w_+ + \alpha\delta^{-1}u - \beta w - \mu w w_+\\
u'_+ &= [f_u(S)(1-c)(1-q) - D]u_+ - \alpha_+ u_+ + \beta_+\delta w_+ + \bar{\mu}uu_+\\
w'_+ &= f_w(S)(1-c)(1-q)w_+ + \alpha_+\delta^{-1}u_+ - \beta_+ w_+ + \mu w w_+
\end{aligned} \tag{18}$$

Key features of the model are summarized as follows:

1. growth and uptake rates of the plasmid-bearing organism are a factor $1 - c$ lower than those for the plasmid-free organism reflecting the cost of bearing plasmid.
2. adhering and sloughing rates for plasmid-bearing (α_+, β_+) and plasmid-free organism (α, β) may differ.
3. fraction q of daughter cells of plasmid-bearing cells do not receive plasmid.
4. plasmid-bearing organisms transmit plasmid via conjugation to plasmid-free organisms in both fluid and wall environments, though perhaps at different rates $(\bar{\mu} \neq \mu)$.
5. all yield coefficients have been taken to be the same (γ).

The model differs from the one in (Imran et al. preprint) where the plasmid-bearing organism's growth rate, but not its uptake rate, was assumed to be reduced by a factor $1 - c$. See the discussion section for an elaboration of this difference.

Our main focus is on conditions under which the plasmid-bearing organism, whose densities are given by u_+, w_+ can survive. The set $u_+ = w_+ = 0$, where they are absent, is invariant and the equations describing the dynamics on this subset are

$$\begin{aligned} S' &= D(S^0 - S) - \gamma^{-1}\left[f_u(S)u + f_w(S)\delta w\right] \\ u' &= (f_u(S) - D)u - \alpha u + \beta\delta w \\ w' &= f_w(S)w + \alpha\delta^{-1}u - \beta w \end{aligned} \tag{19}$$

We refer to it as the plasmid-free system, noting that it is identical to (5). Our main assumptions concern (19) and are collected in the following:

(H) The washout state $(S^0, 0, 0)$ is unstable for (19) (i. e., $s(B(S^0)) > 0$, see (8)) and the survival state $(\bar{S}, \bar{u}, \bar{w})$ attracts all solutions of (19) satisfying $u(0) + \delta w(0) > 0$.

As noted in Theorem 6.3.1 (d), (H) holds when $f_u = f_w$ and $R_0 > 1$. We ignore the case that the washout state for (19) is stable because then it is a global attractor for (19) by Theorem 6.3.1 (a) and, we conjecture, also for (18) although we do not yet have a proof of this.

The structure of (18) implies a restriction on the types of steady states. Obviously, we have the washout state $(S^0, 0, 0, 0, 0)$ and, we will show that the plasmid-free state $(\bar{S}, \bar{u}, \bar{w}, 0, 0)$ exists when the washout state is unstable. However, there is no comparable "plasmid-bearing" state because the segregational loss of plasmid guarantees that where there are plasmid-bearing cells, there will be plasmid-free cells. Especially important are possible coexistence states $(S^*, u^*, w^*, u_+^*, w_+^*)$ which imply plasmid persistence.

The key question is whether or not the plasmid-bearing population can invade the plasmid-free steady state $(\bar{S}, \bar{u}, \bar{w}, 0, 0)$ leading to the persistence

of the plasmid. The answer comes from the linearization of (18) about the plasmid-free state, the jacobian matrix of which, takes the form

$$J := \begin{pmatrix} J_{3\times 3} & X_{3\times 2} \\ 0_{2\times 3} & C_{2\times 2} \end{pmatrix}$$

where $0_{2\times 3}$ is the zero matrix and $J_{3\times 3}$ is a stable matrix because the plasmid-free state is asymptotically stable for (19) by Theorem 6.3.1. Thus, the stability of the plasmid-free state is determined by the eigenvalues of the submatrix C, given by:

$$\begin{pmatrix} f_u(\bar{S})dp - D - \alpha_+ + \bar{\mu}\bar{u} & \beta_+ \\ \alpha_+ & f_w(\bar{S})dp - \beta_+ + \mu\bar{w} \end{pmatrix} \tag{20}$$

where $d = 1 - c$ and $p = 1 - q$. If the stability modulus, $s(C)$, of C (the largest eigenvalue) is negative then the plasmid-free state is locally attracting; if $s(C) > 0$ then the plasmid-free state is unstable. In this case, the plasmid is maintained.

Theorem 6.5.1 *Assume hypothesis (H) holds. If $s(C) > 0$ then the plasmid-bearing population persists. More precisely, there exists $\epsilon > 0$, independent of initial data, such that for all solutions of (18) satisfying $u_+(0) + \delta w_+(0) > 0$, we have*

$$u_+(t) + \delta w_+(t) > \epsilon \tag{21}$$

for all sufficiently large t. In addition, there is at least one coexistence steady state:

$$(S^*, u^*, w^*, u_+^*, w_+^*)$$

with positive components.

Figure 6.4 depicts the invasion of the plasmid-free state by a tiny inoculum of plasmid-bearing organisms. The output has been scaled by S/a, $u/(a\gamma)$, $\delta w/a\gamma$ and similarly for u_+, w_+. Parameter values are the same used in previous section except for μ, which is taken as in (Imran et al. preprint). Initial data are chosen to be near the plasmid-free steady state $(\bar{S}, \bar{u}, \bar{w}, 0, 0) = (.11, 2.21, .93, 0, 0)$ with S, u and w exactly at steady state and $u_+ = 0.001$, $w_+ = 0$. Observe that the simulation tracks the plasmid-free steady state for the first 60 hours then makes a transition to a coexistence state dominated by wall-adherent, plasmid-bearing cells.

Local existence and positivity of solutions of (18) are standard (see Smith and Waltman 1995). A key to proving Theorem 6.5.1 is establishing a uniform ultimate upper bound on solutions.

Lemma 6.5.2 *All nonnegative solutions of (18) are ultimately uniformly bounded in forward time, and thus they exist for all positive time. In fact,*

$$\limsup_{t\to\infty}(S + \frac{u}{\gamma} + \frac{\delta w}{\gamma} + \frac{u_+}{\gamma} + \frac{\delta w_+}{\gamma}) \leq S^0/b \tag{22}$$

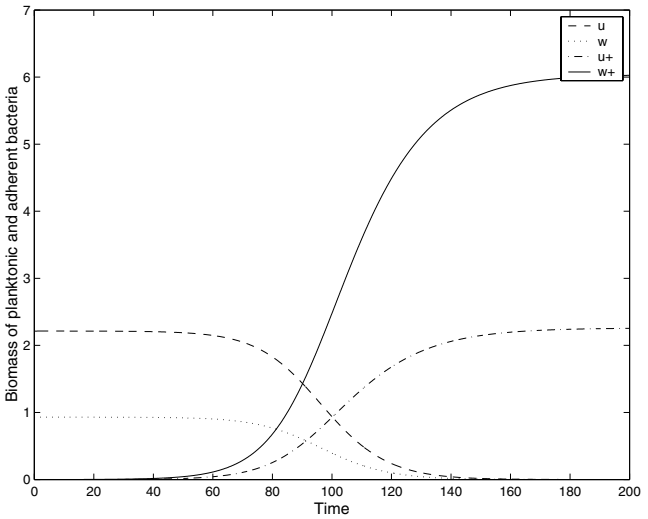

Fig. 6.4. Time series of the invasion of the plasmid-free steady state by inoculum of plasmid-bearing organisms that are better adherers. $\alpha = \alpha+ = \beta = .1, \beta = .4, \mu = 1$ and $\bar{\mu} = .0018$

where $\bar{\beta} = min\{\beta, \beta_+\}$, $d = max\{D + \alpha + \bar{\beta} + f_w(S^0), f_u(S^0)c + D + \alpha_+ + \bar{\beta} + f_w(S^0)\}$, $e = f_u(S^0)$, *and* $b = \frac{-d+\sqrt{d^2+4\bar{\beta}e}}{2e}$.

Proof: From the inequality

$$S' \leq D(S^0 - S)$$

we conclude that $\limsup_{t\to\infty} S \leq S^0$. Monotonicity of f_u and f_w imply that, for given $\epsilon > 0$ we have $f_u(S(t)) \leq f_u(S^0) + \epsilon$ and $f_w(S(t)) \leq f_w(S^0) + \epsilon$, for $t \geq T$

For given a solution we define

$$M(t) = \frac{u + u_+}{u + u_+ + \delta w + \delta w_+} .$$

Then

$$M' = [\frac{(u + u_+)'}{(u + u_+ + \delta w + \delta w_+)}] - [\frac{(u + u_+ + \delta w + \delta w_+)'(u + u_+)}{(u + u_+ + \delta w + \delta w_+)^2}] =: l - n .$$

The first square bracket, l, is

$$l = \frac{(f_u u - Du - \alpha u + \beta\delta w + f_u(1 - c)u_+ - Du_+ - \alpha_+ u_+ + \beta_+\delta w_+)}{(u + u_+ + \delta w + \delta w_+)} ,$$

where $f_u = f_u(S(t))$ and $f_w = f_w(S(t))$. If $a = a(t) := \min\{(f_u - D - \alpha), (f_u(1-c) - D - \alpha_+)\}$, then

$$l \geq aM + \bar{\beta}(1 - M)\,.$$

The second square bracket, n, in M' is

$$\begin{aligned} n &= -\frac{(f_u u - Du + f_w \delta w + f_u(1-c)u_+ - Du_+ + (f_w(1-c)\delta w_+)(u + u_+)}{(u + u_+ + \delta w + \delta w_+)^2} \\ n &\geq \frac{(-f_u u - f_u u_+ - f_w \delta w - f_w \delta w_+)}{(u + u_+ + \delta w + \delta w_+)} M + DM^2 \\ n &\geq -f_w M - f_u M^2 + f_w M^2 + DM^2 \end{aligned}$$

So

$$M' \geq \bar{\beta} + (a - \bar{\beta} - f_w)M + M^2(D + f_w - f_u)\,.$$

Using the result of the first paragraph of the proof, and considering both cases one by one for a, given $\epsilon > 0$, there is $T > 0$ such that

$$\begin{aligned} a - \bar{\beta} - f_w &\geq -d - \epsilon + f_u \\ &\geq -d - \epsilon \end{aligned}$$

for all $t \geq T$. So $M' \geq \bar{\beta} - M(d + \epsilon) - M^2 e/2$. The right hand side of this inequality is a parabola opening down wards. Inside the positive region there is only one stable rest point. Consequently,

$$\frac{-(d+\epsilon) + \sqrt{(d+\epsilon)^2 + 2\bar{\beta}e}}{e} \leq \liminf_{t\to\infty} M$$

and since $\epsilon > 0$ is arbitrary,

$$\frac{-d + \sqrt{d^2 + 2\bar{\beta}e}}{e} \leq \liminf_{t\to\infty} M$$

Let $z = S + \frac{u}{\gamma} + \frac{\delta w}{\gamma} + \frac{u_+}{\gamma} + \frac{\delta w_+}{\gamma}$. Adding the five equations of (1) we find that,

$$z' = D(S^0 - S - \frac{u}{\gamma} - \frac{u_+}{\gamma})$$

For ϵ satisfying $b = \frac{-d+\sqrt{d^2+2\bar{\beta}e}}{e} > \epsilon > 0$, there exists $T > 0$ such that for $t \geq T$

$$u + u_+ \geq [b - \epsilon](u + u_+ + \delta w + \delta w_+)\,.$$

Therefore, for $t \geq T$

$$\begin{aligned} z' &\leq D(S^0 - S) - D\gamma^{-1}(b-\epsilon)(u + u_+ + \delta w + \delta w_+) \\ &\leq D[S^0 - (b-\epsilon)z] \end{aligned}$$

implying that $\limsup_{t\to\infty} z \leq S^0/b$. □

Note that it is critical for the proof that $\beta, \beta_+ > 0$. If, for example, $\beta = 0$, the wall population may grow unboundedly.

Proof of Theorem 6.5.1: We follow a similar argument used in Theorem 5.3 of (Stemmons and Smith 2000), applying Theorem 4.6 in (Thieme 1993). Lemma 22 establishes that (18) has a compact attractor so that the dissipativity requirement of Theorem 4.6 holds.

Using the notation of that result, we set $X = R^5_+$, $X_2 = \{(S, u, w, u_+, w_+) \in X : u_+ = 0 \text{ or } w_+ = 0\}$, and $X_1 = X \setminus X_2$. Observe that solutions of (18) starting in X_2 immediately enter X_1, where $u_+, w_+ > 0$, unless $u_+(0) = w_+(0) = 0$. We want to show that solutions which start in X_1 are eventually bounded away from X_2. Using the notation $x(t) = (S(t), u(t), w(t), u_+(t), w_+(t))$ for a solution of (18), define

$$Y_2 = \{x(0) \in X_2 : x(t) \in X_2, t \geq 0\} = \{x(0) \in X : u_+(0) = w_+(0) = 0\}$$

and Ω_2, the union of omega limit sets of solutions starting in X_2, is, by our hypotheses, the set $\{E_0, E_1\}$ where $E_0 := (S^0, 0, 0, 0, 0)$ and $E_1 := (\bar{S}, \bar{u}, \bar{w}, 0, 0)$. We will show that if $M_0 = \{E_0\}$ and $M_1 = \{E_1\}$, then $\{M_0, M_1\}$ is an isolated acyclic covering of Ω_2 in Y_2 and each M_i is a weak repeller. All solutions starting in Y_2 but not on the S-axis converge to E_1 while those on the axis converge to E_0. E_1, being locally asymptotically stable relative to Y_2, cannot belong to the alpha limit set of any full orbit in X_2 different from E_1 itself. Similar arguments apply to E_0; the only solutions converging to it lie on the S-axis and these are either unbounded or leave X in backward time. Thus $\{M_0, M_1\}$ is an acyclic covering of Ω_2. If M_1 were not a weak repeller for X_1, there would exist an $x(0) \in X_1$ such that $x(t) \to E_1$ as $t \to \infty$. Let $V(t) = (u_+(t), \delta w_+(t))^t$ and define the matrix $P(f(S), u, w)$ $(f = (f_u, f_w))$ by

$$\begin{pmatrix} f_u(S)(1-c)(1-q) - D - \alpha_+ + \bar{\mu}u & \beta_+ \\ \alpha_+ & f_w(S)(1-c)(1-q) - \beta_+ + \mu w \end{pmatrix} \tag{23}$$

Then $A = P(f(\bar{S}), \bar{u}, \bar{w})$ and we may write the equation satisfied by $V(t)$ as

$$\dot{V} = P(f(\bar{S}), \bar{u}, \bar{w})V + [P(f(S), u, w) - P(f(\bar{S}), \bar{u}, \bar{w})]V$$

If $P(f(\bar{S}), \bar{u}, \bar{w})^t W = qW$ where $q = s(P(f(\bar{S}), \bar{u}, \bar{w})) = s(C) > 0$ and $W = (m, n)^t$ with $m, n > 0$ is the Perron–Frobenius eigenvector, then on taking the scalar product of both sides of the differential equation by W and using

that $S(t) \to \bar{S}$ and $w(t) \to \bar{w}$, we have

$$\frac{\mathrm{d}}{\mathrm{d}t}(mu_+ + n\delta w_+) \geq q/2(mu_+ + n\delta w_+)$$

for all large t. But this leads to the contradiction to $x(t) \to E_1$, namely that $mu_+(t)+n\delta w_+(t) \to \infty$ as $t \to \infty$. Thus M_1 is a weak repeller. The argument above together with the fact that E_1 is locally asymptotically stable relative to the subspace $(u_+, w_+) = (0, 0)$ implies that it is an isolated compact invariant set in X. Similar arguments show that M_0 is a weak repeller and an isolated compact invariant set in X. Therefore, Theorem 4.6 in (Thieme 1993) implies our result: there exists $\epsilon > 0$ such that $\liminf_{t\to\infty} d(x(t), X_2) > \epsilon$ for all $x(0) \in X_1$, where $d(x, X_2)$ is the distance from x to X_2.

The existence of at least one coexistence steady state follows from Theorem 1.3.7 of (Zhao 2003). □

6.6 Discussion

Building on previous work, we have constructed a model of gene transfer between micro-organisms in a heterogeneous environment consisting of a biofilm immersed in a fluid medium. The chemostat setting of our model may not be appropriate in many natural environments so we point out here how the model can be modified for different settings (but see also (Imran et al. preprint) for a spatially explicit setting). Equations (1) reflect the chemostat mainly due to the fact that the same term D serves simultaneously as the input rate of supply of fresh substrate, the outflow rate of unused substrate and the removal rates of planktonic cells, both u and u_+. If one replaces the removal rates of planktonic cells by a parameter D', possibly distinct from D, then (1) is extended in a way that may better capture natural environments. Even in the chemostat setting, one may view D' as $D + d$ where d is a death rate of bacteria. Our analysis continues to hold although one must modify slightly Lemma 5.2 and note that the quoted results of Pilyugin and Waltman (1999) have not been established in this setting.

Our model (1) represents a slight modification of the one originally proposed in (Imran et al. preprint). In (Imran et al. preprint), it was assumed that plasmid bearing organisms have the same nutrient uptake rate as plasmid-free organisms but their growth rates are reduced by a factor $1 - c$; bearing plasmid negatively affects growth but not uptake. This implies that the effective yield of plasmid-bearing organisms is reduced by this same factor. In the present work, we follow previous workers by assuming that both uptake and growth of plasmid-bearing organisms are reduced by the factor $1 - c$ so the yield remains the same. In other words, it is assumed that bearing plasmid reduces both uptake and growth rates. This assumption has the effect of greatly improving the mathematical tractability of the model of gene transfer without wall growth (because a conservation relation holds) but does

not significantly affect the analysis of (1). It is likely that uptake and growth rate are affected differently and that the magnitude of each effect depends both on the particular microorganism and on the particular plasmid.

In order to better understand our model, we have reviewed previous work where the key modeling ideas were first developed. These include the work of Levin and Stewart from which most of the mathematical modeling of plasmid transfer can be traced, work of Ryder and DiBiasio (1984) and Stephanopolis and Lapidus (1988) where a more realistic modeling of miss-segregation was proposed, and the work of Pilyugin and Waltman (1999) whose simple biofilm model forms the basis of our model. Our focus in the present chapter, as in these earlier works, is on understanding the conditions that allow the plasmid to persist in a bacterial population despite conferring a growth disadvantage to its bearer and despite the occasional leakage in its vertical transmission from mother to daughter cells. In each of the models considered here, the key to understanding plasmid persistence lies in determining the basic reproduction number R_0, the number of plasmid-bearing progeny that a hypothetical single plasmid-bearing cell would leave if introduced into the plasmid-free steady state environment. These progeny consist of daughter cells born with plasmid and formerly plasmid-free organisms that have acquired the plasmid via conjugation. In the chemostat setting of these models, a cell eventually washes out so a key quantity involved in the calculation of R_0 is the mean residence time (MRT) in the chemostat. Plasmid persistence requires that $R_0 > 1$.

The Stewart and Levin model (10) is chemostat based so MRT $= 1/D$ but a plasmid-bearing cell reverts to a plasmid-free cell at rate q so the mean time our plasmid-bearing cell remains in the chemostat and remains plasmid-bearing is $1/(D+q)$. This leads to

$$R_0 = [f_{u_+}(\lambda) + \bar{\mu}\bar{u}]/(D+q)$$

The term $f_{u_+}(\lambda)/(D+q)$ gives the number of daughter cells born to the single plasmid-bearing cell before washout. The term $\bar{\mu}\bar{u}/(D+q)$ gives the number of plasmid-free cells infected by the plasmid-bearing cell before it washes out. Consequently, the condition $R_0 > 1$ for plasmid persistence just says that the number of plasmid-bearing progeny must exceed one.

Our model of gene transfer without wall growth (13) contains the more realistic modeling of miss-segregation developed by Ryder and DiBiasio (1984) and Stephanopolis and Lapidus (1988). This model does not allow a plasmid-bearing cell to revert to a plasmid-free cell-only one of its daughter cells can be plasmid free. In this case

$$R_0 = [f_u(\lambda)(1-c)(1-q) + \bar{\mu}\bar{u}]/D$$

The interpretation is similar to that above since $f_{u_+}(S) = f_u(S)(1-c)$ but one must discount the progeny $qf_u(\lambda)(1-c)/D$ of our single plasmid-bearing cell that do not carry the plasmid.

We would like to determine the basic reproductive number for our gene transfer model in a biofilm (Berman and Plemmons 1979). How are we to reinterpret the condition for plasmid persistence

$$s(C) > 0$$

in biological terms? As we will see, the Perron–Frobenius theory (see [2]) gives estimates of $s(C)$ which are biologically interpretable. Let

$$Q_+ := \begin{pmatrix} -D - \alpha_+ & \beta_+ \\ \alpha_+ & -\beta_+ \end{pmatrix}$$

In the appendix we show that the mean residence time of a plasmid-bearing cell in the chemostat is given by

$$\mathrm{MRT}_+ = -1/s(Q_+)$$

Define

$$\begin{aligned} k &:= \min\{f_u(\bar{S})dp + \bar{\mu}\bar{u}, f_w(\bar{S})(1-c)(1-q) + \mu\bar{w}\} \\ K &:= \max\{f_u(\bar{S})dp + \bar{\mu}\bar{u}, f_w(\bar{S})(1-c)(1-q) + \mu\bar{w}\} \end{aligned}$$

Then

$$kI + Q_+ \le C \le KI + Q_+$$

which implies that

$$k + s(Q_+) = s(kI + Q_+) \le s(C) \le s(KI + Q_+) = K + s(Q_+)\,.$$

Consequently, we may express $s(C)$ as follows

$$s(C) = F_+[f_u(\bar{S})dp + \bar{\mu}\bar{u}] + (1 - F_+)[f_w(\bar{S})dp + \mu\bar{w}] + s(Q_+)$$

for some F_+ with $0 \le F_+ \le 1$. The condition $0 < s(C)$ for plasmid persistence can then be equivalently expressed as $0 < -s(C)/s(Q_+)$, or as

$$1 < F_+[f_u(\bar{S})dp + \bar{\mu}\bar{u}] + (1 - F_+)[f_w(\bar{S})dp + \mu\bar{w}]) \cdot \mathrm{MRT}_+ \qquad (24)$$

Inequality (24) can be viewed as requiring that a single plasmid-bearing cell, introduced into the plasmid-free steady state, leave more than one plasmid-bearing progeny in order for persistence of the plasmid. Indeed, the term

$$f_u(\bar{S})dp \cdot \mathrm{MRT}_+$$

gives the number of daughter cells carrying plasmid born of the single plasmid-bearing cell before it washes out of the chemostat, assuming that it resides in the fluid during this time. Replacing the first factor by $f_w(\bar{S})dp$

gives the corresponding number of plasmid-bearing progeny assuming that the cell spends its time adhering to the wall. The term

$$\bar{\mu}\bar{u} \cdot \mathrm{MRT}_+$$

gives the number of infectious transfers of plasmid from our single plasmid-bearing cell to plasmid-free cells before washout, assuming that it resides in the fluid during this time. Replacing the first factor by $\mu\bar{w}$ gives the corresponding number of infectious tranfers of plasmid given that the cell resides on the wall during its time in the chemostat.

But we must take into account that our lone plasmid-bearing cell, introduced into the plasmid-free steady state, will spend a certain fraction F_+ of its residence time in the fluid and a complementary fraction $1 - F_+$ on the wall. In this way, the interpretation of (24)is clear-a plasmid-bearing cell must leave more than one plasmid-bearing progeny.

Diekmann and Heesterbeek (2000) (see Thm 6.13) show that

$$s(C) > 0 \Leftrightarrow \rho(-TQ_+^{-1}) > 1$$

where $\rho(A)$ denotes the spectral radius of A,

$$T = \mathrm{diag}[f_u(\bar{S})dp + \bar{\mu}\bar{u}, f_w(\bar{S})dp + \mu\bar{w}]$$

and

$$-Q_+^{-1} = \begin{pmatrix} 1/D & 1/D \\ \alpha_+/D\beta_+ & (\alpha_+ + D)/D\beta_+ \end{pmatrix}$$

See (Imran et al. preprint) for a discussion of the biological meaning for the entries of this matrix.

Thus they are lead to define the basic reproductive number

$$R_0 = \rho(-TQ_+^{-1})\,.$$

We observe that

$$\rho(-Q_+^{-1}) = -1/s(Q_+) = \mathrm{MRT}_+$$

where the subscript "+" denotes that the plasmid-bearing organisms parameters α_+ and β_+ are used. Using the Perron–Frobenius theory, with $\rho(-TQ_+^{-1})$ instead of $s(C)$, as before leads to

$$R_0 = (F_+[f_u(\bar{S})dp + \bar{\mu}\bar{u}] + (1 - F_+)[f_w(\bar{S})dp + \mu\bar{w}]) \cdot \mathrm{MRT}_+ \qquad (25)$$

Let's return to the biology and first ask whether the plasmid could survive if it confers no advantage in biofilm forming ability on its host. That is, assuming:

$$\alpha = \alpha_+\,, \quad \beta = \beta_+\,,$$

can the plasmid survive as a parasite. This question was considered by Stewart and Levin (1977) for the simple chemostat-based model ignoring wall growth. The question boils down to whether or not its advantage in horizontal spread outweighs its growth and segregation disadvantages. In this case, both plasmid-bearing and plasmid-free cells have identical mean residence times in the chemostat, MRT = MRT_+. In order to interpret formula (24) in this case, consider that at the plasmid-free steady state each plasmid-free organism leaves exactly one daughter cell. Thus

$$F \cdot f_u(\bar{S}) \cdot \text{MRT} + (1 - F) \cdot f_w(\bar{S}) \cdot \text{MRT} = 1$$

Not surprisingly, in the absence of horizontal transmission $\mu = \bar{\mu} = 0$, a parasitic plasmid cannot satisfy the persistence condition since $F = F_+$ in that case. In the interesting case that $\mu, \bar{\mu} > 0$, we cannot assert that $F = F_+$ since F_+ depends on μ and $\bar{\mu}$. However, it is reasonable to speculate that $F - F_+$ is small. Putting $F = F_+$, (24) for plasmid persistence becomes:

$$\text{MRT} \cdot [F \cdot \bar{\mu}\bar{u} + (1 - F) \cdot \mu\bar{w}] > 1 - (1 - c)(1 - q) = c + q - cq \qquad (26)$$

The left hand side of the inequality gives the number of plasmid transfers made by a single plasmid-bearing cell before being washed out of the chemostat. It must exceed a positive threshold which depends on the cost of carriage c and the probability of miss-segregation q for the plasmid to survive.

Equation (26) indicates that the conjugation terms must exceed a threshold to maintain a parasitic plasmid. In order to see this more clearly, we fix $\alpha = \alpha_+ = 0.1$ and $\beta = \beta_+ = 0.4$, with all other parameters as in Fig. 6.4, except that $\bar{\mu} = \mu \times 10^{-3}$. We then vary μ and plot the resulting stable steady state value of $u_+ + \delta w_+$ in Fig. 6.5. This was done by integrating the differential equations, starting with a tiny inoculum of plasmid-bearing cells, to steady state. If $u_+ + \delta w_+ = 0$, as it does for small μ, that means the stable steady state is the plasmid-free state; if $u_+ + \delta w_+ > 0$, then we are plotting the coexistence steady state value of $u_+ + \delta w_+$. This bifurcation diagram shows that the critical value $\mu_c \approx 6 \times 10^4$ at which the coexistence steady state appears is much larger than our order of magnitude estimate of a biologically reasonable value of $\mu \approx 1$.

We are particularly interested in the case that the plasmid-free organism cannot form a macroscopically significant biofilm, for example, this may mean that it can form only a monolayer (see e. g. Pratt and Kolter (1998) and O'Toole and Kolter (1998)), while the plasmid-bearing organism can form a healthy biofilm. In this case, it is reasonable to assume that the plasmid-bearing organisms sloughing rate does not exceed that of the plasmid-free organism and that its adhesion rate constant is not less than that of the plasmid-free organism:

$$\beta_+ \leq \beta \quad \text{and} \quad \alpha \leq \alpha_+. \qquad (27)$$

Strict inequality is assumed to hold in at least one of these. In this case, as noted in the appendix, the plasmid-bearing organism has a residence time

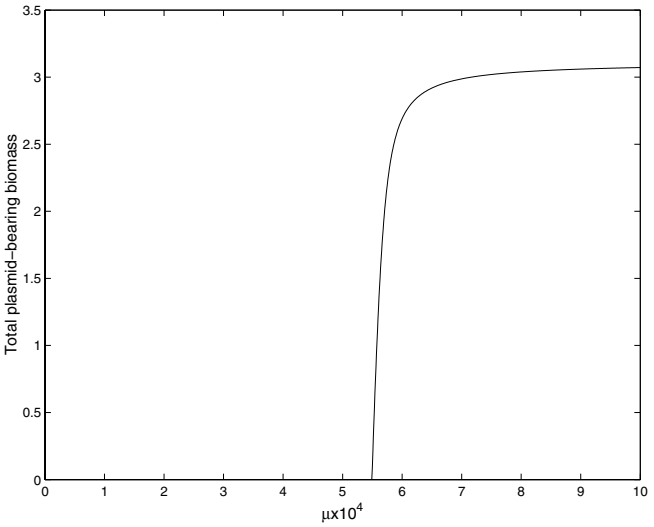

Fig. 6.5. Bifurcation of coexistence steady state from the plasmid-free steady state at a critical value of μ. Vertical axis is the coexistence steady state value of total plasmid-bearing biomass $u_+ + \delta w_+$. Horizontal axis is the conjugation rate μ

advantage over its plasmid-free rival:

$$\mathrm{MRT}_+ > \mathrm{MRT}$$

and it should spend more of this time on the wall than the plasmid-free organism so we conjecture that:

$$F > F_+$$

where, presumably, bacterial densities are higher than in the fluid state and contact rates between organisms are higher:

$$\bar{\mu}\bar{u} >> \mu\bar{w}\,.$$

(24) says that these advantages must outweigh the cost of carriage and segregational loss.

6.7 Appendix: Mean residence times

Consider the linear compartmental system with no inputs

$$x' = Ax \tag{28}$$

where A is a stable, quasi-positive ($a_{ij} \geq 0, i \neq j$), irreducible $n \times n$ matrix satisfying

$$A = B - C$$

where $C > 0$ and B is quasi-positive with zero column sums. Matrix B accounts for the "internal transitions" of mass between compartments while C accounts for the loss of mass to the external environment. To see this, let 1 be the vector of ones. The total mass of x is $1 \cdot x$. Then the rate of loss from the system to the environment is given by

$$(1 \cdot x)' = 1 \cdot Ax = 1 \cdot Bx - 1 \cdot Cx = -1 \cdot Cx \leq 0\,.$$

Let $x_0 > 0$ be such that $1 \cdot x_0 = 1$. We call x_0 a probability vector. If we start the system off at x_0, then $x(t) = \mathrm{e}^{At}x_0$. The probability of still being in the system at time t is given by

$$P(\text{in system at time t}) = \int_t^\infty 1 \cdot Cx(s)\,\mathrm{d}s = 1 \cdot C\int_t^\infty x(s)\,\mathrm{d}s$$

The mean residence time (MRT) is given by

$$\begin{aligned}
\mathrm{MRT}(x_0) &= \int_0^\infty t1 \cdot Cx(t)\,\mathrm{d}t \\
&= \int_0^\infty C\int_t^\infty x(s)\,\mathrm{d}s\,\mathrm{d}t \cdot 1 \\
&= \int_0^\infty C\int_t^\infty \mathrm{e}^{As}\,\mathrm{d}s\,\mathrm{d}t x_0 \cdot 1 \\
&= C\int_0^\infty \mathrm{e}^{At}\int_t^\infty \mathrm{e}^{A(s-t)}\,\mathrm{d}s\,\mathrm{d}t x_0 \cdot 1 \\
&= C\int_0^\infty \mathrm{e}^{At}\int_0^\infty \mathrm{e}^{Ar}\,\mathrm{d}r\,\mathrm{d}t x_0 \cdot 1 \\
&= C(\int_0^\infty \mathrm{e}^{Ar}\,\mathrm{d}r)^2 x_0 \cdot 1 \\
&= C(-A^{-1})^2 x_0 \cdot 1
\end{aligned}$$

where we have used that

$$-A^{-1} = \int_0^\infty \mathrm{e}^{At}\,\mathrm{d}t\,.$$

We see that the $\mathrm{MRT}(x_0)$ depends on x_0, the initial distribution among the compartments. In order to obtain a quantity that is independent of the initial distribution, we might average the above quantity over the standard simplex of probability vectors $\Sigma = \{x_0 \in \mathbb{R}^n : x_0 \geq 0,\ x_0 \cdot 1 = 1\}$, obtaining the result

$$\mathrm{MRT} = \frac{1}{|\Sigma|}\int_\Sigma C(-A^{-1})^2 x_0 \cdot 1\,\mathrm{d}S(x_0) = \frac{1}{n}C(-A^{-1})^2 1 \cdot 1$$

where $|\Sigma|$ denotes the area of Σ.

Instead, we note that the matrix A has a dominant eigenvector v corresponding to $s(A) := \max \Re\lambda$ where the maximum is taken over all eigenvalues of A.

$$Av = s(A)v\,, \quad 1 \cdot v = 1\,, \quad v > 0\,.$$

$s(A)$, the stability modulus of A, is an eigenvalue and our assumptions are that $s(A) < 0$. All non-negative solutions of (28) are asymptotic to $x(t) = \mathrm{e}^{s(A)t}v$.

If $x_0 = v$, then we may easily compute $\mathrm{MRT}(v)$ from the formula above:

$$\mathrm{MRT}(v) = CA^{-2}v \cdot 1 = \frac{1}{s(A)^2} Cv \cdot 1\,.$$

On the other hand, with $x_0 = v$, $x(t) = \mathrm{e}^{s(A)t}v$ so $(1 \cdot x(t))' = s(A)\mathrm{e}^{s(A)t}1 \cdot v$. Thus

$$P(\text{leave system at between } t \text{ and } t + \mathrm{d}t) = -s(A)\mathrm{e}^{s(A)t}1 \cdot v\,\mathrm{d}t$$

so we define MRT for (28) to be:

$$\mathrm{MRT} = \int_0^\infty 1 \cdot \mathrm{e}^{s(A)t}v\,\mathrm{d}t = \int_0^\infty \mathrm{e}^{s(A)t}\,\mathrm{d}t 1 \cdot v = \frac{-1}{s(A)}\,.$$

As an example consider the case

$$A = A(\alpha, \beta, D) := \begin{pmatrix} -D - \alpha & \beta \\ \alpha & -\beta \end{pmatrix} = \begin{pmatrix} -\alpha & \beta \\ \alpha & -\beta \end{pmatrix} - \begin{pmatrix} D & 0 \\ 0 & 0 \end{pmatrix}$$

A simple calculation gives

$$s(A) = -\left[\frac{D + \alpha + \beta - \sqrt{(D + \alpha + \beta)^2 - 4\beta D}}{2}\right]\,. \tag{29}$$

The corresponding eigenvector of unit mass is given by

$$v = [-s(A)/D, (1 + s(A)/D)]^{\mathrm{T}}\,.$$

Using

$$A^{-1} = \frac{1}{D\beta}\begin{pmatrix} -\beta & -\beta \\ -\alpha & -D - \alpha \end{pmatrix}$$

we find that

$$\mathrm{MRT}(x_0) = \frac{(\beta + \alpha)x_0^1 + (\beta + \alpha + D)x_0^2}{D\beta}\,.$$

Obviously,

$$\frac{(\beta+\alpha)}{D\beta} \leq \mathrm{MRT}(x_0) \leq \frac{(\beta+\alpha+D)}{D\beta}$$

where the two extremes arise from starting with all cells in the fluid and starting with all cells on the wall. In particular, we obtain the estimate

$$\frac{1}{D} < \frac{(\beta+\alpha)}{D\beta} \leq \mathrm{MRT} = -\frac{1}{s(A)} \leq \frac{(\beta+\alpha+D)}{D\beta}\,. \tag{30}$$

Recall that $1/D$ is the mean residence time for a bacterial cell in the fluid for the classical chemostat model. The possibility of a cell adhering to the wall obviously has the consequence that the mean residence time in the chemostat increases. The inequality (30) also implies $v > 0$.

Simple calculations give that

$$\frac{\mathrm{d}}{\mathrm{d}\beta}\mathrm{MRT} < 0\,, \quad \frac{\mathrm{d}}{\mathrm{d}\alpha}\mathrm{MRT} > 0\,.$$

It is intuitive that the mean residence time increases with the wall affinity α and decreases with the sloughing rate β.

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