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Long-term dynamics of catabolic plasmids introduced to a microbial community in a polluted environment: a mathematical model

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Abstract

The long-term dynamics of mobile plasmids in natural environments are unclear. This is the first study of the long-term dynamics of introduced plasmids with xenobiotic degradation abilities using a mathematical model that describes the horizontal gene transfer (HGT) of plasmids into indigenous bacteria via conjugation. We focussed on negative feedback between the spread of plasmids and their selective advantage, i.e. the severe competition between plasmid-bearing and plasmid-free bacteria resulting from a decrease in xenobiotic concentration caused by the gene expression of plasmids, favoring plasmid-free bacteria. Two types of HGT enhanced the persistence of plasmids and the degradation of the xenobiotic in different conditions: a relatively low rate of 'intergeneric HGT' from introduced to indigenous bacteria and a high rate of 'intraindigenous HGT' from indigenous to indigenous bacteria. In addition, when the indigenous resource supply rate was high and when the cost of bearing plasmids was low, both types of HGT made large contributions to xenobiotic degradation compared to the contribution of vertical transfer via plasmid replication within the introduced host population. Initial conditions were also important; a higher initial density of introduced plasmid-bearing bacteria led to a lower degradation rate over a long time scale.

Introduction

Some bacteria have rare genes that allow them to degrade xenobiotics that are usually resistant to environmental degradation. These catabolic genes are often located on mobile plasmids (Top *et al*., 2002). Therefore, as a bioaugmentation strategy, the horizontal transfer of catabolic plasmids from an introduced donor host into actively growing indigenous bacteria via conjugation, and the subsequent gene expression have been widely studied since the early 1990s (Fulthorpe & Wyndham, 1991; Bathe *et al*., 2004a, 2005). Most previous studies have focussed on the conditions required for an initial increase in transconjugants (for a review see van Elsas & Bailey, 2002): a nonsterile environment for rapid growth of recipient and donor bacteria to promote frequent conjugation (Top *et al*., 1990; Neilson *et al*., 1994), a sufficiently high transfer rate per conjugation (Neilson *et al*., 1994), a broad host range for plasmids (Pukall *et al*., 1996; Bathe *et al*., 2004b), effective gene expression in transconjugants (Kinkle *et al*., 1993), and a high concentration of the pollutant to create a selective advantage for plasmid-bearing bacteria over other indigenous bacteria (Top *et al*., 1998; Hohnstock *et al*., 2000; de Lipthay *et al*., 2001). These conditions are indicated by the short-term dynamics of plasmids after the introduction of extrinsic donor bacteria; the short-term dynamics of plasmids result in a decrease in the introduced donor bacteria and an increase in transconjugants because of competition and a reduction in the concentration of the xenobiotic (e.g. Top *et al*., 1998). However, over the long term, the competitive advantage of plasmid-bearing bacteria decreases with the spread of plasmids in the bacterial community, because xenobiotic degradation by plasmid-bearing bacteria indirectly facilitates the growth and survival of plasmid-free bacteria that are sensitive to xenobiotics. Thus, plasmids in transconjugants will disappear once the xenobiotic concentration is reduced, if maintenance costs or segregation rate are high, or if horizontal transfer rate is low (Simonsen, 1991; Bergstrom *et al*., 2000). Therefore, the successful spread of transconjugants in short-term experiments does not ensure a sufficient reduction in xenobiotic concentration through the long-term maintenance of transconjugants. Thus, it is necessary to evaluate the long-term dynamics of introduced plasmids and their effects on the indigenous bacterial community to predict the final fate of introduced plasmids in natural environments and determine the

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effective application of bioaugmentation based on conjugal gene transfer. In particular, we focussed on how long plasmids are maintained in transconjugants in xenobiotic-polluted environments, and how effectively plasmid-bearing bacteria degrade the xenobiotic over the long term.

We also examined the self-transmissibility of plasmids within different hosts (self-transmissible vs. mobilizable; see Simonsen, 1991; Hoffmann *et al.*, 1998), i.e. the transfer range, as an important determinant of the long-term persistence of plasmids. In short-term experiments, the increase in plasmid-bearing bacteria is mainly attributed to the vertical transfer of plasmids within the introduced host (i.e. the replication of plasmids within hosts and partitioning of copies to their descendants) or the horizontal transfer of plasmids from the introduced donor host to the indigenous recipient bacteria, or both. However, after this initial phase, further increases in transconjugants may also be attributed to vertical transfer of plasmids within transconjugants and horizontal transfer from transconjugants to indigenous recipients. If plasmids in the transconjugants are self-transmissible, the transconjugants in turn become donors and supply plasmids to new recipients. This will enhance the rate of spread of plasmids in the community (e.g. Dionisio *et al.*, 2002). The comparison of plasmid dynamics among different transfer ranges will clarify the relative importance of vertical transfer within the original introduced host, horizontal transfer from the introduced host to indigenous bacteria, and vertical and horizontal transfer within indigenous bacteria in the spread of plasmids.

Here we considered the introduction of a nonindigenous bacterial host containing catabolic plasmids with genes to degrade xenobiotics and the subsequent horizontal gene transfer (HGT) into the indigenous bacterial community in polluted environments containing indigenous bacteria that cannot degrade xenobiotics. We used a modeling approach to investigate the long-term outcome of such a scenario for two reasons. First, it would be difficult to perform such a long-term experiment (e.g. 10 years) to monitor the behavior and conditions for the successful spread of plasmids. Second, mathematical modeling allows the comparison of multiple scenarios using different values for parameters such as the plasmid transfer rate and cost of bearing plasmids, which are important determinants for the successful spread of plasmids, but are difficult to manipulate experimentally using current technology. In addition, a modeling approach is useful to develop experimentally testable predictions and to suggest directions for future empirical work.

Using the mathematical model, we showed that differences in plasmid dynamics for different transfer ranges largely depend on the transfer rate; we investigated how the relative importance of horizontal and vertical plasmid transfer changes with the rate of gene transfer. In addition, we investigated the effects of other growth-related traits of plasmids (cost of bearing plasmids, segregation rate of plasmids, and their host range) and various environmental factors (a growth-limiting resource supply, initial xenobiotic concentration, xenobiotic degradation rate) on plasmid dynamics. Thus, we clarified how and under what conditions conjugal gene transfer contributes to the long-term spread of introduced genes and the degradation of xenobiotics.

Materials and methods

Model

To model highly diverse natural bacterial communities as simply as possible, we categorized introduced bacteria (X) and indigenous bacteria (Y) into five types (Fig. 1): an introduced plasmid-bearing bacterial strain (x_P); an introduced plasmid-free bacterial strain (x_F); potential recipient indigenous plasmid-free bacteria (y_F); transconjugant bacteria, i.e. indigenous recipients that have received the plasmid (y_P); and nonconjugative groups of bacteria in the indigenous community (y_{NC}), which are not a potential recipient of focal plasmids. Note that the host range of plasmids (HR) can be represented by the initial fraction of potential recipients in indigenous bacteria [$HR = y_F(0)/(y_F(0)+y_{NC}(0)$]. This simplified community, which excludes the diversity and heterogeneity of potential recipients consisting of multiple species and strains, was a first step in understanding the long-term dynamics of plasmids in the natural environment. Note that this model was an expansion of those of Stewart & Levin (1977) and Levin *et al.* (1979).

In addition, we examined the dynamics of an indigenous growth-limiting carbon resource (N_C) and an extrinsic

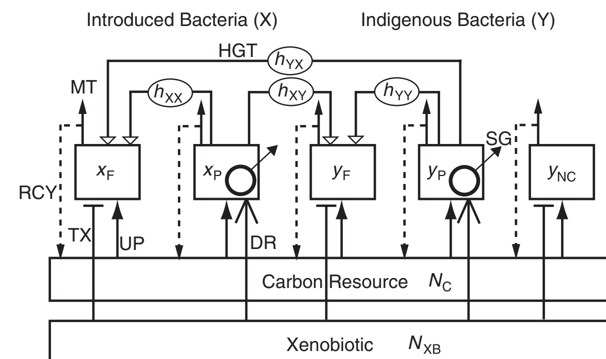

Fig. 1. Model flow diagram. X, introduced bacteria; Y, indigenous bacteria; x_F, plasmid-free introduced bacteria; x_P, plasmid-bearing introduced bacteria; y_F, plasmid-free indigenous bacteria; y_P, plasmid-bearing indigenous bacteria; y_{NC}, plasmid-free nonconjugative bacteria; N_{XB}, the xenobiotic; N_C, the carbon resource. UP, uptake of the carbon resource; TX, the toxicity of the xenobiotic on plasmid-free bacteria; DR, degradation of the xenobiotic; HGT, horizontal gene transfer via conjugation; MT, mortality of bacteria; SG, segregation of plasmids; RCY, recycle of bacterial biomass.

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xenobiotic (N_{XB}) in a batch-like environment. We considered three main processes: consumption of the growth-limiting carbon resource by all types of bacteria and degradation of the xenobiotic by plasmid-bearing bacteria; constant and xenobiotic-concentration-dependent bacterial mortality; and horizontal transfer of the catabolic genes on the plasmid via conjugation. We constructed a simple kinetic model to describe the interactions and dynamics among the seven components (x_P, x_F, y_F, y_P, y_{NC}, N_C and N_{XB}; Fig. 1 and Table 1).

Resource consumption and xenobiotic degradation

We set the uptake rate of the growth-limiting carbon resource (N_C) per introduced and indigenous bacterial cell as $\alpha_X N_C$ and $\alpha_Y N_C$, respectively, where α_X and α_Y are the affinity for the carbon resource of the introduced (X) and indigenous (Y) bacteria, respectively. To simulate the situation in which introduced bacteria are less competitive than indigenous bacteria for the indigenous resource, we assumed $\alpha_X < \alpha_Y$. By contrast, if $\alpha_X > \alpha_Y$, plasmid-bearing introduced bacteria have a selective advantage over indigenous bacteria and easily become dominant in the environment, even without gene transfer to indigenous bacteria. Thus, we examined the case when $\alpha_X < \alpha_Y$.

Introduced and indigenous plasmid-bearing bacteria (x_P and y_P, respectively) took up and degraded the xenobiotic (N_{XB}) at a rate of βN_{XB}, where β is the rate constant of xenobiotic degradation by introduced and indigenous bacteria. The specific growth rates of introduced plasmid-free (x_F) and plasmid-bearing (x_P) bacteria were calculated as $\rho^{-1} e \alpha_X N_C$ and $\rho^{-1}(1-c)(1-s)e(\alpha_X N_C + \beta N_{XB})$, respectively, where ρ is the cell carbon content, e is the growth efficiency, c is the cost of bearing the plasmid, which is assumed to affect growth efficiency negatively (see also eqns 1 and 2 in Table 2), and s is the loss rate of plasmids from hosts, i.e. the relative segregation rate of plasmids (Bhattacharya & Roy, 1995; Ganusov & Brilkov, 2002). We also

Table 1. Definition of parameters and their default values used to run the numerical calculations

Symbol	Definition	Unit	Default
X	Introduced bacteria	–	–
Y	Indigenous bacteria	–	–
x_F	Density of X without plasmids	10^6 cells mL^{-1}	–
x_P	Density of X with plasmids	10^6 cells mL^{-1}	–
y_F	Density of Y without plasmids	10^6 cells mL^{-1}	–
y_P	Density of Y with plasmids	10^6 cells mL^{-1}	–
y_{NC}	Density of Y resistant to conjugation	10^6 cells mL^{-1}	–
N_C	Concentration of indigenous carbon resources	μgC mL^{-1}	–
N_{XB}	Concentration of the xenobiotic	μgC mL^{-1}	–
α_X	Affinity for N_C of X	mL day^{-1} (10^6 cells)$^{-1}$	0.001
α_Y	Affinity for N_C of Y	mL day^{-1} (10^6 cells)$^{-1}$	0.002
β	Degradation rate of N_{XB} by X and Y	mL day^{-1} (10^6 cells)$^{-1}$	0.010
e	Growth efficiency of bacteria	–	0.25*
ρ	Carbon content of bacteria	μgC (10^6 cells)$^{-1}$	0.020†
m_0	Natural loss rate of bacteria	day^{-1}	0.10
m_{XB}	Toxicity of the xenobiotic	day^{-1} (μgC mL^{-1})$^{-1}$	0.10
r	Recycle ratio of dead biomass of bacteria	–	0.50
h_{DR}	Transfer rate from D (X or Y) to R (X or Y)	day^{-1} mL cell^{-1}	–
c	Cost of bearing plasmids	–	1.0e-3‡
s	Relative segregation rate of plasmids	–	1.0e-3‡
P_C	Supply rate of indigenous carbon resources	μgC mL^{-1}	0.010
$N_C(0)$	Initial concentration of indigenous carbon resources	μgC mL^{-1}	§
E_0	Initial concentration of the xenobiotic	μgC mL^{-1}	1.0
$x_F(0)$	Initial value of x_F	10^6 cells mL^{-1}	0.0
$x_P(0)$	Initial value of x_P	10^6 cells mL^{-1}	0.1
$y_F(0)$	Initial value of y_F	10^6 cells mL^{-1}	¶
$y_P(0)$	Initial value of y_P	10^6 cells mL^{-1}	0.0
$y_{NC}(0)$	Initial value of y_{NC}	10^6 cells mL^{-1}	¶
HR	Host range of plasmids	–	0.1¶

*Taken from del Giorgio & Cole (1998).
†Assuming typical value of 20 fgC cell^{-1}.
‡Typical values were assumed to be low based on Freter et al. (1983), Lenski (1998) and Dahlberg & Chao (2003).
§$N_C(0) = \rho(m_0 + m_{XB}E_0)/(e\alpha_Y)$.
¶$y_F(0) = HR \cdot P_C/(\alpha_Y N_C(0) - r\rho(m_0 + m_{XB}E_0))$, $yNC(0) = (1 - HR) \cdot P_C/(\alpha_Y N_C(0) - r\rho(m_0 + m_{XB}E_0))$. Others are assumed values.

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Table 2. The equations for dynamics of five types of bacteria, growth-limiting resource, and the xenobiotic

Plasmid-free subpopulation of introduced bacteria (x_F):

$$\begin{aligned} \mathrm{d}x_F/\mathrm{d}t &= \rho^{-1}e\alpha_X N_C x_F - (m_0 + m_{XB}N_{XB})x_F - (h_{XX}x_P + h_{YX}y_P)x_F + s\rho^{-1}(1-c)e(\alpha_X N_C + \beta N_{XB})x_P \\ &= (\text{growth}) - (\text{mortality}) - (\text{conjugal gene transfer}) + (\text{segregation}) \end{aligned} \quad (1)$$

Plasmid -bearing subpopulation of introduced bacteria (x_P):

$$\begin{aligned} \mathrm{d}x_P/\mathrm{d}t &= \rho^{-1}(1-s)(1-c)e(\alpha_X N_C + \beta N_{XB})x_P - m_0 x_P + (h_{XX}x_P + h_{YX}y_P)x_F \\ &= (\text{growth}) - (\text{mortality}) + (\text{conjugal gene transfer}) \end{aligned} \quad (2)$$

Plasmid-free subpopulation of indigenous recipient bacteria (y_F):

$$\begin{aligned} \mathrm{d}y_F/\mathrm{d}t &= \rho^{-1}e\alpha_Y N_C y_F - (m_0 + m_{XB}N_{XB})y_F - (h_{YY}y_P + h_{XY}x_P)y_F + s\rho^{-1}e(\alpha_Y N_C + \beta N_{XB})y_P \\ &= (\text{growth}) - (\text{mortality}) - (\text{conjugal gene transfer}) + (\text{segregation}) \end{aligned} \quad (3)$$

Plasmid -bearing subpopulation of indigenous recipient bacteria (y_P):

$$\begin{aligned} \mathrm{d}y_P/\mathrm{d}t &= \rho^{-1}(1-s)(1-c)e(\alpha_Y N_C + \beta N_{XB})y_P - m_0 y_P + (h_{YY}y_P + h_{XY}x_P)y_F \\ &= (\text{growth}) - (\text{mortality}) + (\text{conjugal gene transfer}) \end{aligned} \quad (4)$$

Nonconjugative subpopulation of indigenous bacteria (y_{NC}):

$$\begin{aligned} \mathrm{d}y_{NC}/\mathrm{d}t &= \rho^{-1}e\alpha_Y N_C y_{NC} - (m_0 + m_{XB}N_{XB})y_{NC} \\ &= (\text{growth}) - (\text{mortality}) \end{aligned} \quad (5)$$

Growth-limiting carbon resource (N_C):

$$\begin{aligned} \mathrm{d}N_C/\mathrm{d}t =& P_C - \alpha_X N_C(x_F + x_P) - \alpha_Y N_C(y_F + y_P + y_{NC}) \\ &+ r\rho[m_0(x_F + x_P + y_F + y_P + y_{NC}) + m_{XB}N_{XB}(x_F + y_F + y_{NC})] \\ =& (\text{production}) - (\text{uptake by introduced bacteria}) - (\text{uptake by indigenous bacteria}) + (\text{recycle of organic carbon from dead bacterial biomass}) \end{aligned} \quad (6)$$

The xenobiotic (N_{XB}):

$$\begin{aligned} \mathrm{d}N_{XB}/\mathrm{d}t &= -\beta x_P N_{XB} - \beta y_P N_{XB} \\ &= -(\text{degradation by introduced bacteria}) - (\text{degradation by indigenous bacteria}) \end{aligned} \quad (7)$$

assumed that segregation generates viable plasmid-free cells (x_F) at the same rate as segregation. The specific growth rates of the indigenous recipient (y_F and y_P) and nonconjugative (y_{NC}) populations were calculated similarly (see eqns 3–5 in Table 2). The unassimilated portion of N_C and N_{XB}, i.e. $1-e$, was assumed to be respired as CO_2 or converted to nonavailable compounds for bacteria. The possibility that the xenobiotics are transformed into various metabolites and are utilized by different groups of bacteria was not considered for simplicity.

Mortality caused by the xenobiotic and other factors

We assumed that plasmid-free bacteria (introduced and indigenous populations) experienced mortality caused by the xenobiotic at a rate of $m_{XB}N_{XB}$, whereas the xenobiotic did not affect plasmid-bearing bacteria. The parameter m_{XB} represents the toxicity of the xenobiotic. In addition, all types of bacteria experienced constant mortality at a rate of m_0, caused by nonplasmid factors such as predation.

Conjugal gene transfer

We assumed four types of HGT among potential recipients [i.e. plasmid-free introduced (x_F) and plasmid-free indigenous (y_F) recipient bacteria] and potential donors [i.e. plasmid-bearing introduced (x_P) and plasmid-bearing indigenous (y_P) donor bacteria]. We assumed that gene transfer occurs at a rate proportional to the density of both the donor and the recipient, i.e. $h \times$ (donor density) $\times$ (recipient density), where h is the transfer rate constant. In particular, we defined h_{XX}, h_{XY}, h_{YY} and h_{YX} as the plasmid transfer rate constant from introduced donor (x_P) to introduced recipient (x_F), introduced donor (x_P) to indigenous recipient (y_F), indigenous donor (y_P) to

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indigenous recipient (y_F) and indigenous donor (y_P) to introduced recipient (x_F), respectively (Fig. 1). Using h_{XX}, h_{XY}, h_{YY} and h_{YX}, we classified the transfer range and its direction of plasmids into five types: no HGT, plasmids with no ability for transmission to any type of bacteria ($h_{XX} = h_{XY} = h_{YY} = h_{YX} = 0$); HGT from X to X, plasmids in introduced bacteria only transmissible to plasmid-free introduced bacteria ($h_{XX} > 0$; $h_{XY} = h_{YY} = h_{YX} = 0$); HGT from X to Y, plasmids in introduced bacteria transmissible to plasmid-free introduced and indigenous bacteria, but plasmids in indigenous recipients with no ability for transmission to any type of bacteria (h_{XX}, $h_{XY} > 0$; $h_{YY} = h_{YX} = 0$); HGT from Y to Y, plasmids in introduced bacteria transmissible to plasmid-free introduced and indigenous bacteria, and plasmids in indigenous recipients transmissible to plasmid-free indigenous recipients (h_{XX}, h_{XY}, $h_{YY} > 0$; $h_{YX} = 0$); and HGT from Y to X, plasmid-bearing bacteria are donors for all potential recipients (h_{XX}, h_{XY}, h_{YY}, $h_{YX} > 0$).

Carbon resource and xenobiotic dynamics

We assumed that growth-limiting resources are produced constantly at a rate of P_C. They decrease with uptake by bacteria and increase with carbon recycling from dead bacterial biomass. We assumed that a fraction *r* of the dead bacterial biomass is recycled to the growth-limiting carbon resource. For the xenobiotic, we assumed that it has an initial concentration at time $t = 0$ and is thereafter decomposed by plasmid-bearing bacteria.

Model analysis

Based on these processes, we developed a model consisting of differential equations for the seven components (Tables 1 and 2). We simulated 10 years of bacterial community and xenobiotic concentration dynamics, beginning with the introduction of plasmid-bearing bacteria, using computer-aided numerical calculations. Numerical calculations were based on the fourth-order Runge–Kutta method with a fixed time step of 0.005, which is a common algorithm for numerically solving differential equations (Press *et al.*, 1988). Although it is unlikely that all environmental and biological parameters would remain unchanged over 10 years, this type of long-term simulation is still useful for identifying potential factors that affect the long-term dynamics of plasmids. Unless we completely understand the behavior of plasmids in constant environments, we cannot predict their behavior in environments with seasonal and stochastic changes.

We considered the basic case with narrow host range plasmids (i.e. the host range = 10%, $HR = 0.1$). At time $t = 0$, the microbial community began with a small number of plasmid-bearing introduced bacteria [$x_P(t = 0) = 1.0^5$ cell mL^{-1}] and a high density of plasmid-free indigenous bacteria with no plasmid-free introduced or plasmid-bearing indigenous bacteria [i.e. $x_F(t = 0) = y_P(t = 0) = 0$]. The initial concentration of the xenobiotic was $N_{XB}(0)$. We assumed that the initial density of plasmid-free indigenous bacteria [$y_F(0) + y_{NC}(0)$] and the initial concentration of the growth-limiting resource were at equilibrium (Table 1).

In our model, an 'accumulated' density of plasmid-bearing bacteria is directly linked to the final concentration of the xenobiotic, noting that the accumulated density of plasmid-bearing bacteria from $t = 0$ to $t = T$ [$\int_{t=0}^{t=T} (x_P(t) + y_P(t))\mathrm{d}t$] is equal to $(1/\beta)\ln[N_{XB}(0)/N_{XB}(T)]$, which was obtained by the integration of eqn. 7 (Table 2). Therefore, we determined the final concentration of the xenobiotic at $t = 3650$ days, which represented both the long-term spread of the plasmid and its effects on the biodegradation.

We investigated how the transfer range determined the spread of plasmids and the final xenobiotic concentration. We also focussed on the transfer rate and various factors affecting the spread of plasmids and the final xenobiotic concentration. In order to simulate the dynamics of various types of plasmids, our numerical calculations covered broad parameter regions, especially for the transfer rate constant, cost of bearing plasmids, segregation rate and host range, which were based on literature data (Freter *et al.*, 1983; Lenski & Bouma, 1987; Götz *et al.*, 1996; Lenski, 1998; Dahlberg & Chao, 2003; Maisnier-Patin & Andersson, 2004). In addition, we conducted mathematical analyses for equilibrium states of the system ($t \rightarrow \propto$), in order to support the results from numerical calculations for 10-year dynamics, which is available as supplementary material (Appendix).

Results

Effects of the transfer range and its direction

First, we examined the effect of transfer range and its direction on the temporal dynamics of the introduced and indigenous bacterial populations, plasmids and xenobiotic concentrations over 10 years (Fig. 2). When the plasmid was not transmissible (Fig. 2a), the density of plasmid-bearing introduced bacteria increased rapidly (closed triangle), leading to a decrease in xenobiotic concentration (closed circle). This undermined the selective advantage of plasmid-bearing introduced bacteria, leading to an increase in indigenous bacteria (open square) and a decrease in introduced bacteria (open and closed triangle). This prevented further degradation of the xenobiotic and resulted in a high final xenobiotic concentration. We observed a similar trend when the plasmid was only transmissible to introduced

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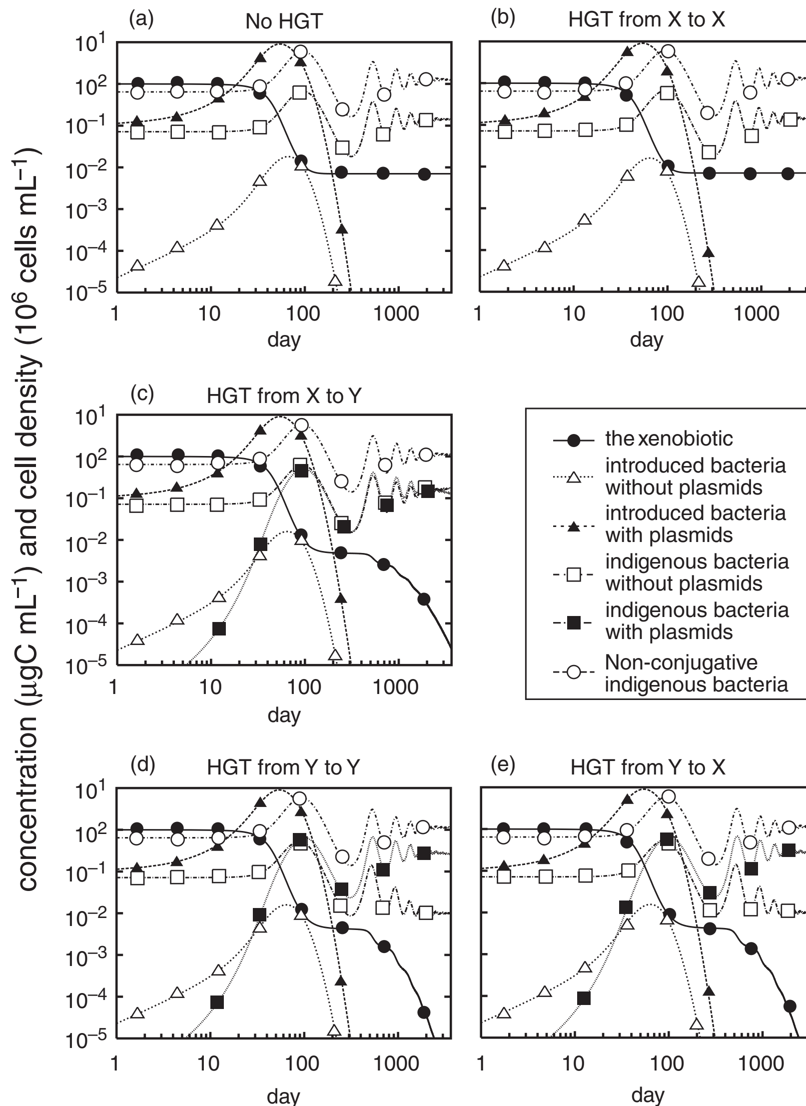

Fig. 2. Time courses of bacterial community depending on donor range. (a) 'No HGT': $h_{XX} = h_{XY} = h_{YY} = h_{YX} = 0$; (b) 'HGT from X to X': h_{XX} = 1.0e-9, $h_{XY} = h_{YY} = h_{YX} = 0$; (c) 'HGT from X to Y': h_{XX} = 1.0e-9, h_{XY} = 1.0e-10, $h_{YY} = h_{YX} = 0$; (d) 'HGT from Y to Y': h_{XX} = 1.0e-9, h_{XY} = 1.0e-10, h_{YY} = 1.0e-8, $h_{YX} = 0$; (e) 'HGT from Y to X': h_{XX} = 1.0e-9, h_{XY} = 1.0e-10 $h_{YY} = h_{YX}$ = 1.0e-8. Parameters used are given in Table 1. [Lines were actual simulation results and marks (e.g. □) were manually added for clarity of presentation.].

bacteria ($h_{XX} = 1.0^{-9}$; Fig. 2b). Although HGT within introduced bacteria may compensate for the loss of plasmids by segregation, it could not mitigate against the selective disadvantage of plasmid-bearing introduced bacteria over indigenous bacteria once the xenobiotic concentration decreased below a particular threshold.

When the plasmid was transmissible from introduced to indigenous bacteria ($h_{XX} = 1.0^{-9}$, $h_{XY} = 1.0^{-10}$; Fig. 2c), the results were quite different. The density of plasmid-bearing indigenous bacteria (closed square) increased with that of plasmid-bearing introduced bacteria. The decrease in xenobiotic concentration led to an increase in plasmid-free indigenous bacteria (open square). However, because plasmid-bearing indigenous bacteria had a higher growth rate than plasmid-bearing introduced bacteria under competition with plasmid-free indigenous bacteria, a high density of plasmids was maintained in the indigenous host for longer periods than in the case with no HGT, resulting in a lower final xenobiotic concentration.

When the plasmid was transmissible from indigenous to indigenous bacteria ($h_{XX} = 1.0^{-9}$, $h_{XY} = 1.0^{-10}$, $h_{YY} = 1.0^{-8}$; Fig. 2d), the negative effects of competition between plasmid-bearing and plasmid-free indigenous bacteria were reduced, allowing the maintenance of plasmids in the indigenous host at a higher density than in the case when the plasmid was not transmissible from indigenous to indigenous bacteria (Fig. 2c). This resulted in a very low final xenobiotic concentration at $t = 3650$ days. We observed a similar trend when the plasmid was transmissible from indigenous to introduced bacteria ($h_{XX} = 1.0^{-9}$, $h_{XY} = 1.0^{-10}$, $h_{YY} = h_{YX} = 1.0^{-8}$; Fig. 2e). The rate of conjugal gene transfer from indigenous to introduced bacteria was not sufficient to compensate for the loss of plasmids from introduced bacteria by segregation because the density of plasmid-free

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introduced bacteria was too low and it was the most inferior competitor in the community.

Further numerical calculations showed that HGT within introduced bacteria and HGT from the indigenous donor to the introduced recipient had little effect on the long-term dynamics of plasmids and the final xenobiotic concentration, even if the transfer rate constants were very high (i.e. h_{XX} and $h_{YX} > 1.0^{-7}$ day^{-1} mL cell^{-1}). Therefore, we had only to focus on two types of HGT that had different effects on the spread of plasmids, i.e. 'intergeneric HGT' (h_{XY}) and 'intraindigenous HGT' (h_{YY}), assuming that other HGT processes were negligible ($h_{XX} = h_{YX} = 0$). Hereafter, we show the results of how intergeneric and intraindigenous HGT contribute to the spread of plasmids and xenobiotic degradation, and how various factors affect the final xenobiotic concentration.

Effects of transfer rate, cost, segregation and host range

We next examined the effect of the rate of gene transfer on the spread of plasmids and on the contribution of intergeneric and intraindigenous HGT (Fig. 3a). The gene transfer rate constant (h_{XY}, h_{YY}) ranged from very low (1.0^{-17} day^{-1} mL cell^{-1}) to very high (1.0^{-7} day^{-1} mL cell^{-1}; Simonsen, 1991; Gordon, 1992; Lilley *et al.*, 2000). We investigated how the transfer rate affected the final xenobiotic concentration (Fig. 3a). Both the intergeneric and the intraindigenous HGT had little effect on the spread of plasmids and xenobiotic degradation when the rate of transfer was very low ($< 1.0^{-16}$ day^{-1} mL cell^{-1}; Fig. 3). An increase in intergeneric HGT led to a lower final xenobiotic concentration (when $h_{XY} > 1.0^{-15}$) (Fig. 3a), whereas an increase in intraindigenous HGT still had little effect on the final xenobiotic concentration (even when $h_{YY} > 1.0^{-15}$). When the rate of intraindigenous HGT was very high ($> 1.0^{-8}$ day^{-1} mL cell^{-1}), the increase in the intraindigenous HGT rate compensated for the negative effects of competition and resulted in a further increase in the activity of plasmids and a further decrease in the final xenobiotic concentration.

We also examined the effects of basic characteristics of plasmids (cost of bearing plasmids, segregation rate and host range of plasmids) on the degradation of the xenobiotic. Negative effects of increasing cost were quantitatively similar to those of increasing segregation rate (Fig. 3b). For example, the final xenobiotic concentration for the case with very low cost ($c = 1.0^{-5}$) and high segregation rate ($s = 0.01$) was almost equal to that for the case with very low segregation rate ($s = 1.0^{-5}$) and high cost ($c = 0.01$). This trend is also obvious from eqns 2 and 4 (Table 2), indicating that the cost and segregation rate reduce the growth rate of plasmid-bearing bacteria in a similar way. Although an increase in the cost of bearing plasmids and a decrease in the host range of

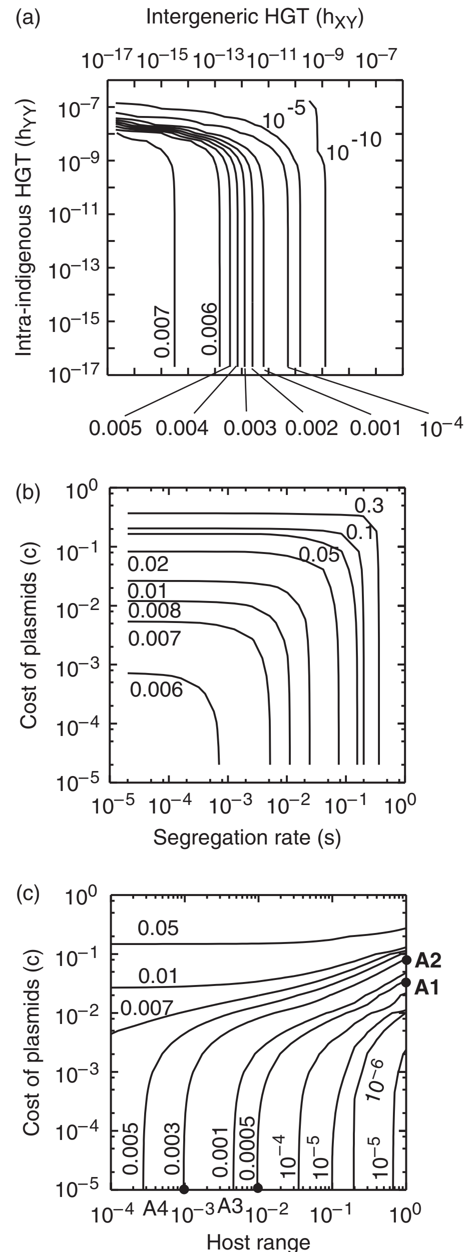

Fig. 3. The effects of transfer rate constant (h_{XY}, h_{YY}), cost of plasmids (c), segregation rate (s) and host range (HR) on the final xenobiotic concentration. The contour plots for (a) the effects of transfer rate constant (day^{-1} mL cell^{-1}), (b) the effects of segregation rate and cost of plasmids, and (c) the effects of host range and cost of plasmids, on the final xenobiotic concentration (μgC mL^{-1}). In (a)–(c), $h_{XX} = h_{YX} = 0$, $h_{XY} = 1.0\text{e-}10$, $h_{YY} = 1.0\text{e-}8$. Parameters used are given in Table 1.

plasmids resulted in a higher final xenobiotic concentration, sensitivities for them were different (Fig. 3c). Negative effects of a slight increase in the cost (e.g. from A1 to A2 in Fig. 3c) were comparable with those of more than a 10-fold difference in the host range (from A3 to A4 in Fig. 3c).

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Effects of initial conditions

We also examined the effects of initial conditions (initial density of introduced bacteria, and initial concentration of the xenobiotic). Surprisingly, a higher initial density of introduced bacteria led to a higher final xenobiotic concentration (Fig. 4: e.g. P1 vs. P2). The final xenobiotic concentration was lowest with intermediate levels of the initial xenobiotic concentration (Fig. 4: e.g. P1 vs. P3 vs. P4). These counterintuitive patterns can be partly explained from the temporal dynamics of introduced and indigenous bacteria. A higher initial density of introduced bacteria led to a higher density of introduced bacteria (Fig. 5a), resulting in a higher degradation rate of the xenobiotic over a short time scale (< 100 days; Fig. 5b). However, after then, a higher density of introduced plasmid-bearing bacteria led to severe competition between introduced and indigenous bacteria, resulting in a lower density of indigenous plasmid-bearing bacteria (Fig. 5a) and a lower degradation rate of the xenobiotic over the long term (Fig. 5b). Complex patterns of effects of initial xenobiotic concentration on the xenobiotic degradation (Fig. 5d) would be also explained by the temporal dynamics of introduced and indigenous bacteria (Fig. 5c).

Sensitivity analysis

In order to assess the sensitivity of the model to parameters values, we increased or decreased by 10-fold the degradation rate of the xenobiotic (β), the supply rate of resources (P_C), the cost of bearing plasmids (c) and the host range (HR). Table 3 shows the effects of these changes on the final xenobiotic concentration. This clarified how each parameter affected the relative contribution of intergeneric HGT and intraindigenous HGT compared with the vertical transfer of plasmids within the original introduced host. An increase in the degradation rate enhanced the vertical transfer of plasmids within introduced bacteria (the case without HGT) and intergeneric and intraindigenous HGT, resulting in a lower final xenobiotic concentration. Although an increase in the indigenous resource supply rate slightly suppressed the vertical transfer of plasmids within introduced bacteria, resulting in a higher final xenobiotic concentration, it had positive effects on intergeneric and intraindigenous HGT. An increase in the cost of plasmids (or the host range) had negative (positive) effects of intergeneric and intraindigenous HGT. In particular, intraindigenous HGT was much more sensitive to changes in the cost and the host range of plasmids compared with intergeneric HGT.

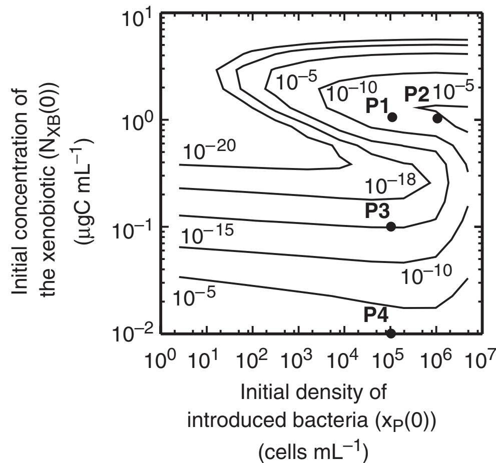

Fig. 4. The effects of initial conditions [$x_P(0)$ and $N_{XB}(0)$] on the final xenobiotic concentration (μgC mL^{-1}). (P1) [$x_P(0)$, $N_{XB}(0)$] = (10^5, 10^0); (P2) [$x_P(0)$, $N_{XB}(0)$] = (10^6, 10^0); (P3) [$x_P(0)$, $N_{XB}(0)$] = (10^5, 10^{-1}); (P4) [$x_P(0)$, $N_{XB}(0)$] = (10^5, 10^{-2}). $h_{XX} = h_{YX} = 0$, $h_{XY} = 1.0^{-10}$, $h_{YY} = 1.0^{-8}$. Parameters used are given in Table 1.

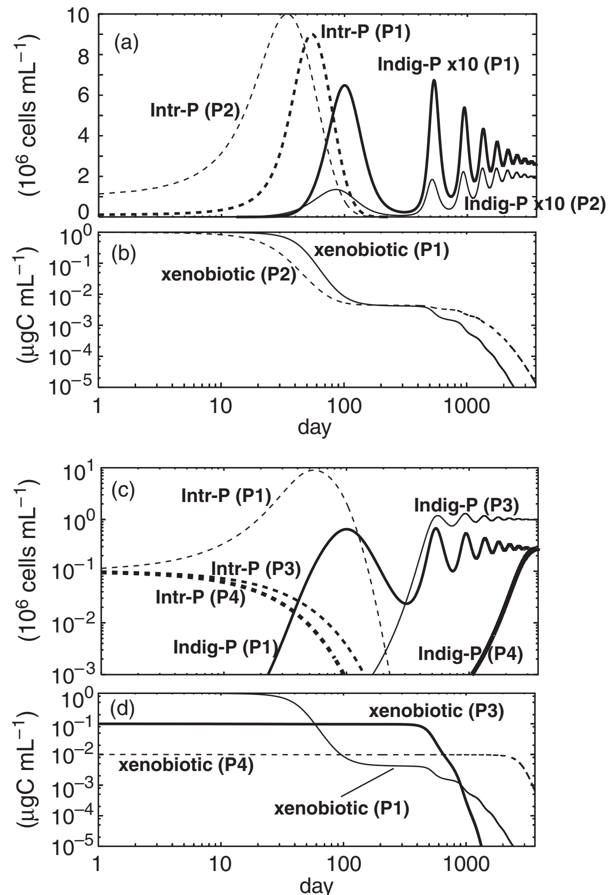

Fig. 5. The effects of initial conditions [$x_P(0)$ and $N_{XB}(0)$] on temporal dynamics of plasmids and the xenobiotic. The effects of initial density of introduced plasmid-bearing bacteria on temporal dynamics of plasmids (a) and the xenobiotic (b). The effects of initial concentration of the xenobiotic on temporal dynamics of plasmids (c) and the xenobiotic (d). Intr-P, the density of introduced plasmid-bearing bacteria (x_P); Indig-P, the density of indigenous plasmid-bearing bacteria (y_P). P1, P2, P3 and P4 correspond to those shown in Fig. 4. Parameters used are as in Fig. 4.

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Table 3. Sensitivity analysis

HGT type	Without HGT	Intergeneric HGT	Intergeneric+intraindigenous HGT
Default value (N_{XB}(3650))	7.03e-3	2.45e-5	3.46e-7
Tenfold increase			
Degradation rate (β)	4.28e-22†	5.36e-23†	1.99e-30†
Supply rate of resource (P_C)	1.83e-2	9.10e-18†	9.18e-41†
Cost (c)	7.94e-3	8.52e-4*	1.68e-4†
Host range (HR)	7.03e-3	8.87e-11*	1.34e-25†
Tenfold decrease			
Degradation rate (β)	6.28e-1*	3.77e-3†	3.34e-3†
Supply rate of resource (P_C)	3.65e-3	1.67e-3*	1.35e-3†
Cost (c)	6.94e-3	1.22e-5	1.05e-7
Host range (HR)	7.03e-3	6.76e-4*	4.15e-4*

*The changes in the final xenobiotic conditions were increased or decreased by more than 10-fold, in response to a 10-fold increase or decrease in the parameter.

†Changes in the final xenobiotic conditions were increased or decreased by more than 100-fold.

Discussion

This is the first application of mathematical modeling to evaluate the effects of HGT on the long-term dynamics of introduced catabolic plasmids encoding the ability to degrade xenobiotics that has considered the negative feedback between the spread and selective advantages of plasmids. Not only do our results support previous experimental studies of bioaugmentation that propose several factors as major determinants of the initial spread of plasmids (Top *et al.*, 1990, 2002; Neilson *et al.*, 1994; de Lipthay *et al.*, 2001), they also clearly reveal the conditions under which these factors enhance the spread and persistence of plasmids over the long term.

Numerically simulated long-term dynamics of plasmids and the xenobiotic revealed how various parameters affected the relative contribution of intergeneric HGT and intraindigenous HGT compared with the vertical transfer of plasmids within the original introduced host. When the xenobiotic degradation rate (β) was high, the contribution of the vertical transfer of plasmids within introduced bacteria was large (Table 3). By contrast, a lower cost of bearing plasmids (c), a higher indigenous resource supply rate (P_C) and a broader host range (HR) resulted in a larger contribution of indigenous and intraindigenous HGT (Table 3); they enhanced intergeneric HGT and the vertical transfer of plasmids within indigenous bacteria, whereas they had little effect on the vertical transfer of plasmids within introduced bacteria because they could not compensate for the negative effects of competition with plasmid-free indigenous bacteria. As a result, the increased density of plasmid-bearing indigenous donor bacteria also enhanced the frequency of intraindigenous conjugal transfer (HGT) of plasmids. Intergeneric and intraindigenous HGT did not always enhance the spread of plasmids, however. Plasmid-bearing introduced and indigenous bacteria always faced competition from plasmid-free indigenous bacteria. A sufficiently high transfer rate (h_{XY}) was necessary for plasmid-bearing introduced bacteria to escape competition by intergeneric HGT (Fig. 3a). For plasmid-bearing indigenous bacteria, a much higher transfer rate (h_{YY}) was necessary for them to escape competition by intraindigenous HGT (Fig. 3a).

We also clarified the relationships among basic characteristics of plasmids that have negative effects on the spread of plasmids. Higher cost, higher segregation rate and narrower host range of plasmids led to a higher final xenobiotic concentration. High cost (or high segregation rate) had severe negative effects on the spread of plasmids than narrow host range of plasmids (Figs 3b and c). Initial conditions also had large effects on the dynamics of plasmid and the final xenobiotic concentration (Fig. 4). Although a rapid increase in introduced plasmid-bearing bacteria led to a rapid decrease in the xenobiotic concentration over a short time scale, this did not always result in a low xenobiotic concentration over a long time scale. It suggested the existence of a trade-off between the rapid degradation and the complete degradation.

Our results provide a clear hypothesis for how plasmids are maintained evolutionarily in bacterial communities, which is a central topic of theoretical studies of plasmid evolution (Simonsen, 1991; Bergstrom *et al.*, 2000). Genes that function in xenobiotic degradation tend to occur on plasmids, rather than chromosomes. Several studies have suggested that repeated transfer to new hosts increases the persistence time of such genes because they only have a selective advantage in transient or spatially limited environments; if these genes occur on chromosomes, they will easily become extinct, together with their original hosts, when the selective pressure on the original host changes (Eberhard, 1989; Lilley *et al.*, 2000). Although these previous studies assumed externally driven changes in selection pressures, we examined autonomous changes in selection pressure driven

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by traits encoded on the focal genes themselves. Our results clearly suggest that these autonomous forces that cause selective disadvantages promote the occurrence of these genes on plasmids for long-term persistence.

Our results also provide some conditions for a successful bioaugmentation using catabolic plasmids (Table 3). For example, in environments polluted by xenobiotics that are easy to degrade (larger β), catabolic plasmids without the ability for HGT will be sufficient for the effective degradation of the xenobiotics. The addition of resources for enhancing bacterial growth (higher P_C) will help biodegradation of pollutant only when catabolic plasmids have the ability for HGT. Similarly, evolution of bearing plasmids and hosts toward a lower cost of bearing plasmids (Lenski, 1998; Dahlberg & Chao, 2003; Maisnier-Patin & Andersson, 2004) will enhance the biodegradation ability of a bacterial community only when catabolic plasmids have the ability for HGT. In addition, as mentioned above, initial conditions will have complex effects on the biodegradation over both a short and a long time scale.

It is clear that HGT plays a major role in bioaugmentation by enhancing the spread of introduced genes that encode the degradation of pollutants such as xenobiotics. As suggested in recent studies (Tschäpe, 1994; Top & Springael, 2003), HGT potentially enhances the adaptive responses of the bacterial community to environmental changes. Incorporating additional empirical evidence into mathematical models will be necessary to develop more general theories. The effects of other major types of HGT, such as transformation and transduction, should also be evaluated (see review in Dröge *et al.*, 1999). Spatial heterogeneity should also be considered to evaluate the occurrence of 'hot spots', where the transfer rate is much higher than in surrounding environments (van Elsas & Bailey, 2002), or where highly structured environments such as soil will prevent the conjugation. Although our model was not spatially explicit and thus not able to evaluate these effects, the negative effect would be partly simulated as reduced conjugative transfer rate of plasmids (h_{XX}, h_{XY}, h_{YY}, h_{YX}). In addition, the food web context should be incorporated, because bacterivorous predators affect the bacterial community composition and their guts supply hot spots for conjugative gene transfer (Otto *et al.*, 1997; Schlimme *et al.*, 1997), and primary producers enhance bacterial growth rates and stimulate natural transformations (Matsui *et al.*, 2003) and conjugal gene transfer (Ueki *et al.*, 2004). These expanded studies will help to provide a better understanding of the behavior of plasmids in natural environments and aid in the development of reliable and safe bioaugmentation technology.

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References

Bathe S, Mohan TVK, Wuertz S & Hausner M (2004a) Bioaugmentation of a sequencing batch biofilm reactor by horizontal gene transfer. *Wat Sci Technol* **49**: 337–344.

Bathe S, Lebuhn M, Ellwart JW, Wuertz S & Hausner M (2004b) High phylogenetic diversity of transconjugants carrying plasmid pJP4 in an activated sludge-derived microbial community. *FEMS Microbiol Lett* **235**: 215–219.

Bathe S, Schwarzenbeck N & Hausner M (2005) Plasmid-mediated bioaugmentation of activated sludge bacteria in a sequencing batch moving bed reactor using pNB2. *Lett Appl Microbiol* **41**: 242–247.

Bergstrom CT, Lipsitch M & Levin BR (2000) Natural selection, infectious transfer and the existence conditions for bacterial plasmids. *Genetics* **155**: 1505–1519.

Bhattacharya P & Roy D (1995) Estimating segregational plasmid instability of recombinant cell cultures: a generalized approach. *J Ferment Bioeng* **80**: 520–521.

Dahlberg C & Chao L (2003) Amelioration of the cost of conjugative plasmid carriage in *Escherichia coli* K12. *Genetics* **165**: 1641–1649.

del Giorgio PA & Cole JJ (1998) Bacterial growth efficiency in natural aquatic systems. *Annu Rev Ecol Syst* **29**: 503–541.

de Lipthay JR, Barkay T & Sorensen SJ (2001) Enhanced degradation of phenoxyacetic acid in soil by horizontal transfer of the *tfdA* gene encoding a 2,4-dichlorophenoxyacetic acid dioxygenase. *FEMS Microbiol Ecol* **35**: 75–84.

Dionisio F, Matic I, Radman M, Rodrigues OR & Taddei F (2002) Plasmids spread very fast in heterogeneous bacterial communities. *Genetics* **162**: 1525–1532.

Dröge M, Pühler A & Selbitschka W (1999) Horizontal gene transfer among bacteria in terrestrial and aquatic habitats as assessed by microcosm and field studies. *Biol Fertil Soils* **29**: 221–245.

Eberhard WG (1989) Why do bacterial plasmids carry some genes and not others. *Plasmid* **21**: 167–174.

Freter R, Freter RR & Brickner H (1983) Experimental and mathematical models of *Escherichia coli* plasmid transfer in vitro and in vivo. *Infect Immun* **39**: 60–84.

Fulthorpe RR & Wyndham RC (1991) Transfer and expression of the catabolic plasmid pBRC60 in wild bacterial recipients in a freshwater ecosystem. *Appl Environ Microbiol* **57**: 1546–1553.

Ganusov VV & Brilkov AV (2002) Estimating the instability parameters of plasmid-bearing cells. I. Chemostat culture. *J Theor Biol* **219**: 193–205.

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Gordon DM (1992) Rate of plasmid transfer among *Escherichia-coli* strains isolated from natural-populations. *J Gen Microbiol* **138**: 17–21.

Götz A, Pukall R, Smit E, Tietze E, Prager R, Tschääpe H, van Elsas JD & Smalla K (1996) Detection and characterization of broad-host-range plasmids in environmental bacteria by PCR. *Appl Environ Microbiol* **62**: 2621–2628.

Hoffmann A, Thimm T, Dröge M, Moore ERB, Munch JC & Tebbe CC (1998) Intergeneric transfer of conjugative and mobilizable plasmids harbored by *Escherichia coli* in the gut of the soil microarthropod *Folsomia candida* (Collembola). *Appl Environ Microbiol* **64**: 2652–2659.

Hohnstock AM, Stuart-Keil KG, Kull EE & Madsen EL (2000) Naphthalene and donor cell density influence field conjugation of naphthalene catabolism plasmids. *Appl Environ Microbiol* **66**: 3088–3092.

Kinkle BK, Sadowsky MJ, Schmidt EL & Koskinen WC (1993) Plasmids pJP4 and r68.45 can be transferred between populations of bradyrhizobia in nonsterile soil. *Appl Environ Microbiol* **59**: 1762–1766.

Lenski RE (1998) Bacterial evolution and the cost of antibiotic resistance. *Int Microbiol* **1**: 265–270.

Lenski RE & Bouma JE (1987) Effects of segregation and selection on instability of plasmid pACYC184 in *Escherichia coli* B. *J Bacteriol* **169**: 5314–5316.

Levin BR, Stewart FM & Rice VA (1979) The kinetics of conjugative plasmid transmission: fit of a simple mass action model. *Plasmid* **2**: 247–260.

Lilley A, Young P & Bailey M (2000) Bacterial population genetics: do plasmids maintain bacterial diversity and adaptation? *The Horizontal Gene Pool: bacterial Plasmids and Gene Spread* (Thomas CM, ed), 287–300. Harwood Academic Pub.

Maisnier-Patin S & Andersson DI (2004) Adaptation to the deleterious effects of antimicrobial drug resistance mutations by compensatory evolution. *Res Microbiol* **155**: 360–369.

Matsui K, Ishii N & Kawabata Z (2003) Release of extracellular transformable plasmid DNA from *Escherichia coli* cocultivated with algae. *Appl Envrion Microbiol* **69**: 2399–2404.

Neilson JW, Josephson KL, Pepper IL, Arnold RB, Di Giovanni GD & Sinclair NA (1994) Frequency of horizontal gene transfer of a large catabolic plasmid (pJP4) in soil. *Appl Environ Microbiol* **60**: 4053–4058.

Otto K, Weichart D & Kjelleberg S (1997) Plasmid transfer between marine *Vibrio* strains during predation by the heterotrophic microflagellate *Cafeteria roenbergensis*. *Appl Environ Microbiol* **63**: 740–752.

Press WH, Teukolsky SA, Vetterling WT & Flannery BP (1988) *Numerical Recipes in C: the Art of Scientific Computing*. Cambridge University Press, Cambridge.

Pukall R, Tschäpe H & Smalla K (1996) Monitoring the spread of broad host and narrow host range plasmids in soil and microcosms. *FEMS Microbiol Ecol* **20**: 53–66.

Schlimme W, Marchiani M, Hanselmann K & Jenni B (1997) Gene transfer between bacteria within digestive vacuoles of protozoa. *FEMS Microbiol Ecol* **23**: 239–247.

Simonsen L (1991) The existence conditions for bacterial plasmids: theory and reality. *Microbiol Ecol* **22**: 187–205.

Stewart FM & Levin BR (1977) The population biology of bacterial plasmids: a priori conditions for the existence of conjugationally transmitted factors. *Genetics* **87**: 209–228.

Top EM & Springael D (2003) The role of mobile genetic elements in bacterial adaptation to xenobiotic organic compounds. *Curr Opin Biotechnol* **14**: 262–269.

Top EM, Mergeay M, Springael D & Verstraete W (1990) Gene escape model: transfer of heavy metal resistance genes from *Escherichia coli* to *Alcaligenes eutrophus* on agar plates and in soil samples. *Appl Environ Microbiol* **56**: 2471–2479.

Top EM, Van Daele P, De Saeyer N & Forney LJ (1998) Enhancement of 2,4-dichlorophenoxyacetic acid (2,4-D) degradation in soil by dissemination of catabolic plasmids. *Anton Leeuwe Int J G* **73**: 87–94.

Top EM, Springael D & Boon M (2002) Catabolic mobile genetic elements and their potential use in bioaugmentation of polluted soils and waters. *FEMS Microbiol Ecol* **42**: 199–208.

Tschäpe H (1994) The spread of plasmids as a function of bacterial adaptability. *FEMS Microbiol Ecol* **15**: 23–31.

Ueki M, Matsui K, Choi K & Kawabata Z (2004) The enhancement of conjugal plasmid pBHR1 transfer between bacteria in the presence of extracellular metabolic products produced by *Microcystis aeruginosa*. *FEMS Microbiol Ecol* **51**: 1–8.

van Elsas JD & Bailey MJ (2002) The ecology of transfer of mobile genetic elements. *FEMS Microbiol Ecol* **42**: 187–197.

Supplementary material

The following supplementary material is available for this article:

Appendix S1. Mathematical analyses for equilibrium.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6941.2007.00357.x (This link will take you to the article abstract).

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