APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1993, p. 3430–3437 Vol. 59, No. 10
0099-2240/93/103430-08$02.00/0

The Specific Growth Rate of *Pseudomonas putida* PAW1 Influences the Conjugal Transfer Rate of the TOL Plasmid

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Received 12 March 1993/Accepted 2 July 1993

The kinetics of the conjugal transfer of a TOL plasmid were investigated by using *Pseudomonas putida* PAW1 as the donor strain and *P. aeruginosa* PAO 1162 as the recipient strain. Short-term batch mating experiments were performed in a nonselective medium, while the evolution of the different cell types was determined by selective plating techniques. The experimental data were analyzed by using a mass action model that describes plasmid transfer kinetics. This method allowed analysis of the mating experiments by a single intrinsic kinetic parameter for conjugal plasmid transfer. Further results indicated that the specific growth rate of the donor strain antecedent to the mating experiment had a strong impact on the measured intrinsic plasmid transfer rate coefficient, which ranged from 1×10^{-14} to 5×10^{-13} ml per cell per min. Preliminary analysis suggested that the transfer rates of the TOL plasmid are large enough to maintain the TOL plasmid in a dense microbial community without selective pressures.

Horizontal transfer of accessory DNA is believed to be a major factor in the adaptation of microbial communities to the environment and the evolution of new metabolic pathways in a community (4, 33). It has been suggested that plasmids, one type of accessory element, are major contributors to the pool of horizontally exchangeable DNA (33). This suggestion is based on the types of genes encoded on plasmids (many plasmids code for traits that confer advantages in stressed and hostile environments [see, e.g., reference 49]), their autonomous replication, and their ability to promote their own transfer (21). Indeed, direct conjugal transfer of naturally occurring or engineered plasmids has resulted in bacteria that possess novel biodegradative capabilities (19, 26, 27, 34, 35, 39). Similarly, continuous cocultivation of different organisms having unique degradative genes on plasmids has led to rearrangements of genetic information within a single organism that resulted in new catabolic functions not shared by or derivable from the separate starter strains (22, 24–26, 37, 44).

One factor that determines the fate of a plasmid in a microbial community is its horizontal transfer by conjugation (41). Therefore, the kinetic events that govern conjugal plasmid transfer must influence their contribution to community adaptation to environmental changes. For example, although the selective pressure for a plasmid-encoded gene may be very small and, therefore, would predict the washout of the plasmid from a community, horizontal transfer within the community may be effective in maintaining the plasmid in the community.

A number of different measures have been used to quantify plasmid transfer in mating experiments. These most commonly are the transconjugant concentration (11, 17, 31, 43), the transconjugant-to-recipient ratio (3, 11), and the transconjugant-to-donor ratio (1, 11, 18). Although such parameters allow comparisons within one study, they do not allow comparisons of different studies, as they are not independent of other variables, such as cell densities, population ratios, and period of incubation. Thus, intrinsic parameters that describe plasmid transfer kinetics independently of such factors are needed.

A small group of researchers (8, 9, 14, 28, 40, 47), characterizing plasmid transfer among *Escherichia coli* strains, developed mechanistic models for conjugational plasmid transfer. All of the models describe transfer by a mass action approach. This means that encounters between plasmid-harboring and plasmidless strains are assumed to occur at random with a frequency jointly proportional to both population densities and that a fraction of these encounters results in transmission of the plasmid.

Most of the kinetic studies of conjugal plasmid transfer were performed with bacteria and plasmids that are medically significant (see, e.g., references 8, 9, 14, 16, 28, 40, and 47), and quantitative data on conjugal transfer of catabolic plasmids for nonenteric organisms is scanty (6, 7). However, conjugal transfer of catabolic plasmids may generally contribute to the acquisition of existing, or the evolution of novel, catabolic traits within communities. If this is so, then control strategies to increase the degradative capability of a microbial community can also be envisioned. From a mass action perspective, such control strategies would benefit from conditions of high microbial density since this favors conjugation (36). For example, very high densities occur in some engineered microbial systems, such as those used for wastewater treatment. Since such systems would be most amenable to control strategies (46), we initially characterized the intrinsic rates of conjugal transfer of a catabolic plasmid, TOL, by *Pseudomonas putida*, an organism encountered in such systems.

MATERIALS AND METHODS

Bacterial strains and plasmid. *P. putida* mt-2 (ATCC 33015), harboring the original TOL plasmid (51), was a gift from D. E. Bradley (Memorial University of Newfoundland, St. John's, Newfoundland, Canada). *P. aeruginosa* PAO 1162 (*leu-38* r^- m^+) (10) was a gift from M. Bagdasarian

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(Michigan State University). Strains were stored at 4°C on agar plates for routine maintenance. Long-term stocks were stored at −80°C in liquid medium supplemented with glycerol at a final concentration of 15%.

Growth media. All bacterial strains were grown in a minimal medium that consisted of the following (in grams per liter of H_2O): glucose, 2.50; $(NH_4)_2SO_4$, 0.472; Na_2HPO_4, 1.42; KH_2PO_4, 1.36; $MgCl_2 \cdot 6H_2O$, 0.248; $CaCl_2 \cdot 2H_2O$, 0.066; and 20 ml of a trace element solution per liter. The trace element solution consisted of the following (in milligrams per liter of H_2O): $FeSO_4 \cdot 7H_2O$, 350; $ZnCl_2$, 100; $MnSO_4 \cdot H_2O$, 80; $CuCl_2$, 20; $CoCl_2 \cdot 6H_2O$, 20; $Na_2MoO_4 \cdot 2H_2O$, 12; H_3BO_3, 5; and 1 ml of H_2SO_4 (96%) per liter. All components were mixed and autoclaved (121°C, 1 atm, 20 min) prior to use. Solid media were supplemented with agar (16 g/liter). *P. putida* was enumerated on minimal medium, designated P. *P. aeruginosa* was enumerated on the minimal medium supplemented with leucine (added from a filter-sterilized stock solution to a final concentration of 20 mg/liter) and kanamycin (30 mg/liter), designated PLK. The medium for specific enumeration of *P. aeruginosa* harboring the TOL plasmid was the medium for *P. aeruginosa* enumeration, except that it contained *m*-toluic acid (0.8 g/liter) instead of glucose; it was designated PTLK. Cell enumeration was performed by serial dilution in phosphate-buffered saline (0.85% NaCl, 10 mM phosphate buffer, pH 7.2) and spread plating. Plates were incubated at 35°C for 2 to 3 days prior to colony counting (Colony Counter; Gallenkamp). Average cell concentrations and standard errors were computed from the colony counts by standard methods (12).

Batch culture. Liquid batch cultures were grown in metal-capped, 13-mm-inside-diameter test tubes. Test tubes contained 2.5 ml of growth medium. Separate colonies from stock plates were used as inocula for batch cultures. *P. putida* (TOL) was routinely grown in the minimal medium with *m*-toluic acid (at 0.8 g/liter) as the sole carbon source (PT), while *P. aeruginosa* was grown in the minimal medium supplemented with leucine (PL). The test tubes were incubated in a constant-temperature water bath (Magniwhirl; Blue M, Blue Island, Ill.) kept at 25°C. The test tubes were shaken vigorously to ensure aeration. The culture's density was monitored by comparing its A_{660} (Spectronic 20; Bausch & Lomb) with that of a distilled-water blank. Growth curves of *P. putida* were fitted with the following delayed-logistic equation (30):

$$\frac{d[X]}{dt} = r[X]\left[1 - \frac{[X](t - \tau)}{K}\right] \quad (1)$$

where $[X]$ is the biomass concentration (measured as A_{660}) and r, t, and K are growth kinetic coefficients. τ represents the growth lag, r is the maximum specific growth rate, and K is the carrying capacity of the growth medium.

A FORTRAN computer program (available upon request) was written to fit the delayed-logistic equation to the experimental data. The differential equation was solved with the DIVPAG subroutine (IMSL, Houston, Tex.); the kinetic coefficients were found by a nonlinear, least-squares error method employing the DUNLSF subroutine (IMSL).

The specific growth rate {i.e., $d[X]/([X]dt)$} was computed for each individual growth curve on the basis of the best-fit growth kinetic coefficients for that growth curve. The specific growth rates were then matched to the corresponding A_{660} values.

Continuous culture. Chemostat cultures were operated at room temperature (20 to 24°C) under aseptic conditions. Chemostats had a 35-ml nominal liquid working volume, and continuous mixing was provided by a stirring bar (45). Aliquots from the chemostat were removed aseptically with a syringe inserted through a sample port capped with a rubber sleeve stopper.

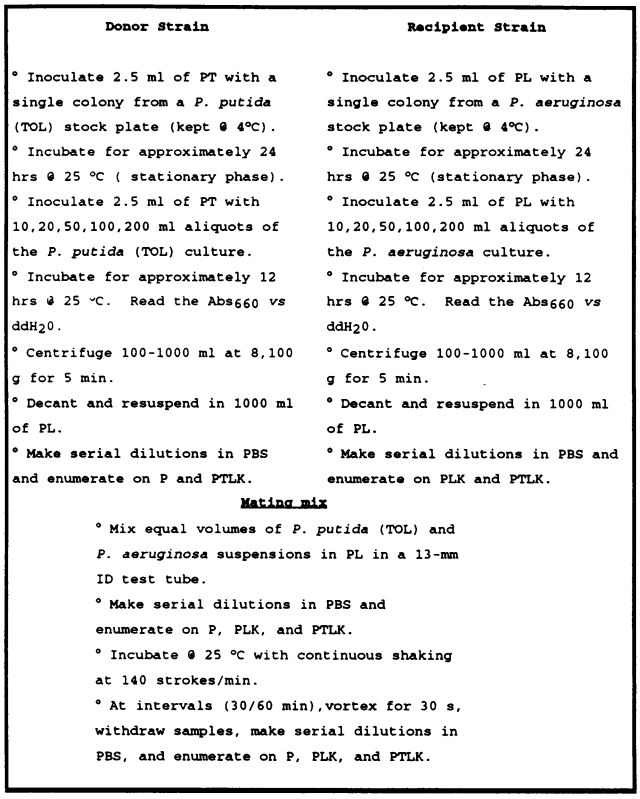

FIG. 1. Experimental protocol for batch mating experiments. PBS, phosphate-buffered saline; ddH_2O, double-distilled H_2O; ID, inside diameter.

Batch mating. All plasmid transfer studies were performed with batch mating experiments. Figure 1 summarizes the steps taken to perform a batch mating experiment. Prior to mating, donor and recipient cells were grown separately in liquid media. After the desired length of incubation, as measured by the A_{660} of the culture, aliquots of the cultures were harvested by centrifugation for 5 min at 8,100 × *g* (Eppendorf 5415 centrifuge). The supernatant was removed by aspiration, and the cell pellet was resuspended by gentle vortexing in PL. Serial dilutions of the cell suspensions were made and plated on the selective media. Both cultures were then mixed in a 13-mm-inside-diameter test tube. After brief mixing, the mating mixture was incubated in a 25°C water bath with shaking. The mixture was periodically sampled and enumerated on the different selective media. Enumeration of donors and recipients was routinely done at 60-min intervals during the first 240 min and at 120-min intervals thereafter. Enumeration of transconjugants was routinely done at 30-min intervals during the first 240 min and at 60-min intervals thereafter. We conducted control experiments in which harvested donor or recipient cultures were separately incubated in the mating medium and plated on the three selective media after different incubation times. Results from the control experiments indicated that PTLK did not support growth of the parental strains and that P and

PLK did not support growth of *P. aeruginosa* PAO 1162 and *P. putida* PAW1, respectively.

Plasmid transfer model. If plasmid transfer is described by a mass action model (28, 46), equations 2 to 4 describe the concentrations of the different cell types during the batch mating experiments.

$$\frac{d[D]}{dt} = f_{gd}[D] \tag{2}$$

$$\frac{d[R]}{dt} = -k_{t1}[D][R] - k_{t2}[T][R] + b_p[T] + f_{gr}[R] \tag{3}$$

$$\frac{d[T]}{dt} = k_{t1}[D][R] + k_{t2}[T][R] - b_p[T] + f_{gt}[T] \tag{4}$$

$[D]$, $[R]$, and $[T]$ refer, respectively, to the concentrations of donor (*P. putida* [TOL]), recipient (*P. aeruginosa*), and transconjugant (*P. aeruginosa* [TOL]) cells, expressed as cells per milliliter. The kinetic coefficients k_{t1} and k_{t2} are expressed in milliliter per cell per minute. The loss coefficient, b_p, is per/minute. The coefficients k_{t1}, k_{t2}, and b_p correspond to γ_1, γ_2, and τ in the original model of Levin et al. (28). f_{gd}, f_{gr}, and f_{gt} are the specific growth functions of the donor, recipient, and transconjugant, respectively, and are per minute. For all batch mating experiments, the transconjugant cell concentration was at least 3 orders of magnitude smaller than the donor or recipient concentration. This allowed the following simplifying statements: $k_{t2}[T][R] \ll k_{t1}[D][R]$ and $b_p[T] \ll k_{t1}[D][R]$. Incorporation of these inequalities reduces equations 3 and 4 to the following:

$$\frac{d[R]}{dt} = -k_{t1}[D][R] + f_{gr}[R] \tag{5}$$

$$\frac{d[T]}{dt} = k_{t1}[D][R] + f_{gt}[T] \tag{6}$$

Additionally, the specific growth functions for recipients and transconjugants could be assumed to be the same: $f_{gr} = f_{gt}$. Because the specific growth rate, f_{gr}, is, by definition, equal to $d[R]/([R]dt)$, equation 6 can be written as follows:

$$\frac{d[T]}{dt} - \frac{1(d[R])}{[R]dt}[T] = k_{t1}[D][R] \tag{7}$$

Division of equation 7 by $[R]$ yields the following:

$$\frac{1(d[T])}{[R]dt} - \frac{1(d[R])}{[R]^2dt}[T] = k_{t1}[D] \tag{8}$$

The left-hand side of equation 8 equals $d([T]/[R])/dt$, yielding a simpler form of equation 8:

$$\frac{d([T]/[R])}{dt} = k_{t1}[D] \tag{9}$$

Integration of equation 9 yields the following:

$$\left(\frac{[T]}{[R]}\right)_{t2} = k_{t1}\int_{t1}^{t2}[D]dt + \left(\frac{[T]}{[R]}\right)_{t1} \tag{10}$$

where the integral is from time 1 to time 2. The discrete approximation to equation 10 is as follows:

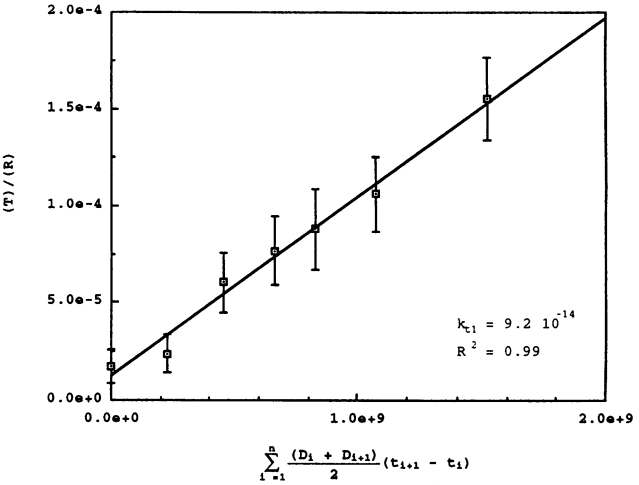

FIG. 2. The evolution of the ratio $[T]/[R]$ as a function of

$$\sum_{i=1}^{n} \frac{(D_i + D_{i+1})}{2}(t_{i+1} - t_i)$$

for batch mating experiments with *P. putida* (TOL) as the plasmid donor and *P. aeruginosa* as the recipient.

$$\left[\frac{[T]}{[R]}\right]_n = k_{t1}\sum_{i=1}^{n} \frac{(D_i + D_{i+1})}{2}(t_{i+1} - t_i) + \left[\frac{[T]}{[R]}\right]_1 \tag{11}$$

where the interval from time 1 to time 2 is divided into n periods and i indicates the time of the current period.

Equation 11 shows that a plot of $\left(\frac{[T]}{[R]}\right)_n$ as a function of

$$\sum_{i=1}^{n} \frac{(D_1 + D_{i+1})}{2}(t_{i+1} - t_i)$$

yields a linear curve with the slope k_{t1}. All data of the short-term batch mating experiments were transformed to allow analysis with equation 11.

RESULTS AND DISCUSSION

Initial experiments were conducted to determine whether equation 11 is a valid descriptor of the events in the batch mating experiment. Figure 2 shows typical results of a mating experiment after transformation of the $[D]$, $[R]$, $[T]$, and time data to allow analysis with equation 11. The results in Fig. 2 show a clear linear profile, with a high coefficient of determination ($R^2 = 0.99$), which indicates that a single kinetic parameter, k_{t1}, suffices to describe plasmid transfer events in the short-term plasmid transfer experiments. Additionally, the slope of the linear best fit to the transformed data provides an estimate for k_{t1}. Numerical simulations have indicated that growth rate differences between transconjugant and recipient cells, which were not considered in the current model, can complicate k_{t1} estimates during batch mating experiments (42). However, in the short-term plasmid transfer experiments performed in this study, data were collected before onset of significant growth of the recipient population (i.e., the recipient population had never increased by more than approximately 10% at the end

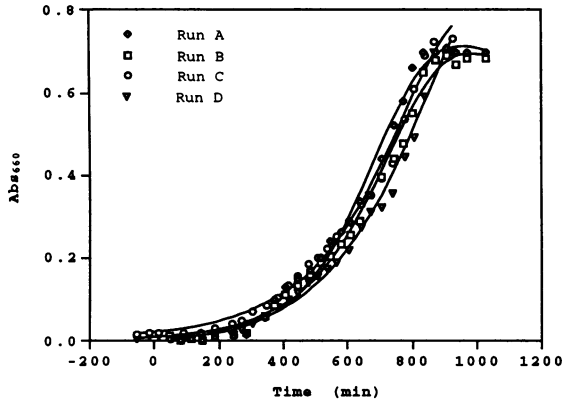

FIG. 3. Growth curves of *P. putida* (TOL) in PT at 25°C.

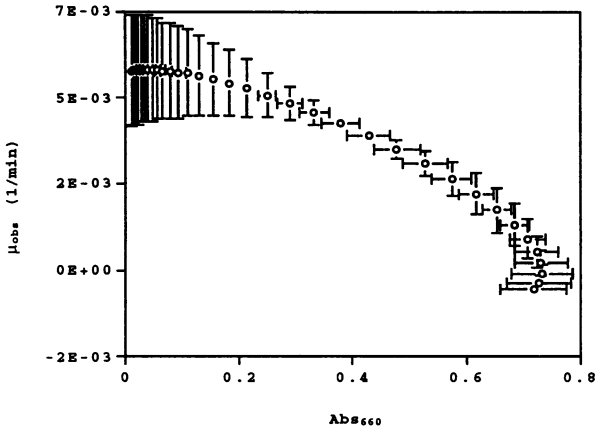

FIG. 4. Specific growth rate profile as a function of *P. putida* (TOL) culture density in PT.

of the experiment). Under such conditions, the impact of growth rate differences on k_{t1} estimation is minimal.

Although individual experiments were adequately described by equation 11, each experiment yielded a different estimate for k_{t1}. Thus, the effect of the donor's specific growth rate upon k_{t1} was examined. For these experiments, *P. putida* (TOL) was grown in batch culture to desired densities, corresponding to known specific growth rates. The relationship between growth rate and culture density was determined by several batch growth experiments. Four of these growth curves are shown in Fig. 3. However, growth did not follow the characteristic logistic curve typical of most batch bacterial cultures (2). Rather, there was a protracted period with a low growth rate, a short transition phase to a high growth rate, and then a typical entry into the stationary phase. This pattern is consistent with the results of Hugouvieux-Cotte-Pattat et al. (23), who observed growth phase-dependent expression of the *meta* pathway: at the early exponential phase, poor expression of the catabolic operons was reported, in contrast to strong expression at the late exponential phase.

The best-fit solution to each individual growth curve is presented in Fig. 3. From the growth curve equations, a specific growth rate was computed as a function of culture density (measured as A_{660}). Figure 4 shows the average specific growth rate profile. The standard deviation reflects the deviation of the four individual curves from this average growth rate profile. The deviations are highest at the very low culture densities (approximately at an A_{660} of <0.15), where the specific growth rates are highest. Figure 4 was used to estimate the specific growth rate (with standard deviation) for a given A_{660} of a *P. putida* batch culture.

Table 1 lists the results of all batch mating experiments. The donor culture was harvested at widely varying growth phases: from the early exponential phase (A_{660} = 0.10) through the stationary phase (A_{660} = 0.75). The recipient culture was harvested from a narrow range of growth phases: most cultures had an A_{660} between 0.2 and 0.35 during the exponential growth phase. However, two experiments were run with recipient cultures from the late exponential phase (A_{660} = 0.37), and in four experiments, continuously pregrown recipient cultures (μ_{obs} [the net or observed specific growth rate] = 0.10 to 0.13/h) were used.

A clear trend is apparent in Table 1. The highest plasmid

TABLE 1. k_{t1} values measured in different batch mating experiments[a]

P. putida (TOL)		*P. aeruginosa*	k_{t1}, ml/cell/min (SE)	$P > F$[c]
A_{660}	μ, 1/h (SE)	A_{660}[b]		
0.10	0.343 (0.076)	0.26	3.06×10^{-13} (3.94×10^{-14})	1.21×10^{-2}
0.17	0.333 (0.060)	0.26	3.35×10^{-13} (8.27×10^{-14})	5.58×10^{-2}
0.19	0.330 (0.056)	0.29	3.36×10^{-13} (6.51×10^{-14})	6.67×10^{-3}
0.24	0.316 (0.043)	0.20	4.95×10^{-13} (7.67×10^{-14})	2.96×10^{-3}
0.26	0.310 (0.038)	0.30	1.90×10^{-13} (2.61×10^{-14})	7.69×10^{-4}
0.32	0.289 (0.024)	CC (0.11/h)	4.07×10^{-13} (1.28×10^{-13})	5.00×10^{-2}
0.33	0.285 (0.021)	0.23	5.37×10^{-13} (7.63×10^{-14})	2.16×10^{-3}
0.34	0.280 (0.019)	0.37	2.65×10^{-13} (3.17×10^{-14})	3.97×10^{-4}
0.36	0.272 (0.014)	0.37	2.42×10^{-13} (8.25×10^{-15})	1.16×10^{-3}
0.42	0.242 (0.008)	CC (0.11/h)	8.68×10^{-14} (1.36×10^{-14})	7.83×10^{-3}
0.42	0.242 (0.008)	0.22	1.01×10^{-13} (1.00×10^{-14})	9.76×10^{-3}
0.45	0.225 (0.012)	CC (0.10/h)	1.38×10^{-13} (9.38×10^{-14})	2.17×10^{-1}
0.57	0.146 (0.027)	0.26	9.19×10^{-14} (4.96×10^{-15})	8.37×10^{-6}
0.69	0.048 (0.006)	CC (0.13/h)	1.17×10^{-14} (1.16×10^{-15})	9.59×10^{-3}
0.75	−0.001 (0.003)	0.27	1.03×10^{-14} (1.15×10^{-15})	6.93×10^{-5}

[a] The A_{660}s of both parent cultures at the time of harvest are reported.
[b] CC (x/h) means that the recipient strain was obtained from a chemostat culture operated at a dilution rate of x per hour.
[c] $P > F$ reflects the probability that the null hypothesis—the correlation coefficient in the sample population is zero—is correct (15). Thus, small values of $P > F$ indicate a significant correlation in the data.

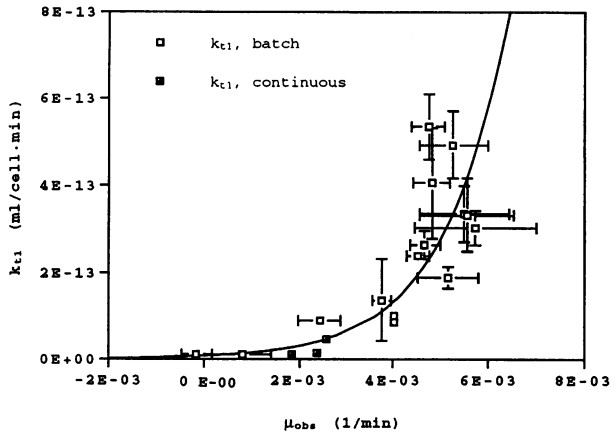

FIG. 5. Donor-to-recipient plasmid transfer rate coefficient, k_{t1}, as a function of the donor's observed specific growth rate at the time of harvest of the culture.

transfer rates were observed with donor cultures harvested in the exponential phase, and lower rates were observed with stationary-phase donor cultures. Others have reported similar findings for F plasmid transfer in *E. coli* (8, 14, 28) and RP4 transfer (29) in a *Pseudomonas* sp.

A more striking correlation was observed when the rate coefficient for plasmid transfer was expressed as a function of the observed specific growth rate of *P. putida* (TOL) just antecedent to the batch mating experiment. The function presented in Fig. 4 was used to calculate the donor culture's observed specific growth rate from the absorbance value. The result of this transformation of data is presented in Fig. 5. Figure 5 also shows the results of three experiments with chemostat-grown *P. putida* (TOL), for which the donor culture's observed specific growth rate was the dilution rate (0.111, 0.143, and 0.154/h).

Three conclusions can be drawn from Fig. 5. (i) The trend is clear: k_{t1} values increased dramatically with increasing values for μ_{obs}. (ii) Although lower k_{t1} values were measured at lower growth rates, even cells harvested from a slowly growing to stationary-phase culture maintained the ability to transfer the plasmid at measurable rates. (iii) The k_{t1} values measured with donor cells grown in continuous culture fairly closely matched the measured k_{t1} values for batch-grown cultures when harvested at the same specific growth rate. The latter conclusion indicates that kinetic coefficients determined for batch-grown cells can be predictive of their behavior under continuous-growth conditions. However, definite support for such a hypothesis awaits the testing of cultures grown continuously under a wider range of dilution rates.

So far, a formal relationship between the plasmid transfer rate and the cell growth rate has only been proposed by Simonsen et al. (42). The following equality can be derived from their kinetic expressions:

$$k_{t1}[S] = \frac{k_{t1,\,max}}{\mu_{max}}\mu[S] \qquad (12)$$

where $[S]$ is the substrate concentration and $k_{t1,\,max}$ is the maximal plasmid transfer coefficient (i.e., k_{t1} at μ_{max} [the maximal specific growth rate]). This expression dictates a linear relationship between k_{t1} and μ, but the experimental data in Fig. 5 do not support a linear relationship.

In its dependence on plasmid-determined factors (e.g., the expression of transfer genes) and host-determined factors (e.g., the availability of DNA polymerase and deoxynucleoside triphosphates [50]), plasmid transfer resembles plasmid replication. Therefore, a general kinetic expression for plasmid transfer can be derived from an expression for plasmid replication as developed by Satyagal and Agrawal (38):

$$k_{t1} = k_{t1p}\frac{k_{t1h}{}^{0}\,\mu_{obs}}{\mu_{obs} + K_{t1h}} \qquad (13)$$

where k_{t1p} is the intrinsic plasmid transfer coefficient and represents the effect that plasmid-encoded genes have on plasmid transfer, while the other terms represent the influence the host cell has on plasmid transfer. The host cell effects are postulated to depend on the metabolic activity in a Michaelis-Menten fashion (38). A further assumption is that the metabolic activity and μ_{obs} are proportional. K_{t1h} is a measure of the dependence of the plasmid on the host cell for transfer, and $k_{t1h}{}^{0}$ represents the maximum positive impact host cell functions can have on plasmid transfer at high metabolic activity. Equation 13 indicates that the plasmid transfer rate is a hyperbolic function of the net growth rate. The experimental results (Fig. 5) do not follow a hyperbolic function. In fact, the saturation effect predicted by equation 13 is the opposite of the trend seen in Fig. 5.

Although the dependence of plasmid-related functions on the metabolic status of the host cells, as postulated by Satyagal and Agrawal (38) and Simonsen et al. (42), is borne out qualitatively by the present experimental results (Table 1 and Fig. 5), both of the proposed functional expressions fail to describe the experimentally observed relationship between the plasmid transfer rate constant, k_{t1}, and the observed specific growth rate, μ_{obs}. Therefore, the following new function is proposed to take into account a finite plasmid transfer rate at zero net specific growth rate and an exponential dependence of k_{t1} on μ_{obs}:

$$k_{t1} = k_{t1}{}^{0}\exp(\alpha\mu_{obs}) \qquad (14)$$

In this equation, $k_{t1}{}^{0}$ is the plasmid transfer rate coefficient at an observed specific growth rate of zero and α is a coefficient that determines the degree of exponential dependence of k_{t1} on μ_{obs}. α therefore measures how the rate of conjugal transfer of a plasmid depends on the availability of host factors and is probably plasmid specific (e.g., small plasmids may have a smaller dependence on host factors, measured by a smaller α, than large plasmids). The continuous curve in Fig. 5 is a plot of equation 14 with $k_{t1}{}^{0} = 6.6 \times 10^{-15}$ ml per cell per min, $\alpha = 740$, and μ_{obs} is per minute. The curve fit is reasonable, with a coefficient of determination (R^2) of 0.85. We attempted to keep the antecedent specific growth rate of the recipient strain in all experiments constant by sampling at similar culture densities (Table 1). However, differences in the recipient's specific growth rate may have contributed to the unexplained variability in the experimental data, as it can be surmised that the metabolic status of the receiving strain also has an impact on the plasmid transfer kinetics.

The only other reported values for TOL transfer kinetics by *P. putida* PAW1 are from Bradley and Williams (6), who measured transfer frequencies of 1.2×10^{-2} to 5×10^{-3} transconjugants donor^{-1} h^{-1}. These values, assuming a recipient concentration of 10^9/ml in their experiments, convert to values of 2×10^{-13} to 8.3×10^{-14} ml per cell per min and are therefore comparable to our results. To what extent

the range of their values is indicative of different specific growth rates in the experiments could not be determined. Bradley and Williams (6) additionally found that the rates of TOL plasmid transfer were several orders of magnitude lower for another *P. putida* strain; they concluded that TOL plasmid transfer was derepressed in *P. putida* PAW1, which was confirmed by a sex pilus-specific phage assay (5, 6).

Comparison of the k_{t1} values measured in this study with plasmid transfer rates measured by other researchers is difficult because, as indicated before, the measured parameters frequently were not intrinsic, and provided enough information is available, back calculation is necessary to provide an estimate of k_{t1} (46). Estimated values (45, 46) varied widely, and it was not clear how much of the range was due to differences in the organisms and plasmids used, bias from model assumptions, or experimental variability.

Most of the better-defined studies were made for *E. coli*, which reduces probability because of the organism type. With exponentially growing *E. coli* K-12, reported k_{t1} values were 1.6×10^{-14} to 1.2×10^{-13} ml per cell per min for R1 (28, 42), 6.3×10^{-11} ml per cell per min for derepressed R1-*drd-19* (28), and 2.6×10^{-11} ml per cell per min for F-*lac-pro* (28). Gordon (16), studying *E. coli* plasmids isolated from natural populations, measured a range of values from 8.7×10^{-13} to 1.8×10^{-20} ml per cell per min, with an average of 2.2×10^{-17} ml per cell per min. A wide range of k_{t1} values was obtained even within *E. coli*, and that may reflect differences in the intrinsic transfer abilities of specific plasmids. In comparison with the k_{t1} values obtained in this study (1×10^{-14} to 5×10^{-13} ml per cell per min), the *E. coli* data, however, suggest that in nonselective environments the conjugal transfer of catabolic plasmids (e.g., a TOL plasmid) and naturally occurring resistance plasmids may occur at similar rates.

The stability and transfer of plasmids in natural or managed environments has received considerable attention in studies evaluating the benefits and risks associated with deliberate or accidental release of bioengineered and resistance plasmids (13, 48). In a detailed fate analysis of conjugal plasmids, Simonsen (41) evaluated the conditions necessary to maintain a plasmid in a bacterial population. Her results indicated that conjugal plasmid transfer rates as high as 1.7×10^{-10} to 5×10^{-12} ml per cell per min would be necessary to maintain a nonselective plasmid in a fecal *E. coli* population. This derivation assumed a population density of 10^6 cells per ml, plasmid loss by segregation at a rate of 1.7×10^{-5} to 1.7×10^{-7} min^{-1}, and a 1 to 10% decrease in the net specific growth rate of cells that harbored the plasmid. Although the effect of TOL plasmid presence on cell growth kinetics was not quantified in this study, the measured loss rate of the TOL catabolic genes of 4.2×10^{-6} min^{-1} (45) was within the reported range. The plasmid transfer rates measured in this study are lower than those reported by Simonsen (41), but the possibility for control of microbial systems through horizontal transfer of catabolic plasmids remains under conditions of high microbial densities (41, 46, 47). Microbial densities of 10^7 to 10^9 cells per ml can be obtained in engineered microbial systems, such as wastewater treatment systems (where conjugal plasmid transfer has been observed [1, 31]), and the increased biomass concentration should compensate for a lower plasmid transfer rate in maintaining a plasmid in a microbial community (41, 46, 47).

The range of measured values for conjugal TOL plasmid transfer (5×10^{-13} and 1×10^{-14} ml per cell per min) and the dependence of the measured value of the specific growth rate have two major implications. (i) Microbial cell densities that ensure maintenance of a TOL plasmid in managed systems appear feasible. Assuming that all other conditions are comparable to those reported by Simonsen (41), microbial densities of 10^9 cells per ml must accompany k_{t1} values of 1.7×10^{-13} to 5×10^{-15} ml per cell per min for steady-state presence of the plasmid in a microbial system. (ii) Because k_{t1} values are correlated with the specific growth rate, the required microbial density depends on the specific growth rate. For example, higher specific growth rates would favor maintenance of the TOL plasmid. Under typical operating conditions, specific growth rates in engineered microbial systems are very low, putting the transfer rates in the low range: 10^{-14} ml per cell per min. The analysis of Simonsen (41) suggests that even at these rates, plasmid maintenance in a microbial community is feasible at a microbial density of 10^9 cells per ml. These results suggest that for dense microbial communities, in the absence of any selective pressure, conjugal transfer rates of catabolic plasmids may be high enough to maintain a plasmid-encoded gene in a community by horizontal spread.

Many questions must be answered before a full picture on the fate of conjugal plasmids in microbial communities can emerge. (i) Our evaluation of plasmid transfer kinetics was done under suspended-growth conditions, while most of the microbial biomass in many natural or engineered systems occurs in aggregates or on surfaces. No mathematical model has been developed to describe plasmid transfer kinetics on surfaces, where, because of the high localized microbial densities and protection of the cells from shear, conditions for plasmid transfer may be more favorable (5, 6) than in suspension. (ii) The plasmid fate analyses performed by Simonsen (41) and by us (46) assume that the entire community can partake in plasmid transfer. Such an assumption may be warranted only when the community consists of closely related genera or the plasmid has a broad host range. For many plasmids, however, the transfer may be limited (20). For plasmids with a narrower host range, such as TOL plasmids (32), the fraction of the community that can participate will likely vary from community to community. (iii) Plasmid transfer kinetics are usually measured under nonselective conditions, i.e., without a selective force for maintenance of the plasmid. A very important, and essentially unstudied, issue in this regard is whether the presence of low substrate concentrations confers selective pressure.

In conclusion, k_{t1}, an intrinsic parameter of conjugal plasmid transfer, was successfully measured for *P. putida* transfer of a TOL plasmid. This value ranged from 5×10^{-13} to 1×10^{-14} ml per cell per min and was highly dependent on the donor's observed specific growth rate prior to the mating experiment. An exponential function adequately described the dependence of k_{t1} on μ_{obs}. The magnitude of k_{t1} for the TOL plasmid was comparable to the k_{t1} values determined for some resistance plasmids in *E. coli*, and a preliminary evaluation suggests that the conjugal transfer rates of TOL plasmids are high enough to maintain a TOL plasmid in a dense microbial community without a selective advantage.

ACKNOWLEDGMENTS

This research was funded in part by the U.S. Environmental Protection Agency through cooperative agreement CR-812582-03 with the Advanced Environmental Control Technology Research Center. Additional funding was provided by the Amoco Oil Company.

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