# Mobilization of Broad Host Range Plasmid from *Pseudomonas putida* to Established Biofilm of *Bacillus azotoformans.* I. Experiments

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**Abstract:** A strain of *Pseudomonas putida* harboring plasmids RK2 and pDLB101 was exposed to a pure culture biofilm of *Bacillus azotoformans* grown in a rotating annular reactor under three different concentrations of the limiting nutrient, succinate. Experimental results demonstrated that the broad host range RSF1010 derivative pDLB101 was transferred to and expressed by *B. azotoformans.* At the lower concentrations, donor mediated plasmid transfer increased with increasing nutrient levels, but the highest nutrient concentration yielded the lowest rate of donor to recipient plasmid transfer. For transconjugant initiated transfer, the rate of transfer increased with increasing nutrient concentrations for all cases. At the lower nutrient concentrations, the frequency of plasmid transfer was higher between donors and recipients than between transconjugants and recipients. The reverse was true at the highest succinate concentration. The rates and frequencies of plasmid transfer by mobilization were compared to gene exchange by retrotransfer. The initial rate of retrotransfer was slower than mobilization, but then increased dramatically. Retrotransfer produced a plasmid transfer frequency more than an order of magnitude higher than simple mobilization. © 1998 John Wiley & Sons, Inc. *Biotechnol Bioeng* **57:** 272–279, 1998.
**Keywords:** biofilm; plasmid transfer; conjugation; retrotransfer

## INTRODUCTION

The potential for using biotechnology in the treatment of toxic waste in the United States is considerable. More than 24,000 industrial plants in the United States generate over 260 million tons of hazardous waste per year (EPA, 1993) of which 25–50% can be biologically treated (McCormick, 1985). Biotechnology has allowed the creation of genetically engineered microorganisms (GEMs) capable of degrading single compounds or mixtures of similar chemicals more completely and efficiently than natural bacterial isolates (Haugland et al., 1990). Before these bioengineered organisms can be released into the environment, the fate and effects of novel microorganisms and their genetically altered plasmid or chromosomal DNA on the natural ecosystem must be assessed. In particular, the survival, establishment, and growth of recombinant organisms, and the transfer of their plasmid DNA in these environments must be investigated before the government will permit the release of recombinant organisms.

It has been shown that the survival and genetic transfer abilities of microorganisms depend on several factors: the nature of the bacterial host and cloning vector, the final ecological niches of the organisms, the transmissibility of the recombinant DNA (rDNA) to other bacteria, and the selective advantage that the DNA can confer to the host in order to survive harsh environments (Cruz–Cruz et al., 1988; Stotzky and Babich, 1984). Most bacteria found in nature are attached to surfaces and grow as surface-bound microcolonies. These surface-attached cells and their extracellular polymeric substances (EPSs) form what is called a biofilm. It had been assumed that plasmid transfer in natural environments would not occur because suspended bacterial population densities are usually lower than those employed in laboratory experiments. However, biofilms found in natural environments can support dense and active microbial communities. Many of these biofilm ecosystems are stable and resist newly introduced microorganisms from colonizing them. This has been shown to hold true even when the newly introduced species was originally one of the indigenous population (Freter et al., 1983).

Because little information is available on the dynamics of gene transfer to and between biofilm organisms, improved knowledge of these gene transfer patterns will contribute to the understanding of the effect of GEMs in natural ecosystems. This is especially important because bacteria isolated

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from the environment have been shown to possess plasmids that are conjugative (Bender and Cooksey, 1986; Diels et al., 1989; Focht et al., 1996; Fry and Day, 1990; Guiney and Lanka, 1989; Hill et al., 1992; Lilley et al., 1992; Shoemaker et al., 1992; Wickham and Atlas, 1988) or have been shown to mobilize other plasmids (Hill et al., 1992; Mergeay et al., 1987). Angles et al. (1993) found transfer of the broad host range plasmid RP1 to be higher in biofilms than in the aqueous phase. However, in their experiments they used a recipient strain that had been cultured and optimized for plasmid transfer and they measured plasmid transfer by replica plating, which has been shown to give artificially high numbers (Walter et al., 1991).

This study investigates and quantifies the transfer of a plasmid by mobilization in a biofilm as a function of ambient nutrient level and the mechanism of transfer. Estimates of the rates and kinetics of transfer are made for each condition. In the companion article to this work, theoretical models of biofilm accumulation are coupled with the kinetic expression for plasmid transfer to give a more quantitative description of the dynamics of plasmid transfer in biofilm systems.

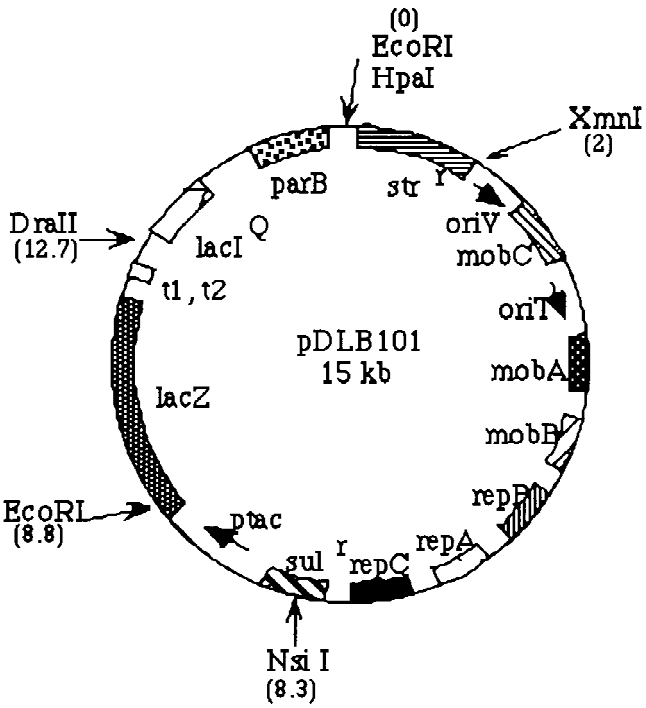


**Figure 1.** Restriction map of mobilizable plasmid pDLB101 used in transfer experiments.

## MATERIALS AND METHODS

### Strains and Plasmids

The donor strain used in all experiments was *Pseudomonas putida* PB2440 (Bagdasarian et al., 1981). This Gram-negative soil bacterium was chosen because it has been widely characterized for use in biodegradation due to its metabolic capabilities. This strain was made nalidixic acid resistant by plating onto Luria–Bertani (LB) agar containing 50 μg/mL nalidixic acid and selecting spontaneous mutants. For these experiments the recipient strain was selected based on a different cell morphology than the donors, the ability to express and maintain the broad host range plasmid RSF1010, and an inability to metabolize lactose. *Bacillus azotoformans*, a large Gram-positive rod (1 × 3–10 μm) originally isolated from soil, fulfilled these criteria (Pichinoty et al., 1978).

The plasmid being transferred in these experiments, pDLB101, combines the broad host range and mobilization abilities of RSF1010 and the stability and selection markers of pTKW106 (see Fig. 1). RSF1010's mode of replication renders it independent of any host replication functions, unlike other plasmids (Scholz et al., 1985). This Gram-negative plasmid has been shown to be stably maintained, replicated, and expressed in several Gram-positive species (Gormley and Davies, 1991; Hermans et al., 1991; Mermet–Bouvier et al., 1993; Nesvera et al., 1994). The inducible expression system is based on the *lacZ* gene that codes for the production of the marker protein, β-galactosidase. This tightly regulated expression vector originally derived from plasmid pMJR1750 (Stark, 1987) includes the strong *tac* promoter and, for complete repression, the $lacI^Q$ gene. The plasmid also contains the *hok/sok* (formerly *parB*) locus that eliminates the presence of plasmid-free bacteria generated by plasmid segregational instability. Thus, plasmid pDLB101 provides a system from which transfer can be readily observed and serves as an upper limit for the potential for plasmids to transfer to indigenous organisms.

Because plasmid pDLB101 is only capable of mobilization and not conjugation, a helper plasmid must be provided to facilitate transfer. The broad host range plasmid RK2 served as this helper plasmid in the experiments (Thomas, 1989).

The strains were grown on a minimal salts medium supplemented with casamino acids, a carbon source, and a trace metal solution. Formulation for the medium was per liter distilled water: 2.68 g $Na_2HPO_4 \cdot 7\ H_2O$, 0.98 g $KH_2PO_4$, 0.30 g $MgSO_4 \cdot 7\ H_2O$, 0.50 g $NH_4Cl$, 1.00 g $NH_4SO_4$, 0.10 g $NaH_2PO_4$, and 0.13 g $K_2HPO_4$. These components were autoclaved for 20 min. After cooling, the following presterilized solutions were added: 600 μL 8% (w/v) casamino acids, 500 μL 5% (w/v) succinate, 250 μL 10% (w/v) lactose, and 200 μL trace metal solution (2.20 g $ZnSO_4 \cdot 7\ H_2O$, 5.54 g $CaCl_2$, 5.06 g $MnCl_2 \cdot 4\ H_2O$, 4.79 g $FeSO_4 \cdot 7\ H_2O$, 1.10 g $NH_4Mo_7O_{24} \cdot 4\ H_2O$, 1.57 g $CuSO_4 \cdot 5\ H_2O$, 1.60 g $CoCl_2 \cdot 6\ H_2O$, 0.05 g $H_3BO_3$, and 50.0 g EDTA/L $dH_2O$).

Growth rates and Monod kinetic parameters were determined in independent experiments (data not shown). Suspended cultures of all three strains were grown in the above media across the range of succinate concentrations employed in these experiments. Concentrations were determined from colony counts on LB plates. All measurements

were done in triplicate. It was assumed that growth rates in biofilms and in suspensions were the same even though there is conflicting evidence supporting and refuting this.

## Reactor Description

Biofilms were cultivated in a rotating annular reactor (see Fig. 2) comprising two concentric cylinders creating a small annular gap separating the two. This reactor design provides a high surface area to volume ratio, thereby promoting biofilm growth and minimizing suspended cell growth. The outer cylinder remains stationary while the inner one is rotated to affect a desired surface velocity or shear stress. Twelve polycarbonate removable slides were machined to fit into the wall of the outer cylinder for sampling.

To determine the effect of nutrient concentration, and concomitantly bacterial growth rate, on plasmid transfer, the succinate concentration in the media delivered to the rotating annular reactor was varied. For the base case, 25 μg/mL (1×) succinate was used. The other cases used either 12.5 μg/mL (0.5×) or 75 μg/mL (3×) succinate. The type of plasmid transfer was also varied. Mobilization, in which the conjugative helper plasmid RK2 is transferred with the mobilizable pDLB101 following after, was the primary mode of plasmid transfer examined. However, in one retrotransfer experiment the donor cells (*P. putida* PB2440) contained only pDLB101 and the recipient cells (*B. azotoformans*) harbored the conjugative plasmid RK2. During retrotransfer *B. azotoformans* should transfer RK2 to PB2440 and then pDLB101 could be transferred back to *B. azotoformans.*

## Experimental Protocol

The reactor was inoculated with 500 mL of an overnight culture of the recipient strain (*B. azotoformans*) grown in mineral salts medium at 30°C. The suspended culture was allowed to grow in batch mode for 6 h. At this point the reactor was switched to continuous mode and fresh nutrients were delivered at a flow rate to affect a dilution rate of 1.0/h. This dilution rate exceeds the maximum growth rate of the cells ($\mu = 0.365$/h), thereby minimizing suspended growth in the reactor. After 3 days of biofilm accumulation, the biofilm and reactor effluent were sampled and the nutrient flow was stopped. Immediately after sampling, 500 mL of *P. putida* donor cells ($3 \times 10^9$ cells/mL) were added to the reactor and allowed to contact the recipient biofilm for 3 h before being washed out by resuming continuous influent nutrient media flow, giving the donor cells adequate time to contact the biofilm and attach themselves. Both the biofilm and effluent were then sampled periodically over the next 4 days.

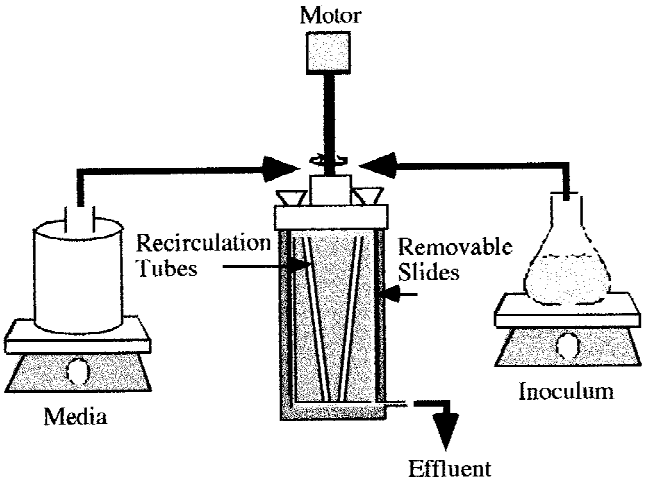


**Figure 2.** Rotating annular reactor used for growing biofilms in plasmid transfer experiments.

## Analytical Techniques

At each sampling time, a Plexiglas slide was removed from the reactor to collect a sample of the biofilm; a sample of the reactor effluent was also taken. A portion (28 $cm^2$) of the biofilm was scraped from the slide into 8 mL of sterile buffer and resuspended by vigorous vortexing for 2 min. Liquid samples, both the resuspended biofilm sample and reactor effluent sample, were fixed with 2 mL of 20 mg/mL 1-ethyl-3-(3-dimethylaminopropyl-)-carbodiimide HCl (EDC, Sigma Chemical Co.) in phosphate buffer and stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma) to indicate total cell numbers and with fluorescein di-β-galactopyranoside (FDG, Sigma) to indicate cells expressing β-galactosidase.

For DAPI staining, 900 μL of fixed, suspended cells (serially diluted if necessary) were added to 100 μL (10 μg/mL) DAPI in a microfuge tube. The contents were vortexed briefly to mix, then allowed to stand 20 min at room temperature. At this time, the stained cells were filtered onto a 25-mm Poretics 0.2-μm pore size black PCTE membrane and washed by filtering 4 mL of sterile distilled $H_2O$. The filter was placed on a drop of immersion oil on a microscope slide, topped with a coverslip, and a minimum of 10 grids were counted using an Olympus BH-2 microscope with epifluorescence illumination (100-W mercury lamp). An Olympus U excitation filter cubic unit with excitation filter (UG-1), a dichroic mirror (DM400), and a barrier filter (L420) were used to visualize the DAPI-stained cells.

For FDG staining, 980 μL of fixed, suspended cells (serially diluted if necessary) were added to 20 μL of stock FDG solution (50 mg/mL) in a microfuge tube. The contents were vortexed briefly to mix then allowed to stand 30 min at room temperature. The cells were then filtered, washed, and counted as above.

The remainder of the biofilm on the slide (6 $cm^2$) was cryoembedded and cryosectioned via the method of Yu et al. (1994), then stained and examined under the microscope to visually determine the spatial location of bacterial cells containing and expressing the pDLB101 plasmid. First a slide was removed from the reactor and placed flat on a brick of dry ice. A thick layer of Tissue-Tek OCT com-

pound (Miles Incorporated) was poured on top of the biofilm, embedding the biofilm and freezing it completely (sample turns opaque white). The biofilm was removed from the slide by gently bending the polycarbonate slide. The sample was placed OCT-side down onto the block of dry ice, another thick layer of OCT was poured over the exposed biofilm side, and the sample was allowed to freeze. Samples were wrapped in aluminum foil and stored at −70°C until ready to cryosection. Frozen sections were placed in a cryostat (Reichert–Jung Cryocut 1800, Leica) operated at −19°C for 15 min before sectioning to allow thermal equilibration. After equilibration, the samples were cut into 5-μm slices and collected on positively charged microscope slides. To each biofilm slice a 10-μL aliquot of one of the following staining solutions was added: 1.0 μg/mL DAPI in 4.0 mg/mL EDC fixative buffer or 1.0 μg/mL FDG in 4.0 mg/mL EDC fixative buffer. *P. putida* (0.5 × 1 μm) and *B. azotoformans* (1 × 10 μm) are easily distinguished by relative cell sizes. FDG stains β-galactosidase in only those cells containing a plasmid expressing *lacZ* activity. Both stains were allowed to penetrate the biofilm sample slice for 10 min. Excess solution was then absorbed onto a Kimwipe tissue. Each biofilm sample slice was then covered with a coverslip and examined using an epifluorescence microscope.

### Statistical Analysis

Each sample was counted under the microscope on a minimum of 10 grids. The numbers plotted represent the average values and the error bars showing the standard deviation.

## RESULTS AND DISCUSSION

There are two possible mechanisms of plasmid transfer in the experiments above. Initially, only the donors are capable of transferring their DNA to the recipient cells. However, as the number of transconjugants increases, it is expected that the frequency of plasmid transfer from the transconjugants to the recipients also increases. It has been shown that the rate of plasmid transfer in suspension can be described mathematically by $k_1DR$ (or $k_2TR$) where $k_1$ and $k_2$ are kinetic constants and $D$, $R$, and $T$ represent, respectively, the concentrations of donors, recipients, and transconjugants in the suspension (cells/mL) (Levin et al., 1979; MacDonald et al., 1992). In biofilm systems, however, there is contact only between individual layers of cells (see Fig. 3A) and not between all cells in the biofilm volume. Therefore, the rate expression for plasmid transfer in biofilms should use the number of donors, recipients, or transconjugants per unit biofilm surface area instead of per unit volume.

### Effect of Nutrient Concentration

In these experiments the donor strain was *P. putida* PB2440 (RK2, pDLB101), the recipient strain was *B. azotoformans*, and the resulting transconjugant was *B. azotoformans* (RK2, pDLB101). As mentioned above, three different levels of succinate were fed to the biofilm reactor: the base case (25 μg/mL), 3× succinate (75 μg/mL), and 0.5× succinate (12.5 μg/mL).

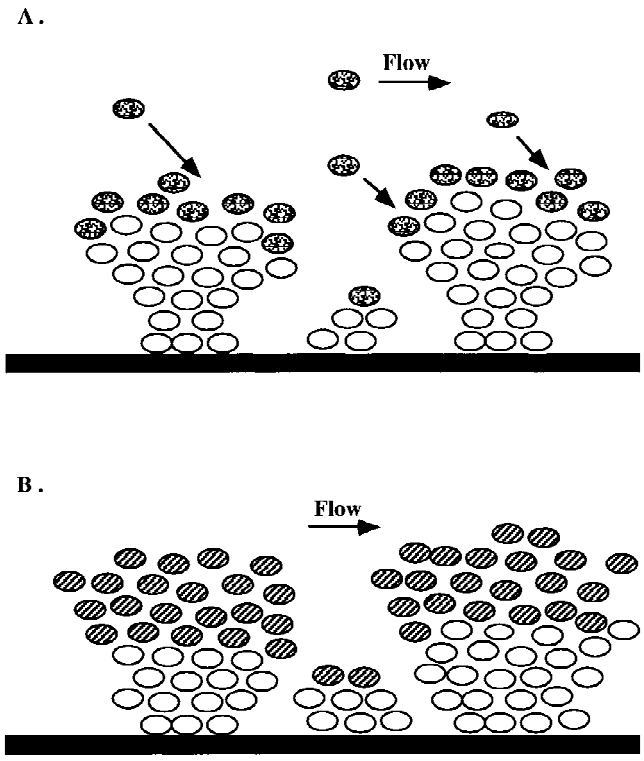


**Figure 3.** (A) Layering nature of bacterial species in biofilm for plasmid transfer. The shaded cells are donors; open cells are recipients. (B) Distribution of donor, recipient, and transconjugant cells in biofilm microcolonies resulting from plasmid transfer between an established biofilm and a released donor. The shaded cells are donors, open cells are recipients, and striped cells are transconjugants.

As stated previously, at short times plasmid transfer occurs exclusively between donor cells and the recipients. Growth of the transconjugants is assumed to be small compared to their formation by plasmid transfer. By plotting the initial transconjugant concentrations over time, the rate of plasmid transfer between donors and recipients can be determined from the slope of this plot. A change in the plasmid transfer rate would indicate a shift from donor-mediated plasmid transfer to transfer initiated by the transconjugants and transconjugant growth.

Figure 4 shows the short time transconjugant concentrations measured for each of the three succinate concentrations. As the plot indicates, there is very little difference in the rate of plasmid transfer for either the base case or 0.5× succinate. For the 3× succinate case, however, the rate of plasmid transfer from donors to recipients decreases significantly. The transfer rates for these cases are given in Table I.

At longer times, it is speculated that the majority of plasmid transfer is carried out between transconjugant cells and the recipients (see Fig. 3B). During this time, cell growth is also a major factor in the net accumulation of transconju-

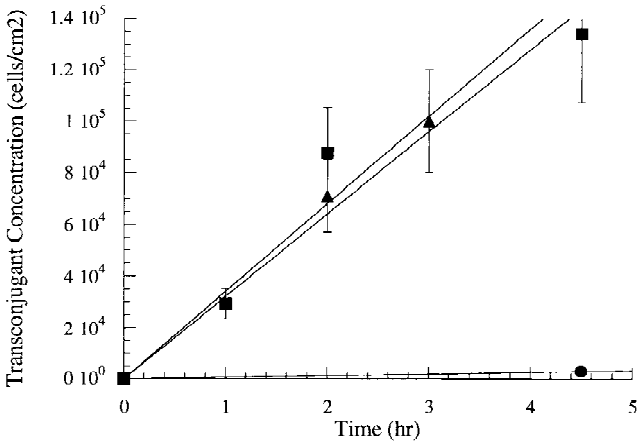


**Figure 4.** Early transconjugant concentration profiles resulting from plasmid transfer between *P. putida* PB2440 (RK2, pDLB101) and *B. azotoformans* using three different succinate concentrations: (■) 25 μg/mL succinate, (▲) 12.5 μg/mL succinate, and (●) 75 μg/mL succinate.

gants. By subtracting the rate of transconjugant cell growth (measured in independent experiments, data not shown) from the net accumulation of transconjugants, an estimate of the rate of plasmid transfer between transconjugants and recipients can be made.

The measured concentrations of transconjugants over the course of the entire experiment for all three succinate concentrations are shown in Figure 5A. Figure 5B shows the approximate transconjugant concentrations if cell growth is subtracted from each case. The rate of plasmid transfer from transconjugants to recipients is slightly higher for 3× succinate compared to the base case, with the 0.5× succinate case not producing any further transconjugants via plasmid transfer, probably due to consumption of the limited nutrients for maintaining cell growth. These values are an order of magnitude lower for the base case and an order of magnitude higher for 3× succinate compared to the transfer rates measured for donor initiated transfer.

According to MacDonald et al. (1992), plasmid transfer is an energy intensive process, with higher transfer rates occurring at higher nutrient concentrations in batch suspension. In a well-mixed batch system, the cells divide rapidly, leading to a higher frequency of collisions between cells. In a biofilm system, however, this is not necessarily true. In the microcolony structure of a biofilm, rapidly dividing cells may actually inhibit the formation of efficient mating pairs

**Table I.** Measured plasmid transfer rates for donors and transconjugants for various influent nutrient concentrations.

| Succinate concentration (mg/mL) | Rate of plasmid transfer (cells/cm$^2$/h) | |
|---|---|---|
| | Donor mediated | Transconjugant mediated |
| 0.025 | 3.0E + 04 | 4.5E + 03 |
| 0.0125 | 3.4E + 04 | 0 |
| 0.075 | 7.0E + 02 | 5.6E + 03 |

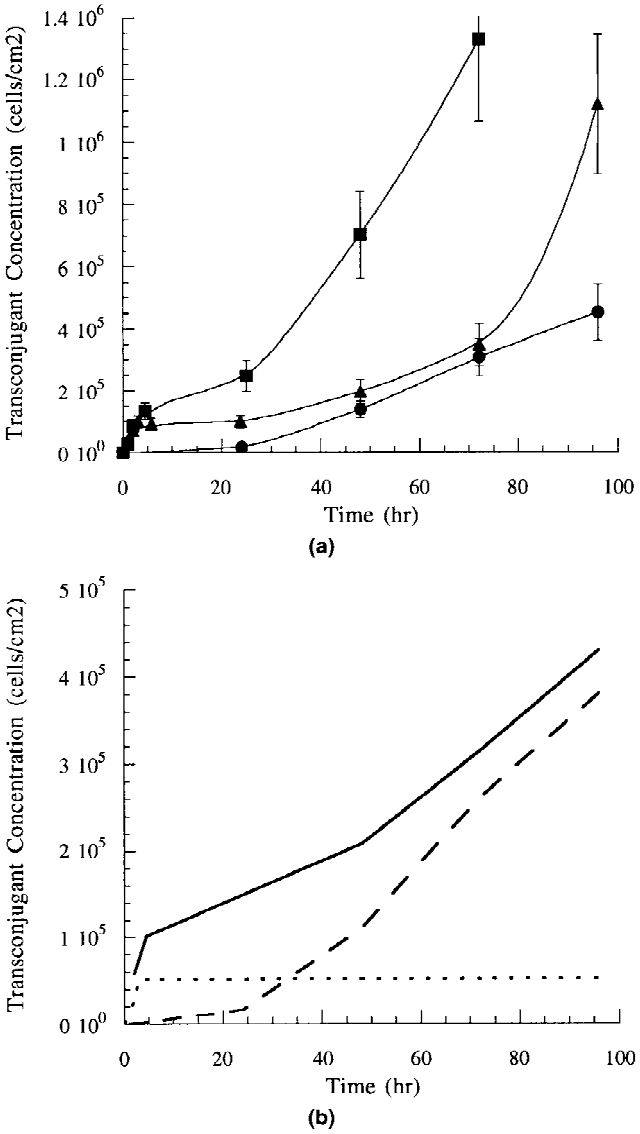


**Figure 5.** Transconjugant concentration profiles resulting from plasmid transfer between *P. putida* PB2440 (RK2, pDLB101) and *B. azotoformans* using three different succinate concentrations. (A) Measured concentrations; (■) 25 μg/mL succinate, (▲) 12.5 μg/mL succinate, and (●) 75 μg/mL succinate. (B) Approximate concentrations when cell growth is subtracted: (—) 25 μg/mL succinate, (· · ·) 12.5 μg/mL succinate, and (- - -) 75 μg/mL succinate.

by pushing neighboring cells away from each other. The time between a donor cell and recipient cell coming in contact and establishing a mating pair may be interrupted by cell division pushing them apart. It is hypothesized that this may account for the large decrease in the donor to recipient transfer rate for 3× succinate because these cells are in the outer region of the microcolony and have a large amount of free space for microcolony expansion. This is not seen in the inner region of the microcolony where transconjugant initiated transfer occurs. This region has less space available for cells to be pushed apart, so disruption of the mating pairs may be minimized.

MacDonald et al. (1992) also found that transconjugants

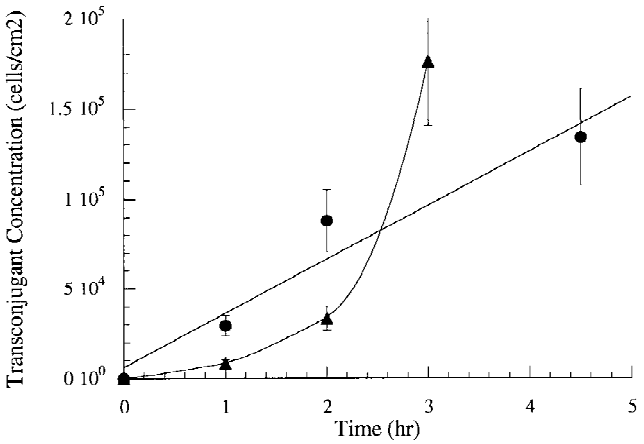


**Figure 6.** Early transconjugant concentration profiles resulting from plasmid transfer between *P. putida* PB2440 (RK2, pDLB101) and *B. azotoformans* and between *P. putida* PB2440 (pDLB101) and *B. azotoformans* (RK2). (●) Normal conjugation and (▲) retrotransfer.

are equally if not more efficient at plasmid transfer than donors. In these experiments, this theory holds true for the 3× succinate case but not for the base case or for 0.5× succinate. This discrepancy in donor versus transconjugant initiated transfer indicates that plasmid transfer may not be just a strain related phenomenon, but also a result of the nutrient level. DAPI and FDG images of the biofilms resulting from each of these nutrient levels (photos not shown) indicate that the 1× and 0.5× succinate cases produce a denser biofilm than the 3× succinate case based on the intensity of the fluorescence. These dense microcolonies may affect the transport and consumption of nutrients. Reduced local nutrient concentration in the microcolony interior due to mass transfer limitations may create gradients in cell growth rates, reducing the cells' ability to transfer plasmid DNA, especially because piliation and donor ability are known to be functions of a cell's growth phase (Brinton, 1965).

## Effect of Retrotransfer

During retrotransfer, the recipient, *B. azotoformans*, harbors the conjugative RK2 plasmid and the donor, *P. putida*, contains plasmid pDLB101. Results of retrotransfer and mobilization at 1× succinate are plotted in Figures 6 and 7. As Figure 6 indicates, the rate of formation of transconjugants during the first few hours of contact is initially slower for retrotransfer compared to mobilization, but at 2 h retrotransfer rates dramatically increase and surpass direct mobilization rates. This change in the transfer rate may be attributed to the time required for RK2 to be transferred to and replicated in PB2440 before pDLB101 is transferred back to *B. azotoformans*. The exponential increase in transconjugant cell numbers after the initial 2 h of retrotransfer is likely a result of transconjugant growth. Plasmid transfer rates for these two cases are given in Table II. These results are in agreement with retrotransfer experiments carried out by

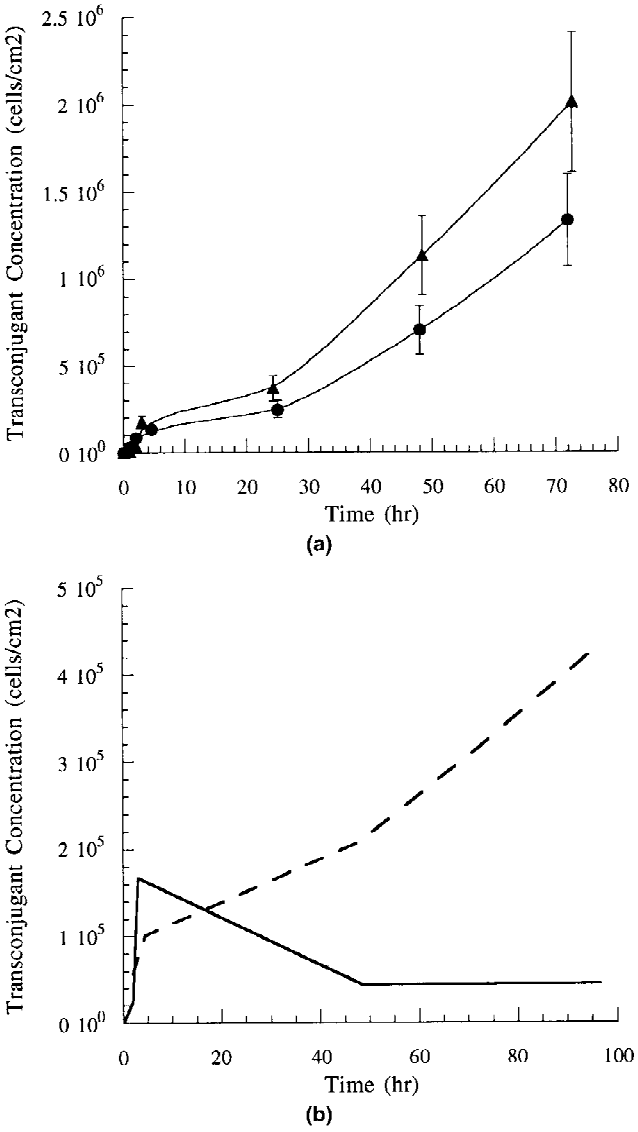


**Figure 7.** Transconjugant concentration profiles resulting from plasmid transfer between *P. putida* PB2440 (RK2, pDLB101) and *B. azotoformans* and between *P. putida* PB2440 (pDLB101) and *B. azotoformans* (RK2) using 25 μg/mL succinate. (A) Measured concentrations: (●) normal conjugation and (▲) retrotransfer. (B) Approximate concentrations when cell growth is subtracted out: (- - -) normal conjugation and (—) retrotransfer.

Perkins et al. (1994). In their experiments they found a 45–60 min lag before detecting transconjugants.

During retrotransfer there can only be exchange between the donor, PB2440 (pDLB101), and the recipient, *B. azoto-*

**Table II.** Measured plasmid transfer rates for donors and transconjugants for simple mobilization and retrotransfer.

| | Rate of plasmid transfer (cells/cm$^2$/h) | |
|---|---|---|
| | Donor mediated | Transconjugant mediated |
| Mobilization | 3.0E + 04 | 4.5E + 03 |
| Retrotransfer | 1.2E + 04 | 0 |

*formans* (RK2). Resulting transconjugants, *B. azotoformans* (RK2, pDLB101), and recipients both harbor the conjugative RK2 plasmid. Surface exclusion prevents two cells harboring the same conjugative plasmid from forming a mating pair. Once all of the recipient cells in contact with donors have become transconjugants, plasmid transfer stops and the transconjugant population increases only due to cell growth. Figure 7 shows the measured transconjugant concentrations for mobilization and retrotransfer over the course of the entire experiment as well as the transconjugant concentrations if cell growth is neglected. As expected, after some initial plasmid transfer from the donors to recipients, the rate of retrotransfer goes to zero.

## CONCLUSIONS

Experiments show that plasmid transfer and subsequent gene expression do occur when a GEM is released into an established biofilm community, even between Gram-negative and Gram-positive organisms. This means that special attention should be paid to the types of genes placed on plasmids used for bioremediation.

Previous research has shown that the rate of plasmid transfer in suspension is higher for higher nutrient concentrations and when transfer is initiated by the transconjugants as opposed to the original donors (MacDonald et al., 1992). This study indicates that during the initial contact between donors and recipients in a biofilm, plasmid transfer appears to be inhibited by high concentrations of the limiting carbon source. However, this may be a result of the nature of the biofilm matrix and not a physiological response. Rapidly dividing cells may actually inhibit the formation of efficient mating pairs by pushing neighboring cells away from each other before a donor cell and recipient cell can establish a mating pair. At longer times, when plasmid transfer is between transconjugants and recipients, the anticipated correlation between higher nutrient concentrations (more actively growing cells) and faster rates of plasmid transfer is seen. In these experiments, the rate of plasmid transfer from transconjugants to recipients appears to be slower than that for donor initiated transfer at the lower nutrient concentrations. This latter result may be an artifact of the biofilm matrix and not a function of the bacterial species.

Many of the bacteria in nature harbor conjugative plasmids, making retrotransfer of mobilizable plasmids a significant mechanism of genetic exchange. In measuring plasmid transfer resulting from retrotransfer in biofilms, the extent of transfer was limited by the number of donors in contact with recipients. However, the transconjugants continued to grow and multiply, yielding transconjugant concentrations similar to those measured for mobilization. Therefore, if the ultimate intent is to restrict the distribution of a phenotype in a specific ecosystem, then it is imperative that plasmids do not contain genes for any of the transfer functions.

A companion article to this work combines a theoretical model of biofilm accumulation with a kinetic expression for plasmid transfer and compares the data presented above with model simulations. This model includes attachment and detachment of cells between the bulk fluid phase and the biofilm matrix, the rates of mass transport of nutrients from the bulk fluid, the net rate of conversion of these substrates into new biomass, and bulk biomass convection due to growth. Results of these simulations are in excellent agreement with the experimental results.

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