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Frequency of Horizontal Gene Transfer of a Large Catabolic Plasmid (pJP4) in Soil

J. W. NEILSON, K. L. JOSEPHSON, I. L. PEPPER,* R. B. ARNOLD, G. D. DI GIOVANNI, AND N. A. SINCLAIR

Department of Soil and Water Science, University of Arizona, Tucson, Arizona 85721

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Limited work has been done to assess the bioremediation potential of transfer of plasmid-borne degradative genes from introduced to indigenous organisms in the environment. Here we demonstrate the transfer by conjugation of the catabolic plasmid pJP4, using a model system with donor and recipient organisms. The donor organism was *Alcaligenes eutrophus* JMP134 and the recipient organism was *Variovorax paradoxus* isolated from a toxic waste site. Plasmid pJP4 contains genes for mercury resistance and 2,4-dichlorophenoxyacetic (2,4-D) acid degradation. A transfer frequency of approximately $1/10^3$ donor and recipient cells (parent cells) was observed on solid agar media, decreasing to $1/10^5$ parent cells in sterile soil and finally $1/10^6$ parent cells in 2,4-D-amended, nonsterile soil. Presumptive transconjugants were confirmed to be resistant to Hg, to be capable of degrading 2,4-D, and to contain a plasmid of size comparable to that of pJP4. In addition, we confirmed the transfer through PCR amplifications of the *tfdB* gene. Although transfer of pJP4 did occur at a high frequency in pure culture, the rate was significantly decreased by the introduction of abiotic (sterile soil) and biotic (nonsterile soil) stresses. An evaluation of the data from this model system implies that the reliance on plasmid transfer from a donor organism as a remediative strategy has limited potential.

The introduction of genetically engineered microorganisms and/or bacteria with uncommon physiological capabilities has been considered for the improvement of crop yield, control of insects (16), frost prevention, and the degradation of a variety of persistent, organic compounds that would otherwise accumulate to hazardous levels in the environment (10). Generally, such microbial additions are known as bioaugmentation. As a remediative strategy, bioaugmentation offers the most promise in situations in which physicochemical treatment processes are impractical and pollutants are impervious to indigenous microbiota, e.g., in groundwater aquifers and soils (13).

Bioaugmentation strategies uniformly suffer from a lack of information regarding the stability of introduced genes and corresponding physiological traits in the environment. Halvorson et al. (11) listed the persistence of introduced organisms and rates of horizontal gene transfer to indigenous species as areas requiring scientific attention en route to the development of reliable biotechnology for environmental managements. Many studies have evaluated the survival of the heterotrophic aerobic gram-negative organism *Rhizobium* spp. when introduced into soil as an inoculant for legumes (19, 22). On the basis of these studies, the probability of long-term survival of an introduced organism in soil is low, thus limiting the bioremediation potential of introduced degraders (30). Horizontal transfer of plasmid-borne antibiotic and heavy metal resistance genes on solid media and in soil environments has been well documented (23, 24, 26, 27, 33), yet limited work has been done to assess the mobility of plasmid-borne degradative genes to indigenous microorganisms in the environment (9).

Research shows that the transfer and expression of plasmid-borne degradative genes are unique in comparison with the transfer of antibiotic and heavy metal resistance genes for various reasons. Degradative genes are typically found on larger, low-copy-number plasmids, which may have transfer behavior different from that of the smaller, high-copy-number antibiotic resistance plasmids. In addition, certain plasmid-borne degradative genes such as those for 2,4-dichlorophenoxyacetic acid (2,4-D) degradation are not expressed when transferred into organisms which lack the chromosomally encoded maleylacetate reductase vital to the organism's survival on 2,4-D as a sole carbon source (14). Research by Kinkle et al. (14) which monitored the transfer of plasmid pJP4 bearing 2,4-D degradative genes to soil populations of rhizobia detected the transconjugants by the expression of plasmid-borne mercury resistance genes, but 2,4-D degradative genes were not expressed. Similarly, Don and Pemberton (5) monitored transfer of pJP4 in pure culture and found that the 2,4-D genes were expressed in *Alcaligenes eutrophus*, *Variovorax paradoxus*, and *Pseudomonas putida* but not in *Escherichia coli*, *Rhodopseudomonas sphaeroides*, *Agrobacterium tumefaciens*, *Rhizobium* sp., *Pseudomonas fluorescens*, or *Acinetobacter calcoaceticus*. Again, resistance to mercury was expressed in all of the above organisms. Background information concerning the occurrence of maleylacetate reductase genes among different genera in the indigenous population is not available; thus, the transfer of 2,4-D degradative genes must be studied concurrent with the phenotypic expression of those genes in order to evaluate the remediation potential.

The influence of various abiotic factors on conjugation has been evaluated in terms of their influence on transfer frequency. Although gene transfer can occur by conjugation, transduction, or transformation, information on gene transfer in soil is limited. Optimal transfer frequencies have normally been observed at incubation temperatures, soil pH, nutrient concentrations, and moisture contents near those required for optimal bacterial growth (4, 26, 28).

In this study, soil microcosms were used to evaluate conjugation frequency in a model system, under simulated environmental conditions, in order to assess the potential of gene transfer as a bioremediation strategy. Microcosms were constructed of Brazito sandy loam, a typical desert soil. Donor and recipient organisms were selected to evaluate the transfer potential of the pJP4 plasmid carrying genes capable of degrading 2,4-D to an organism capable of expressing the degradative capacity. Under optimal growth conditions, con-

* Corresponding author.

jugation frequency was evaluated under the increasingly restrictive conditions of pure culture, sterile soil, and finally nonsterile soil. The influence of plate mating on artifactual gene transfer was also evaluated in the soil studies.

The donor microorganism was *A. eutrophus* JMP134, a pink, gram-negative soil bacterium containing plasmid pJP4 with genes coding for Hg resistance and the degradation of 2,4-D (5, 6, 18, 25). Plasmid pJP4 is a well-characterized (80-kb), catabolic, low-copy-number, broad-host-range P1 incompatibility group plasmid (5). Genes for the degradation of 2,4-D to 2-chloromaleylacetate are located on this plasmid and have been sequenced by Perkins et al. (18). Genes coding for the degradation of 2-chloromaleylacetate are located on the chromosome, thus allowing this organism to use 2,4-D as a sole carbon source.

The recipient microorganism was *V. paradoxus*, a yellow, kanamycin-resistant, gram-negative bacterium isolated from contaminated soils at Hill Air Force Base in Utah. This bacterium was chosen as the recipient because it was a non-2,4-D-degrading species and was indigenous to a polluted site and in addition, the lack of detectable plasmids precluded the possibility of subsequent plasmid incompatibility. The phenotypic and morphological differences between donor and recipient were sufficient to distinguish among donor, recipient, and transconjugant organisms in conjugation experiments. However, in these studies all presumptive transconjugants were confirmed.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The donor organism was *A. eutrophus* JMP134 (Km^s), containing plasmid pJP4 ($2,4\text{-}D^+$ Hg^r). *A. eutrophus* JMP134 was grown and maintained on brain heart infusion medium (BBL, Cockeysville, Md.) with 25 μg of Hg ml^{-1} as $HgCl_2$. The recipient, *A. paradoxus* (Km^r), was grown on PY medium (0.5% peptone, 0.3% yeast extract, and 10 mM $CaCl_2$) with 100 μg of kanamycin ml^{-1}. Both donor and recipient organisms were grown at 23°C with shaking at 100 rpm.

All cells were washed and diluted in sterile 0.85% saline (NaCl) for use in conjugation experiments. The extraction solution used to remove bacterial cells from soils contained 6 μM Zwittergent detergent (Calbiochem Corp., La Jolla, Calif.) and 0.2% sodium hexametaphosphate (Pfaltz and Bauer, Waterbury, Conn.) (3). The indicator broth used for detection of 2,4-D degradation contained minimal salts broth, supplemented with 500 μg of 2,4-D ml^{-1} and 0.004% bromthymol blue, with the pH adjusted to 7.0 (15). Minimal salts broth contained the following: $MgSO_4$ $7H_2O$, 112 mg; $ZnSO_4$ $7H_2O$, 5 mg; Na_2MoO_4 $2H_2O$, 2.5 mg; KH_2PO_4, 340 mg; Na_2HPO_4, 355 mg; $CaCl_2$ $2H_2O$, 14 mg; $FeCl_3$ $6H_2O$, 0.22 mg; and NH_4Cl, 0.5 mg in 1 liter of distilled water. Acid produced by the release of chloride ions during 2,4-D degradation caused a color change from green to yellow. The color change correlated with disappearance of 2,4-D as measured by a decrease in A_{283} (DU-6 spectrophotometer; Beckman, Fullerton, Calif.) (12).

Soil. Brazito sandy loam (Typic Torrifluvent) collected from the surface horizon at the University of Arizona's Campus Agricultural Center in Tucson was used for all microcosm experiments. Soil samples were air dried, ground, sieved (<2 mm), and stored at 4°C. Physical and chemical properties of the soil were as follows: pH, 8.2; total nitrogen, 0.01%; organic carbon, 0.12%; and electrical conductivity measured on a saturated paste, 0.3 dS m^{-1} (19). For Brazito soil, water potentials of −0.002, −0.004, and −0.006 MPa correspond to gravimetric water contents (grams per gram) of 21.5, 17.3, and 15.4%, respectively. Soil used in sterile microcosms was autoclaved at 121°C and 21 lb/in^2 pressure for 90 min. All soils presumed sterile were plated on PY media to confirm sterility.

Mating on solid agar media. Donor and recipient cells were mated on solid agar media to establish the maximum conjugation frequency under optimal conditions and to confirm the ability of transconjugants to degrade 2,4-D. Late-log-phase cultures of donor and recipient cells were centrifuged for 10 min at 10,780 × *g*, washed once, and resuspended in 0.85% sterile saline. Duplicate 5-μl aliquots of donor and recipient cells containing 9×10^5 CFU ml^{-1} each were combined in a mating patch on PY agar by a modification of the procedure of Walter et al. (32). Separate 5-μl patches of donor and recipient cells were also included as controls. Cultures were incubated at 27°C for 18 h. Each patch was then scraped into separate 1-ml saline solutions, diluted, and plated on the following selective media: PY agar with 25 μg of Hg ml^{-1} (PYM) for enumeration of donors and transconjugants, PY agar with 100 μg of kanamycin ml^{-1} (PYK) for enumeration of recipients and transconjugants, and PY agar with 100 μg of kanamycin and 25 μg of Hg ml^{-1} (PYMK) for enumeration of transconjugants only. Numbers of donors, recipients, and transconjugants were determined from CFU per plate on selective media.

Confirmation of transconjugants. Complete transfer of pJP4 plasmid with associated phenotypic traits was confirmed by the following procedure. Bacterial colonies growing on each replicate plate of PYMK were transferred individually to separate brain heart infusion broth cultures containing 25 μg of Hg and 100 μg of kanamycin ml^{-1} and grown to a cell density of 1.2×10^9 CFU ml^{-1}. Cells from turbid broths were centrifuged, resuspended in saline, and tested for the ability to degrade 2,4-D, the presence of the *tfdB* gene, and complete transfer of the 80-kb plasmid. The ability to degrade 2,4-D was confirmed by growth in the indicator broth. The presence of the *tfdB* gene from pJP4 was demonstrated by the amplification of a portion of the gene by PCR followed by visualization by agarose gel electrophoresis and ethidium bromide staining (17). Plasmid DNA from presumptive transconjugants was isolated by using a modified miniscreen for large plasmids (21) and then visualized by vertical agarose gel electrophoresis and ethidium bromide stained. Transconjugants with plasmids of the same size as pJP4 that could degrade 2,4-D, were resistant to Hg, and contained the *tfdB* gene were assumed to represent complete transfer of the pJP4 plasmid to recipients.

Assessment of artifacts due to plate mating. A modification of the procedure of Smit and van Elsas (23) was implemented to evaluate overestimations due to mating on selective agar plates. Late-log-phase cultures of donor and recipient cells were washed, resuspended on 0.85% saline, and serially diluted. Equal aliquots of donor and recipient cells from the same dilution were combined, vortexed, and plated on selective media PYM, PYK, and PYMK. All procedures were carried out at room temperature (23°C). Plate cultures were incubated at 28°C. The above experiment was then repeated at 4°C as described by Smit and van Elsas (23) with the exception that plates were incubated at 4°C for 24 h after inoculation before incubation at 28°C. The above dilution and plating procedure was then repeated at 23 and 4°C in soil in order to evaluate the influence of soil on these observations.

Mating in sterile soil. The effect of soil as a medium for bacterial conjugation was evaluated with sterile soil microcosms. Microcosms consisted of 50-g samples of air-dried, sterile Brazito soil in sterile, airtight glass jars. Late-log-phase cultures of donor and recipient organisms were washed and suspended in 2 ml of sterile saline. The recipient cell suspen-

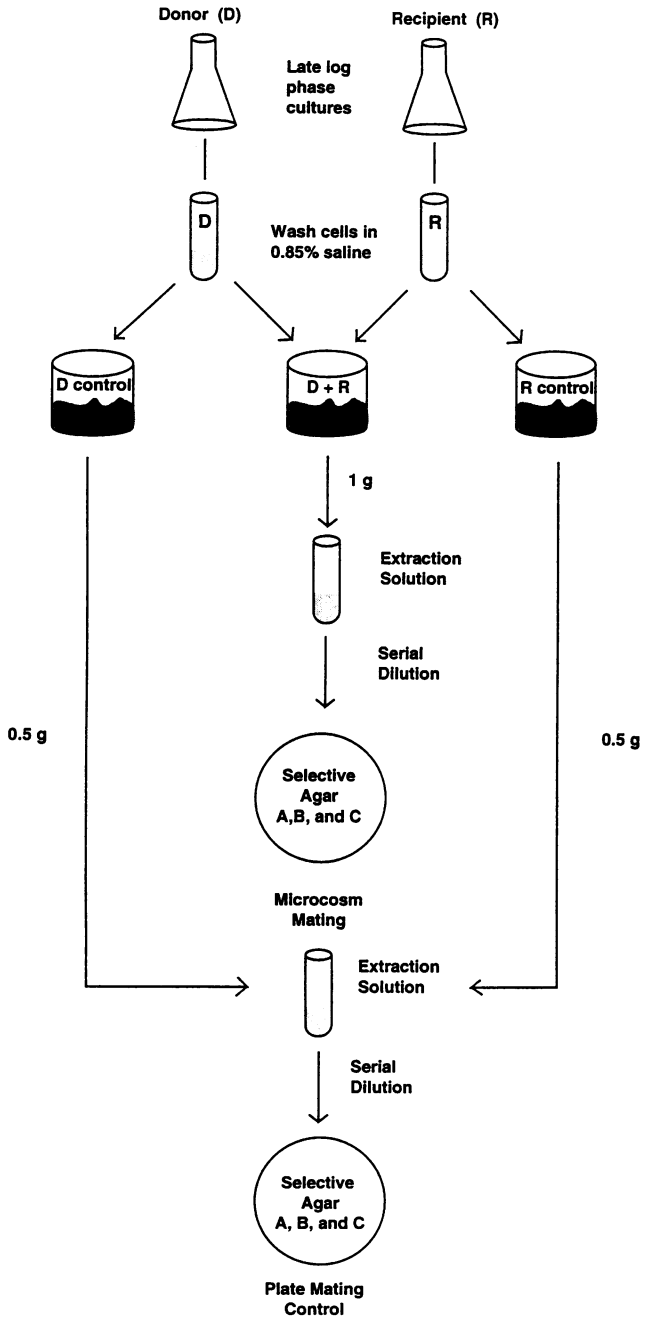

FIG. 1. Procedure for determination of conjugation frequency in soil. Sampling, dilution, and plating were done at 4°C.

sion was applied to the soil surface with a syringe fitted with a 26-gauge needle and mixed thoroughly to give an approximate inoculum of 10^8 CFU g of dry soil^{-1}. After 1 h, donor cells were added in the same manner and at the same concentration. Sterile saline was then sprayed over the soil surface to bring the gravimetric moisture content to 19% (percent saturation, 51.6%). Two control microcosms were also established under the same conditions, each inoculated with either donor or recipient cells. Moisture contents remained constant (±2%) over the entire period of incubation.

TABLE 1. Conjugation on solid agar medium

Cell	Expt 1	Expt 2
Donor	2.0×10^9	1.0×10^9
Recipient	1.9×10^9	2.0×10^9
Transconjugant	2.4×10^6	2.4×10^6

All microcosms were sampled immediately to give initial counts of donor, recipient, and transconjugant cells. Triplicate 1-g samples were removed from different areas of the jointly inoculated microcosm. Each 1-g sample was vortexed vigorously for 1 min in 9.5 ml of extraction solution, diluted, and then plated on selective media PYM, PYK, and PYMK. Overestimations due to plate mating were eliminated by processing soil samples and preincubating plates at 4°C prior to incubation at 28°C (as described previously). As an additional control for plate mating, separate 0.5-g samples were removed in triplicate from the donor and recipient control microcosms, combined in 9.5 ml of extraction solution, diluted, and plated as described above (Fig. 1). Any transconjugants found on these plates could only have resulted from conjugation during extraction or plating, and the absence of growth on PYMK would indicate the absence of conjugation on plates.

The above sampling procedure was repeated on days 1, 2, 3, and 8. Twenty-seven presumptive transconjugants were randomly isolated from the day 8 sample and were confirmed as described previously. Plasmid miniscreens were conducted with 14 of the 27.

Mating in nonsterile soil. The effect of competition from indigenous bacteria was evaluated by repeating the above experiments in nonsterile soil. Transfer frequencies were evaluated in unamended and 2,4-D-amended nonsterile microcosms. Microcosms containing 50 g of dry Brazito soil were brought to a gravimetric moisture content of 10% and preincubated at 28°C for 48 h to stimulate metabolic activity of indigenous organisms. These preincubated microcosms were then inoculated with donors and recipients each suspended in 1 ml of sterile saline as described for the sterile microcosms. Microcosms were sampled on days 0, 1, and 2 and cultured as described above.

The above procedure was repeated with a 2,4-D soil amendment to select for transconjugants. Soils in both the experimental and control microcosms were amended with 100 μg of 2,4-D g of dry soil^{-1}. In addition, bacteria from 1-g soil samples were extracted in 4.5 ml of extraction solution to increase the sensitivity of detection. All presumptive transconjugants found on replicates of PYMK media were transferred to selective broth and tested for plasmid transfer by growth in 2,4-D indicator broth, PCR, and plasmid profile.

RESULTS

Gene transfer on solid agar medium. Large numbers of transconjugants were generated when *A. eutrophus* JMP134 pJP4 and *V. paradoxus* were mated on nonselective solid media (Table 1). Frequency of transfer was approximately 1 transconjugant per 10^3 parent cells. No attempt was made to maximize conjugation frequency, only to demonstrate that transfer is frequent on solid agar media between this mating pair and that the number of transconjugants generated was consistent after repeated trials under optimum conditions. Transfer is assumed to have occurred by conjugation, although transformation cannot be totally ruled out.

From the solid medium transfer experiments, 75 presump-

TABLE 2. Effect of temperature on plate mating of bacteria extracted from soil samples

Cells	No. of bacteria g of dry soil^{-1} at °C:	
	23	4
Donors	6.1×10^9	8.2×10^9
Recipients	2.5×10^9	2.0×10^9
Transconjugants	10^{3a}	BDL[b]

[a] A minimum of 10^3 transconjugants was obtained, but enumeration was difficult because of diffuse overlapping colonies.
[b] BDL, below detection limit of 100 CFU g of dry soil^{-1}.

tive transconjugants were subsequently selected and further tested for confirmation of plasmid transfer and gene expression, with 2,4-D screening broth, PCR, and plasmid profile analysis (as described previously). As shown in Table 5, all presumptive transconjugants tested were positive and confirmed as true transconjugants.

Influence of plate mating on the determination of conjugation frequency. A minimum of 10^6 donor and recipient cells from broth cultures were required per plate for transconjugants to form when plate mating experiments were conducted at 23°C. When experiments were repeated at 4°C, the number of donor and recipient cells required per plate for plate mating to occur increased to 10^7. Results from plate mating experiments with donor and recipient cells extracted from soils are presented in Table 2. At 23°C, the number of donor and recipient cells required per plate for conjugation to occur was 10^7. At 4°C, transconjugants were not detected, implying that more than 10^7 donor and recipient cells were required for plate mating to occur. Thus, plate mating was reduced to an insignificant level for bacterial cells extracted from soil microcosms containing 10^8 donor and recipient cells when samples were processed at 4°C and the detection limit was 100 CFU/g of soil.

Gene transfer in sterile soil microcosm. When gene transfer studies were conducted in sterile soil, plasmid transfer was significantly less than that observed on culture media (Table 3). Over the 8-day incubation period, numbers of donor and recipient cells remained fairly constant. Transconjugant numbers increased from 0 to approximately 3×10^3 CFU g of dry soil^{-1} over the initial 24-h period and remained constant over the 8-day period. Mating frequency was approximately 1 transconjugant per 10^5 parent cells and was substantially less than the $1/10^3$ ratio observed on solid agar media (Table 1). It appears that conjugation occurred within the first 24-h period and that numbers of transconjugants remained stable over the 8-day period. Twenty-seven randomly selected presumptive transconjugants from the day 8 sample were confirmed positive for plasmid transfer as described above (see Table 5).

TABLE 3. Conjugation in sterile soil microcosm

Time and sample[a]	No. of bacteria g of dry soil^{-1}					
	Donors (10^8 CFU)		Recipients (10^8 CFU)		Transconjugants (10^3 CFU)[b]	
	Avg	SD	Avg	SD	Avg	SD
Day 0						
Joint	6.2	0.4	1.7	0.6	BDL	0
Control	5.6	0.9	1.5	0.5	BDL	0
Day 1						
Joint	6.2	0.6	2.6	0.7	3.8	2.0
Control	8.5	2.2	3.6	0.5	BDL	0
Day 2						
Joint	5.7	0.7	2.5	0.3	2.9	1.2
Control	8.0	2.8	3.2	0.4	BDL	0
Day 3						
Joint	6.9	1.6	2.2	0.6	2.0	0.5
Control	8.4	1.7	3.1	0.4	BDL	0
Day 8						
Joint	5.0	1.1	2.7	0.5	3.3	2.0
Control	8.3	0.5	3.5	0.2	BDL	0

[a] Joint refers to microcosm mating, while control refers to samples from control microcosms combined to determine occurrences of plate mating.
[b] BDL, below detection limit of 100 CFU g of dry soil^{-1}.

TABLE 4. Conjugation in 2,4-D-amended nonsterile soil microcosm

Time and sample[a]	No. of bacteria g of dry soil^{-1}					
	Donors (10^8 CFU)		Recipients (10^8 CFU)		Transconjugants (10^2 CFU)[b]	
	Avg	SD	Avg	SD	Avg	SD
Day 0						
Joint	2.4	0.3	1.8	0.2	BDL	0
Control	1.5	0.7	1.9	0.4	BDL	0
Day 1						
Joint	1.7	0.7	1.7	0.6	BDL	0
Control	1.1	0.1	0.9	0.3	BDL	0
Day 2						
Joint	0.6	0.3	1.1	0.2	1.1	0
Control	0.4	0.1	0.3	0.03	BDL	0

[a] Joint refers to microcosm mating, while control refers to samples from control microcosms combined to determine occurrences of plate mating.
[b] BDL, below detection limit of 50 CFU g of dry soil^{-1}.

Gene transfer in nonsterile soil microcosm. Nonsterile microcosms inoculated and incubated under the same conditions as the sterile soil microcosm produced no detectable transconjugants within 6 days. Initial donor and recipient counts were 2.8×10^8 and 3.4×10^8 CFU g of dry soil^{-1}, respectively, and numbers remained fairly constant through the first 48 h. By day 6, the donor and recipient counts had decreased to 1.4×10^6 and 2.2×10^7 CFU g of dry soil^{-1}, respectively.

The experiment was repeated with the addition of 100 μg of 2,4-D g^{-1} as a carbon source to enrich for donors and transconjugants. In addition, the extraction solution volume was decreased to increase the detection limit from 100 to 50 CFU g of dry soil^{-1}. Under these conditions, 1.1×10^2 transconjugants g of dry soil^{-1} were isolated after 48 h of incubation (Table 4). Frequency of transfer was approximately 1 transconjugant per 10^6 parents and was an order of magnitude less than that observed in sterile soil even though initial numbers of donors and recipients were comparable. No transconjugants were detected from control microcosm soils that were combined, extracted, and plated, thus eliminating plate mating as a source of the transconjugants. Five presumptive transconjugants were isolated and confirmed for plasmid transfer (Table 5).

DISCUSSION

The gene transfer studies conducted on solid agar demonstrated that plasmid pJP4 was transferred from *A. eutrophus* to *V. paradoxus* via plate matings. The transfer frequency of 1/1,000 parent cells was similar to that reported by Friedrich et al. (7). They examined the transfer of pJP4 between pure cultures of *A. eutrophus* and *E. coli* and reported transfer frequencies of between 1/100 and 1/1,000 donor cells. Our data confirmed that the entire plasmid was transferred as follows.

TABLE 5. Confirmation of presumptive transconjugants

Growth environment	No. positive/no. tested			
	Selective broth (Hg^r Km^r)	Indicator broth ($2,4\text{-}D^+$)	PCR (*tfdB* gene)	Plasmid profile (80-kb plasmid)
Solid agar medium	75/75	75/75	75/75	28/28
Sterile soil	27/27	27/27	27/27	14/14
Nonsterile soil	5/5	5/5	5/5	5/5

Genes responsible for Hg resistance were transferred as indicated by growth on selective antibiotic-Hg plates and by growth in Hg-enriched selective broth. The transfer and expression of genes for 2,4-D degradation were demonstrated by growth in indicator broth and by the presence of the *tfdB* gene as determined by PCR analyses. Finally, plasmid profile analyses showed that transconjugants contained plasmids of size similar to that of pJP4.

Preliminary studies in soil showed that gene transfer could arise from conjugation either in soil or on the surface of agar plates (plate matings). This latter phenomenon would result in an overestimation of the true rate of gene transfer in soil. Plate mating has also been identified as producing artifacts in other gene transfer studies in soil (23, 31). Walter et al. (31) tried to evaluate gene transfer between *Pseudomonas cepacia* strains, but initially over 99% of their transconjugants were due to plate matings. Smit and van Elsas (23) showed that plate mating did not occur when the number of parent cells was low ($<10^4$ cells of each per plate). They also showed that plate mating could be reduced by preincubation at 4°C prior to the normal higher incubation temperatures. Our studies agreed with those of Smit and van Elsas, since no plate matings were found after a preincubation at 4°C.

Many studies have evaluated gene transfer in broth or on agar plates. However, data on the transfer and expression of large, catabolic plasmids in soil are more limited, particularly in nonsterile soil. Studies utilizing sterile soil are useful to evaluate the influence of abiotic environmental parameters on gene transfer and are easier to conduct than those with nonsterile systems. Gene transfer in sterile soil was significantly less than that on agar medium, perhaps because of physical separation of donor and recipient cells by soil particles, or because of less active growth of parent cells in soil. Conjugation occurred during the initial 24 h of incubation, with numbers remaining constant over the remainder of the incubation period. However, it is unclear whether the initial population of transconjugants survived, or whether the rate of production and death of transconjugants was similar. Richaume et al. (20) evaluated transfer from donor *E. coli* cells to a *Rhizobium fredii* recipient in a sterile sandy loam soil. They showed that maximum transfer occurred after 5 days, at an optimum temperature of 28°C. Soil organic matter, pH, and soil moisture all influenced transfer, illustrating that transfer rates in any particular soil are unique to that soil. Bleakley and Crawford (2), using *Streptomyces lividans* donors and recipients in a sterile silt loam, also showed that soil abiotic factors influenced transfer rates.

Although useful, sterile soil studies do not evaluate true gene transfer rates since they do not take into account the influence of biotic stress. In nonsterile systems, donors, recipients, and transconjugants must be able to be detected and distinguished from indigenous organisms. In our nonsterile microcosms, parent cells and transconjugants were detected through antibiotic and heavy metal resistances. The influence of biotic stress resulted in the population of parent cells decreasing after 6 days, and no transconjugants were found. When the experiment was repeated, giving the introduced donor a competitive advantage by the addition of 2,4-D, a few transconjugants were detected. However, the transfer frequency in nonsterile soils was lower than that in sterile soil, illustrating the influence of indigenous soil organisms. Under sterile conditions, transconjugants were first detected after 24 h, compared with 48 h under nonsterile conditions. Trevors and Starodub (29) looked at R-plasmid transfer between *E. coli* strains in nonsterile soil. They also showed that transfer was significantly decreased under nonsterile conditions and that transfer was influenced by soil conditions. Smit and van Elsas (23) also evaluated gene transfer in nonsterile soil and showed that transfer was dependent on the number of parent cells. Similar to our study, the number of transconjugants in their study peaked at 2 days.

The data presented here show that transfer of large catabolic plasmids does occur under ideal growth conditions in nonsterile soil but that the transfer frequency is greatly reduced compared with transfer in sterile soil or on solid agar media. Thus, the use of plasmid transfer from a donor organism as a remediative strategy has a limited potential in the model system evaluated. Overall, gene transfer between different bacterial genera is possible but is likely to occur at low frequency rates (8). Thus, in an environment such as soil, over long periods of time, gene transfer is likely to be an important mechanism to enhance microbial diversity. However, in this model system the potential for bioremediation by transconjugation arising from introduced recipients it is not likely to be significant.

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