Gene Transfer of *Alcaligenes eutrophus* JMP134 Plasmid pJP4 to Indigenous Soil Recipients

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This study evaluated the potential for gene transfer of a large catabolic plasmid from an introduced organism to indigenous soil recipients. The donor organism *Alcaligenes eutrophus* JMP134 contained the 80-kb plasmid pJP4, which contains genes that code for mercury resistance. Genes on this plasmid plus chromosomal genes also allow degradation of 2,4-dichlorophenoxyacetic acid (2,4-D). When JMP134 was inoculated into a nonsterile soil microcosm amended with 1,000 μg of 2,4-D g^{-1} , significant (10^6 g of soil $^{-1}$) populations of indigenous recipients or transconjugants arose. These transconjugants all contained an 80-kb plasmid similar in size to pJP4, and all degraded 2,4-D. In addition, all transconjugants were resistant to mercury and contained the *tfdB* gene of pJP4 as detected by PCR. No mercury-resistant, 2,4-D-degrading organisms with large plasmids or the *tfdB* gene were found in the 2,4-D-amended but uninoculated control microcosm. These data clearly show that the plasmid pJP4 was transferred to indigenous soil recipients. Even more striking is the fact that not only did the indigenous transconjugant population survive and proliferate but also enhanced rates of 2,4-D degradation occurred relative to microcosms in which no such gene transfer occurred. Overall, these data indicate that gene transfer from introduced organisms is an effective means of bioaugmentation and that survival of the introduced organism is not a prerequisite for biodegradation that utilizes introduced biodegradative genes.

Biodegradation of organics within soil requires that indigenous soil microbes possess the appropriate degradative genes. If such organisms are not present, one strategy is to “introduce” microbes that do contain the necessary genes. This process is known as bioaugmentation, but it often suffers from two problems. The first problem is that the introduced organism often dies within a period of a few weeks as a result of abiotic and biotic stress. Second, it is difficult to get the introduced organism dispersed throughout the soil medium. These problems could, however, be overcome if gene transfer of the degradative genes from the introduced organism to indigenous soil recipients occurred. Genes for the degradation of many contaminants are often plasmid encoded (9, 11). Transfer of plasmids by conjugation with pure cultures in model and sterile soil systems has been well documented (14, 15, 19, 22, 23, 26–31, 33). The majority of these studies have focused on risk assessment of the release of engineered DNA into the environment rather than on the potential for bioaugmentation. In addition, few have found significant rates of transfer of catabolic plasmids in nonsterile soil from introduced organisms to indigenous organisms. Finally, it is important to note that little is known concerning the dissemination, persistence, and expression of introduced catabolic plasmids in natural microbial populations. Pertsova et al. (22) observed transfer of a 3-chlorobenzoate plasmid from *Pseudomonas* strains introduced into soil columns to indigenous pseudomonads. Fulthorpe and Wyndham (8) observed transfer of the 3-chlorobenzoate plasmid pBRC60 from *Alcaligenes* sp. strain BR60 to indigenous aquatic microorganisms in freshwater flowthrough mesocosms, but the design of the experiment did not permit the monitoring of growth and survival of specific recipient organisms.

The present study has focused on degradation of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), which is another

compound for which the catabolic genes are often located on plasmids (1, 4, 5, 18). One extensively studied 2,4-D-degrading plasmid is pJP4 (5–7, 10, 16, 21), a broad-host-range IncP1 plasmid, 80 kb in size, which encodes enzymes for the partial degradation of 2,4-D and confers mercury resistance. Plasmid pJP4 contains the genes for the degradation of 2,4-D to chloromaleylacetic acid, while chromosomal genes of the host are necessary for complete mineralization of the compound (16). Recently, Neilson et al. (19) used a model system to demonstrate transfer of plasmid pJP4 in nonsterile soil from *Alcaligenes eutrophus* JMP134 to an introduced *Variovorax paradoxus* strain. However, rates of transfer were low, with a frequency of 1 transconjugant per 10^6 parent cells in nonsterile soil amended with 100 μg of 2,4-D per g of dry soil.

In this study, we examined gene transfer of plasmid pJP4 to indigenous microorganisms in nonsterile soil microcosms. Microcosms were inoculated with *A. eutrophus* JMP134(pJP4) and enriched with 2,4-D. The isolation, identification, and characterization of transconjugants capable of expressing the *tfd* genes are described.

MATERIALS AND METHODS

Donor bacterium and maintenance. The donor organism *A. eutrophus* JMP134, which contains plasmid pJP4 (Tfd^+ Hg^r), was kindly provided by Kevin Short. The organism was grown and maintained on PH medium, which contained the following: peptone, 5.0 g; yeast extract, 3.0 g; CaCl_2 , 1.1 g; HgCl_2 , 25 mg; and 15 g of agar per liter of distilled water. Cultures were incubated at 28°C unless stated otherwise.

Microcosm design and treatments. Microcosms consisted of 150 g (dry weight) of nonsterile soil in 0.5-liter wide-mouth screw-cap glass jars. Surface soil, characterized as an acidic sandy loam with a pH of 5.5, was obtained from Madera Canyon Recreational Area of the Coronado National Forest, approximately 60 miles south of Tucson, Ariz. To our knowledge, this soil has never been cultivated or previously exposed to 2,4-D. Soil was sieved (2-mm mesh), brought to and maintained at a gravimetric moisture content of 18% with 0.85% NaCl, and incubated for 1 week at 28°C prior to treatments. A 1% 2,4-D stock solution was used to amend microcosms and prepared as follows. Ten grams of 2,4-D (Sigma Chemical Co., St. Louis, Mo.) was dissolved in 900 ml of distilled water by the

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addition of 10 ml of 5 N NaOH, adjusted to pH 7.0 with concentrated HCl, brought to a total volume of 1 liter, and filter sterilized (0.45- μ m pore size). The 2,4-D was added dropwise with a pipette while the soil was vigorously stirred. The donor inoculum, *A. eutrophus* JMP134, was prepared by resuspending colonies from a 48-h PH medium plate in 0.85% NaCl saline. The donor cell suspension was applied dropwise with a syringe fitted with a 26-gauge needle while the soil was vigorously stirred to give an approximate inoculum of 10^5 CFU g of dry soil⁻¹. The microcosms were treated as follows: microcosm I was an unamended, uninoculated control; microcosm II was amended with 500 ppm of 2,4-D and was uninoculated; microcosm III was amended with 500 ppm of 2,4-D and inoculated with JMP134; and microcosm IV was amended with 1,000 ppm of 2,4-D and inoculated with JMP134. After 5 weeks, microcosm IV received an additional 500 ppm of 2,4-D. Microcosms were sampled in triplicate every 7 days beginning at week 1 and terminating with week 9. At each sampling time, soils were stirred vigorously prior to sampling, 2,4-D levels were quantified, and mercury-resistant and 2,4-D-degrading organisms were enumerated.

At the conclusion of the experiment described above, the study was partially repeated to verify the results observed in microcosm IV. Microcosm IV remained the same, but in addition, an uninoculated control amended with 1,000 ppm of 2,4-D was included. Microcosms were sampled as described previously beginning at week 0 and proceeding for 6 weeks.

Quantitation of 2,4-D biodegradation. The utilization of 2,4-D was measured spectrophotometrically. Triplicate 0.5-g (dry weight) soil samples were removed, mixed with 4.75 ml of distilled water, and vortexed for 1 min. A 1.0-ml aliquot of this slurry was placed immediately in a microcentrifuge tube and centrifuged at $16,000 \times g$ for 5 min. A 500- μ l aliquot of supernatant was added to 500 μ l of distilled water, and the A_{230} was determined with a model DU-6 spectrophotometer (Beckman Instruments, Fullerton, Calif.). A decrease in A_{230} indicated 2,4-D metabolism. A standard curve generated from soils amended with 2,4-D standards was used to calculate 2,4-D concentrations from absorbance readings. Recovery of 2,4-D from soils was determined to be 100%, with an average standard deviation of 8%. Analysis of unamended soils revealed no significant absorbance by soil components at this wavelength.

Enumeration of mercury-resistant (Hg^r) and 2,4-D-degrading microorganisms. Microbial cells were extracted from soils and enumerated as follows. Triplicate 1-g soil samples were added to 9.5 ml of extraction solution which contained 6 μ M Zwittergent detergent (Calbiochem Corp., La Jolla, Calif.) and 0.2% Na hexametaphosphate (Pfaltz and Bauer, Waterbury, Conn.) (2). Samples were vortexed vigorously for 1 min and serially diluted. To enumerate mercury-resistant bacteria, diluted samples were spread plated on PH medium supplemented with 100 μ g of cycloheximide ml⁻¹ to inhibit fungal growth. Cultures were incubated for 1 week, and viable plate counts (in CFU milliliter⁻¹) were calculated.

A five-tube most-probable-number (MPN) method was used for the enumeration of 2,4-D-degrading microbes. The growth medium for the MPN tubes was an indicator broth which contained the following: $MgSO_4 \cdot 7H_2O$, 112 mg; $ZnSO_4 \cdot 7H_2O$, 5 mg; $Na_2MoO_4 \cdot 2H_2O$, 2.5 mg; KH_2PO_4 , 340 mg; Na_2HPO_4 , 355 mg; $CaCl_2$, 14 mg; $FeCl_3 \cdot 6H_2O$, 0.22 mg; NH_4Cl , 0.5 g; 2,4-D, 500 mg; and 0.04 g of bromothymol blue dye liter of distilled water⁻¹ (17) with the pH adjusted to 6.8. Cultures were incubated at 23°C with shaking at 150 rpm for 2 to 6 weeks, and change in color from green to yellow was scored as a positive reaction. The color change correlated with the disappearance of 2,4-D as measured by loss of A_{283} (13). Bacterial densities were calculated from MPN tables (12).

Isolation, identification, and characterization of transconjugants. In this paper, recipients of plasmid pJP4 are called transconjugants by convention (5). Expression of the Hg resistance gene found on plasmid pJP4 was used to identify potential transconjugant colonies. Colonies which developed from the highest dilutions on PH medium spread plates used for enumeration of mercury-resistant bacteria and which differed in morphology from the donor, *A. eutrophus* JMP134, were presumed to be transconjugants. These were isolated on PH medium. All isolates were gram-negative rods and were confirmed not to be the donor organism with GN microplates (Biolog Inc., Hayward, Calif.). Presumptive transconjugants were tested for the ability to degrade 2,4-D and for the presence of an 80-kb plasmid and the *tfdB* gene. The *tfdB* gene was utilized as a marker confirming the presence of pJP4 genes (20) presumably from the donor organism. 2,4-D degradation was confirmed by growth and acid production in the indicator broth. Plasmids were isolated by a modified miniscreen (24), and the size was confirmed by visualization by vertical agarose gel electrophoresis and ethidium bromide staining with the pJP4 plasmid as a size marker. Vertical gel conditions were 0.7% agarose and 20 mA of constant current per gel for 16 h at 4°C. The presence of the *tfdB* gene was confirmed by amplification of a 205-bp central portion of the gene by PCR (20). All Hg^r Tfd⁺ indigenous isolates containing the *tfdB* gene in conjunction with an 80-kb plasmid were considered to be transconjugants when isolates with the same combination of phenotypic and genotypic traits could not be isolated from control microcosms.

Effect of pH on 2,4-D soil extract cultures. The effect of pH on the degradation of 2,4-D by pure cultures of *A. eutrophus* JMP134 and selected transconjugants was determined by growing the organisms in phosphate-buffered soil extract supplemented with 2,4-D. Soil extract was prepared as follows: 400 g of sieved Madera Canyon soil was added to 960 ml of tap water, stirred well, prefiltered through paper towels, centrifuged at $8,000 \times g$ for 10 min, and finally filter

FIG. 1. Biodegradation of 2,4-D in soil microcosms. The data are the means and standard deviations for three replicate samples of microcosm I (■), microcosm II (●), microcosm III (▲), and microcosm IV (◆) (the left and right sets of datum points for this microcosm represent the 2,4-D amendments of 1,000 ppm at week 0 and 500 ppm at week 5, respectively). Values at week 0 are theoretical values based on amendment concentration.

sterilized (0.2- μ m pore size). Phosphate buffers (0.07 M) were prepared with the soil extract to achieve final reactions at pH values of 5.3, 5.9, and 7.0, representing a range from the acidic soils of Madera Canyon to neutral soil conditions. The medium was then amended with sterile 1% 2,4-D solution to give a final concentration of 1,000 μ g of 2,4-D ml⁻¹. Cultures were incubated at 23°C with shaking at 150 rpm, and 2,4-D concentrations were monitored for 7 days.

RESULTS

Biodegradation of 2,4-D. In microcosms inoculated with *A. eutrophus* JMP134 (microcosms III and IV), 2,4-D was degraded within 4 weeks irrespective of the initial concentration (Fig. 1). In addition, in microcosm IV, which was amended twice with 2,4-D, the second amendment of 500 ppm was completely degraded within 3 weeks. In the uninoculated microcosm (microcosm II), 2,4-D was degraded slowly and incompletely even after 9 weeks of incubation. When the experiment was repeated, microcosm IV showed complete degradation of 1,000 ppm of 2,4-D after 15 days. In comparison, an uninoculated control resulted in only 20% degradation of the 1,000 ppm after 6 weeks (data not shown).

Enumeration and characterization of mercury-resistant and 2,4-D-degrading microorganisms. Populations of mercury-resistant (Hg^r) organisms in the unamended and uninoculated microcosm I and the amended and uninoculated microcosm II remained relatively constant at approximately 3×10^3 CFU g of dry soil⁻¹ over the 9-week sampling period (Fig. 2). Randomly selected Hg^r isolates from these uninoculated controls were confirmed to be unable to degrade 2,4-D (Table 1). In the microcosm amended with 500 ppm of 2,4-D and inoculated with JMP134 (microcosm III), the numbers of Hg^r bacteria declined gradually from the initial JMP134 inoculum density of 4.8×10^5 to 3.1×10^3 CFU g of dry soil⁻¹ after 6 weeks. Throughout the 9-week sampling period, all colonies which grew on PH medium spread plates were morphologically identical to *A. eutrophus* JMP134 and randomly picked colonies were confirmed to be *A. eutrophus* with Biolog GN microplates. In the microcosm amended with 1,000 ppm of 2,4-D and inoculated with JMP134 (microcosm IV), the results were markedly different. One week after inoculation, the numbers of Hg^r microbes were at background levels and no JMP134 colonies could be detected. By weeks 2 and 3, increases to 1.9×10^5 CFU g of dry soil⁻¹ and subsequently 4.4×10^6 CFU g of dry soil⁻¹, respectively, were observed. Moreover,

FIG. 2. Enumeration of mercury-resistant bacteria in soil microcosms. The data are the means and standard deviations for three replicate samples of microcosm I (■), microcosm II (●), microcosm III (▲), and microcosm IV (◆). Values at week 0 are theoretical values based on inoculum density.

morphologies of colonies on PH medium plates at these times were different than that of *A. eutrophus* JMP134. Two different colony types were observed: one was an opaque, tan morphology type I (approximately 10^6 CFU g of dry soil⁻¹), and the other was a translucent, cream morphology type II (approximately 10^5 CFU g of dry soil⁻¹). By week 4, type II was dominant. At week 5, a third colony type with an opaque, white morphology type III became dominant (approximately 10^6 CFU g of dry soil⁻¹). Type III remained predominant throughout the remainder of the study.

Several colonies of each presumptive transconjugant were purified for identification and characterization. All of the presumptive transconjugants isolated from microcosm IV PH medium plates degraded 2,4-D, were Hg^r as indicated by growth on PH medium plates, contained an 80-kb plasmid equal in size to *A. eutrophus* JMP134 plasmid pJP4 (Fig. 3), and were positive for the *tfdB* gene (Fig. 4) of pJP4 (Table 1). The isolates were identified by Biolog as follows: colony type I, designated strain GDD1, was identified as *Pseudomonas glathei* (0.613 similarity value); colony type II, designated strain GDD2, was identified as *Burkholderia caryophyllii* (0.575 similarity value); and colony type III, designated strain GDD3, was identified as *Burkholderia cepacia* (0.631 similarity value).

TABLE 1. Confirmation of presumptive transconjugants

Soil amendment (ppm of 2,4-D)	Inoculum (CFU of <i>A. eutrophus</i> JMP134 g of dry soil ⁻¹)	Description of Hg ^r isolates ^a by:			
		Screening broth ^b	Biolog ID ^c	PCR ^d	Plasmid profile ^e
0	0	—	ND ^f	ND	ND
500	0	—	ND	ND	ND
500	10 ⁵	+	<i>A. eutrophus</i>	+	+
1,000	0	—	ND	ND	ND
1,000	10 ⁵	+	<i>P. glathei</i> <i>B. caryophyllii</i> <i>B. cepacia</i>	+	+

^a Isolates were taken from highest-dilution PH medium plates from week 2 to week 6 when 2,4-D was being actively metabolized. Only Hg^r isolates capable of degrading 2,4-D were identified and screened for the 80-kb plasmid and the *tfdB* gene.

^b —, 2,4-D negative; +, 2,4-D positive.

^c ID, identification (of strain).

^d +, presence of *tfdB* gene.

^e +, presence of 80-kb plasmid.

^f ND, not done.

FIG. 3. Plasmid profile of selected isolates visualized by vertical agarose gel electrophoresis and ethidium bromide staining. Lanes: 1, *P. glathei* GDD1; 2, *B. caryophyllii* GDD2; 3 and 4, *B. cepacia* GDD3; 5, indigenous 2,4-D degrader isolated from microcosm II; 6 and 7, bacteria from positive MPN tubes (10^{-3} dilution), microcosms I and II, respectively; 8, *A. eutrophus* JMP134(pJP4) recovered from microcosm III; 9, 80-kb plasmid isolated from *A. eutrophus* JMP134(pJP4) for size marker; 10, *V. paradoxus* DS17 negative control.

Changes in numbers of 2,4-D degraders in microcosms as determined by MPN are shown in Fig. 5. Data for microcosm II (amended and uninoculated) indicated an increase in numbers of indigenous degraders in response to the 2,4-D, but this increase was not paralleled by a similar increase in numbers of Hg^r microbes (Fig. 2). In addition, as shown in Fig. 3 and 4, indigenous 2,4-D degraders isolated from microcosms I and II and cells harvested from positive MPN cultures of microcosms I and II did not contain plasmids and were negative for the *tfdB* gene. Data concerning 2,4-D degraders for microcosms III and IV were similar to the Hg^r plate counts in that changes in numbers of 2,4-D degraders corresponded with changes in Hg^r populations. In microcosm III, numbers of 2,4-D degraders (*A. eutrophus* JMP134) as well as Hg^r organisms declined over the 9-week period. In contrast, in microcosm IV, the detection of only background levels of 2,4-D degraders at week 1 paralleled the lack of culturable JMP134 on PH selective medium. By week 2, the numbers of degraders increased sharply, and they remained at approximately 10^5 CFU g of dry soil⁻¹ for the remainder of the study.

Similar results were documented when the experiment was repeated. In this second study, presumptive indigenous transconjugants (Hg^r and Tfd⁺) were found at 10^6 CFU g of dry soil⁻¹ by day 15 in the inoculated microcosm amended with 1,000 ppm of 2,4-D. In contrast, in the uninoculated control soil amended with 1,000 ppm of 2,4-D, indigenous 2,4-D degraders remained below the detection limit of 10^2 CFU g of dry soil⁻¹. As in the previous study, presumptive transconjugants degrading 2,4-D were Hg^r, contained the 80-kb plasmid and the *tfdB* gene, and were confirmed by Biolog not to be the

FIG. 4. PCR detection of the *tfdB* gene of *A. eutrophus* JMP134 plasmid pJP4. Amplification products (size, 205 bp) were visualized by agarose gel electrophoresis and ethidium bromide staining. (A) Lanes: 1 to 4, *P. glathei* GDD1; 5 to 7, *B. caryophyllii* GDD2; 8 to 11, *B. cepacia* GDD3; 12 to 15, indigenous 2,4-D-degrading isolates from microcosm II. (B) Lanes: 1 to 4, bacteria from positive MPN tubes (10^{-3} dilution) from microcosms I to IV, respectively; 5 to 9, *A. eutrophus* JMP134(pJP4) recovered from microcosm III; 10, 205-bp amplified product from *A. eutrophus* JMP134(pJP4) positive control; 11, *V. paradoxus* DS17 negative control; 12, 205-bp molecular weight marker; 13 and 14, blank.

donor organism, *A. eutrophus* JMP134 (data not shown). Mercury-resistant organisms were present in the control soil at levels averaging 10^3 CFU g of dry soil $^{-1}$ throughout the incubation period, but none were able to degrade 2,4-D. Thus, the limited degradation observed in the control microcosm was

FIG. 5. Enumeration of 2,4-D-degrading bacteria by the MPN method (data at the 95% confidence level) for microcosm I (■), microcosm II (●), microcosm III (▲), and microcosm IV (◆). Values at week 0 are theoretical values based on inoculum density.

FIG. 6. Degradation of 2,4-D by *A. eutrophus* JMP134 (open symbols) and *B. cepacia* GDD3 (closed symbols) in soil extract broth containing 1,000 ppm of 2,4-D. Symbols: squares, pH 5.3; circles, pH 5.9; triangles, pH 7.0.

attributed to indigenous 2,4-D degraders rather than indigenous transconjugants.

Effect of pH on 2,4-D degradation. The rate and extent of degradation of 2,4-D by the predominant transconjugant *B. cepacia* GDD3 were essentially the same at all pH levels tested, i.e., pH 5.3, pH 5.9, and pH 7.0 (Fig. 6). In contrast, *A. eutrophus* JMP134 did not degrade 2,4-D at pH 5.3 and degraded the compound at pH 5.9 and 7.0 only after a 1- to 2-day lag period. JMP134 never degraded 2,4-D as effectively as strain GDD3 did at any of the pH values tested.

DISCUSSION

The results of this study indicate that significant gene transfer of a large catabolic plasmid from an introduced organism to indigenous soil recipients can occur. Previous studies have demonstrated plasmid transfer to introduced recipients in non-sterile soil (19, 30, 32), but transconjugant numbers remained extremely low. Transfer to indigenous recipients has also been observed (8), but the growth and survival of such transconjugants over time in soil have not been documented. In contrast, the data here clearly show that large populations of indigenous recipients can result from the introduction of donor genes into a soil system. Equally impressive is the enhanced ability of the transconjugants to degrade 2,4-D under soil conditions which inhibit the donor organism. Although the specific mechanism of transfer has not been identified, the significance of these results to bioaugmentation as a remediation strategy is clear.

Transfer of pJP4 was observed only in microcosm IV in which JMP134 was undetectable by culturable methods, 1 week after its introduction and prior to the appearance of transconjugants. Three different species of transconjugants within two genera appeared successively and were isolated and identified as *P. glathei* GDD1, *B. caryophyllii* GDD2, and *B. cepacia* GDD3. All contained an 80-kb plasmid identical in size to *A. eutrophus* JMP134 plasmid pJP4 and expressed the pJP4 phenotype, i.e., utilization of 2,4-D and mercury resistance. Finally, all contained pJP4 gene *tfdB* as detected by PCR.

A comparison of the results from each of the microcosm treatments justifies the assumption that isolates with the ability to degrade 2,4-D in conjunction with Hg r and the presence of an 80-kb plasmid and the *tfdB* gene are in fact indigenous transconjugants (Table 1). No strains similar to GDD1, GDD2, and GDD3 and coincidentally having the same genotypic and phenotypic characteristics as JMP134 could be isolated from microcosm I or II (uninoculated-unamended and uninocu-

lated-amended with 500 ppm of 2,4-D, respectively). In addition, none of the 2,4-D degraders isolated from these control microcosms contained an 80-kb plasmid or the *tfdB* gene of plasmid pJP4 as detected by plasmid isolation and PCR. Similarly, the uninoculated control amended with 1,000 ppm of 2,4-D in the second study contained no organisms that displayed the phenotypic characteristics identifying the transconjugants, i.e., that were capable of 2,4-D degradation and exhibited mercury resistance. Since the 80-kb plasmid and the *tfdB* gene common to all the transconjugants was not found in any of the degrading or Hg^r organisms isolated from the uninoculated soils, these genes could have originated only from the donor organism.

Transfer of plasmid pJP4 occurred in soil in microcosm IV and not on plating media. This conclusion is supported by the fact that it has been demonstrated that a minimum of 10⁷ donor and recipient cells per g of soil are required for plate mating to occur (19). This is because high concentrations of donor and recipient cells increase the probability of cell-to-cell contact on the plate. Thus, by isolating only presumptive transconjugant colonies from the high-dilution (>10⁻⁴) spread plates, the potential numbers of donor and recipient cells per plate are below the minimum required for plate mating to occur.

The apparent decline of JMP134 levels in microcosm IV was most likely due to a combination of low soil pH (pH 5.5) and high 2,4-D concentration (1,000 ppm), although other soil biotic and abiotic factors may have also contributed. It has been observed that only actively metabolizing JMP134 cells can be recovered from soils on 2,4-D- and Hg-selective media; thus, it is also possible that the JMP134 population did not decrease below detection levels as a result of cell death but rather remained present in the soil in an unculturable state. Data in Fig. 6 show that in soil extract, JMP134 did not metabolize 1,000 ppm of 2,4-D at values near soil pH (pH 5.3 and 5.9) and metabolized it only poorly at its optimum of pH 7.0. It has been established that JMP134 is able to degrade 85% of an initial 1,000 ppm of 2,4-D within 48 h when grown at pH 7.0 in minimal salts broth (data not shown), thus suggesting that other factors in the soil extract broth may be inhibitory along with pH. In contrast, the transconjugants were well suited to the soil conditions as evidenced by their growth and prolonged survival in microcosm IV and further exemplified by the ability of strain GDD3 to rapidly metabolize 2,4-D at pH 5.3 and 5.9 in soil extract. Thus, the transconjugants are not only acid tolerant but also well suited to other factors in the soil extract solution which appear to be inhibitory to the donor organism.

Despite the convention of referring to recipients of conjugal plasmids as transconjugants, the mechanism of transfer of plasmid pJP4 in microcosm IV is unclear. Recently, Romanowski et al. (25) showed that plasmid DNA introduced into nonsterile soil persisted for 60 days and that intact plasmids extracted from this soil retained *in vitro* transforming activity. Chamier et al. (3) also showed that plasmid DNA adsorbed on sand and groundwater aquifer substrate was able to transform *Acinetobacter calcoaceticus*. Whether *A. eutrophus* JMP134 lyses and releases plasmid DNA, which in turn may transform indigenous *Pseudomonas* and *Burkholderia* species, remains unknown and the subject of planned research. It is also unclear whether plasmid DNA passed sequentially from one strain to the next as they appeared during the 9-week incubation or whether the plasmid transferred directly from the donor organism to each of the three recipients. In the latter case, the sequential appearance of each transconjugant could be attributed to a number of factors, including either differences in the transfer frequency or the growth rate of each of the organisms.

Presumptive transconjugants were isolated only from the highest dilutions; thus, all three recipients may have been present at reduced numbers from the time the transconjugants were first detected.

The influence of gene transfer on biodegradation of 2,4-D is clearly shown by the increased rates of biodegradation in microcosm IV. This is particularly in evidence after the second amendment of 500 µg of 2,4-D g⁻¹, which was degraded very rapidly and correlated with the establishment of transconjugant populations. These data show that gene transfer of degradative genes from an introduced organism to indigenous recipients is an effective means of bioaugmentation and that survival of the introduced organism is not a prerequisite for the success of this strategy.

This article documents significant gene transfer of a catabolic plasmid from an introduced donor to indigenous soil recipients. To date, few studies have illustrated gene transfer to indigenous bacterial strains followed by growth and survival at levels significant enough (10⁶ g of soil⁻¹) to impact the soil system. Irrespective of mechanism, the transfer of desirable abilities from introduced strains to indigenous strains better fit for growth and survival in the environment may allow successful *in situ* bioremediation of contaminated soil.

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