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Conjugal Gene Transfer to Aquatic Bacteria Detected by the Generation of a New Phenotype†

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An experimental approach based on the assembly of genes of a catabolic pathway was used to detect transconjugants in aquatic communities. Resistance to phenylmercury acetate was established in transconjugants when wide-host-range conjugal plasmids containing *merB*, the gene encoding organomercurial lyase, were transferred to strains from aquatic communities that had been acclimated to inorganic mercury and thus enriched for populations containing *merA*, the gene encoding mercuric reductase (T. Barkay, Appl. Environ. Microbiol. 53:2725–2732, 1987). Conjugation was confirmed by using the plasmids' encoded antibiotic resistance patterns and by hybridization with a eukaryotic gene. Three *merB*-conjugal plasmids, belonging to incompatibility groups W (pGTE16), P1 (pGTE26), and N (pGTE25), were prepared. Transfers by filter matings of pGTE16 and pGTE26 from *Pseudomonas aeruginosa* PAO1 to indigenous strains were at efficiencies of 4.5×10^{-2} and 4.8×10^{-3} transconjugant per potential recipient, respectively. These efficiencies were from 1 to 2 orders of magnitude below those observed for intraspecies matings with genetically marked recipients. The third plasmid, pGTE25, was not stably maintained in *P. aeruginosa* donors, and its transfer from *Escherichia coli* donors was below the level of detection. Characterized transconjugant strains were shown to be *Pseudomonas* spp. Potential applications of the described experimental approach in the creation of bacterial populations with new catabolic capabilities in hazardous waste sites and in the detection of transfer of recombinant DNA from engineered microorganisms to indigenous bacteria are discussed.

The transfer of genetic material among microorganisms has been known for almost 50 years (1). Transduction (bacteriophage-mediated transfer [26]) and conjugation (transfer by direct contact between donor and recipient cells [21]) were later added to the original observation of transformation (uptake of extracellular DNA [53]). More recently, mechanisms that facilitate physical changes within a genome (34) and their potential to expand metabolic functions (43) have been described. Together, gene transfer (between genomes [32]) and gene change (within genomes [34]) give the microbial genome an extraordinary plasticity.

The role of gene transfer and change in the ecology of microbial communities is an intriguing issue (41, 59). Gene change and exchange are mechanisms that promote physiological diversity. The potential to alter metabolic functions is advantageous, because most microbes in nature live in changing environments. Moreover, genes that enable survival and proliferation in new and/or hostile environments (tolerance to antibiotics and toxic metals [55], utilization of chemically complex growth substrates [13], and colonization of plants [24] and animals [11]) are often located on mobile genetic elements. Thus, gene change and exchange may play an important role in adaptation of microbial communities (13, 51).

Investigations on the role of gene transfer in the ecology of natural microbial communities depend on the development of experimental approaches and methods to monitor gene flow in environmental samples. Achievement of this goal can be assisted by novel applications of molecular biological approaches to microbial ecology (20, 22, 38, 57). Here we report the detection of transconjugants among aquatic bacteria by the generation of a new phenotype when the *merB* gene (encoding organomercurial lyase [OL]) is conjugally transferred to strains carrying genes that encode resistance to inorganic mercury [Hg(II)], resulting in the establishment of resistance to phenylmercury acetate (PMA).

This approach is based on an understanding of the genetics and biochemistry of mercury resistance in bacteria (47, 55) and on our previous studies on acclimation of aquatic microbial communities to mercury (3, 5–7). Mercury resistance is a detoxification mechanism whereby toxic mercurial compounds are converted to the less toxic volatile form, Hg^0. It is specified by the *mer* operon, which encodes enzymes that transform Hg(II) and organic mercurial compounds to Hg^0, a mercury transport system, and functions needed for the regulation of *mer* expression. The enzymes are OL and a mercuric reductase (MR), the gene product of *merA*. In bacteria with both *merA* and *merB* (carrying a broad-spectrum *mer* operon), organomercury is first degraded by the OL to Hg(II) and a reduced organic moiety, and Hg(II) is then reduced by MR (Fig. 1). Bacteria that do not specify OL (carrying a narrow-spectrum *mer* operon) are resistant to inorganic mercury and a few organomercurials but can reduce only inorganic mercurial compounds. In the absence of OL, organomercurials can not be degraded and are therefore toxic (47, 55). Obviously, an Hg(II)-resistant recipient bacterium could become tolerant to organomercury upon supplementing its *merA* with a conjugally transferred *merB* gene. We therefore used strains with recombinant conjugal plasmids carrying *merB* as donors.

The source for *merA*-carrying recipients among indigenous bacteria was created by acclimation to Hg(II). Exposure to Hg(II) results in an enrichment of Hg(II)-resistant bacteria (3, 7). These strains carry *merA* genes (5, 6) and produce the MR enzyme (5). Transfer events were scored by

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† Dedicated to the memory of Steve M. Cuskey.

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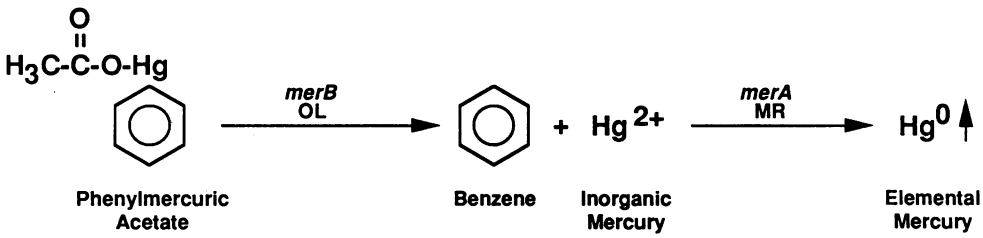

FIG. 1. Catabolic pathway for the degradation of PMA. The carbon-mercury bond in PMA is cleaved by the activity of the *merB* gene product, OL. One product of this reaction, mercuric mercury, is subsequently reduced by the *merA* gene product, MR, to volatile elemental mercury.

the appearance of PMA-resistant (PMA^r) bacteria following incubations of donor strains with strains from Hg(II)-acclimated communities.

MATERIALS AND METHODS

Bacterial strains, plasmids, and bacteriophages: sources and growth conditions. Bacterial strains, plasmids, and bacteriophages used in this study are listed in Table 1. Plasmids were the gifts of Anne Summers (University of Georgia, Athens), Dennis Ohman (University of Tennessee, Memphis), Joanne Horn (University of West Florida, Pensacola), Ron Olsen (University of Michigan, Ann Arbor), and Robert Miller (Oklahoma State University, Clearwater). *Escherichia coli* strains and *Pseudomonas aeruginosa* PAO1 were grown in Luria-Bartani (LB) medium (tryptone [Difco Laboratories, Detroit, Mich.], 10 g/liter; yeast extract [Difco], 5 g/liter; NaCl, 10 g/liter; glucose [Difco], 1 g/liter; pH 7.2 to 7.4) at 37°C. Agar (Difco; 15 g/liter) was added to solid media. Filter-sterilized stock solutions of mercurial compounds and antibiotics were added to autoclaved, cooled (55°C) media. Mercuric chloride (Sigma Scientific Products, St. Louis, Mo.) and PMA (Sigma) were added to a final concentration of either 50 or 25 μM. Antibiotics (ampicillin [AP], chloramphenicol [CM], tetracycline [TC], trimethoprim [TP], kanamycin [KM], and carbenicillin [CB] [Sigma]) were added to the concentrations indicated in the text. AP and CB were used for selection of β-lactamase-producing (*bla*$^+$) *E. coli* and *P. aeruginosa*, respectively.

Male-specific bacteriophages were the gift of R. Miller, and phage lysates were prepared according to the method of Miller and Ku (36) with PAO1 RM2004(RP4) as a host.

Construction of plasmids. Three plasmids representing three incompatibility groups (Inc) of wide-host-range conjugal plasmids in gram-negative bacteria were prepared by subcloning a *merB*-plant DNA cassette. Each of these plasmids also carried antibiotic resistance genes.

The *merB*-plant DNA cassette was prepared as follows. A recombinant plasmid, pGTE15 (Fig. 2), containing *merB* and a 350-bp *Eco*RI-*Bam*HI fragment from the napin gene of

TABLE 1. Bacterial strains, plasmids, and bacteriophages[a]

Bacterium, plasmid, or bacteriophage	Relevant genotype or phenotype	Comments	Reference or source
Bacteria			
E. coli JM109	*thi-1 proAB*		ERL/GB
E. coli HMS174			ERL/GB
P. aeruginosa PAO1	Prototroph		ERL/GB
P. aeruginosa PAO1 RM4002 (RP1)	Km^r	Positive control for male-specific phage assays	R. B. Miller
Plasmids			
p#7	Ap^r	pUC19 derivative with 0.35-kb *B. napus* DNA	12; this paper
pCT12	Ap^r *merB*	Contains 1.5-kb *Eco*RV fragment with *merB* from R831	A. O. Summers (46)
pGTE15	Ap^r	p#7 with the *merB* from pCT12	This paper
pGTE22	Ap^r	pGTE15 with an additional multicloning site	This paper
pACYC184::Tn*501*	Cm^r Hg resistant		A. O. Summers
R388::Tn*501*	Tp^r Hg resistant		R. Olsen
pGTE27	*tra* Tp^r	Conjugation-negative derivative of R388::Tn*501*	This paper
pKSM7::Tn*501*	Tc^r Hg resistant IncP1	pLAFR3 with Tn*501*	D. Ohman, J. Horn
R388	Tp^r IncW		R. Olsen (58)
pGTE16	Tp^r *merB*	*merB*-plant DNA derivative of R388	This paper
pKM101	Ap^r IncN		R. B. Miller (28)
pGTE25	Ap^r *merB*	*merB*-plant DNA derivative of pkM101	This paper
RP1	Ap^r Tc^r Km^r IncP1		R. B. Miller (16)
pGTE26	Ap^r Tc^r Km^r *merB*	*merB*-plant DNA derivative of RP1	This paper
Bacteriophages			
PRD1		Male-specific phage	R. B. Miller (39)
PR4		Male-specific phage	R. B. Miller (52)

[a] Abbreviations: ERL/GB, Environmental Research Laboratory, Gulf Breeze culture collection; *tra*, conjugal transfer deficiency; *thi*, thiamine deficiency; *pro*, proline deficiency; r, resistance.

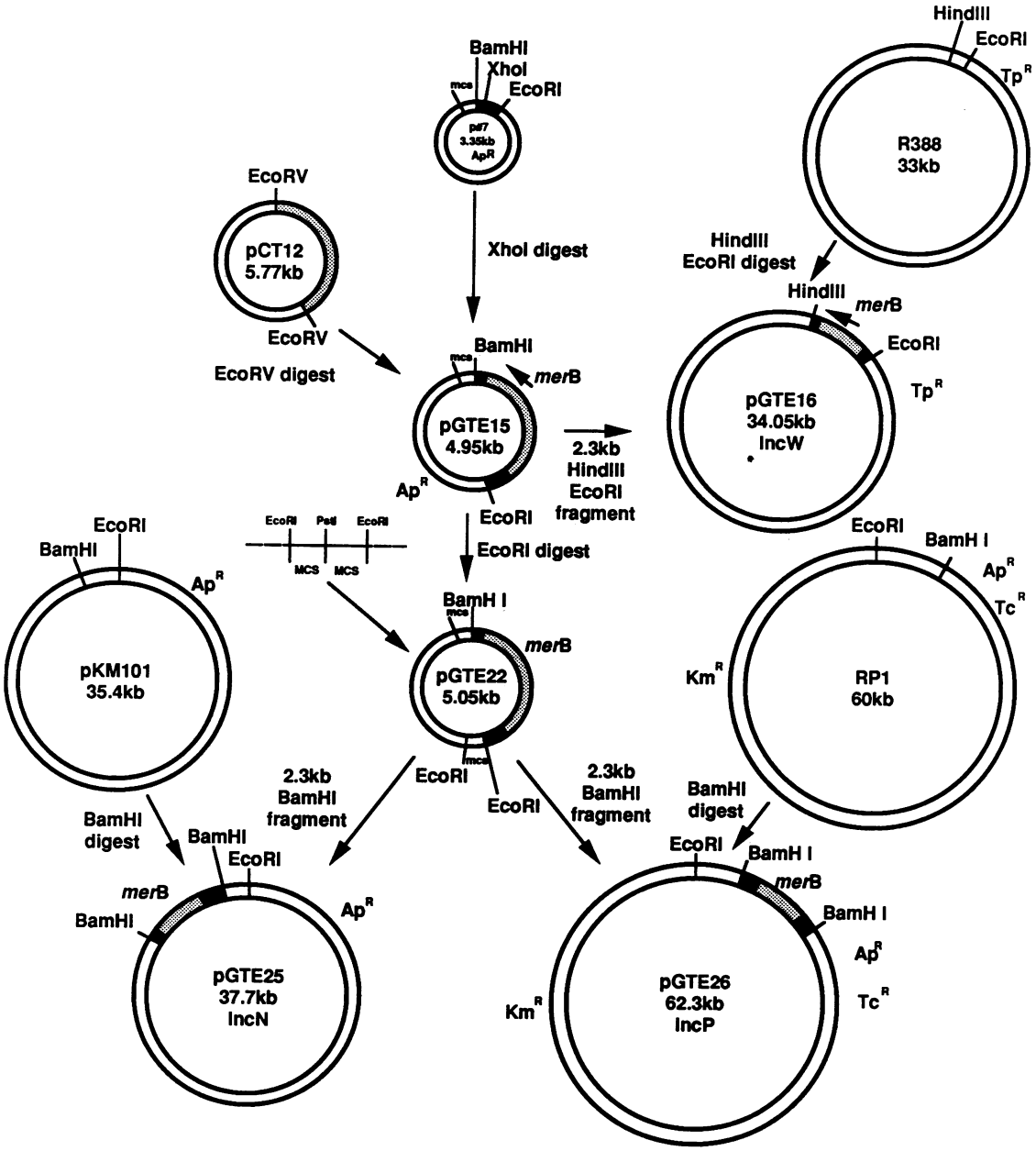

FIG. 2. Construction of *merB*-plant DNA broad-host-range plasmids. Vector sequences are indicated by open frames, filled areas denote plant DNA sequences, and grey areas represent *merB*. Relevant restriction sites and locations of antibiotic resistance genes are indicated. MCS, multicloning site.

Brassica napus (12) was prepared. The source of *merB* was pCT12, which is a recombinant plasmid containing a *merR* gene, the *mer* operator-promoter region, a truncated *merA* gene, and a *merB* gene from the broad-spectrum mercury resistance plasmid R831 (46) on a 1.6-kb *Eco*RV fragment (54). This *Eco*RV fragment was cloned in an *Xho*I-digested pUC19 derivative (p#7) that contained the *B. napus* sequence. In pGTE15 the plant DNA brackets *merB*, and this cassette is bordered on one side by a portion of the pUC19 multicloning site. A second multicloning site was inserted in the single *Eco*RI site of pGTE15. The insert consisted of an 84-bp fragment containing most of the restriction sites of the multicloning site of pUC19 and pUC18, and it was formed by ligating *Pst*I-digested pUC19 and pUC18 (35) in a mirror image orientation and by subsequent *Eco*RI digestion. The resulting plasmid, pGTE22, is a source for the *merB*-plant DNA cassette that can be conveniently cloned in a variety of DNA vectors (Fig. 2).

Plasmid pGTE16 (IncW) was prepared by subcloning a *Hin*dIII-*Eco*RI fragment from pGTE15 to plasmid R388 (58). Plasmids belonging to IncP1 (pGTE26) and IncN (pGTE25) were prepared by subcloning the *merB*-plant DNA cassette on a *Bam*HI fragment in plasmids RP1 (16) and pKM101 (28), respectively. Although available restriction maps (29) suggest that *bla* would be inactivated by an insertion in the *Bam*HI site of RP1, subsequent analysis indicated that both

E. coli and *P. aeruginosa* carrying pGTE26 were fully resistant to AP (CB).

Routine procedures for the isolation of plasmid DNA, gene cloning, transformations of *E. coli* strains, visualization of small plasmids (<30 kb) in *E. coli* transformants, and agarose gel electrophoresis were as described by Maniatis et al. (33) and Sambrook et al. (45). Restriction enzyme digestions were performed as recommended by the manufacturer (New England Biolabs, Inc., Beverly, Mass.). Cell transformations of strain PAO1 were performed as described by Bagdasarian and Timmis (2), and small plasmids in PAO1 hosts were visualized as described by Gonzalez et al. (15). Large plasmids (>30 kb) in *E. coli* and PAO1 strains were extracted and visualized by a scaled-up modification of the alkaline lysis procedure (33), starting with 8 ml of late-log-phase cultures.

Following the construction of recombinant derivatives of conjugal plasmids (i.e., pGTE16, pGTE25, and pGTE26 [Fig. 2]), the presence of plasmids was confirmed by growth on selective media, visualization of plasmids, and plaque formation following infection with male-specific bacteriophages PR4 and PRD1 (36).

Conjugal transfer between pure cultures. Conjugations were performed on solid surfaces because the three conjugal plasmids studied here coded for a rigid pilus morphology and had been shown to be transferred at higher frequencies on plates as compared with broth matings (9). Donor strains contained the recombinant *merB* plasmids or their parent plasmids. Recipient strains contained plasmids that were compatible with the conjugal *merB* plasmids, had a narrow-spectrum *mer* operon from Tn*501*, and coded for at least one antibiotic resistance marker not present on the conjugal plasmid (Table 1).

Plasmids were transferred to new hosts by patch conjugation. Cultures of donor and recipient strains were grown together on LB agar, and the resulting mixture of cells was streaked on media selecting for transconjugants. Negative controls consisted of donor and recipient strains cultured separately. Patch conjugations overlaid with 200 U of DNase I (Sigma) were performed to rule out transformation as the mechanism of plasmid transfer.

Filter matings, performed to measure efficiency of plasmid transfer, followed the procedure of Simon et al. (48) except that LB medium was used and conjugations were performed for 2 h at 37°C. After cells were dislodged from the filters (by 30-s vortexing), suspensions were induced by incubation in the presence of 0.1 μM $HgCl_2$ for 30 min at 37°C with shaking (200 rpm). Induced cell suspensions were diluted and plated on selective media, and plates were incubated at 37°C overnight. Transconjugants were enumerated on medium with PMA and on medium with a combination of antibiotics that selected for the transferred plasmid and the resident *merA* plasmid. Recipients were enumerated on LB agar containing appropriate antibiotics. The efficiency of transfer was expressed as the number of transconjugants per number of recipients.

Conjugal transfer of plasmids to aquatic bacteria. Transfer of *merB* plasmids to indigenous flora was detected following filter matings between PAO1 donor strains and bacteria from Hg(II)-acclimated microbial communities. Samples collected at a freshwater pond (Pensacola Beach, Fla.) were exposed to 250 μg of Hg(II) per liter, as $HgCl_2$, to enrich for Hg(II)-resistant populations (3). A suspension consisting of 20 ml of acclimated water samples was mixed with 2×10^6 cells (as CFU) of the donor strain and filtered through a 0.22-μm-pore-size type GS filter (Millipore Corp., Bedford, Mass.). Matings were performed as described above for pure cultures, except that filters were incubated on half-strength plate count agar (1/2PCA [tryptone, 2.5 g/liter; yeast extract, 1.25 g/liter; glucose, 0.5 g/liter; agar, 15 g/liter; pH 7.0]) for 4 h at 30°C. No growth of indigenous bacteria occurred under these conditions (data not shown). Following matings, cells were suspended in half-strength plate count broth, incubated in the presence of 0.1 μM $HgCl_2$ at 30°C for 30 min, and plated on 1/2PCA containing 25 μM PMA to select for transconjugants and on 1/2PCA containing 25 μM $HgCl_2$ to enumerate potential recipients. Donors were enumerated in some mating experiments by plating on LB with the appropriate antibiotics. Plates were incubated at 30°C for 24 to 48 h. The efficiency of transfer was expressed as the number of transconjugants (PMA^r CFU) per number of potential recipients [Hg(II)-resistant CFU]. Bacteria from unacclimated communities (3) were used as a control for the role of acclimation in the generation of a pool of potential recipients. Acclimated and unacclimated water samples that did not contain donors were used to enumerate indigenous PMA^r bacteria.

The transfer of *merB* plasmids was confirmed by subculturing PMA^r colonies to 1/2PCA plates containing the antibiotics that were encoded by the test conjugal plasmid and by colony hybridization with a eukaryotic DNA probe. Labelling of the probe (a 350-bp *Eco*RI-*Bam*HI restriction fragment from plasmid p#7; Fig. 2), hybridization, and preparation of autoradiograms were as previously described (3). Transconjugants were characterized by API Rapid NFT strips (Analytab Products, Plainview, N.Y.). An attempt to preserve transconjugants, according to the method described by Maniatis et al. (33), for characterization and further study was largely unsuccessful.

RESULTS

Conjugal transfer of broad-host-range plasmids and their *merB*-plant DNA derivatives. Transfer of recombinant plasmids and their progenitors between pure cultures of donors and recipients was performed to test whether cloning of the *merB*-plant DNA had affected conjugal transfer functions and to determine mating efficiencies as a reference for comparison with transfer to aquatic bacteria (see below).

Transconjugants were enumerated by growth in the presence of antibiotics (resistances to which were encoded by the conjugal plasmid and the resident *merA* plasmid) and on medium with 50 μM PMA (Table 2). Initially, efficiency of transfer as determined by antibiotic selection was 3 to 4 orders of magnitude higher compared with that determined by PMA selection. Because all antibiotic-resistant transconjugants grew upon a secondary transfer to PMA medium, indicating the presence of active MR and OL, we assumed that expression of *merA* and *merB* in newly formed transconjugants was insufficient to overcome the toxicity of PMA. Preliminary experiments showed that the efficiency of selection on PMA was reduced because of the absence of prior induction of *mer* and the lack of an outgrowth period. It was therefore necessary to modify the mating protocol to include a 0.5-h incubation period in the presence of subtoxic, yet inducing, concentrations of Hg(II) (0.1 μM) (55) prior to plating on selective media. This treatment resulted in an improved selection of PMA^r *E. coli* transconjugants, with a difference of less than 1 order of magnitude between transfer efficiencies on antibiotics and on PMA media. Complete recovery of transconjugants on PMA media was obtained with *P. aeruginosa* transconjugants (Table 2). Preliminary

TABLE 2. Efficiency of intraspecies transfer of broad-host-range plasmids and their *merB*-plant DNA derivatives among pure cultures of *E. coli* and *P. aeruginosa*

Species and plasmid	Antibiotics[a] (μg/ml)	Efficiency of transfer (transconjugant/recipient)[b] with selection on:	
		Antibiotics	PMA (50 μM)
E. coli[c]			
R388	TP (1,000), CM (100)	4.6×10^{-2}	$<4.0 \times 10^{-9}$
pGTE16	TP (1,000), CM (100)	8.3×10^{-2}	2.3×10^{-2}
pKM101	AP (25), CM (50)	3.4×10^{-1}	$<4.7 \times 10^{-9}$
pGTE25	AP (25), CM (50)	3.6×10^{-1}	2.6×10^{-1}
RP1	AP (100), CM (50)	9.0×10^{-1}	$<2.4 \times 10^{-9}$
pGTE26	AP (100), CM (50)	2.6×10^{-1}	1.8×10^{-1}
P. aeruginosa[d]			
R388	TP (600), TC (5)	8.4×10^{-1}	$<4.6 \times 10^{-9}$
pGTE16	TP (600), TC (5)	1.9×10^{-1}	2.0×10^{-1}
RP1	CB (600), TP (1,000)	5.1×10^{-1}	$<3.1 \times 10^{-9}$
pGTE26	CB (600), TP (1,000)	8.1×10^{-1}	9.0×10^{-1}

[a] The first drug indicates selection for the conjugal plasmid; the second indicates selection for the resident *merA* plasmid.
[b] Results were obtained with the means of CFU per milliliter of two to three replicate samples. Standard errors were ≤30% of the means.
[c] In all matings, donor and recipient *E. coli* strains were JM109 and HMS174(pACYC184::Tn*501*), respectively.
[d] Strain PAO1 served as a donor. PAO1(pGTE27) was the recipient in matings with PAO1(RP1) and PAO1(pGTE26). Strain PAO1(pKSM7::Tn*501*) was the recipient in matings with PAO1(R388) and PAO1(pGTE16).

experiments indicated that there was no growth of either transconjugant or recipient strains that might have affected the efficiency-of-transfer determinations during the 0.5-h incubation prior to plating of mating mixtures.

Plasmids were transferred among *E. coli* and *P. aeruginosa* strains at similar efficiencies (10^{-1} to 10^{-2} transconjugant per recipient). The efficiency of interspecies conjugations between *E. coli* donors and *P. aeruginosa* recipients was approximately 1 order of magnitude lower (range, 10^{-2} to 10^{-3}) than that of intraspecies matings (data not shown).

Our attempts to obtain stable donor strains of *P. aeruginosa* with pKM101 and pGTE25 were unsuccessful. These included conjugation from *E. coli* donors, transformation, and electroporation. The few Cb^r clones of PAO1 that were selected did not transfer the plasmids to secondary recipients, and agarose gel electrophoresis analyses of DNA preparations showed no recognizable plasmid patterns. Apparently, similar to findings of Tardif and Grant (56), our IncN plasmids pKM101 and pGTE25 could not be stably maintained in *P. aeruginosa*.

Conjugal transfer to indigenous flora. Conjugations were performed under optimal conditions to evaluate our approach of detecting gene transfer from specific donors to indigenous bacteria. Conjugations were performed at least twice for each cross, using triplicate crosses for acclimated communities. Results (the means for CFU; standard errors were below 50% of the means) of one experiment for each plasmid are presented here (Table 3).

Transfers from PAO1 donors were demonstrated for pGTE16 and pGTE26 (Table 3). Transfer of pGTE16 was performed with an acclimated community that contained 1.0×10^3 Hg(II)-resistant CFU/ml and 5.1×10^1 PMA^r CFU/ml (data not shown). These numbers represent an increase of 2 orders of magnitude over Hg-resistant and PMA^r counts of control unacclimated communities. The efficiency of pGTE16 transfer was 4.5×10^{-2} transconjugant per potential recipient. This is approximately an order of magnitude lower than efficiencies observed in matings between PAO1 strains (Table 2). All PMA^r colonies were TP^r and hybridized with the plant DNA probe, confirming their identity as transconjugants. Two colony morphologies, small (1 to 2 mm in diameter) cream colored and medium (3 to 4 mm) cream colored, were apparent among pGTE16 transconjugants. Two representative cultures of the medium-sized colonies were characterized as *Pseudomonas* spp. Although some PMA^r strains were present in the acclimated community (see above), no PMA^r colonies were detected following conjugations between PAO1(pGTE16) and an unacclimated community or in acclimated communities to which the donor strain was not added (i.e., background indigenous PMA^r). The absence of PMA^r colonies in control crosses following conjugation could be explained by the sensitivity of plating.

Transfer of pGTE26 was performed with an acclimated community that contained 1.8×10^3 Hg(II)-resistant CFU/ml (an increase of 2 orders of magnitude over control counts) and <10 PMA^r CFU/ml. The efficiency of pGTE26 transfer was 4.8×10^{-3} transconjugant per potential recipient (Table 3), almost 2 orders of magnitude lower than those recorded for transfer between pure PAO1 strains (Table 2). Donors were not enumerated in pGTE26 conjugations. The number of PMA^r colonies in control crosses was below the level of detection. Transconjugants belonged to two colonial morphology groups, a spreading colony and a small cream colony. All were resistant to TC, CB, and KM, and all hybridized with the plant DNA probe. None of these colonies survived for further characterization.

High numbers of donors were employed in these experiments (Table 3). Subsequent experiments have shown that at lower donor densities no conjugal transfer could be detected.

Two attempts to transfer pGTE25 to indigenous flora with *E. coli* JM109 as a donor were unsuccessful. Thus, determination of the efficiency of transfer of this plasmid awaits its stable inheritance in a suitable donor strain.

TABLE 3. Conjugal transfer of *merB*-plant DNA plasmids from *P. aeruginosa* PAO1 to Hg(II)-acclimated freshwater bacteria[a]

Plasmid	Source of recipients[b]	No. of CFU/ml				Efficiency (transconjugant/recipient)
		Recipients [Hg(II) resistant]	Donors	Transconjugants (PMA^r)	Background (PMA^r)[c]	
pGTE16	Hg(II) acclimated	9.6×10^3	1.5×10^7	4.4×10^2	<1.4	4.5×10^{-2}
	Control	$<10^2$	1.2×10^7	<1.4	<1.4	$<1.4 \times 10^{-2}$
pGTE26	Hg(II) acclimated	1.8×10^4		8.6×10^1	<1	4.8×10^{-3}
	Control	10^2		<1	<1	$<10^{-2}$

[a] All numbers are for the final suspensions obtained following conjugations and incubations in the presence of 0.1 μM $HgCl_2$.
[b] Hg(II) acclimated, from Hg(II)-exposed microcosm; Control, from unexposed microcosm.
[c] In control matings in which no donor strains were added.

DISCUSSION

Conjugal transfer to aquatic bacteria was successfully detected by the formation of a new phenotype following the assembly of catabolic genes. Experiments were performed under optimal conditions, using broad-host-range plasmids and laboratory incubations. Under these conditions, transfer was readily measurable, indicating that recipients are present in natural waters. Efficiencies were lower than those observed for conjugations with pure laboratory strains, suggesting that studies with pure cultures may overestimate transfer efficiencies among naturally occurring strains. Although only a limited number of transconjugants were characterized in this study, it is likely that recipients were mostly pseudomonads. Previous studies (4, 5) have shown that the majority of *merA*$^+$ aquatic populations were *Pseudomonas* spp.

Results showed that enumeration of transconjugants was not affected by the occurrence of background PMA^r populations. PMA^r bacteria are abundant in natural waters (55), and they were enriched following exposure to Hg(II) in this study. However, only on one occasion were PMA^r colonies selected in controls (conjugation with unacclimated communities and no donor addition) of an experiment employing PAO1(pGTE26) as a donor (data not shown). This background did not interfere with the estimation of conjugal transfer efficiency, because transconjugants were easily distinguished from background PMA^r colonies by their antibiotic resistance patterns and by hybridization with the plant DNA probe, and the data could be normalized accordingly.

The approach described here could be used to study other gene exchange mechanisms as well as the role of gene change in the ecology of natural microbial communities. As such, it could have several applications in microbial ecology and environmental management.

It has long been suggested that gene transfer contributes to acclimation for the detoxification and degradation of pollutants (8, 51). Although the central role of enrichment in acclimation cannot be disputed (10), horizontal gene transfer may be a principal mechanism to promote metabolic diversity in acclimated communities. This question can now be addressed by looking at rates of degradation of compounds for which catabolic pathways are assembled following gene exchange. This principle could be used in the management of polluted sites by expanding the catabolic range of indigenous microbial communities. The idea of expanding catabolic functions by genetic manipulations is not new. It is based on observations suggesting that catabolic pathways evolved, at least to some extent, by genetic exchange (18, 30, 59). Catabolic pathways have been expanded and modified in vitro (31, 40, 42, 44) and in vivo (14, 23, 27). However, a major hurdle to using introduced bacterial strains (genetically modified or unmodified) is their inability to invade existing microbial communities (probably due to a competitive disadvantage [37]). Thus, although inoculation as a means of bioremediation has been discussed for almost a decade (17), very few examples of successful introductions are available. Directed transmission of genes encoding enzymes that would expand the substrate range of indigenous biodegrading populations may be a superior alternative to the introduction of specialized strains. The usefulness of this approach may be best suited to pathways for the degradation of recalcitrant xenobiotic organic pollutants (50).

Another application of the ability to detect gene transfer in indigenous microbial communities is in assessing the risk of genetically engineered microorganisms (GEMs) in the environment. The prospect of using GEMs in environmental management raises the issue of gene transfer (17, 25). The potential risk posed by introduced recombinant DNA may be magnified upon its inheritance by indigenous flora. Numerous studies (32) have documented gene transfer, albeit at very low efficiencies, among microorganisms (mostly bacteria) in environmental samples and in situ with environmental chambers. The experimental approach employed in these studies was based on addition of genetically marked donor and recipient strains to sterile and nonsterile environmental samples. Thus, in most cases no information regarding transfer from a potential GEM to indigenous microbial populations was collected. The reason for this deficiency is the difficulty of distinguishing transconjugants in the absence of selection for a recipient phenotype (i.e., selection against donors). Two approaches to circumvent this problem have recently been reported. The first (19) employed *E. coli* strains as donors. Because *E. coli* survived poorly in inoculated soils, indigenous antibiotic-resistant transconjugants were distinguished. In the second, a highly specific lytic bacteriophage was used by Smit et al. (49) for counterselection against a *Pseudomonas fluorescens* donor. Subsequently, indigenous transconjugants were identified by antibiotic resistance and by the presence of a eukaryotic DNA sequence. Formation of transconjugants with phenotypes resulting from the assembly of catabolic genes is an additional approach to detect the spread of genes from GEMs to indigenous flora.

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