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TOM, a New Aromatic Degradative Plasmid from *Burkholderia* (*Pseudomonas*) *cepacia* G4

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Burkholderia* (*Pseudomonas*) *cepacia* PR1$_{23}$ has been shown to constitutively express a toluene catabolic pathway distinguished by a unique toluene *ortho*-monooxygenase (Tom). This strain has also been shown to contain two extrachromosomal elements of <70 and >100 kb. A derivative strain cured of the largest plasmid, PR1$_{23}$ Cure, was unable to grow on phenol or toluene as the sole source of carbon and energy, which requires expression of the Tom pathway. Transfer of the larger plasmid from strain G4 (the parent strain inducible for Tom) enabled PR1$_{23}$ Cure to grow on toluene or phenol via inducible Tom pathway expression. Conjugal transfer of TOM$_{23c}$ from PR1$_{23}$ to an antibiotic-resistant derivative of PR1$_{23}$ Cure enabled the transconjugant to grow with either phenol or toluene as the sole source of carbon and energy through constitutive expression of the Tom pathway. A cloned 11.2-kb *Eco*RI restriction fragment of TOM$_{23c}$ resulted in the expression of both Tom and catechol 2,3-dioxygenase in *Escherichia coli*, as evidenced by its ability to oxidize trichloroethylene, toluene, *m*-cresol, *o*-cresol, phenol, and catechol. The largest resident plasmid of PR1 was identified as the source of these genes by DNA hybridization. These results indicate that the genes which encode Tom and catechol 2,3-dioxygenase are located on TOM, an approximately 108-kb degradative plasmid of *B. cepacia* G4.

Five unique bacterial pathways that result in oxygenase-catalyzed hydroxylation of toluene have been described (26). One involves the oxidation of toluene through benzyl alcohol, benzaldehyde, and benzoate to catechol and is the only route known to be encoded by a catabolic plasmid, TOL (31). The remaining pathways initiate toluene oxidation through the hydroxylation of aromatic ring carbons via either mono- or dioxygenases. Only one toluene dioxygenase, the toluene 2,3-dioxygenase of *Pseudomonas putida* F1, has been described. Toluene 2,3-dioxygenase produces *cis*-toluene-2,3-dihyrodiol from toluene through the addition of a single diatomic oxygen (8). Toluene monooxygenases that hydroxylate the aromatic nucleus at all three possible positions, producing *ortho*-, *meta*-, or *para*-cresol, have been described. These include the toluene *ortho*-monooxygenase (Tom) of *Burkholderia* (*Pseudomonas*) *cepacia* G4 (25), the toluene *meta*-monooxygenase of *Pseudomonas pickettii* PK01 (13), and the toluene *para*-monooxygenase of *Pseudomonas mendocina* KR1 (24). The genes that encode these oxygenases have all been cloned and studied in greater detail (13, 30, 35), with the last two fully sequenced (4, 34). Toluene 2,3-dioxygenase has been shown to be a complex of three proteins, a reductase, a ferredoxin reductase, and an iron sulfur oxidase (products of *todA*, *todB*, and *todC1* and *todC2* genes, respectively) (35). The toluene *para*-monooxygenase has been shown to be slightly more complex, requiring the products of five genes (*tmoABCDE*) for activity (34). Both *tod* and *tmo* genes are chromosomally encoded.

Several TOL-type plasmids which share the same route of toluene oxidation via benzyl alcohol have been described. These include XYL (6), pKJ1 (33), pDK (18), pWW53 (15), pTK0 (16), pDTG501 (29), pGB (1), and several less well-defined plasmids (5, 17).

We previously described a Tn*5* mutant of *B. cepacia* G4 that constitutively expresses Tom and catechol 2,3-dioxygenase (C23O) and reported its ability to cooxidize trichloroethylene (TCE) (27). We report here that the genes for Tom (which also hydroxylates cresol to 3-methylcatechol) and C23O (*tomA* and *tomB*, respectively) reside on a large self-transmissible plasmid native to G4. We also describe different locations of Tn*5* in various mutants that affect the Tom pathway (all but one of which are on the native plasmid). We designate this new toluene catabolic plasmid TOM because of its novel catabolic pathway initiated by the *ortho* hydroxylation of toluene.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains used in this study are listed in Table 1. *Escherichia coli* strains were grown and maintained on either Luria-Bertani (LB) broth or M9 minimal medium (20). *B. cepacia* and its derivatives were grown on either LB broth or basal salts medium (10) that contained a single carbon source (20 mM lactate or 2 mM phenol). Antibiotic selection was carried out with 50 μg of kanamycin sulfate per ml, 30 μg of chloramphenicol per ml, 100 μg of ampicillin per ml, 25 μg of tetracycline per ml, 20 μg of streptomycin per ml, 100 μg of nalidixic acid per ml, or 50 μg of rifampin per ml, as required.

Bacterial matings. Plate matings between bacterial strains were carried out by pipetting 5 μl of overnight LB cultures of each donor and recipient separately and in combination to LB agar plate surfaces and incubating at 30°C. On the following day, colony material was transferred to selective medium. Donor and recipient inocula alone served as negative controls.

PR1$_{23}$ Cure. A 2,4-dichlorophenoxyacetic acid (2,4-D)-degradative plasmid (pRO101) (14) was conjugally transferred to PR1$_{23}$, thus enabling growth on 2,4-D and tetracycline resistance. Following extended growth on 2,4-D, a spontaneous derivative that lacked TOM$_{23c}$ was obtained (7) (presumably because of replication or partition incompatibility with the IncP1 plasmid pRO101).

Rif^r Nal^r PR1$_{23}$ Cure. A spontaneous Rif^r colony of PR1$_{23}$ was isolated on LB-rifampin. This strain was likewise selected on LB-nalidixic acid. The resulting Nal^r strain, PR1$_{23}$ Cure^{NR}, was Nal^r and Rif^r through spontaneous mutation and Km^r through the continued presence of Tn*5* in the chromosome of this strain.

Molecular techniques. *E. coli* and *B. cepacia* plasmids were isolated by an alkaline lysis technique (2). Genomic *B. cepacia* DNA was isolated by the technique of Marmur (21). Restriction endonuclease digestion, molecular cloning, Southern blot to Nytran (Schleicher and Schuell, Keene, N.H.), nick translation with [α-^{32}P]dCTP (Amersham Corp.), and autoradiography were performed according to the methods of Maniatis et al. (20). DNA fragments for nick translation were derived from digested DNA that had been separated by and recovered from low-melting-point agarose as described by Maniatis et al. (20). The stringency of hybridization was controlled through the following membrane

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description[a]	Reference or source
Strains		
E. coli		
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-proAB*) (F′ *traD36 proAB*$^+$ *lacI*^{q} *lac* ΔM15)	32
DH5α	*supE44* Δ*lacU169* (Φ80 *lacZ* ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Bethesda Research Laboratories
HB101	Str^r, *supE44 hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	3
B. cepacia		
G4(TOM)	Phe$^+$ C23O$^+$ Tom^i	25
G4 5220(TOM_{20})	Km^r Phe$^+$ C23O$^-$ Tom^i; *tomB* nonrevertable Tn*5* mutant of G4(TOM)	27
G4 5223(TOM_{23})	Km^r Phe^r C23O$^+$ Tom$^-$; *tomA* revertable Tn*5* mutant of G4(TOM)	27
G4 5227(TOM_{27})	Km^r Phe$^-$ C23O$^+$ Tom$^-$; *tomA* nonrevertable Tn*5* mutant of G4(TOM)	27
G4 5231(TOM_{31})	Km^r Phe^r C23O$^+$ Tom$^-$; *tomA* revertable Tn*5* mutant of G4(TOM)	27
$PR1_{23}(TOM_{23c})$[b]	Km^r Phe$^+$ C23O$^+$ Tom^c; phenol-utilizing revertant of G4 5223(TOM_{23})	27
$PR1_{23}$ Cure	Km^r Phe$^-$ C23O$^-$ Tom$^-$; $PR1_{23}$ lacking TOM_{23c}	This article
$PR1_{31}(TOM_{31c})$	Km^r Phe$^+$ C23O$^+$ Tom^c; phenol-utilizing revertant of G4 5231(TOM_{31})	This article
Plasmids		
pRO101	pJP4::Tn*1721* derivative that encodes 2,4-dichlorophenoxyacetic acid utilization and Tc^r	13
pRO1614	Tc^r Cb^r broad-host-range cloning vector	23
pGEM4Z	Ap^r cloning vector for *E. coli*	Promega
pRZ102	ColE1::Tn*5* (*mob*$^+$ Km^r) suicide vector for delivery of Tn*5* into *Pseudomonas* spp. (27)	12

[a] Abbreviations: Phe$^+$, capable of phenol utilization; Phe$^-$, incapable of phenol utilization; Phe^r, revertable to phenol utilization; Tom^i, Tom inducible expression; Tom^c, Tom constitutive expression.
[b] Formerly G4-5223-PR1 (27).

wash conditions: twice for 1 h each in 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% sodium dodecyl sulfate at 65°C (20).

Enzyme assays and TCE analysis. Assays for C23O activity and trifluoroheptadienoic acid (TFHA) production were performed spectrophotometrically at A_{386} as previously described (26). TCE degradation was quantified by gas chromatographic analyses of pentane extracts as previously described (26). Expression of Tom in JM109(pMS64) was confirmed following growth in M9 medium that contained 0.1% glucose, 0.05 mM thiamine, and 0.5 mM toluene, phenol, *o*-cresol, or *m*-cresol. Aliquots were removed at 0-, 1-, 2-, 3-, 5-, and 7-h intervals and analyzed by high-performance liquid chromatography (HPLC) as previously described (25).

RESULTS

Conjugal transfer of TOM. (i) Transfer of TOM_{23c} to *B. cepacia*. Stability studies of pRO101 in $PR1_{23}(TOM_{23c})$ revealed that several isolates had retained the ability to utilize 2,4-D but had lost the ability to grow on phenol (Phe$^-$). These isolates, although Km^r, were unable to oxidize trifluoromethylphenol (TFMP) to TFHA or to degrade TCE. Plasmid DNA preparations of Phe$^-$ isolates revealed the absence of the largest native plasmid (Fig. 1A). To determine if phenol utilization could be reintroduced, G4(TOM) (Km^s Phe$^+$) was mated with one of these isolates, $PR1_{23}$ Cure (Km^r Phe$^-$). Km^r Phe$^+$ transconjugants were selected, and TOM was physically demonstrated (Fig. 1). Though they were able to grow on phenol, these strains oxidized TFMP and TCE only after preexposure to phenol. Therefore, TOM retained its Tom-inducible phenotype in $PR1_{23}$ Cure(TOM) (Table 2). It was therefore of interest to determine if constitutive Tom expression could be transferred through conjugation with a constitutive derivative of G4. The Tom constitutive strain $PR1_{23}(TOM_{23c})$ was mated with $PR1_{23}$ $Cure^{NR}$, and transconjugants were selected for growth on basal salts medium-phenol-rifampin-nalidixic acid plates. One such strain, $PR1_{23}$ $Cure^{NR}(TOM_{23c})$, constitutively oxidized TFMP and TCE (Table 2). This indicated that, in the case of TOM_{23c}, the determinant for constitutive Tom pathway expression remains plasmid associated.

(ii) Transfer of TOM_{31c} to *E. coli*. Following successful TOM_{23c} transfer between *B. cepacia* strains, it was of interest to determine if intergeneric transfer and expression of the Tom operon were feasible. Since *E. coli* would not be expected to grow on phenol, another selectable phenotype was necessary to allow detection of an *E. coli* transconjugant. $PR1_{31}(TOM_{31c})$, a Tom constitutive revertant of G4 5231(TOM_{31}) (27), was selected since hybridization evidence indicated that Tn*5* was located on TOM_{31c} (see below) and should therefore encode a readily selectable Km^r phenotype in *E. coli*. $PR1_{31}(TOM_{31c})$ was mated with Tc^r *E. coli* JM109(pRO1614). DNA preparations of putative Km^r Tc^r JM109 transconjugants revealed the

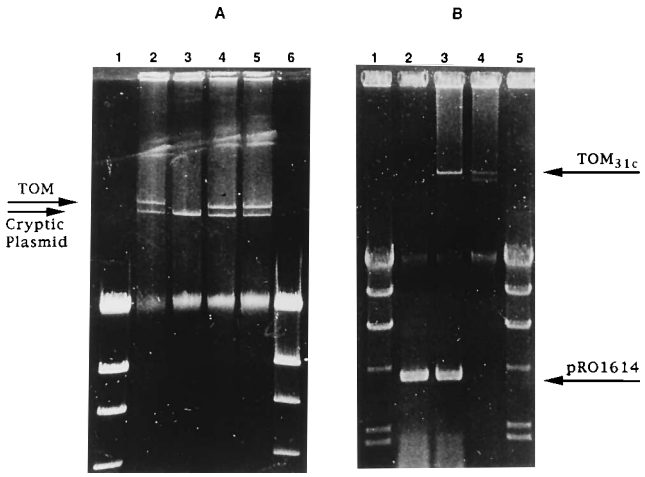

FIG. 1. Plasmid profiles of donor, recipient, and transconjugant strains. (A) Lanes: 1 and 6, lambda × *Hin*dIII; 2, $PR1_{23}(TOM_{23c})$; 3, $PR1_{23}$ Cure; 4, $PR1_{23}$ Cure(TOM); 5, G4(TOM). (B) Lanes: 1 and 5, lambda × *Hin*dIII; 2, JM109(pRO1614); 3, JM109(pRO1614, TOM_{31c}); 4, $PR1_{31}(TOM_{31c})$.

TABLE 2. Degradative phenotypes of parental and transconjugant strains

Mating[a]	Selection	Oxidation[b]	
		TFMP	TCE
I			
G4(TOM)	Phe^+ Km^s	I	I
$PR1_{23}$ Cure	Phe^- Km^r	—	—
$PR1_{23}$(TOM)	Phe^+ Km^r	I	I
II			
$PR1_{23}(TOM_{23c})$	Phe^+ Nal^s Rif^s	C	C
$PR1_{23}$ $Cure^{NR}$	Phe^- Nal^r Rif^r	—	—
$PR1_{23}$ $Cure^{NR}(TOM_{23c})$	Phe^+ Nal^r Rif^r	C	C
III			
$PR1_{31}(TOM_{31c})$	Km^r Tc^s	C	C
JM109(pRO1614)	Km^s Tc^r	—	—
JM109(pRO1614, TOM_{31c})	Km^r Tc^r	—	—

[a] Each set of mating strains consists of a donor, recipient, and transconjugant (in that order).
[b] I, inducible; C, constitutive; —, not detected.

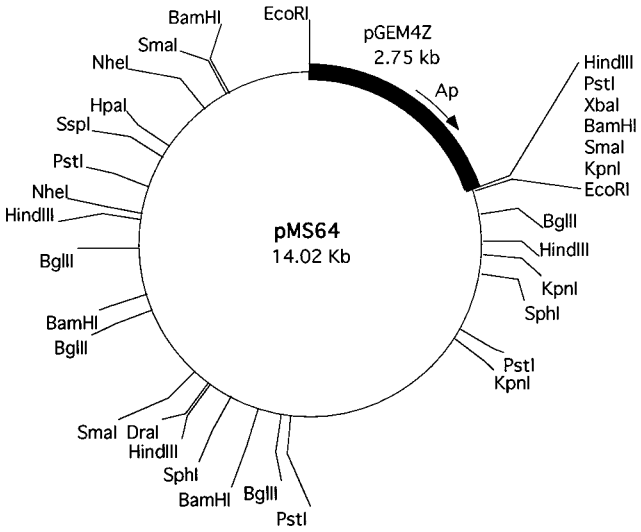

FIG. 2. Restriction map of pMS64, which encodes Tom and C23O.

presence of TOM_{31c} (Fig. 1B). *E. coli* JM109(pRO1614, TOM_{31c}) failed to oxidize any of the following four known Tom substrates: phenol, toluene, TFMP, and TCE (Table 2). This possibly indicates a lack of TOM_{31c} constitutive promoter recognition in *E. coli.*

Cloning of *tomA* and *tomB* from TOM_{23c}. Genetic evidence based on curing and subsequent reintroduction of TOM suggested that *tomA* and *tomB* were located on the large plasmid. Therefore, direct cloning of DNA from TOM_{23c} was attempted by using *Eco*RI, *Bam*HI, and *Hin*dIII single digestions for ligation into the same unique sites of pGEM4Z. Transformants were initially selected on LB, ampicillin, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), and IPTG (isopropyl-β-D-thiogalactopyranoside), and white putative recombinants were selected. Detection of clones that carried *tomA* and *tomB* was attempted by pulling colonies to TFMP-impregnated nitrocellulose discs as previously described for *B. cepacia* G4 (27), with negative results. They were also tested by growth on M9-glucose agar that contained 2 mM phenol and by spraying colonies grown on M9-glucose agar with a 2 mM solution of catechol. In the first instance, cells capable only of hydroxylating phenol to catechol would probably be detectable as brown colonies because of accumulated catechol oxidation (26). Those that produced catechol and subsequently cleaved it to C23O, but no further, would be yellow because of ring fission aldehyde accumulation.

Clone pMS64 (Fig. 2), which contained an 11.2-kb *Eco*RI fragment of TOM_{23c} inserted into the unique *Eco*RI site of pGEM4Z, was isolated. *E. coli* JM109(pMS64) produced a yellow product, whose UV-visible light (UV-VIS) spectral scan indicated maximal absorbance at A_{386} when grown on 20 mM glucose in the presence of 2 mM phenol. The absorption spectrum of growth medium that contained this product was characteristic of the catechol *meta*-fission product, 2-hydroxymuconic semialdehyde, by C23O (a known phenol metabolite in *B. cepacia* [26]).

HPLC analysis confirmed *o*-cresol production from toluene and 3-methylcatechol production from *o*- and *m*-cresol by JM109(pMS64) (data not shown). For unknown reasons, JM109(pMS64) is unable to convert TFMP to TFHA when tested in liquid medium or colonies by TFMP-impregnated filters.

TCE degradation by recombinant *E. coli.* The results shown in Table 3 clearly indicate that LB-grown *E. coli* JM109 was unable to affect TCE levels in basal salts medium during an overnight incubation assay, whether or not phenol was present as an inducer. Suspended in either LB or M9 with or without phenol, JM109(pMS64) was capable of degrading TCE, indicating constitutive expression of Tom by this construct. Since the constitutive promoter present in TOM_{31c} failed to produce Tom expression in JM109(TOM_{31c}), it seems likely that Tom expression by JM109(pMS64) is due to transcriptional activity of the cloning vector. The relatively better performance with LB as the growth substrate may be a function of the nutritional state of cells, additional cellular growth during the assay, or both. In both media, the absence of phenol resulted in relatively greater TCE removal.

Locations of *tomA*, *tomB*, and Tn*5*. To further establish the location of *tomA*, plasmid DNA minipreps of G4 and G4 Tn*5* mutants (27) were prepared, electrophoresed on 0.7% agarose, denatured, and transferred to a nitrocellulose membrane. This blot was sequentially hybridized with the 11.2-kb *Eco*RI fragment of pMS64 (Fig. 2) and the 2.7-kb *Bgl*II fragment of pRZ102 (i.e., the internal *Bgl*II fragment of Tn*5*) (12). The 11.2-kb *Eco*RI fragment of pMS64 hybridized specifically with each undigested large plasmid in G4 and derivative strains (Fig. 3). G4(TOM) served as the negative control for Tn*5* hybridization. *E. coli*(pRZ102) DNA preparations were used in these experiments but were not included in the composite figure. They served as positive controls for Tn*5*-specific hybridizations and as negative controls for pMS64 insert-specific hybridizations. There were no unexpected hybridizations to these controls.

TABLE 3. TCE degradation by recombinant *E. coli*

Strain	TCE remaining (μM)[a]			
	LB	LB-phenol	M9-glucose	M9-glucose-phenol
JM109	16.81	17.73	14.51	15.24
JM109(pMS64)	ND	0.04	0.77	2.93
Uninoculated	16.55	NT	NT	NT

[a] Data are amounts of TCE remaining in solution following overnight incubation at 30°C with cells previously grown as indicated and are the means of triplicate determinations. ND, not detected; NT, not tested.

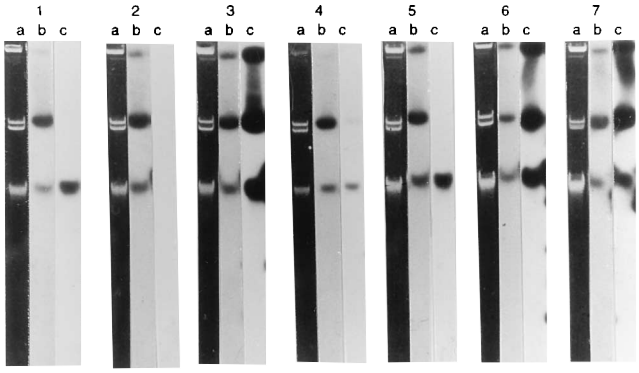

FIG. 3. Hybridization profiles of G4 strains. Ethidium bromide-stained 0.7% agarose gel of plasmid minipreps (a) and Southern blots hybridized with 11.2-kb insert of pMS64 that contained *tomA* and *tomB* (b) and 2,784-bp *Bgl*II fragment of Tn*5* (c). Lanes: 1, $PR1_{23}(TOM_{23})$; 2, G4(TOM); 3, G4 5220(TOM_{20}); 4, $PR1_{23c}(TOM_{23c})$; 5, G4 5223(TOM_{23}); 6, G4 5227(TOM_{27}); 7, G4 5231 (TOM_{31}).

The secondary hybridization signals are interpreted to be unresolved, fragmented plasmid DNA that has comigrated with chromosomal DNA. This conclusion is strongly supported by the presence of an identical signal from hybridization between the same 11.2-kb *Eco*RI probe and TOM_{31c} from *E. coli* HB101(TOM_{31c}) and the disappearance of all detectable signals from DNA prepared from $PR1_{23}$ Cure (data not shown).

Since the initial mutation in the Tom pathway for each mutant was caused by the introduction of Tn*5*, its location in each mutant strain was also examined. Hybridization to the 2,784-bp *Bgl*II fragment of Tn*5* revealed no homology with DNA isolated from the parent strain, G4. Hybridization to chromosomal DNA of G4 5223(TOM_{23}), $PR1_{23}$ Cure, and $PR1_{23}$(TOM) indicated that despite evidence for locating the Tom genes on TOM, Tn*5* is chromosomally located in G4 5223. However, Tn*5* hybridization reveals a very weak autoradiographic signal with TOM_{23c}, indicating probable partial homology following conversion to constitutivity.

Strong hybridization between the *Bgl*II fragment of Tn*5* and TOM_{20}, TOM_{27}, and TOM_{31} (in G4 5220, 5227, and 5231, respectively) is clear evidence for TOM as the location of Tn*5* in these strains.

Sizing of TOM. *E. coli* JM109(pRO1614, TOM_{31c}) was subsequently mated with *E. coli* HB101 (Str^r) in order to allow isolation of TOM_{31c} free of F′*lac* and pRO1614. TOM_{31c}, isolated in this manner, was digested with *Eco*RI, *Bam*HI, and *Hin*dIII. Summation of individual restriction fragment sizes derived by single digestion and double digestion with these enzymes revealed a mean size of approximately 114 kb for TOM_{31c} (data not shown). Subtracting 5.8 kb for Tn*5* yields a size estimate of approximately 108 kb for TOM.

DISCUSSION

We have previously described the Tn*5* mutagenesis which yielded *tomA* mutant strains, G4 5223(TOM_{23}) and G4 5231(TOM_{31}), which were unable to utilize phenol. These mutants were shown to spontaneously regain the ability to grow on phenol; in doing so, they became constitutive for *tomA* and *tomB* gene expression and consequently were able to oxidize TCE and related isomers without aromatic induction (27). Preliminary evidence for a plasmid location of the *tom* operon and a chromosomal location of Tn*5* in $PR1_{23}$ came from the isolation of a strain, derived from $PR1_{23}$, that retained Km^r, was unable to utilize phenol, and was cured of TOM_{23c} ($PR1_{23}$ Cure). Hybridization with Tn*5* indicates a chromosomal location for this transposon in G4 5223(TOM_{23}). However, there appear to be newly derived plasmid sequences homologous to Tn*5* following its conversion to the constitutive strain $PR1_{23}(TOM_{23c})$. Hybridization evidence indicates that conversion to constitutive expression of Tom is the result of plasmid acquisition of at least some Tn*5*-associated sequences by TOM_{23c}. The precise nature of the movement of Tn*5* sequences between chromosome and plasmid remains the subject of current research. The loss of detectable plasmid DNA in $PR1_{23}$ Cure was shown to be an authentic cure through the lack of hybridization between this strain and the 11.2-kb *Eco*RI fragment cloned from $PR1_{23}(TOM_{23c})$ that contained both *tomA* and *tomB*. The remaining *tomA* [G4 5227(TOM_{27}) and G4 5231(TOM_{31})] and *tomB* [G4 5220(TOM_{20})] mutants demonstrated strong hybridizations between their respective TOM plasmids and both Tn*5* and *tomAB* probes.

It is unclear how Tn*5* can have such similar effects at such different locations. Two likely possibilities are that (i) in G4 5223, Tn*5* interrupted a *trans*-acting chromosomally encoded factor necessary for Tom pathway expression in TOM or (ii) the original site of Tn*5* insertion in G4 5223 was the plasmid, but subsequent rearrangement resulted in transfer to the chromosome. The evidence presented here that argues against the former interpretation is the inducible expression of Tom by $PR1_{23}$ Cure(TOM) and constitutive expression by $PR1_{23}$ Cure(TOM_{23c}). This leads us to the conclusion that both inducible and constitutive determinants are plasmid encoded.

Because TOM_{31c} apparently contains an entire Tn*5*, Km^r was available as a selective marker for TOM_{31c} conjugal transfer to *E. coli*. *E. coli* JM109(TOM_{31c}) expressed Tn*5*-encoded Km^r but remained unable to degrade TCE or produce TFHA from TFMP. One explanation for this apparent lack of Tom activity may be *E. coli* failure to express Tom from the native plasmid promoters of TOM_{31c}. This is supported by the observation that JM109(pMS64) effectively degrades TCE and produces the same oxidative products of toluene, phenol, *o*-cresol, catechol, and 3-methylcatechol as G4 does. Therefore, *E. coli* is capable of accurate translation and assembly from *tomA* and *tomB*. In this case, expression is most likely due to nonspecific transcriptional activity from pGEM4Z vector promoters. The inability of JM109(pMS64) to oxidize TFMP under the same assay conditions that allow oxidation of TFMP to TFHA by constitutive G4 mutants is somewhat puzzling in view of its demonstrated ability to oxidize nonfluorinated analogs. This may reflect the failure of TFMP transport into JM109 or merely greatly differing relative rates of activity, compared with that of a *B. cepacia* host.

As a species, *B. cepacia* has frequently been shown to contain a single plasmid or multiple plasmids of greater than 200 kb (19). Other studies have reported the presence of plasmids in up to 94% of the *B. cepacia* strains surveyed (9, 22). In this study, we have introduced mating, cloning, and hybridization evidence to indicate that the source of toluene-, phenol-, and TCE-degradative capabilities in *B. cepacia* G4 is the large (approximately 108-kb) self-transmissible catabolic plasmid TOM.

The only other known plasmid-encoded toluene catabolic pathway is that of the archetypal TOL, in which toluene oxidation proceeds through a benzoate intermediate (31). TOM, however, encodes a pathway that results in toluene hydroxylation which occurs sequentially at the *ortho* and *meta* positions to yield 3-methylcatechol, which is in turn oxidized by TOM-encoded C23O to 2-hydroxy-6-oxohepta-2,4-dienoic acid (25). Insofar as TCE degradation by such pathways is concerned, the only other plasmid-encoded aromatic oxygenase capable of

TCE cooxidation that has thus far been described is the 2,4-dichlorophenol hydroxylase of pJP4 (11). A similar plasmid with the capacity to metabolize both phenol and methyl-substituted phenols (but not toluene) via an *ortho*-monooxygenase and C23O pathway is the dimethylphenol-degradative plasmid pVI150 (28). Genetic and cometabolic similarities between pVI150 and TOM remain to be determined.

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