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Activation of the *lac* Genes of Tn*951* by Insertion Sequences from *Pseudomonas cepacia*

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We have identified three transposable gene-activating elements from *Pseudomonas cepacia* on the basis of their abilities to increase expression of the *lac* genes of the broad-host-range plasmid pGC91.14 (pRP1::Tn*951*). When introduced into auxotrophic derivatives of *P. cepacia* 249 (ATCC 17616), this plasmid failed to confer the ability to utilize lactose. The *lac* genes of Tn*951* were poorly expressed in *P. cepacia* and were not induced by isopropyl-β-D-thiogalactopyranoside. Lac$^+$ variants of the pGC91.14-containing strains which formed β-galactosidase at high constitutive levels as a consequence of transposition of insertion sequences from the *P. cepacia* genome to sites upstream of the *lacZ* gene of Tn*951* were isolated. Certain of the elements also increased gene expression in other bacteria. For example, IS*407* strongly activated the *lacZ* gene of Tn*951* in *Pseudomonas aeruginosa* and *Escherichia coli*, and IS*406* (but not IS*407*) did so in *Zymomonas mobilis*. The results indicate that IS elements from *P. cepacia* have potential for turning on the expression of foreign genes in a variety of gram-negative bacteria.

Although *Pseudomonas cepacia* is notable for its extraordinary nutritional versatility (4, 20, 26, 27, 29), most soil isolates of this bacterium are unable to utilize lactose as a sole source of carbon and energy (4, 20, 21). Cornelis and co-workers identified a 17-kilobase (kb) transposon, Tn*951* (Tn*lac*), in *Yersinia enterolitica* which carried *lacI*, *lacZ*, and *lacY* genes seemingly identical to those of the *Escherichia coli lac* operon and isolated several variants of the broad-host-range plasmid pRP1 (30) containing this element (12–14). We transferred one of these, pGC91.14 (Fig. 1) into auxotrophic derivatives of *P. cepacia* 249 (ATCC 17616) by conjugation from *E. coli* JC3272. Although unable to utilize lactose themselves, the pGC91.14-containing transconjugants gave rise to Lac$^+$ variants (M. S. Wood, C. Lory, and T. G. Lessie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, H23, p. 143). In these strains, transposable gene-activating elements from the *P. cepacia* genome had inserted into Tn*951* and increased expression of its *lac* genes. The results were similar to those we reported earlier for activation of the *bla* gene of Tn*1* in *P. cepacia* (20, 28), which restored ability of β-lactamase-deficient strains of this bacterium to utilize penicillin as a sole source of carbon and energy.

We report here data describing the transposition of three *P. cepacia* insertion sequences, IS*406*, IS*407*, and IS*415*, to different sites within Tn*951* and their effect on formation of *lac*-specific mRNA and of β-galactosidase. We also describe the influence of *P. cepacia* insertion sequences on *lac* gene expression in other gram-negative bacteria.

MATERIALS AND METHODS

Bacterial strains and plasmids. Table 1 lists strains and plasmids used in this study. Bacteria were grown in inorganic salts medium (33) supplemented with 1% (wt/vol) yeast extract or Casamino Acids or 0.5% of the specified carbon sources. Plasmid pGC91.14 was transferred from *E. coli* JC3272 to *P. cepacia* 249-2 (pTGL6$^-$) and 249-42-3 by plating ca. 10^7 donor and recipient bacteria on citrate medium containing 2% agar, tetracycline (100 μg/ml), and amino acids (50 μg/ml) required by the recipient strain. Tetracycline-resistant transconjugants were picked and purified on the same medium. Plasmid DNA was isolated by the methods of Birnboim (9) and of Currier and Nester (16). Restriction enzyme digestions, agarose gel electrophoresis, Southern hybridization experiments, and autoradiography were carried out by using standard protocols (3, 24).

Construction of pBR329 derivatives carrying unactivated and activated *lac* genes of pGC91.14. The *lac* gene-containing restriction fragments of pGC91.14, pTGL66, pTGL67, and pTGL75 were cloned into the plasmid vector pBR329 by standard procedures (3, 24). Recombinant plasmids were introduced into the Lac$^-$ strain *E. coli* JC3272 by transformation (19), and Lac$^+$ colonies were identified on yeast extract plates supplemented with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and with 100 μg of ampicillin and 50 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) per ml.

Determination of β-galactosidase activity. Cell-free extracts were prepared as described previously (33). Assay mixtures (0.5 ml) containing phosphate buffer (10 mM Na_2HPO_4 and 10 mM KH_2PO_4), pH 6.8, 10 mM 2-mercaptoethanol, appropriately diluted bacterial extract, and 2 mM *ortho*-nitrophenylgalactoside were incubated at 37°C for 30 min. The reactions were stopped by addition of 1 ml of 2% (wt/vol) Na_2CO_3 containing 0.1 N NaOH, and the formation of *ortho*-nitrophenol was determined by measuring the A_{420}. Protein content was determined by the Lowry procedure (23).

Measurement of *lacZ* mRNA. Bacteria were grown in 200 ml of Casamino Acids medium, harvested at a cell density of ca. 10^9 cells per ml, and suspended in 5 ml of 100 mM sodium acetate buffer, pH 4.5. The bacteria were disrupted by sonic treatment for 90 s, and cell debris and unbroken cells were removed by centrifugation. Sodium dodecyl sulfate was added to the supernatant to a final concentration of 1%, and the preparation was extracted with phenol at 65°C. Nucleic acids were precipitated with ethanol, and RNA was purified by centrifugation through a cesium chloride cushion (24). The RNA samples were applied to GeneScreen Plus nylon membranes (Dupont, NEN Research Products, Boston, Mass.) and probed with a ^{32}P-labeled preparation of the 3-kb

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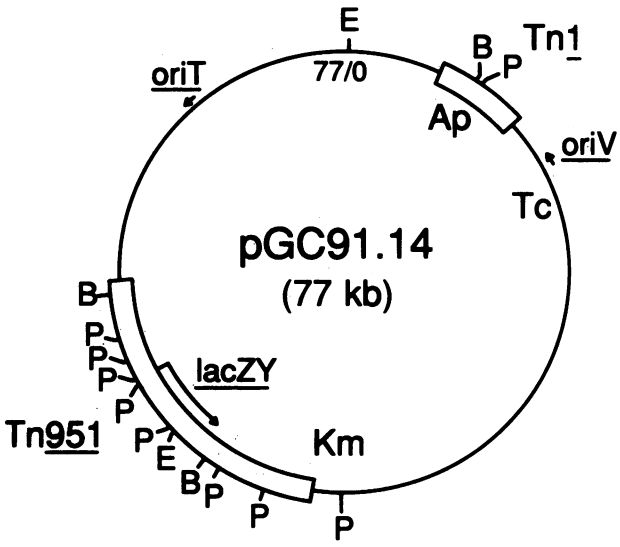

FIG. 1. Schematic representation of pGC91.14. The figure shows the distribution of *Pvu*II (P), *Bam*HI (B), and *Eco*RI (E) sites within pGC91.14 and the locations of Tn*1* and Tn*951* on the plasmid. Also shown are the positions of genes conferring resistance to tetracycline (Tc), kanamycin (Km), and ampicillin (Ap) as well as the origins of replication (*oriV*) and plasmid transfer (*oriT*) and the *lac* genes of Tn*951*.

lacZ gene-containing *Pst*I fragment of pMC1871 (obtained by nick translation with [α-^{32}P]dGTP). Prehybridization, hybridization, and subsequent washing steps were carried out as recommended by the manufacturer.

RESULTS

Expression of the *lac* genes of pGC91.14 in *P. cepacia*. In an attempt to derive *P. cepacia* 249 variants able to grow on lactose, we transferred the broad-host-range plasmid pGC91.14 (Fig. 1) from *E. coli* JC3272 to the *P. cepacia* auxotroph 249-42-3 by conjugation, selecting for the ability of pGC91.14 to confer resistance to tetracycline. The pGC91.14-containing derivative of 249-42-3 failed to utilize lactose. However, it was possible to isolate Lac$^+$ variants from it by spreading ca. 2×10^8 bacteria on inorganic salts medium containing lactose as the sole carbon source. Approximately 50 colonies appeared on each plate after 4 days of incubation at 37°C. The Lac$^+$ variants also acquired the ability to utilize lactulose (4-*O*-β-D-galactopyranosyl-D-fructose). No such Lac$^+$ or lactulose$^+$ variants were obtained from *P. cepacia* 249-42-3 lacking pGC91.14.

The Lac$^+$ phenotype of the above lactose-utilizing derivatives of strain 249-42-3 (pGC91.14) was transferred to the lysine auxotroph 249-2 (pTGL6$^-$) by conjugation in experiments in which selection was for the tetracycline resistance marker of pGC91.14. This suggested that the capacity of the Lac$^+$ derivatives to utilize lactose was due to alteration of pGC91.14. The Lac$^+$ transconjugants from three independently isolated Lac$^+$ derivatives of strain 249-42-3 (pGC91.14) were chosen for further characterization. The pGC91.14 variants in these strains were designated pTGL66, pTGL67, and pTGL75.

Table 2 shows a comparison of the levels of β-galactosidase in extracts of the Lac$^-$ strain 249-2 (pTGL6$^-$, pGC91.14) and its corresponding Lac$^+$ pTGL66-, pTGL67-, and pTGL75-containing derivatives. Similar results were obtained for the corresponding Lac$^+$ derivatives of strain 249-42-3 (data not shown). The Lac$^+$ strains had between 10- and 30-fold-higher levels of enzyme activity than their Lac$^-$ parent. In each case, β-galactosidase was formed constitutively. Addition of IPTG (1 mM) did not increase enzyme formation. Extracts of strains 249-42-3 and 249-2 (pTGL6$^-$) lacking pGC91.14 exhibited no β-galactosidase activity (results not shown).

TABLE 1. Strains and plasmids

Strain or plasmid	Pertinent characteristics	Source or reference
Strain		
P. cepacia 249 (ATCC 17616)	Wild type; contains the 170-kb cryptic plasmid pTGL1	4, 29
P. cepacia 249-UM	Contains the pTGL1 derivative pTGL6	17, 20
P. cepacia 249-2 (pTGL6$^-$)	*lys-2*; cured of pTGL6	20
P. cepacia 249-42-3	*arg-1 ileA4*; contains pTGL6	8
P. aeruginosa PAO	Wild type	P. V. Phibbs, Jr., East Carolina University, Greenville, N.C.
Z. mobilis CP4	Wild type	10
E. coli JC3272	Δ*lacX74 his trp lys*	13, 25
E. coli JC3272-1	Δ*lacX74 his trp*; Lys$^+$ revertant of strain JC3272	This study
Plasmid		
pGC91.14	pRP1::Tn*951* (Tn*lac*)	13
pTGL66	pGC91.14::IS*406*	This study
pTGL67	pGC91.14::IS*407*	This study
pTGL75	pGC91.14::IS*415*	This study
pBR329	Ap^r Cm^r Tc^r	15
pTGL260	pBR329 derivative carrying the 10.5-kb *Bam*HI fragment of pGC91.14	This study
pTGL263	pBR329 derivative carrying the 11.8-kb, IS*406*-containing *Bam*HI fragment of pTGL66	This study
pTGL265	pBR329 derivative carrying the 11.7-kb, IS*407*-containing *Bam*HI fragment of pTGL67	This study
pTGL269	pBR329 derivative carrying the 13-kb, IS*415*-containing *Hin*dIII fragment of pTGL75	This study
pMC1871	pBR322 derivative carrying the *E. coli lacZ* gene on a 3-kb *Pst*I fragment	M. Berman, Bionetics Research Inc., Rockville, Md.

TABLE 2. IS*406*-, IS*407*-, and IS*415*-dependent expression of the *lacZ* gene of Tn*951* in different bacteria[a]

Strain	β-Galactosidase sp act with plasmid[b]:			
	pGC91.14	pTGL66	pTGL67	pTGL75
P. cepacia 249-2 (pTGL6$^-$)	29	302	845	320
P. aeruginosa PAO	136	493	4,546	330
E. coli JC3272-1	84	300	1,107	180
Z. mobilis CP4	78	1,385	32	61

[a] Except for strain CP4, the bacteria were grown in inorganic salts medium supplemented with Casamino Acids (Difco). CP4 was grown in a medium consisting of 1% (wt/vol) each of yeast extract and D-glucose and 300 μg of Na_2HPO_4 per ml. The medium was supplemented with 100 μg of tetracycline per ml, except for strain CP4, for which the concentration was 40 μg/ml.

[b] Variants of pGC91.14 containing the pertinent elements were transferred into the indicated strains by selecting for tetracycline resistance conferred by this plasmid. Enzyme activities are expressed in nanomoles of *ortho*-nitrophenylgalactoside cleaved per min per mg of cell extract protein.

Increased formation of *lacZ*-specific mRNA in Lac$^+$ variants of *P. cepacia*. The increase in β-galactosidase activity in Lac$^+$ derivatives of *P. cepacia* was associated with a comparable increase in *lac* gene transcription. The results of a DNA-RNA hybridization experiment comparing the amounts of *lacZ*-specific mRNA in preparations of total cellular RNA from the Lac$^-$ strain 249-2 (pTGL6$^-$), its Lac$^-$ pGC91.14-containing variant, and the Lac$^+$ strain 249-2 (pTGL6$^-$, pTGL67) are shown in Fig. 2. There was a marked increase of *lacZ*-specific mRNA in the pTGL67-containing strain compared with the corresponding pGC91.14-containing strain. No *lacZ*-specific mRNA was detected in strain 249-2 (pTGL6$^-$).

Plasmid alteration associated with *lac* gene activation. We had previously demonstrated that mutations leading to high-level constitutive expression of the *bla* gene of pRP1 in *P. cepacia* were a consequence of the transposition of insertion sequences from the *P. cepacia* genome to sites upstream of the activated gene (20, 28). To determine whether the *lac* gene activation we had observed was related to insertion of similar elements into pGC91.14, we characterized the plasmids from the three Lac$^+$ variants of strain 249-2 (pTGL6$^-$ pGC91.14) listed in Table 2. Use of these particular strains facilitated our analyses, since they were devoid of the 170-kb cryptic plasmid ordinarily present in derivatives of *P. cepacia* 249 (17, 20).

The schematic in Fig. 1 shows the distribution of *Bam*HI and *Pvu*II sites in pGC91.14. Digestion with *Bam*HI cleaves

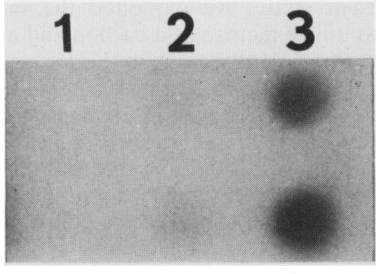

FIG. 2. IS*407*-dependent transcription of the *lacZ* gene of pTGL67. Samples of total cellular RNA were applied to a nylon membrane and probed with denatured ^{32}P-labeled *lacZ* DNA. Columns 1 to 3 of the autoradiogram contained, respectively, RNA from strains 249-2 (pTGL6$^-$), 249-2 (pTGL6$^-$, pGC91.14) and 249-2 (pTGL6$^-$, pTGL67). The upper set of samples contained 10 μg of RNA, and the lower set contained 20 μg of RNA.

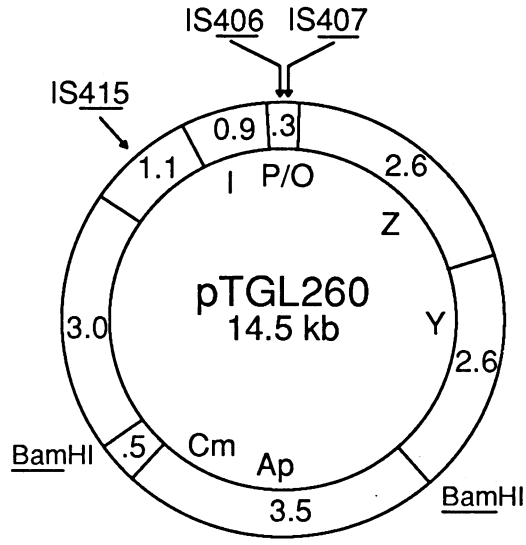

FIG. 3. Distribution of *Pvu*II sites within pTGL260. This recombinant plasmid was formed by cloning the 10.5-kb *lac* gene-containing *Bam*HI fragment of pGC91.14 into the *E. coli* vector pBR329. The schematic shows the 0.3-kb *lac* promoter-containing fragment of Tn*951* into which IS*406* and IS*407* were inserted and the upstream 1.1-kb *Pvu*II fragment which was the target for insertion of IS*415*. The chloramphenicol (Cm) and ampicillin (Ap) resistance genes are located within the 4.0-kb *Bam*HI fragment contributed by pBR329. Five of the six *Pvu*II sites on this plasmid (indicated by bars between the inner and outer circles) were located within the 10.5-kb *Bam*HI fragment contributed by Tn*951*.

pGC91.14 into three fragments of ca. 10.5, 27, and 40 kb. The *lac* genes of Tn*951* were located on the 10.5-kb *Bam*HI fragment. These genes were also contained on an overlapping 10-kb *Hin*dIII fragment which is not shown in Fig. 1. We cloned the *lac* gene-containing *Bam*HI and *Hin*dIII fragments of pGC91.14, pTGL66, pTGL67, and pTGL75 into the *E. coli* vector pBR329 (15). The distribution of *Pvu*II sites within pTGL260, the pBR329 derivative carrying the 10.5-kb *Bam*HI fragment of pGC91.14, is shown in Fig. 3. A comparison of the *Pvu*II fragments of pTGL260 with those of pTGL265, the pBR329 derivative carrying the 11.7-kb *lac*-gene-containing *Bam*HI fragment of pTGL67, is shown in Fig. 4. The results indicated that the 0.3-kb *Pvu*II fragment of pTGL260, which is an internal fragment of Tn*951*, was replaced in pTGL265 by a 1.5-kb fragment. Similar data (not shown) were obtained for pTGL263 and pTGL269, which are pBR329 derivatives carrying, respectively, the 11.8-kb *Bam*HI fragment of pTGL66 and the 13-kb *Hin*dIII fragment of pTGL75. In pTGL263, the 0.3-kb *Pvu*II fragment of pTGL260 was replaced by a 1.6-kb fragment. In the case of pTGL269, the 0.3-kb *Pvu*II fragment of Tn*951* was still intact, but its 1.1-kb *Pvu*II fragment was replaced by four new fragments of 1.7, 1.5, 0.5, and 0.4 kb.

Lanes 4 and 5 of Fig. 4 show the results of a Southern hybridization experiment in which a ^{32}P-labeled preparation of the 1.5-kb fragment of pTGL265 was used as a probe to confirm its relationship with the 0.3-kb *Pvu*II fragment of pTGL260. The results indicated that the 1.5-kb *Pvu*II fragment of pTGL265 (and, accordingly, that of pTGL67) had been formed by insertion of a 1.2-kb element, designated IS*407*, into the 0.3-kb *Pvu*II fragment of pGC91.14. Similar experiments (not shown here) confirmed that a 1.3-kb element, designated IS*406*, had been inserted into the 0.3-kb

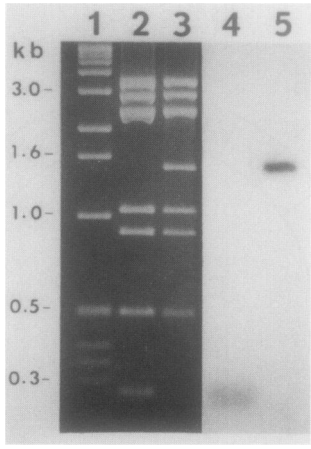

FIG. 4. Insertion of IS*407* into the 0.3-kb *Pvu*II fragment of Tn*951*. Plasmids pTGL260 and pTGL265 (derivatives of pBR329 carrying, respectively, the 10.5- and 11.7-kb *Bam*HI fragments of pGC91.14 and pTGL67) were digested with *Bam*HI and *Pvu*II (IS*407* contains no *Bam*HI or *Pvu*II sites), and the resulting DNA fragments were resolved electrophoretically on a 1.2% agarose gel. Lanes 1, 2 and 3 contained, respectively, kilobase ladder DNA size markers (Bethesda Research Laboratories, Inc., Gaithersburg, Md.), pTGL260 fragments, and pTGL265 fragments. Lanes 4 and 5 show the results of a Southern hybridization experiment in which the same DNA fragments of pTGL260 (lane 4) and pTGL265 (lane 5) were transferred to a nitrocellulose membrane and probed with a ^{32}P-labeled preparation of the 1.5-kb *Pvu*II fragment of pTGL265. The amount of pTGL260 DNA used in this experiment (6 μg) was 30-fold higher than that of pTGL265.

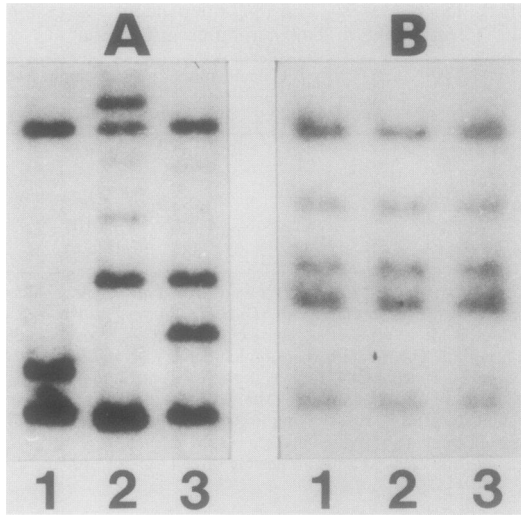

FIG. 5. Southern hybridization experiments showing the distribution of chromosomal fragments exhibiting homology to IS*407* and IS*406*. Lanes 1 to 3 contained *Eco*RI digests of chromosomal DNA from *P. cepacia* 249 (ATCC 17616), 249-UM, and 249-2 (pTGL6$^-$), respectively. The chromosomal fragments were probed with ^{32}P-labeled preparations of the 1.5-kb IS*407*-containing *Pvu*II fragment of pTGL265 (A) and of the 1.6-kb IS*406*-containing *Pvu*II fragment of pTGL263 (B).

*Pvu*II fragment of pGC91.14 to create the 1.6-kb *Pvu*II fragment of pTGL66. The 1.7- and 1.5-kb fragments of pTGL75 were shown to be fusion fragments containing portions of DNA from an element designated IS*415* and of the 1.1-kb *Pvu*II fragment of Tn*951*.

The results suggested that the constitutive expression of the *lac* genes of pTGL66, pTGL67, and pTGL75 was related to the upstream insertion of different IS elements from the *P. cepacia* genome into pGC91.14. To confirm that the inserted DNA originated from the *P. cepacia* genome and to estimate the number of chromosomal copies of each element, we carried out Southern hybridization experiments to identify homologous fragments in restriction digests of *P. cepacia* DNA. For these experiments we used ^{32}P-labeled fragments of pTGL263, pTGL265, and pTGL269 containing IS*406*, IS*407*, and IS*415* as probe DNA. The results of a representative Southern hybridization experiment in which the 1.5-kb, IS*407*-containing *Pvu*II fragment of pTGL265 was used to probe *Eco*RI digests of genomic DNA from *P. cepacia* 249 (ATCC 17616) and two strains derived from it, 249-UM and 249-2 (pTGL6$^-$), are shown in Fig. 5A. Since IS*407* contained no *Eco*RI sites, each band in the autoradiogram represents one or more copies of this element. Strains 249-ATCC, 249-UM, and 249-2 (pTGL6$^-$) contained, respectively, three, five, and four copies of IS*407*. Data obtained by using the 1.6-kb fragment of pTGL263 as the probe indicated that these strains all contained five copies of IS*406* (Fig. 5B). Similar experiments (not shown here) indicated that the above three strains contained, respectively, six, eight, and six copies of IS*415*.

The differences in copy number, genomic distribution, and sizes of IS*406*, IS*407*, and IS*415* indicated that they were distinct elements. On the basis of these parameters, they also differed from the *P. cepacia* insertion sequences identified previously on pTGL6 (17) as well as those isolated on the basis of their ability to activate the *bla* genes of pRP1 (20, 28). The results of Southern hybridization experiments not shown here indicated that pTGL6, the cryptic plasmid in strain 249-UM, contained no copies of IS*406*, IS*407*, or IS*415*.

IS*406*-, IS*407*-, and IS*415*-dependent expression of the *lac* genes of Tn*951* in other bacteria. The transposable elements we identified on the basis of their abilities to increase expression of the *lacZ* gene of pGC91.14 in *P. cepacia* also did so in other gram-negative bacteria we tested. Table 2 summarizes the results of experiments in which we compared β-galactosidase levels in derivatives of *E. coli*, *Pseudomonas aeruginosa*, and *Zymomonas mobilis* into which we transferred pGC91.14, pTGL66, pTGL67, and pTGL75 by conjugation. The data indicated that IS*406* strongly activated *lacZ* expression in *Z. mobilis* and that IS*407* did so in *E. coli* and *P. aeruginosa*.

DISCUSSION

In the present study, we exploited the inability of *P. cepacia* 249 to utilize lactose as a carbon and energy source and its failure to efficiently express the *lac* genes Tn*951* to isolate transposable gene-activating elements from this bacterium. We identified three new IS elements from strain 249 whose insertion upstream of the *lac* genes of Tn*951* increased their expression and conferred the ability to utilize lactose and lactulose. The ability to utilize lactulose appeared to be related to its uptake as a consequence of *lacY* expression (20, 22). DNA sequence analyses, to be reported elsewhere, have confirmed that two of the elements, IS*406* and IS*407*, contain terminal inverted repeats and generate short directly duplicated sequences of target DNA at the site of insertion (M. S. Wood, A. Ferrance, A. Byrne, and T. G. Lessie, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, H-143, p. 193), consistent with their designation as insertion sequences.

RNA-DNA hybridization experiments indicated that the increase in *lac* gene expression was a consequence of transcriptional activation; however, the mechanism of such activation remains to be determined. Nucleotide sequence data on hand suggest that IS*406* and IS*407* may provide new promoters from which transcription of the activated genes proceeds (22). However, we still need to define the site(s) at which transcription is initiated to confirm whether or not the putative promoters are active in vivo. IS*415* may activate gene expression by a different mechanism. Nucleotide sequence data has indicated that IS*415* is inserted 102 base pairs upstream of the Tn*951* *lacI* gene (M. S. Wood, unpublished data). In this case it seems reasonable that the activation of *lacZ* might involve communication by DNA looping (1).

The approach we have used to identify *lac* gene-activating elements in *P. cepacia* might also be used to search for similar elements in other Lac$^-$ bacteria. Other investigators have reported that the *lac* genes of Tn*951* are expressed poorly in *Pseudomonas fluorescens* (7), *Z. mobilis* (10), and *Rhodobacter sphaeroides* (25), and we have found this also to be the case for *Acetobacter xylinum* and *Acinetobacter calcoaceticus* (22). An important advantage of using the *lac* genes of the broad-host-range plasmid pGC91.14 as a target for insertional activation is that the activated plasmids can be transferred and subsequently used to examine the influence of the gene-activating elements on *lac* gene expression in other bacteria. This approach might lead to the identification of broad-host-range transposable gene-activating elements that could be used in vivo to turn on the expression of foreign genes in a variety of gram-negative bacteria.

Insertion sequence-dependent activation of foreign genes is not restricted to *P. cepacia*. This phenomenon has been observed for *E. coli*, but the number of insertion sequences identified in this bacterium is relatively low (2, 6, 11, 18, 32, 34). We have identified more than a dozen different transposable gene-activating elements in *P. cepacia* 249 (22). A. Chakrabarty and his co-workers have isolated still other such elements in strain AC1100 (31; R. A. Haugland, U. M. X. Sangodkar, and A. M. Chakrabarty, Mol. Gen. Genet., in press). The large number of transposable elements in *P. cepacia* may be a major factor contributing to its unusual catabolic potential and adaptability. Certain of the insertion sequences we have identified have been shown to promote replicon fusions as well as to activate gene expression (5, 20, 22). Thus, they have the potential to mediate the incorporation of foreign genes into the *P. cepacia* genome and simultaneously turn on their expression. It seems reasonable that the complex repertoire of catabolic functions carried out by *P. cepacia* might have evolved in part by using such a mechanism for the recruitment of foreign genes.

ACKNOWLEDGMENTS

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