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Insertion-Sequence-Dependent Rearrangements of *Pseudomonas cepacia* Plasmid pTGL1

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Pseudomonas cepacia **249 (ATCC 17616) harbors a 170-kilobase (kb) plasmid designated pTGL1. We identified three insertion sequences, IS*405*, IS*408*, and IS*411*, on this plasmid. Various prototrophic and auxotrophic derivatives in our collection contained variants of pTGL1 formed by accretion and deletion of other elements. Plasmid pTGL6, the variant in one prototroph, evolved from pTGL1 by the addition of three copies of IS*401* (1.3 kb) and one of IS*402* (1 kb), to generate pTGL5, and recombination between two of the copies of IS*401* on pTGL5 to form pTGL6. The latter event entailed loss of one copy of IS*401* and an additional 5.4 kb of plasmid DNA. Derivatives of the broad-host-range plasmid pRP1 carrying the above insertion sequences and recombinant plasmids carrying fragments of plasmids pTGL6 and pTGL5 were used as probes to ascertain the extent of reiteration of the various elements in the *P. cepacia* genome. The data indicate a high frequency of genomic rearrangements which presumably contributes to the extraordinary adaptability of this bacterium.**

Pseudomonas cepacia is notable for the extraordinary number of compounds it can utilize as sole carbon and energy sources (1, 4, 17, 21, 26, 27, 36). It is both an opportunistic pathogen for humans and a phytopathogen (1, 14, 26). Its unusual catabolic potential and wide distribution in the environment suggest the possibility of novel mechanisms of genetic adaptability. We describe here the identification of various insertion sequences in this bacterium and their involvement in rearrangements of its genome. The first suggestion of the presence of such elements came from our studies of plasmid alterations in various derivatives of *P. cepacia* 249. This strain harbors a 170-kilobase (kb) cryptic plasmid which we have designated pTGL1. Approximately 60% of the auxotrophic derivatives in our collection harbor variant forms of this plasmid (3). We originally considered that the phenotypes of the mutant isolates might be a consequence of recombination between the chromosome and the plasmid. The results described here indicate that no strict correlation exists between the plasmid alterations and chromosomal mutation. We propose that the high frequency of plasmid rearrangements reflects a more general genomic plasticity related to the prevalence of insertion sequences in this bacterium and suggest that the extraordinary adaptability of this organism is at least in part related to the presence of such elements.

MATERIALS AND METHODS

Bacterial strains and plasmids. *P. cepacia* 249 (ATCC 17616), which contains pTGL1, was obtained from the American Type Culture Collection, Rockville, Md. Our laboratory stock of *P. cepacia* 249, designated 249-UM and containing pTGL6, was obtained from the Berkeley collection (36) in 1967. Mutant derivatives of strain 249 isolated in our laboratory and used in this study included: 249-21, a glucose dehydrogenase-deficient mutant containing pTGL5, and 249-13-5 (*ilvA1 lys-4*), which contains the pTGL1-related plasmid pTGL10 (21). We also used derivatives of the mutants 249-2 (a lysine auxotroph) and 249-30 (a strain blocked in pyruvate and glucose utilization) which were cured of plasmid pTGL6. *Escherichia coli* SK1592 (18) was used for the isolation of recombinant derivatives of plasmids pACYC184 (8) and pBR325 (7) carrying fragments of pTGL5 and pTGL6. These and other plasmids described in this paper are listed in Tables 1 and 2. Bacteria were grown at 37°C with shaking in flasks filled to one-fifth of their nominal capacity. For *P. cepacia* the medium consisted of inorganic salts solution (20) supplemented with 1% (wt/vol) Casamino Acids (Difco Laboratories, Detroit, Mich.). Strain SK1592 was grown in LB medium supplemented with either ampicillin (25 μg/ml) or chloramphenicol (20 μg/ml) depending on the vector used and the insert location.

Isolation of DNA. Plasmid DNA was isolated by the procedure of Currier and Nester (12). When used as probes in Southern hybridization experiments the samples were subjected to two rounds of cesium chloride centrifugation. In some experiments plasmid DNA from *E. coli* was isolated by the procedure of Birnboim and Doly (6). Isolation of chromosomal DNA was essentially as described by Thomas et al. (38). Restriction enzyme digestion of DNA was carried out under conditions specified by the suppliers (Bethesda Research Laboratories, Gaithersburg, Md., and International Biotechnologies, Inc., New Haven, Conn.). Agarose gel electrophoresis was carried out in horizontal gels containing Tris borate buffer (pH 8.3) (24), and the gels were poststained with ethidium bromide (0.5 μg/ml). Molecular weights of DNA fragments were determined by comparing their mobilities with those of a kilobase ladder (from Bethesda Research Laboratories) consisting of fragments ranging in size between 0.2 and 12.2 kb pairs.

Cloning of plasmid fragments. Fragments from *Eco*RI and *Hin*dIII digests of plasmids pTGL5 and pTGL6 were ligated with similarly digested preparations of pACYC184 and pBR325 DNA. In some cases fragments were resolved on agarose gels and recovered electrophoretically by the procedure of Heckman and RajBhandary (15) before ligation. The conditions for ligation of DNA fragments were essentially those described by Maniatis et al. (23). T4 DNA ligase was obtained from New England Nuclear Corp., Boston,

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TABLE 1. List of plasmids able to replicate in *P. cepacia*

Plasmid	Size (kb)	Pertinent characteristics
pTGL1	170	Present in *P. cepacia* 249 (ATCC 17616)
pTGL5	175	Derivative of pTGL1 containing three copies of IS*401* and one of IS*402*
pTGL6	168	Derivative of pTGL5 in which recombination between two of the three copies of IS*401* on pTGL5 resulted in loss of 6.7 kb of DNA containing the equivalent of one copy of IS*401*
pRP1	60	IncP1 (39), confers resistance to tetracycline when introduced into strain 249
pTGL62	61	pRP1::IS*402* (21, 34)
pGC91.14	77	pRP1::Tn*951*, carries an intact *lac* operon (10)
pTGL68	80	pGC91.14::IS*408* (21)
pMR5	60	Derivative of RP1 unable to replicate in *P. cepacia* at 47°C (2, 21, 31)
pTGL105	61	pMR5::IS*401* (2)
pTGL109	63	Derivative of pMR5 containing IS*411* (2)

Mass. Recombinant plasmids were introduced into *E. coli* SK1592 essentially as described by Lederberg and Cohen (19).

DNA hybridization experiments. DNA samples used as probes were prepared by nick translation (30), using α-$^{32}PO_4$-labeled dCTP and kits obtained from Bethesda Research Laboratories. The specific activities of the preparations were between 3×10^7 and 10^8 cpm/μg of DNA. DNA fragments were transferred from agarose gels to Gene Screen Plus nylon membranes (New England Nuclear Corp.) by the procedure of Southern (35). Hybridization conditions were those recommended by the supplier. Autoradiography was performed at −70°C with Kodak XAR-5 film and Du Pont Cronex Lightning-Plus intensifying screens.

Chemicals. Cesium chloride and ethidium bromide were obtained from Sigma Chemical Co., St. Louis, Mo. Agarose was purchased from Bio-Rad Co., Rockville Center, N.Y.

RESULTS

Evolution of pTGL6 from pTGL1. We discovered that the strain of *P. cepacia* 249 maintained in the American Type Culture Collection (ATCC 17616) harbors a 170-kb plasmid, pTGL1, which differs in several ways from the plasmid, pTGL6, isolated from our laboratory strain, *P. cepacia* 249-UM. The latter strain was obtained almost 20 years ago from the same stock deposited in the American Type Culture Collection by Stanier and his co-workers (36). The glucose dehydrogenase-deficient mutant 249-21 (22) contains another pTGL1 variant, pTGL5.

TABLE 2. Recombinant derivatives of pACYC184[a] carrying inserts of *Hin*dIII fragments of pTGL5 and pTGL6

Plasmid	Inserted *Hin*dIII fragment
pTGL104	5.8-kb fragment of pTGL6 containing IS*401* and IS*408*
pTGL214	5.85-kb fragment of pTGL5 containing IS*401*
pTGL217	6.0-kb fragment of pTGL5 containing IS*402*
pTGL219	4.1-kb fragment of pTGL5 containing IS*401* and IS*408*
pTGL223	2.95-kb fragment of pTGL5 containing IS*401*
pTGL229	2.5-kb fragment of pTGL5 deleted in formation of pTGL6

[a] *Hin*dIII fragments of plasmids pTGL5 and pTGL6 were ligated into the tetracycline resistance determinant of the 4-kb multicopy vector pACYC184 (8) and introduced into *E. coli* SK1592 by transformation (18). Chloramphenicol-resistant, tetracycline-sensitive transformants were subsequently screened to identify the appropriate recombinant plasmids (16).

TABLE 3. Complement of *Eco*RI, *Hin*dIII, and *Sma*I fragments of *P. cepacia* plasmid pTGL1

Restriction enzyme fragments[a]					
*Eco*RI		*Hin*dIII		*Sma*I	
Fragment	Size (kb)	Fragment	Size (kb)	Fragment	Size (kb)
1	17.8[b]	1	28.0	1	17.5
2	11.5[c]	2	24.0	2	15.5 (2)
3	7.2	3	18.5	3	11.8
4	6.8 (2)	4	16.0	4	9.7
5	6.6	5	12.0	5	8.5 (2)
6	6.3	6	8.9	6	7.3
7	5.95	7	8.55	7	6.9
8	5.8	8	5.65	8	6.7
9	5.25	9	5.05 (2)	9	6.1
10	5.05	10	4.95	10	5.5
11	4.75 (2)	11	4.55 (2)	11	5.2 (2)
12	4.2 (4)	12	3.75	12	5.1
13	4.0	13	2.8	13	4.8
14	3.9	14	2.6	14	4.7
15	3.8	15	2.5	15	4.6
16	3.5[d]	16	2.1	16	3.9
17	3.0	17	1.85	17	3.4
18	2.95	18	1.65	18	2.7
19	2.9 (2)	19	1.45	19	2.6
20	2.75	20	1.2	20	2.5
21	2.6 (2)	21	1.05 (2)	21	2.2
22	2.4	22	0.85	22	2.0
23	2.15	23	0.6 (2)	23	0.6
24	1.7 (3)			24	0.45
25	1.4			25	0.35
26	1.35			26	0.3
27	1.3				
28	1.2 (3)				
29	0.95				
30	0.85 (3)				
31	0.75 (2)				
32	0.55				
33	0.45 (2)				
34	0.4				

[a] Numbers in parentheses indicate the number of restriction enzyme fragments of the size specified. There were 49 total *Eco*RI fragments, 27 total *Hin*dIII fragments, and 29 total *Sma*I fragments.
[b] Contains IS*408*.
[c] Contains IS*405*.
[d] Contains IS*411*.

Plasmid pTGL1 was cleaved into 49, 27, and 29 fragments, respectively, by treatment with the restriction enzymes *Eco*RI, *Hin*dIII, and *Sma*I (Table 3). Restriction enzyme digests of pTGL5 were missing three *Eco*RI fragments (E-1, E-12a, and E-24a) and four *Hin*dIII fragments (H-9a, H-11a, H-13, and H-18) present in pTGL1. Digests of pTGL5 contained three new *Eco*RI fragments of 20.4, 5.15, and 3.0 kb and four new *Hin*dIII fragments of 6.0, 5.85, 4.1, and 2.95 kb not present in pTGL1. *Sma*I digests of pTGL5 were missing four pTGL1 fragments (S-4, S-5, S-9, and S-11) and contained eight new fragments of 6.7, 5.7, 5.3, 3.8, 3.6, 3.5, 3.1, and 2.7 kb.

The plasmid pTGL6 from the prototroph *P. cepacia* 249-UM appeared to be more closely related to pTGL5 than to pTGL1. Except for the substitution in pTGL6 of a 13.7-kb fragment for the 20.4-kb fragment of pTGL5, the *Eco*RI fragment patterns of these two plasmids were the same. *Sma*I digests of pTGL6 were missing the 3.6- and 3.1-kb

fragments of pTGL5 (Fig. 1). Otherwise, the complement of *Sma*I fragments was identical. The *Hin*dIII patterns of the two plasmids differed in several respects. Digests of pTGL6 were missing three pTGL5 *Hin*dIII fragments of 5.85, 2.5, and 4.1 kb and contained a new fragment of 5.8 kb.

We carried out Southern hybridization experiments using fragments of pTGL5 as probes to determine the relationships between the different fragments of pTGL1, pTGL5, and pTGL6. We also probed restriction enzyme digests of the three plasmids with variants of the broad-host-range drug resistance plasmid pRP1 carrying IS*401*, IS*402*, and IS*408*, three insertion sequences present in the *P. cepacia* genome (2, 21, 34). The results, which are described below, are consistent with the interpretation that pTGL5 evolved from the IS*408*-containing plasmid pTGL1 by insertion of IS*401* and IS*402* at multiple sites on pTGL1 and that pTGL6 was formed from pTGL5 as a consequence of a single deletion event involving recombination between two IS*401* elements.

Figure 2A shows the results of an experiment in which pTGL105 (2), a variant of the drug resistance plasmid pMR5 (31) carrying IS*401*, was used to probe *Hin*dIII digests of plasmids pTGL1, pTGL5, and pTGL6. Plasmid pTGL105 failed to hybridize with pTGL1, but exhibited strong homology with the 5.85- and 4.1-kb fragments of pTGL5, the 2.95-kb fragment common to both pTGL5 and pTGL6, and the 5.8-kb fragment of pTGL6.

Panels B, C, and D of Fig. 2 show hybridization experiments in which *Hin*dIII digests of pTGL1, pTGL5, and pTGL6 were probed with pTGL214, pTGL219, and pTGL223 (derivatives of pACYC184 containing, respectively, the 5.85-, 4.1-, and 2.95-kb *Hin*dIII fragments of pTGL5). The data showed that the 5.85-, 4.1-, and 2.95-kb *Hin*dIII fragments of pTGL5 were related, respectively, to the 4.55 (H-11a)-, 2.8 (H-13)-, and 1.65 (H-18)-kb *Hin*dIII fragments of pTGL1.

Similar experiments with pTGL68 (pGC91.14::IS*408*) as a probe indicated that IS*408* was present in the respective 2.8-, 4.1-, and 5.8-kb *Hin*dIII fragments of pTGL1, pTGL5, and pTGL6 (Fig. 3). Neither pMR5 nor pGC91.14 exhibited homology with pTGL1, pTGL5, or pTGL6 (data not shown).

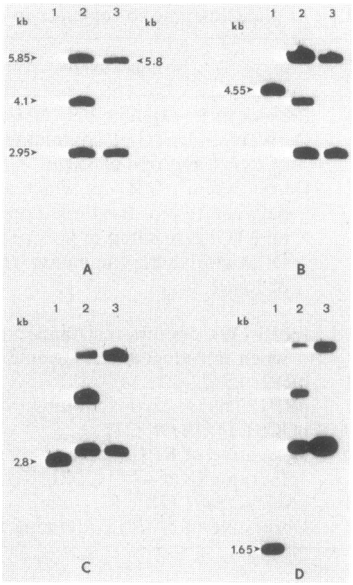

FIG. 2. Southern hybridization experiments defining IS*401*-containing *Hin*dIII fragments in plasmids pTGL5 and pTGL6 and their corresponding precursor fragments in pTGL1. Lanes 1, 2, and 3 in panels A through D contained, respectively, digests of plasmids pTGL1, pTGL5, and pTGL6. The ^{32}P-labeled probe DNAs in the four experiments were: in panel A, pTGL105 (pMR5::IS*401*); and in panels B through D, plasmids pTGL214, pTGL219, and pTGL223, respectively. The latter three plasmids were derivatives of pACYC184 carrying, respectively, the 5.85-, 4.1-, and 2.95-kb IS*401*-containing *Hin*dIII fragments of pTGL6.

The most reasonable interpretation of the above data is that the 5.85-, 4.1-, and 2.95-kb *Hin*dIII fragments of pTGL5 arose by the addition of copies of IS*401* to *Hin*dIII fragments H-11a, H-13, and H-18 of pTGL1. This 1.3-kb element contains no *Hin*dIII site (2). The presence of IS*401* thus accounts for the differences in size between the pTGL5 and

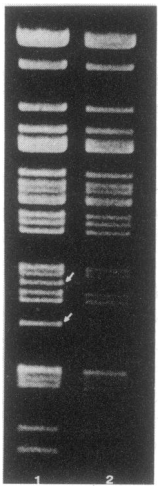

FIG. 1. *Sma*I restriction fragment patterns of plasmids pTGL5 (lane 1) and pTGL6 (lane 2). The 6.7-kb difference in size between these two plasmids is accounted for entirely by the elimination of the 3.6- and 3.1-kb fragments present in digests of pTGL5 but absent from those of pTGL6. The pertinent pTGL5 fragments in lane 1 are indicated by the arrows.

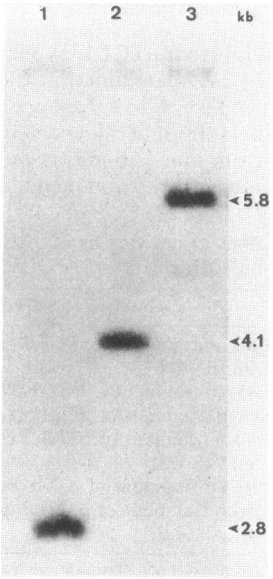

FIG. 3. Hybridization of plasmid pTGL68 (pGC91.14::IS*408*) with IS*408*-containing fragments of plasmids pTGL1 (lane 1), pTGL5 (lane 2), and pTGL6 (lane 3).

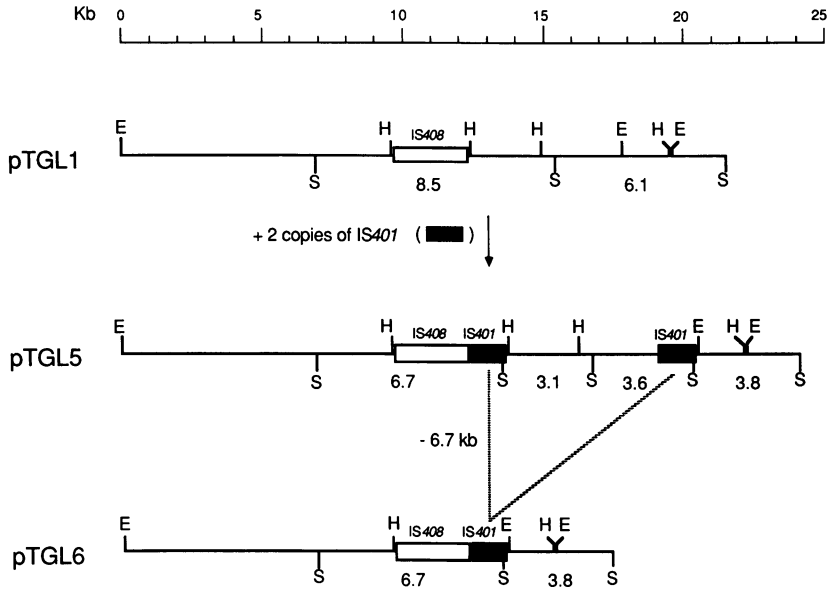

FIG. 4. Restriction maps of the IS*408*-containing regions of plasmids pTGL1, pTGL5, and pTGL6 showing the locations of *Eco*RI (E), *Hin*dIII (H), and *Sma*I (S) sites. Distances between pertinent *Sma*I sites are indicated in kilobases. The 6.7 kb of DNA deleted from pTGL5 in formation of pTGL6 included 5.4 kb of DNA also present in pTGL1 and the equivalent of one copy of IS*401* (see text).

pTGL1 fragments and the fact that the three pTGL5 fragments cross-hybridized with each other. It also explains the homology of IS*408* with the 4.1-kb *Hin*dIII fragment of pTGL5, since this fragment was derived by insertion of IS*401* into the IS*408*-containing 2.8-kb *Hin*dIII fragment of pTGL1.

Southern hybridization experiments also were carried out with the probes pTGL52 (pRP1::IS*402*) and pTGL217 (pACYC184 carrying the 6-kb *Hin*dIII fragment of pTGL5). Plasmid pTGL52 failed to hybridize with pTGL1, but exhibited strong homology with the 6-kb *Hin*dIII fragments of both pTGL5 and pTGL6. Plasmid pTGL217 hybridized with the 5.05-kb fragment of pTGL1 (H-9a) and the 6-kb *Hin*dIII fragment of pTGL6 (data not shown). Like IS*401*, IS*402* contained no *Hin*dIII site (2). The addition of this 1-kb element to fragment H-9a of pTGL1 thus explains the appearance of the new 6-kb *Hin*dIII fragment in pTGL5 and pTGL6. The above data, combined with the data of Fig. 2, indicate that pTGL5 arose from the IS*408*-containing plasmid pTGL1 by the addition of three copies of IS*401* and one of IS*402*.

Figure 4 shows restriction maps of the IS*408*-containing regions of pTGL1, pTGL5, and pTGL6. The maps for pTGL1 and pTGL5 include the two *Sma*I fragments missing from pTGL6 (Fig. 1). The maps are based on restriction analyses of various cloned fragments and the use of overlapping cloned fragments as hybridization probes to define adjacent restriction fragments. They incorporate the results of the hybridization experiments in which pTGL105 and pTGL68 were used to identify IS*401*- and IS*408*-containing fragments of the three plasmids. Not shown are the regions of pTGL5 and pTGL6 containing IS*402* or the other copy of IS*401* on pTGL5 and pTGL6.

The simplest interpretation of the data of Fig. 1 to 4 is that pTGL6 arose from pTGL5 by a deletion involving recombination between the two IS*401* elements located in the 4.1- and 5.85-kb *Hin*dIII fragments of the latter plasmid. The net effect was fusion of portions of these two fragments (to form the 5.8-kb *Hin*dIII fragment of pTGL6) with loss of the 2.5-kb *Hin*dIII fragment situated between them and elimination of one copy of IS*401*. A Southern hybridization experiment in which pTGL229 (pACYC184 carrying the 2.5-kb *Hin*dIII fragment of pTGL5) was probed against total cellular DNA from the pTGL6-containing strain 249-UM indicated that the deleted 2.5-kb fragment was not present in the genome of this bacterium (data not shown). This ruled out the possibility that the deleted DNA had been transferred from pTGL5 to the chromosome in an illegitimate recombination event.

IS*401* contains a *Sma*I site close to one of its ends (2). Figure 4 shows how the 3.1- and 3.6-kb *Sma*I fragments deleted in formation of pTGL6 extended from the *Sma*I sites within the directly repeated copies of IS*401* involved in the recombination event. It should be noted that the 3.1-kb *Sma*I fragment overlapped the 2.5-kb *Hin*dIII fragment lost in the formation of pTGL6 and that the 5.8-kb *Hin*dIII fragment generated in this event contained copies of both IS*401* and IS*408*.

Other insertion sequences present on pTGL1. As a step toward identifying any additional insertion sequences carried by pTGL1-related plasmids, ^{32}P-labeled chromosomal DNA from a derivative of the glucose nonutilizing mutant 249-30 which had been cured of pTGL6 (21) was hybridized with resolved fragments from *Eco*RI digests of pTGL6. Five *Eco*RI pTGL6 fragments exhibited significant homology with chromosomal DNA from this strain (data not shown). These included the 13.7- and 3.0-kb fragments, each of which contained IS*401*, and the 5.15-kb fragment which contained IS*402*. However, the most prominent signals came from *Eco*RI fragments of 11.5 and 3.5 kb.

Plasmids pTGL200 (pBR325 carrying the 11.5-kb *Eco*RI fragment of pTGL6) and pTGL113 (pACYC184 carrying the 3.5-kb *Eco*RI fragment of pTGL6) were constructed and used to probe *Eco*RI-digested chromosomal DNA from strain 249-30 (pTGL6$^-$). Plasmid pTGL200 hybridized with 12 different chromosomal fragments ranging in size from 20 to 2.5 kb, while pTGL113 hybridized with 7 chromosomal fragments ranging in size from 18.5 to 2 kb (data not shown).

The subsequent isolation of plasmids pTGL55 and pTGL109 (2, 21, 34) allowed us to demonstrate that the 11.5-kb *Eco*RI fragment of pTGL6 contained IS*405* and that the 3.5 *Eco*RI fragment of this plasmid contained IS*411*. Plasmid pTGL55 hybridized with the 11.5-kb fragment of pTGL6 and the same 12 chromosomal *Eco*RI fragments as did pTGL200. Plasmid pTGL109 hybridized with the 3.5-kb fragment of pTGL6 and the same seven chromosomal *Eco*RI fragments as did pTGL113.

The first indication that the 13.7-kb *Eco*RI fragment of pTGL6 and the 5.8-kb *Hin*dIII fragment which it overlapped contained an element in addition to IS*401* came from experiments in which pTGL104 (pACYC184 carrying the 5.8-kb *Hin*dIII fragment of pTGL6) was hybridized against chromosomal DNA from a derivative of the lysine auxotroph 249-2 cured of pTGL6. Six chromosomal *Eco*RI fragments of 8.6, 6.5, 5.8, 5.2, 4.5, and 2.7 kb exhibited homology (data not shown). In contrast, the copy of IS*401* in pTGL105 (pMR5::IS*401*) exhibited homology with only two of the fragments (6.5 and 4.5 kb). The IS*408* element in pTGL68 (pGC91.14::IS*408*) was later shown to hybridize to the other four, non-IS*401*-containing chromosomal fragments. The *Eco*RI fragments of pTGL1 containing IS*408*, as well as those containing IS*405* and IS*411*, are indicated in Table 1. All three of these elements were conserved in pTGL5 and pTGL6.

Chromosomal copies of elements present on pTGL6. We used derivatives of pRP1 carrying the different insertion sequences identified on pTGL6 as hybridization probes to estimate the number of copies of these elements in the chromosome of a derivative of strain 249-30 cured of pTGL6. Plasmids pTGL105 (pMR5::IS*401*), pTGL52 (pRP1::IS*402*), pTGL55 (pRP1::IS*405*), pTGL68 (pGC91.14::IS*408*), and pTGL109, a pRP1 derivative carrying IS*411*, were hybridized against *Eco*RI fragments of chromosomal DNA from this strain. The number of *Eco*RI fragments exhibiting homology with IS*401*, IS*402*, IS*405*, IS*408*, and IS*411* were, respectively, 3, 2, 12, 4, and 7. Thus, there appear to be more copies of IS*405* than of the other elements on pTGL6.

Several strains in our collection of *P. cepacia* 249 mutants blocked in various biosynthetic and catabolic pathways differed in their complement of chromosomal copies of IS*401* and IS*402*. Figure 5 shows the results of a Southern hybridization experiment in which pTGL105 (pMR5::IS*401*) was hybridized against *Eco*RI fragments of chromosomal DNA from the prototroph 249-ATCC and four different mutant strains. Chromosomal DNA from both the prototroph (lane 1) and the Ilv$^-$ Lys$^-$ strain 13-5 (lane 2) contained only one fragment (6.5 kb) homologous with the IS*401* element on this probe. In contrast, DNA from a derivative of the Lys$^-$ strain 249-2 which had been cured of pTGL6 (lane 3), from a similarly cured derivative of strain 249-30, a mutant blocked in glucose utilization (lane 4), and from a Glu$^+$ revertant of the latter strain (lane 5) contained, respectively, two, three, and four such fragments. Similar results (not shown here) indicated that there were rearrangements of IS*402* in the chromosome of strain 249-13-5 and of IS*405* in the chromosome of strain 249-30 (pTGL6$^-$).

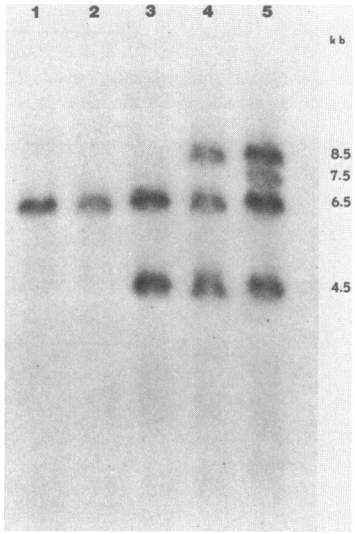

FIG. 5. Different chromosomal distributions of IS*401* in mutants derived from strain 249 (ATCC 17616). Plasmid pTGL105 (pMR5::IS*401*) was used to probe *Eco*RI digests of chromosomal DNA from strains 249-ATCC (lane 1), 249-13-5 (lane 2), and mutants cured of pTGL6 (lanes 3 to 5). These were, respectively, 249-2 (pTGL6$^-$), 249-30 (pTGL6$^-$), and 249-30-1 (pTGL6$^-$), a Glu$^+$ revertant of strain 249-30 (pTGL6$^-$). DNA digests from strains 249-ATCC and 249-13-5 contained one copy of IS*401* in a fragment of ca. 6.5 kb. (IS*401* contains no *Eco*RI site.) The 4.5-, 8.5-, and 7.5-kb fragments in digests of DNA from the other strains represent new copies of this element.

DISCUSSION

Insertion sequences which we detected in *P. cepacia* 249 (ATCC 17616) mediated rearrangements of pTGL1, a 170-kb cryptic plasmid harbored by this bacterium. The evolution of pTGL5 from pTGL1 involved the transposition of three copies of IS*401* and one of IS*402* to pTGL1, increasing its size by 4.9 kb. The exact order of these transposition events is unknown, since *P. cepacia* strains containing putative intermediates have not been identified. However, IS*402* and at least one copy of IS*401* presumably transposed to pTGL1 from the chromosome, since neither of these insertion sequences was located on pTGL1. Both IS*401* and IS*402*, as well as the three elements which were present on pTGL1 (IS*405*, IS*408*, and IS*411*), were detected in the *P. cepacia* chromosome.

The deletion that formed pTGL6 from pTGL5 occurred in the vicinity of the IS*408* element conserved in the overall evolution of pTGL6 from pTGL1. It extended between two directly repeated copies of IS*401* which were separated by 5.4 kb of plasmid DNA. As a consequence, adjacent 3.1- and 3.6-kb *Sma*I fragments spanning the region of pTGL5 between the *Sma*I sites in these two elements were lost, and one copy of IS*401* was eliminated. Presuming the deletion involved homologous recombination between the two elements, the copy of IS*401* resulting from this event should be intact and functional. That this was the case was demonstrated by the fact that this element promoted the fusion of pTGL6 and pMR5 to form the cointegrate plasmid pTGL101 (2).

It should be emphasized that the plasmid variants described in this study were isolated without direct selection. They were identified in the course of screening various mutant derivatives for altered plasmids. This suggests that the insertion sequences implicated in their formation transpose at a high frequency. Our detection in Southern hybridization experiments of differences in the number of chromosomal copies of IS*401* and IS*402* between strains is consistent with this conclusion. A number of investigators have observed a similarly high frequency of unselected plasmid

TABLE 4. Summary of insertion elements identified on pTGL1-related plasmids

Element	Size (kb)	No. of chromosomal copies[a]	Characteristics
IS*401*[b]	1.3	2	Present on pTGL5 and pTGL6 but not on pTGL1; promotes replicon fusions (2)
IS*402*[b]	1.0	2	Present on pTGL5 and pTGL6 but not on pTGL1; promotes replicon fusions (2); *bla* gene activating (21, 34)
IS*405*	1.5	12	Present on pTGL1; *bla* gene activating (21, 34)
IS*408*	2.7	4	Present on pTGL1; *lac* gene activating (21)
IS*411*	2.0	7	Present on pTGL1; transposed to pMR5 (2)

[a] Determined by Southern hybridization experiments with *Eco*RI digests of chromosomal DNA from a derivative of strain 249-30 cured of pTGL6. None of the five elements described in this table contained an *Eco*RI site.
[b] IS*401* and IS*402* were the only elements containing a *Sma*I site.

rearrangements in other bacteria (5, 11, 25, 28, 37). Insertion sequences or repetitive elements were also identified in these organisms (9, 13, 28, 29, 32, 33).

Characteristics of the insertion sequences described in this study are summarized in Table 4. All the elements have been shown to transpose to derivatives of the drug resistance plasmid pRP1 (2, 21). Some have the capacity to activate expression of genes introduced into *P. cepacia* on the broad-host-range plasmids pRP1 and pGC91.14. These include IS*402* and IS*405*, which were shown to activate expression of the *bla* gene of the Tn*1* element on pRP1 (34), and IS*408*, which was shown to insert into the *lacZ* gene of the Tn*951* element on pGC91.14 (pRP1*lac*) and activate its *lacY* gene (21). IS*402* has been demonstrated both to mediate replicon fusions between pTGL6 and pMR5 and to activate expression of the *bla* gene of the latter plasmid (2, 21, 34). Additional *bla* and *lac* gene-activating elements not present on pTGL1 have been identified (21, 34). At this point we estimate that there are at least 12 different transposable elements contained in the *P. cepacia* genome.

The large number of such elements suggests a considerable potential for rearrangement of the *P. cepacia* genome. It seems reasonable that many of these insertion sequences (in addition to IS*402*) will prove able both to activate expression of foreign genes and to mediate their incorporation into the genome of this bacterium. The unusual adaptability of *P. cepacia* may be related to the genomic plasticity provided by these elements and their ability to activate gene expression.

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