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Genetic Homology Between Independently Isolated Chlorobenzoate-Degradative Plasmids

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Two chlorobenzoate-degradative plasmids were studied by the hybridization of the restriction endonuclease-generated fragments of one plasmid after transfer to a nitrocellulose filter with nick-translated radioactive DNA of the other plasmid as a probe. Two strains harboring the 3-chlorobenzoic acid-degradative plasmids were isolated in two different parts of the world at two different times. The plasmids are now found to be closely related to each other by hybridization studies. The chlorobenzoate-degradative plasmid from *Pseudomonas* sp. strain B13 (termed pB13) has a 6-kilobase deletion but otherwise is homologous with previously described plasmid pAC25.

We have previously reported the isolation and characterization of plasmid pAC25 in a strain of *Pseudomonas putida* specifying biodegradation of 3-chlorobenzoic acid (2). Knackmuss and colleagues reported the isolation of another *Pseudomonas* sp. strain, B13, capable of utilizing 3-chlorobenzoic acid as a sole source of carbon (3, 4, 9). Although the genetic evidence suggests that a conjugative plasmid is involved in specifying 3-chlorobenzoic acid degradation in strain B13, there is no physical evidence for the presence of any plasmid. Despite attempts by several workers (6, 9, 10), physical demonstration of the presence of plasmid DNA in strain B13 has remained elusive. In this report, we describe the isolation and restriction enzyme digestion profiles of the chlorobenzoate-degradative plasmid in strain B13 and demonstrate the homology of this plasmid with another independently isolated chlorobenzoate-degradative plasmid, pAC25.

Cultures of *Pseudomonas* species strain B13 and *P. putida* AC858, harboring the plasmid pAC25 (2), were grown in basal synthetic medium (8) containing 1.5 mg of 3-chlorobenzoic acid (sodium salt) per ml as the sole source of carbon and energy. Transfer of the restriction endonuclease (New England Biolabs)-generated fragments of plasmid DNAs from agarose gel (0.7%) to nitrocellulose filters (Schleicher & Schuell Co.) was done essentially as described by Southern (11). Plasmid DNA was nick translated with [^{32}P]CTP (Amersham Corp.) by the procedure of Maniatis et al. (7).

Attempts to isolate plasmid DNA from strain B13 by procedures commonly used by others (5, 12) always met with failure. By using a modification (2) of the procedure described by Casse et al. (1), we were finally able to isolate both pAC25 and the plasmid DNA from strain B13. The yield of plasmid DNA was, however, low (about 60 μg of B13 plasmid DNA from a 2-liter culture grown with 3-chlorobenzoate as a sole source of carbon; the yield of pAC25 was somewhat higher, about 100 μg of DNA under similar conditions). We isolated plasmid DNA from two different B13 slants, sent to us on two different occasions by W. Reineke and H.-J. Knackmuss, and demonstrated transmissibility of the plasmid from strain B13 by transferring the chlorobenzoate-degradative ability to our laboratory strain of *P. putida* AC10. The isolation of a single type of plasmid from both B13 isolates suggested that there is a single plasmid present in strain B13. This plasmid is termed pB13.

A profile of fragments generated after *Eco*RI digestion can be seen in Fig. 1. Both pAC25 and pB13 generated 10 fragments after *Eco*RI digestion. Only two of the fragments showed altered mobilities in the agarose gel electrophoresis. Fragments *Eco*RI-B and *Eco*RI-E of pAC25 were replaced in pB13 (Table 1). Plasmid pAC25 was 6 kilobases (kb) larger than pB13.

To find out whether the 6-kb deletion from pAC25 could also be obtained after digestion with various other restriction endonucleases, we digested pAC25 as well as pB13 with *Hin*dIII and *Bgl*II and compared the digestion profiles side by side after agarose gel electrophoresis. The size of the deletion from pAC25 was about 6 kb (Table 1). The mean molecular mass of pAC25 and pB13 were calculated to be about 117 and 111 kb, respectively.

To determine whether all the fragments of pAC25 and pB13 generated by various restriction endonucleases are homologous to one an-

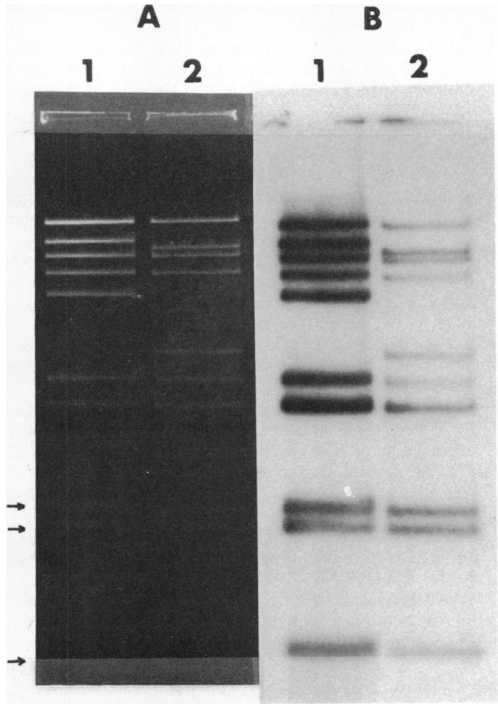

FIG. 1. Agarose gel electrophoresis of *Eco*RI-digested pAC25 (lane 1) and pB13 (lane 2). (A) Ethidium bromide-stained agarose gel. The molecular weights of the fragments are given in Table 1. (B) Autoradiogram of the gels shown in (A) after hybridization with ^{32}P-labeled pAC25 as a probe. The arrows indicate the faint bands visible in the original gel.

TABLE 1. pAC25 and pB13 fragments generated after restriction endonuclease digestions

Digestion	Kilobase pairs	
	pAC25	pB13
*Eco*RI	27.5	27.5
	22.5	20.8
	18.5	18.5
	15.5	15.5
	12.0	7.8
	6.8	6.8
	5.7	5.7
	3.6	3.6
	3.2	3.2
	1.8	1.8
*Hin*dIII	≤93	≤93
	15.0	9.1
	8.1	8.1
	1.1	1.1
*Bgl*II	≤64	≤64
	19.5	19.5
	16.0	10.0
	8.8	8.8
	4.3	4.3
	3.8	3.8
	1.4	1.4

other, the fragments were transferred to a nitrocellulose paper and hybridized with nick-translated ^{32}P-labeled pAC25 DNA as a probe. All the fragments of pB13 were shown to be hybridizable with pAC25 (Fig. 1B and 2). The smaller fragments of pB13 hybridized as well as the fragments of identical mobility with probe pAC25 DNA, suggesting that these fragments were derived by a 6-kb deletion of the larger fragments of pAC25.

Our results clearly demonstrate that the 3-chlorobenzoate-utilizing *Pseudomonas* sp. strain B13 harbors a plasmid, termed pB13, which is homologous to the 3-chlorobenzoate-degradative plasmid, pAC25. These two plasmids are not only homologous as judged by a DNA-DNA hybridization study, but also have related restriction endonuclease digestion profiles. Since both the chlorobenzoate-degradative plasmids are conjugative (2, 9, 10), these results

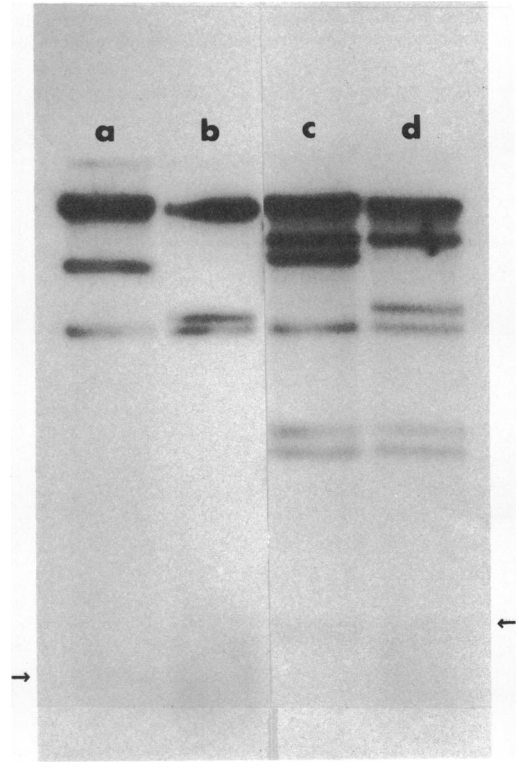

FIG. 2. Autoradiograms of the fragments generated after restriction endonuclease digestions of pAC25 and pB13 after hybridization with ^{32}P-labeled pAC25 as a probe. a and b, *Hin*dIII-digested pAC25 and pB13, respectively; c and d, *Bgl*II-digested pAC25 and pB13, respectively. The arrows indicate the smaller fragments of pAC25 or pB13 obtained after *Hin*dIII or *Bgl*II digestions which were clearly visible in the original autoradiogram.

suggest that the extra 6-kb portion present in pAC25 is not essential for 3-chlorobenzoic acid degradation or transfer of the plasmids. Strain B13 was isolated in Germany in the early 1970s, whereas the strain with the pAC25 plasmid was isolated in the United States in the late 1970s; it is interesting that they harbor closely related plasmids.

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LITERATURE CITED

1. **Casse, F., C. Boucher, J. S. Julliot, M. Michel, and J. Denarie.** 1979. Identification and characterization of large plasmids in *Rhizobium meliloti* using agarose gel electrophoresis. J. Gen. Microbiol. **113**:229–242.
2. **Chatterjee, D. K., S. T. Kellogg, S. Hamada, and A. M. Chakrabarty.** 1981. Plasmid specifying total degradation of 3-chlorobenzoate by a modified *othro* pathway. J. Bacteriol. **146**:639–646.
3. **Dorn, E., M. Hellwig, W. Reineke, and H.-J. Knackmuss.** 1974. Isolation and characterization of a 3-chlorobenzoate degradating pseudomonad. Arch. Microbiol. **99**:61–70.
4. **Dorn, E., and H.-J. Knackmuss.** 1978. Chemical structure and biodegradability of halogenated aromatic compounds. Two catechol 1,2-dioxygenases from a 3-chlorobenzoate grown pseudomonad. Biochem. J. **174**:73–84.
5. **Hansen, J. B., and R. H. Olsen.** 1978. Isolation of large bacterial plasmids and characterization of the P2 incompatibility group plasmids pMGI and pMG2. J. Bacteriol. **135**:227–238.
6. **Jeenes, D. J., and P. A. Williams.** 1982. Excision and integration of degradative pathway genes from TOL plasmid pWWO. J. Bacteriol. **150**:188–194.
7. **Maniatis, T., A. Jeffery, and D. G. Kleid.** 1975. Nucleotide sequence of the rightward operator of phage λ. Proc. Natl. Acad. Sci. U.S.A. **72**:1184–1188.
8. **Phibbs, P. V., Jr., T. W. Feary, and W. T. Blevins.** 1974. Pyruvate carboxylase deficiency in pleiotropic carbohydrate-negative mutant strains of *Pseudomonas aeruginosa*. J. Bacteriol. **118**:999–1099.
9. **Reineke, W., and H.-J. Knackmuss.** 1979. Construction of haloaromatics utilizing bacteria. Nature (London) **277**:385–386.
10. **Shapiro, J. S., A. Charbit, S. Benson, M. Caruso, R. Laux, R. Meyer, and F. Banuett.** 1981. Perspectives for genetic engineering of hydrocarbon oxidizing bacteria, p. 243–272. *In* A. Hollaender (ed.), Trends in the biology of fermentation for fuels and chemicals. Plenum Publishing Corp., New York.
11. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
12. **Wheatcroft, R., and P. A. Williams.** 1981. Rapid methods for the study of both stable and unstable plasmids in *Pseudomonas*. J. Gen. Microbiol. **124**:433–437.