JOURNAL OF BACTERIOLOGY, Nov. 1981, p. 413-418
0021-9193/81/110413-06$02.00

Vol. 148, No. 2

Molecular Cloning of Gene *xylS* of the TOL Plasmid: Evidence for Positive Regulation of the *xylDEGF* Operon by *xylS*

SACHIYE INOUYE, ATSUSHI NAKAZAWA, AND TERUKO NAKAZAWA*

Department of Biochemistry, Yamaguchi University School of Medicine, Ube, 755 Japan

Received 27 April 1981/Accepted 30 June 1981

The *xylDEGF* operon and the regulatory gene *xylS* of the TOL plasmid found in *Pseudomonas putida* mt-2 were cloned onto *Escherichia coli* vector plasmids. A 9.5-kilobase fragment, derived from the TOL segment of pTN2 deoxyribonucleic acid, carried the *xyl* genes *D*, *E*, *G*, and *F*, which encode toluate oxygenase, catechol 2,3-oxygenase, 2-hydroxymuconic semialdehyde dehydrogenase, and 2-hydroxymuconic semialdehyde hydrolase, respectively. The enzymes were non-inducible unless a 3-kilobase *Pst*I fragment, derived also from the TOL segment, was provided in either *cis* or *trans*. The *Pst*I fragment appeared to contain the regulatory gene *xylS*, which produced a positive regulator. The regulator was activated by *m*-toluate or benzoate, but not by *m*-xylene or *m*-methylbenzyl alcohol. The map positions of *xylG* and *xylF* were also determined.

Members of the bacterial genus *Pseudomonas* are renowned for their capacity to utilize an enormous range of compounds as carbon sources. It is now known that the ability of these organisms to degrade certain substrates is specified by genes borne on plasmids. The plasmids carry the structural genes of degradative enzymes as well as their regulatory genes, and can be purified and subjected to DNA structural investigations and manipulations.

The TOL plasmid of *Pseudomonas putida* mt-2 encodes a pathway for catabolism of toluene and *m*- and *p*-xylenes by oxidizing a methyl substituent to a carboxylic acid followed by oxidative ring-cleavage via the *meta* pathway to central metabolites (17). From the analysis of the induction of degradative enzymes, Worsey et al. (16) proposed a model for the regulation of the early enzymes. A modified model based on this study is shown in Fig. 1. The enzymes are organized in at least two regulatory blocks, associated respectively with the oxidation of the methyl group (*xylABC*) and the further oxidation and degradation of the carboxylic acids (*xylDEGF*). Two regulatory genes, *xylR* and *xylS*, are involved; *xylR* plays a role in the induction of both regulatory blocks by the hydrocarbons and the alcohol metabolites (and probably also the aldehyde metabolites), whereas *xylS* is responsible for the induction of only the second block by the carboxylic acids. Recently the positive regulation of the enzymes by the product of the *xylR* gene has been demonstrated by constructing a partial diploid of the TOL genes (9, 13).

The structure of the TOL plasmid has been analyzed on pWWO, a natural plasmid, and also on pTN2, an in vivo recombinant of TOL and RP4 (7, 13). The pTN2 plasmid, consisting of the entire region of RP4 and the 56-kilobase (kb) TOL segment, appears to contain all of the structural and regulatory genes of the pathway. To analyze the genetic organization of the pathway, we have carried out gene cloning of *xylB* coding for benzyl alcohol dehydrogenase and *xylE* coding for catechol 2,3-oxygenase (EC 1.13.11.2) in *Escherichia coli* (11). The cloned genes were expressed mainly from the vector promoter of pBR322 and were not inducible. On the other hand, the cells of *E. coli* carrying pTN2 showed the induction of the *xylB* and *xylE* products by a specific inducer. Therefore, the regulatory regions involved in the expression of these genes did not appear to be located in the vicinity of the structural genes. Thus, we searched for the regulatory genes and the operator regions by molecular cloning.

In the present study, the *xylDEGF* operon and the *xylS* gene were cloned onto pBR322 and pACYC177, respectively. Induction of the enzymes encoded by *xyl* genes *D*, *E*, *G*, and *F* occurred only when the cells of *E. coli* carried both the *xylDEGF* operon and the *xylS* gene. Analysis of the induction of the enzymes in these cells has demonstrated that the *xylS* gene codes for a positive regulator which is activated specifically by the inducer.

MATERIALS AND METHODS

Bacterial strain and plasmids. The bacterial strain used was *E. coli* 20SO (*thi lac mal mtl ara xyl rpsL*) (1), a derivative of *E. coli* K-12. The plasmids

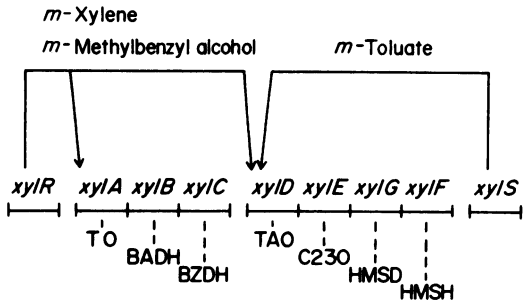

FIG. 1. *Model for the regulation of early enzymes of the TOL-encoded degradative pathway. The product of the xylR gene combines with m-xylene or m-methylbenzyl alcohol and induces both xylABC and xylDEGF. The product of the xylS gene combines with m-toluate and induces only xylDEGF. Enzyme abbreviations: TO, toluene oxygenase; BADH, benzyl alcohol dehydrogenase; BZDH, benzaldehyde dehydrogenase; TAO, toluate oxygenase; C23O, catechol 2,3-oxygenase; HMSD, 2-hydroxymuconic semialdehyde dehydrogenase; HMSH, 2-hydroxymuconic semialdehyde hydrolase. The locations of xylR, xylA, and xylC are tentative.*

used were pBR322 (3), pACYC184 (5), pACYC177 (5), and pTN2 (12).

Media and culture conditions. The medium used throughout the experiment was L broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl). Agar was added to 1.5% in the L broth medium to prepare L agar plates. Antibiotic concentrations used for selection of transformants were 100 or 25 μg of ampicillin, 10 μg of tetracycline, 10 μg of chloramphenicol, and 25 μg of kanamycin per ml. Benzoate plates contained 5 mM benzoate in L agar. Agar plates were incubated at 37°C for selection of transformants and at 30°C for detection of toluate oxygenase.

Enzyme assays. Cells were grown overnight in L broth containing ampicillin (25 μg/ml), chloramphenicol (5 μg/ml) or ampicillin (25 μg/ml), and kanamycin (25 μg/ml) at 30 or 37°C. Induction of enzymes was achieved as described previously (13). Inducers were added to the growth medium at 5 mM, except *m*-xylene which was added in vapor. Preparation procedures and assay conditions of catechol 2,3-oxygenase, 2-hydroxymuconic semialdehyde dehydrogenase, 2-hydroxymuconic semialdehyde hydrolase, and β-lactamase were described previously (13, 14). One milliunit corresponds to formation of 1 nmol of the product or the degradation of 1 nmol of the substrate per min at 27°C.

Detection of toluate oxygenase and catechol 2,3-oxygenase. Since the toluate oxygenase levels in *E. coli* cells were too low to be assayed by the polarographic method (13), the enzyme in the cells was detected by the color of the colonies on benzoate plates. When the cells had the induced toluate oxygenase and catechol 2,3-oxygenase, the colonies were yellow due to 2-hydroxymuconic semialdehyde produced from benzoate via catechol. Catechol 2,3-oxygenase in cells was indicated also by a yellow color after spraying a solution of 0.1 M catechol on the colonies.

Purification of plasmid DNA. Isolation of covalently closed circular plasmid DNA has been described previously (13). Crude plasmid DNA was prepared by alkaline extraction methods (2).

Restriction enzyme analysis and gel electrophoresis. Restriction endonucleases *Xho*I and *Kpn*I were products of Bethesda Research Laboratories, Bethesda, Md. *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I were purchased from Takara Shuzo Co. Ltd., Kyoto, Japan. Digestion with restriction endonucleases was carried out under the conditions described previously (13) or according to the instructions of the manufacturers of the enzymes. Analysis of restriction fragments was made on vertical 1.2% agarose or 5% polyacrylamide slab gels in Tris-borate buffer. Purification of digested DNA fragments by electrophoretic elution was described previously (13).

Ligation and transformation. Ligation and transformation were carried out as described previously (11). Transformants were selected on L agar containing antibiotics.

RESULTS

Molecular cloning of the *xylDEGF* operon. The physical and functional maps of pTN2 and hybrid plasmids constructed in this study are summarized in Fig. 2. We previously described the locations of *xylD*, the structural gene of toluate oxygenase, and *xylE*, the structural gene of catechol 2,3-oxygenase, on fragments BE and BD of pTN2, respectively (13). The *xylE* gene was mapped more precisely in the 1.8-kb region shared by fragments BD and XI and was shown to be transcribed from left to right on the pTN2 map (11). These results suggested that the operator-promoter region of the proposed *xylDEGF* operon is located on the left of *xylD*.

Since the *xylE* product in cells was easily detected as described above, we attempted to clone *xylD* and *xylE* together with the complete operator-promoter region of the *xylDEGF* operon. The *Hin*dIII fragment HA in the middle of the TOL segment appeared to be large enough to cover the above region. Thus pTN2 DNA was digested with *Hin*dIII, ligated to the *Hin*dIII site of pACYC184, and used for transformation. Chloramphenicol-resistant transformants were selected, and a solution of catechol was sprayed to detect catechol 2,3-oxygenase-positive transformants. All transformants producing the enzyme were tetracycline sensitive. The plasmid pTS66 in these transformants contained the HA fragment. Deletion derivatives of pTS66 were isolated by partial digestion with *Bam*HI and ligation. Chloramphenicol-resistant and catechol 2,3-oxygenase-positive transformants were selected. Plasmid DNAs were analyzed by cleavage with *Bam*HI or *Eco*RI. Thus, pTS69 con-

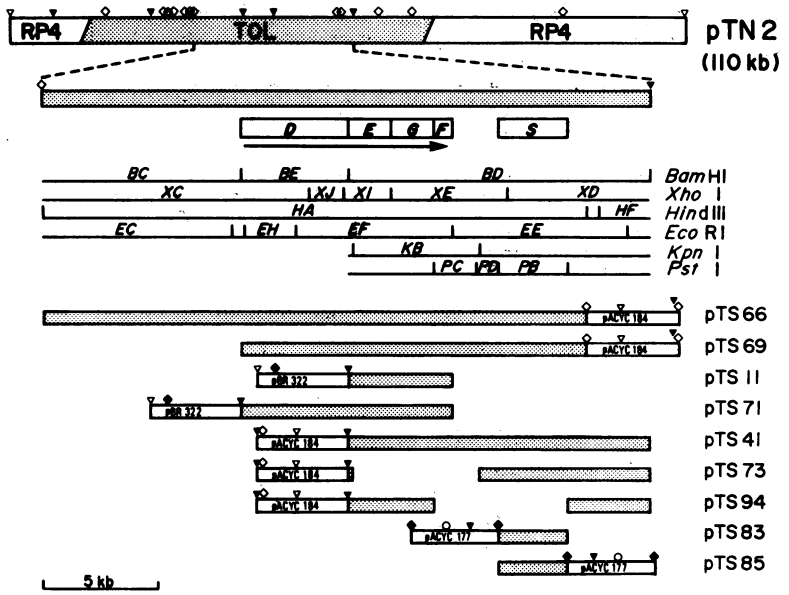

FIG. 2. *Physical and functional map of the TOL segment around the xylDEGF operon. The positions of genes of toluate oxygenase (xylD), catechol 2,3-oxygenase (xylE), 2-hydroxymuconic semialdehyde dehydrogenase (xylG), 2-hydroxymuconic semialdehyde hydrolase (xylF) and the regulatory gene (xylS) are indicated as D, E, G, F, and S, respectively, in open boxes. The arrow indicates the direction of transcription (11, 13). For each restriction endonuclease indicated on the right, the cleavage sites are marked by a vertical line. The positions of the inserted TOL segments in derivatives of pACYC184, pBR322, and pACYC177 are shown in the lower part of the figure. Stippled boxes indicate the TOL segment, and open boxes indicate the region of vectors. Symbols for restriction sites are as follows: ▼, BamHI; ○, XhoI; ◇, HindIII; ▽, EcoRI; ◆, PstI.*

taining the *Bam*HI fragment BE and a part of BD was obtained and was found to carry the complete *xylDEGF* operon as well as the *xylS* gene, as described below.

In an attempt to clone the operon separately from *xylS*, the purified BE fragment containing *xylD* was ligated to the *Bam*HI-digested pTS11, which has a part of fragment BD on pBR322 and contains *xylE* (11). Plasmid DNAs of ampicillin-resistant and catechol 2,3-oxygenase-positive transformants were analyzed by cleavage with *Bam*HI or *Xho*I. Thus, pTS71 was isolated in which the fragments BE and a part of BD were arranged in the same orientation as in pTN2. It was found that pTS71 carried *xylD*, *xylE*, *xylG* and *xylF*, but not *xylS*, as shown below.

Restriction endonuclease cleavage map of the BD fragment. Since the above results indicated the presence of not only *xylE* but also *xylG*, *xylF*, and *xylS* in fragment BD of pTN2, the fragment was analyzed in detail. The purified BD fragment was inserted into the *Bam*HI site of pACYC184 to construct pTS41, and the restriction endonuclease cleavage sites of *Kpn*I and *Pst*I were mapped. Digestion of pTS41 DNA with *Kpn*I gave only two fragments: 12.3 and 5.6 kb (KB). KB was cleaved into 4.0- and 1.6-kb fragments by *Xho*I and into 4.4- and 1.2-kb fragments by *Eco*RI. The vector plasmid, pACYC184, had no *Kpn*I site. Thus, the *Kpn*I sites in the BD fragment were mapped as shown in Fig. 2. Digestion of pTS41 DNA with *Pst*I gave four fragments: 11.8, 3.0 (PB), 1.9 (PC), and 0.8 kb (PD). By double digestion with *Pst*I and either *Bam*HI, *Eco*RI, *Xho*I, or *Kpn*I, the order of the *Pst*I fragments in BD was deduced to be PC, PD, and PB from left to right on the pTN2 map (Fig. 2).

Isolation of pTS41 derivatives. To map further the *xyl* genes in the BD fragment, deletion derivatives of pTS41 were isolated. pTS73 was made after the KB fragment was removed by digesting pTS41 DNA with *Kpn*I. The plasmid was found to contain *xylS*, as shown below. To locate *xylS* more precisely, pTS73 DNA was digested with *Pst*I and ligated to the *Pst*I site of pACYC177, and kanamycin-resistant and ampicillin-sensitive transformants were selected. Crude plasmid DNAs of the transformants were digested with *Pst*I or *Xho*I, and the size and the orientation of the inserted fragment were determined. Two kinds of hybrid plasmids containing the PB fragment in opposite orientations were isolated (pTS83 and pTS85). pTS94 was obtained by removing the *Pst*I fragments PB, PC,

and PD from pTS41. The plasmid was found to contain *xylE* and *xylG*, as shown below.

Locations of the *xylDEGF* operon and *xylS* on the pTN2 map. The noninduced and induced levels of TOL enzymes in crude extracts of *E. coli* carrying various hybrid plasmids are summarized in Table 1. Cells carrying pTS66 synthesized catechol 2,3-oxygenase, 2-hydroxymuconic semialdehyde dehydrogenase, and 2-hydroxymuconic semialdehyde hydrolase. In the presence of *m*-toluate, a specific inducer of the *xylDEGF* operon, the synthesized catechol 2,3-oxygenase level was more than 30 times the noninduced level. 2-Hydroxymuconic semialdehyde dehydrogenase and 2-hydroxymuconic semialdehyde hydrolase were also induced. In addition, toluate oxygenase was detected in these cells as judged by the yellow color of colonies on benzoate plates. *m*-Methylbenzyl alcohol and *m*-xylene, which are proposed to induce both *xylABC* and *xylDEGF* operons in the presence of *xylR*, did not cause induction of the enzymes. From these results, both the *xylDEGF* operon and *xylS* were found to be in HA. Deletion of a part of fragment BC did not affect the expression of the *xylDEGF* operon (pTS69). Deletion of a part of fragment EE did not cause a loss of the enzymes, but abolished the induction of the enzymes (pTS71). Double transformants of pTS71 and pTS73, however, showed the induction of the enzymes by *m*-toluate, although cells carrying pTS73 alone did not produce the enzymes at all (data not shown). Toluate oxygenase in cells carrying pTS71 alone was undetectable on benzoate plates, but the enzyme became detectable when pTS73 was introduced. Since the noninduced levels of the enzymes were essentially the same in cells carrying pTS71 alone and in those carrying pTS71 and pTS73 (Table 1), it was suggested that the *xylS* product is a positive regulator which has no repressor function. pTS83 and pTS85 contained fragment PB in opposite orientations on the vector. In cells carrying either pTS71 and pTS83 or pTS71 and pTS85, the enzymes were induced by *m*-toluate, and toluate oxygenase was detectable on benzoate plates. Therefore, *xylS* is located in the PB fragment (3.0 kb) (Fig. 2).

β-Lactamase encoded by the vector plasmid pBR322 was essentially constant in cells carry-

TABLE 1. *Activities of plasmid-encoded enzymes in E. coli 20SO carrying hybrid plasmids*[a]

Plasmid	Inducer	Sp act (mU/mg)			
		C23O	HMSD	HMSH	β-Lactamase
pTS66	None	10	0.1	<0.1	
	m-Xylene	2	<0.1	<0.1	
	m-Methylbenzyl alcohol	7	0.1	<0.1	
	m-Toluate	370	3.4	6.5	
pTS69	None	8	0.1	0.2	
	m-Xylene	6	0.1	0.3	
	m-Methylbenzyl alcohol	10	0.2	0.3	
	m-Toluate	520	4.1	1.5	
pTS71	None	21	0.1	0.3	730
	m-Xylene	4	0.1	0.2	430
	m-Methylbenzyl alcohol	30	0.1	0.4	920
	m-Toluate	25	0.1	0.4	920
pTS71 and pTS73	None	24	0.2	0.5	1,040
	m-Xylene	25	0.5	0.8	410
	m-Methylbenzyl alcohol	160	1.7	1.8	1,220
	m-Toluate	1,050	20	17	1,860
pTS71 and pTS83	None	15	0.1	0.2	730
	m-Xylene	15	<0.1	0.2	750
	m-Methylbenzyl alcohol	19	<0.1	0.2	960
	m-Toluate	330	2.2	1.6	1,180
pTS71 and pTS85	None	11	<0.1	0.1	890
	m-Xylene	10	<0.1	0.2	950
	m-Methylbenzyl alcohol	12	<0.1	0.2	1,050
	m-Toluate	320	2.6	4.4	1,370

[a] Cells were grown in L broth at 30°C. Enzyme abbreviations are given in the legend to Fig. 1.

ing pTS71 singly or together with another plasmid in the absence of *m*-toluate. In the presence of the inducer, however, the synthesis of the enzyme was slightly enhanced in cells bearing the *xylS* gene.

Various substrates of the TOL pathway enzymes were tested for their inducer activity in cells carrying pTS71 and pTS85 (Table 2). *m*-Toluate and benzoate induced the enzymes. Other substrates were inactive, except *m*-tolualdehyde, which appeared to be slightly active. These results were consistent with the model shown in Fig. 1.

In each double transformant of the above experiments, copy numbers of the plasmids derived from both pACYC184 (pTS73) and pACYC177 (pTS83 and pTS85) were approximately one-third of that derived from pBR322 (pTS71) as judged by plasmid DNAs stained by ethidium bromide on agarose gel. However, the copy numbers of each plasmid did not change in the absence or presence of an inducer.

Locations of *xylG* and *xylF* on the pTN2 map. From the results described above, the genes *xylE*, *xylG*, and *xylF* were located in the 4.6-kb region of pTN2 extending from the left end of BD to the right end of EF. Cells carrying pTS41 synthesized catechol 2,3-oxygenase, 2-hydroxymuconic semialdehyde dehydrogenase, and 2-hydroxymuconic semialdehyde hydrolase, whereas those carrying pTS94 which lost PC, PD, and PB synthesized the dehydrogenase but not the hydrolase (Table 3). Therefore, the *xylG* gene was located in the region next to the *xylE* gene and extended to the left end of PC. It was concluded that the order of the genes in the operon is *xylD*, *xylE*, *xylG*, and *xylF* from left to right on the pTN2 map, and the locations of these genes are as shown in Fig. 2.

TABLE 2. *Inducer activity of substrates in E. coli 20SO carrying pTS71 and pTS85*[a]

Inducer	Sp act (mU/mg)			
	C23O	HMSD	HMSH	β-Lactamase
None	11	<0.1	0.1	890
m-Xylene	10	<0.1	0.2	950
m-Methylbenzyl alcohol	12	<0.1	0.2	1,050
Benzyl alcohol	13	<0.1	0.2	920
m-Tolualdehyde	26	0.2	0.3	1,010
Benzaldehyde	20	<0.1	0.2	950
m-Toluate	320	2.6	4.4	1,370
Benzoate	50	0.4	0.3	1,030

[a] Cells were grown in L broth at 30°C. Enzyme abbreviations are given in the legend to Fig. 1.

TABLE 3. *Activities of plasmid-encoded enzymes in E. coli 20SO carrying pTS41 or pTS94*[a]

Plasmid	Sp act (mU/mg)		
	C23O	HMSD	HMSH
pTS41	35.3	0.17	0.16
pTS94	50.3	0.18	<0.02

[a] Cells were grown in L broth at 37°C. Enzyme abbreviations are given in the legend to Fig. 1.

DISCUSSION

In the present study we have described the molecular cloning of the *xylDEGF* operon and the *xylS* gene in *E. coli* and direct evidence for the positive control of the operon by *xylS*. The *xylDEGF* operon was cloned on pBR322, and the *xylS* gene was cloned on pACYC177 which is compatible with pBR322. The enzymes encoded by the operon were inducible when *xylS* was provided either in *cis* or *trans*. In addition, the order of genes in the operon was determined to be *xylD*, *xylE*, *xylG*, and *xylF*. We previously reported that a recombinant of TOL and RP4 (pTN2) specifies inducible synthesis of TOL enzymes in *E. coli* (11). We also reported the molecular cloning of *xylE* in *E. coli* and showed that catechol 2,3-oxygenase encoded by the cloned fragment was not inducible. The operator-promoter region of the *xylDEGF* operon appeared to be absent in the hybrid plasmid, and *xylE* was expressed from the vector promoter.

Cells carrying pTS66 or pTS69 synthesized toluate oxygenase, catechol 2,3-oxygenase, 2-hydroxymuconic semialdehyde dehydrogenase, and 2-hydroxymuconic semialdehyde hydrolase, which were induced by *m*-toluate but not by *m*-xylene or *m*-methylbenzyl alcohol. On the other hand, the enzymes were not inducible in cells carrying pTS71. Therefore, TOL fragments in pTS66 and pTS69 contained both *xylDEGF* operon and *xylS*, whereas *xylS* was missing in pTS71. The enzymes were induced by *m*-toluate in cells carrying pTS71 and either pTS73, pTS83 or pTS85. From these findings *xylS* was mapped on fragment PB. The noninduced level of catechol 2,3-oxygenase in cells carrying pTS71 alone was not affected markedly by the presence of plasmids containing *xylS*. These results indicate that the *xylS* product is a positive regulator and appears to have no repressor function. The concept of positive control of gene expression mainly evolved from the work of Englesberg and Wilcox on the L-arabinose system in *E. coli* (8). In the presence of L-arabinose (the inducer) the product of gene *araC* stimulates the transcription of the *araBAD* operon, whereas it acts as a repressor without the inducer (15). In contrast to the L-arabinose system, the maltose system in *E. coli* is regulated by the product of gene *malT* in

a strictly positive manner (6). The product of gene *xylS* appears to be functionally similar to that of gene *malT*.

The results of inducer specificity on the cells carrying pTS71 and pTS85 revealed that carboxylic acids act as the inducer, and the product of gene *xylS* has the characteristics expected from the model in Fig. 1. *m*-Tolualdehyde showed a slight but significant inducer activity, in accord with the results on induction of TOL enzymes with an *xylR* mutant (16). It is not clear whether *m*-tolualdehyde per se has an inducer function, since the possibility could not be excluded that *xylC* coding for benzaldehyde dehydrogenase is contained in pTS71, which converts the aldehyde to the carboxylic acid.

The direction of transcription of *xylS* is not known at present. Both pTS83 and pTS85, which have the PB fragment inserted into the *Pst*I site within the β-lactamase gene of the vector in opposite orientations, activated the *xylDEGF* operon of pTS71 to a similar extent (Table 1). These results would indicate that the PB fragment carries *xylS* with the intact promoter, and a read-through transcription from the vector promoter is not necessary for *xylS* expression.

We have previously reported that expression of *xyl* genes in *E. coli* is less than 5% of that in *P. putida* (11, 13). Such low expression of *Pseudomonas* genes in *E. coli* was reported also on salicylate degradative genes of the SAL plasmid (4) and *trp* genes of *Pseudomonas aeruginosa* (10). Nevertheless, molecular cloning of *Pseudomonas* genes in *E. coli* as used in this study is a useful technique to elucidate the molecular basis of the control of their expression.

ACKNOWLEDGMENTS

We are indebted to S. N. Cohen and K. Matsubara for providing pACYC177 and pACYC184.

This work was supported by a Grant-in-Aid G 457126 for Scientific Research from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K 12. Bacteriol. Rev. **36**:525–557.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
4. **Chakrabarty, A. M., D. A. Friello, and L. H. Bopp.** 1978. Transposition of plasmid DNA segments specifying hydrocarbon degradation and their expression in various microorganisms. Proc. Natl. Acad. Sci. U.S.A. **75**:3109–3112.
5. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
6. **Debarbouille, M., H. A. Shuman, T. J. Silhavy, and M. Schwarty.** 1978. Dominant constitutive mutation in *Escherichia coli*. J. Mol. Biol. **124**:359–371.
7. **Downing, R. G., and P. Broda.** 1979. A cleavage map of the TOL plasmid of *Pseudomonas putida* mt-2. Mol. Gen. Genet. **177**:189–191.
8. **Englesberg, E., and G. Wilcox.** 1974. Regulation: positive control. Annu. Rev. Genet. **8**:219–242.
9. **Franklin, F. C. H., and P. A. Williams.** 1980. Construction of a partial diploid for the degradative pathway encoded by the TOL plasmid (pWWO) from *Pseudomonas putida* mt-2: evidence for the positive nature of the regulation of *xylR* gene. Mol. Gen. Genet. **177**:321–328.
10. **Hedges, R. W., A. E. Jacob, and I. P. Crawford.** 1977. Wide ranging plasmid bearing the *Pseudomonas aeruginosa* tryptophan synthase genes. Nature (London) **267**:283–284.
11. **Inouye, S., A. Nakazawa, and T. Nakazawa.** 1981. Molecular cloning of TOL genes *xylB* and *xylE* in *Escherichia coli*. J. Bacteriol. **145**:1137–1143.
12. **Nakazawa, T., E. Hayashi, T. Yokota, Y. Ebina, and A. Nakazawa.** 1978. Isolation of TOL and RP4 recombinant by integrative suppression. J. Bacteriol. **134**: 270–277.
13. **Nakazawa, T., S. Inouye, and A. Nakazawa.** 1980. Physical and functinal mapping of RP4-TOL plasmid recombinants: analysis of insertion and deletion mutants. J. Bacteriol. **144**:222–231.
14. **Nakazawa, T., and T. Yokota.** 1973. Benzoate metabolism in *Pseudomonas putida* (*arvilla*) mt-2: demonstration of two benzoate pathways. J. Bacteriol. **115**:262–267.
15. **Ogden, S., D. Haggerty, C. M. Stoner, D. Kolodrubets, and R. Schleif.** 1980. The *Escherichia coli* L-arabinose operon: binding sites of the regulatory proteins and a mechanism of positive and negative regulation. Proc. Natl. Acad. Sci. U.S.A. **77**:3346–3350.
16. **Worsey, M. J., F. C. H. Franklin, and P. A. Williams.** 1978. Regulation of the degradative pathway enzymes coded by the TOL plasmid (pWWO) from *Pseudomonas putida* mt-2. J. Bacteriol. **134**:757–764.
17. **Worsey, M. J., and P. A. Williams.** 1975. Metabolism of toluene and xylenes by *Pseudomonas putida* (*arvilla*) mt-2: evidence for a new function of the TOL plasmid. J. Bacteriol. **124**:7–13.