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Cosmid Cloning of Five *Zymomonas trp* Genes by Complementation of *Escherichia coli* and *Pseudomonas putida trp* Mutants†

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A library of *Zymomonas mobilis* genomic DNA was constructed in the broad-host-range cosmid pLAFR1. The library was mobilized into a variety of *Escherichia coli* and *Pseudomonas putida trp* mutants by using the helper plasmid pRK2013. Five *Z. mobilis trp* genes were identified by the ability to complement the *trp* mutants. The *trpF*, *trpB*, and *trpA* genes were on one cosmid, while the *trpD* and *trpC* genes were on two separate cosmids. The organization of the *Z. mobilis trp* genes seems to be similar to the organization found in *Rhizobium* spp., *Acinetobacter calcoaceticus*, and *Pseudomonas acidovorans*. The *trpF*, *trpB*, and *trpA* genes appeared to be linked, but they were not closely associated with *trpD* or *trpC* genes.

Zymomonas mobilis is a gram-negative bacterium with good potential for industrial fermentation of ethanol, but it is also interesting for its unusual biology (25, 35, 37). It is obligatorily fermentative, using only an Entner-Doudoroff pathway leading to the production of ethanol and CO_2. The catabolism of carbohydrates appears to be limited, since only glucose, fructose, or sucrose will support growth. However, *Z. mobilis* is able to synthesize its own amino acids and nucleotides (37).

Genetic analysis of *Z. mobilis* has been limited by inefficient methods of gene transfer (4, 25, 35, 37). Conjugation with broad-host-range plasmids is possible (4, 25, 35), but the plasmids are often unstable in *Z. mobilis* (25). The *Z. mobilis* chromosome can be mobilized by using the broad-host-range plasmid R68.45 (27, 35), but genetic mapping has not been reported. Since classical genetic mapping techniques are limited, it may be necessary to study the organization of *Z. mobilis* genes by molecular cloning and physical mapping. A few of the genes necessary for glycolysis and ethanol production have been cloned and sequenced (3, 7–9). It appears that glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase are encoded in an operon in *Z. mobilis* (7, 8).

As an initial step in the analysis of how biosynthetic genes are organized and expressed in *Z. mobilis*, genes involved in tryptophan biosynthesis have been cloned. The organization of genes involved in tryptophan biosynthesis has been investigated in a variety of organisms. The reactions required to synthesize tryptophan from chorismic acid are the same in all organisms investigated (10, 11). However, the number, organization, and regulation of the genes vary considerably (10, 11). For example, in enteric bacteria there are five or six genes arranged in an operon (10, 41). In other gram-negative bacteria, such as *Pseudomonas putida* (17), *Pseudomonas aeruginosa* (10, 18), *Acinetobacter calcoaceticus* (32), *Rhizobium* spp. (21), *Pseudomonas acidovorans* (5), and *Caulobacter crescentus* (39), there are seven genes, some of which are clustered or in an operon but unlinked to other *trp* genes. In many organisms there are three separate linkage groups encoding one to four of the *trp* genes.

In this study, five *Z. mobilis trp* genes were cloned by complementation of *E. coli* and *P. putida trp* mutants. The *trpD* and *trpC* genes were on two different cosmids, while *trpF*, *trpB*, and *trpA* were on a third cosmid.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Strains and plasmids used in this study are listed in Table 1. *Z. mobilis* was grown at 30°C without shaking in rich medium containing (per liter) 10 g of yeast extract, 20 g of glucose, and 2 g of KH_2PO_4. *E. coli* and *P. putida* were grown with shaking in LB (24) or Vogel Bonner medium (38) at 37 and 30°C, respectively. Streptomycin (100 μg/ml) and tetracycline (20 μg/ml for *E. coli* and 5 μg/ml for *P. putida*) were added to media for selection. When necessary, 20 μg of tryptophan per ml and 0.5% methionine were added to minimal media.

Isolation of chromosomal DNA. *Z. mobilis* and *E. coli* genomic DNA was isolated by a modification of a method described by Sato and Miura (31). A 1-g (wet weight) sample of cells was suspended in 5 ml of buffer containing 0.1 M EDTA and 0.15 M NaCl. Lysozyme was added to a final concentration of 12 mg/ml for *Z. mobilis* or 2 mg/ml for *E. coli*. Cells were incubated for 30 min at 37°C. *Z. mobilis* cells were subjected to two freeze-thaw cycles in a dry ice–ethanol bath. A 25-ml solution containing 1% sodium dodecyl sulfate and 0.1 M Tris (pH 9.0) was added, and cells were subjected to additional freeze-thaw cycles until lysis occurred. *E. coli* cells were lysed as previously described (33).

The lysed cells were mixed with an equal volume of phenol and were shaken gently for 15 min at 4°C. The phenol and aqueous phases were separated by centrifugation for 5 min at 3,000 × *g* in a Sorvall SS34 rotor. After additional extractions with phenol and phenol-chloroform, the DNA was precipitated with 2 volumes of cold ethanol. High-molecular-weight DNA was recovered by spooling onto a glass rod. DNA was rinsed in 70% ethanol and dissolved in TE-8 (10 mM Tris [pH 8.0], 1 mM EDTA). The DNA was treated with 100 μg of RNase per ml at 37°C for 30 min and extracted several times with equal volumes of phenol and phenol-chloroform. Ammonium acetate (5 M) was added to a final concentration of 2.5 M, and DNA was precipitated by adding 2 volumes of isopropanol.

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† Dedicated to the memory of O. H. Smith (deceased 12 June 1985).

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Source or reference
Zymomonas mobilis 29192	Wild type	ATCC[a]
Escherichia coli		
W3110	Wild type	
T-3	*trpE3*	Yanofsky collection
T-58	*trpD2*	Yanofsky collection
T-80	*trpD3*	Yanofsky collection
T1073	*trpC2*	36
T1028	*trpC5*	36
T1227	*trpC9*	36
T1153	*trpC8*	36
T-41	*trpB4*	Yanofsky collection
WRT-4	*trpB8*	Yanofsky collection
T-8	*trpA2*	Yanofsky collection
K12, *trp*	*trpA23*	Yanofsky collection
HB101	*recA str hsdR hsdM pro leu*	2
TC4	*recA hsdR pro thi*	7
BH2688	In vitro packaging strain	19
BH2690	In vitro packaging strain	19
Pseudomonas putida		
2	*trpE602*	17
22	*met-601 trpD611*	17
19	*trpF221*	17
18	*trpC615*	17
20	*trpC31*	17
21	*trpA655*	17
6	*trpB661*	17
Plasmids		
PLAFR1	Tet^r *mob cos*	15
pRK2013	Km^r *mob tra*	14
pC2	pLAFR1 *trpC*	This study
pC5	pLAFR1 *trpC*	This study
pD3	pLAFR1 *trpD*	This study
pD4	pLAFR1 *trpD*	This study
pFBA9	pLAFR1 *trpF trpB trpA*	This study
pF20	pLAFR1 *trpF*	This study
pA1	pLAFR1 *trpA*	This study
pF17	17-kb *Eco*RI fragment from pFBA9 in pLAFR1	This study
pF5.8	5.8-kb *Eco*RI fragment from pFBA9 in pLAFR1	This study

[a] ATCC, American Type Culture Collection.

Preparation of plasmid DNA. Plasmids were isolated from *E. coli* by using the alkaline lysis method (23).

Restriction enzymes and ligase. Restriction enzymes, purchased from International Biochemicals Inc., Bethesda Research Laboratories, or Pharmacia, and T4 DNA ligase, purchased from Bethesda Research Laboratories, were used according to the instructions of the manufacturer.

Lambda in vitro packaging. Packaging extracts were prepared as described by Rodriguez and Tait (29) by using *E. coli* BH2688 and BH2690. Aliquots (25 μl) were stored at −70°C in 1.5-ml Eppendorf tubes. In vitro packaging was performed as described by Rodriguez and Tait (29) with the following modifications. After the packaging extract and DNA were incubated for 30 min at 37°C, a second packaging extract containing 5 μg of DNase I and 2.5 μl of 0.5 M $MgCl_2$ was added. The reaction mixture was incubated for an additional 30 min at 37°C and stopped by the addition of 0.5 ml of SM buffer (100 mM NaCl, 5 mM $MgSO_4$, 50 mM Tris hydrochloride [pH 7.5], 0.01% gelatin) and 2 drops of chloroform. The extract was mixed gently and centrifuged for 30 s in a microfuge. The supernatant was stored at 4°C. Alternatively, Gigapack Plus packaging extracts from Stratagene Cloning Systems were used according to the instructions of the manufacturer.

Construction of a cosmid library. *Z. mobilis* total genomic DNA was partially digested with *Eco*RI and electrophoresed through a 0.4% low-melting-point agarose gel. Fragments of 20 to 30 kilobases (kb) in length were isolated from the agarose gel (23). The purified genomic DNA was mixed with pLAFR1, previously cut with *Eco*RI, at a ratio of 5 to 1. T4 DNA ligase was added, and the mixture was incubated at 4°C for 48 h.

The ligation mix was packaged in vitro and used to transfect *E. coli* HB101. Aliquots of the transfected cells were plated on LB plates containing tetracycline to determine the number of cells that received a cosmid. The rest of the transfected cells were grown overnight in 5 ml of liquid LB with tetracycline to enrich for cells containing cosmids. Permanent cultures were made by mixing 0.8 ml of the enriched cultures with 0.2 ml of 80% glycerol in glass vials and freezing in a dry-ice–ethanol bath. The library was stored at −70°C.

Identification of *Z. mobilis trp* genes by conjugation. Cosmids were transferred from HB101 to the *E. coli* and *P.*

putida trp mutants by triparental mating using pRK2013 as a helper plasmid. The donor, helper, and recipient strains were grown overnight in LB. For crosses into the *E. coli* mutants, 0.1-ml samples of each of the three overnight cultures were mixed on an LB plate, which was incubated for 4 h at 37°C. To transfer cosmids into *P. putida trp* mutants, 0.15 ml of the *E. coli* donor and helper strains were mixed with 0.05 ml of the *P. putida* mutant in a sterile Eppendorf tube. The cells were collected by centrifugation. All but about 50 μl of the supernatant was removed. The cells were resuspended in the remaining broth and spread onto a nitrocellulose filter (25-mm diameter, 45-μm pore size) previously placed on an LB plate.

All crosses were suspended and washed twice in 5 ml of saline. Dilutions of these suspended crosses were plated for single colonies on the following media: LB with tetracycline and streptomycin to determine the number of donors; minimal medium with tryptophan to determine the number of recipients; minimal medium with tetracycline to select for complemented colonies; and minimal medium with tetracycline and tryptophan to determine the titers of the transconjugants. Each time a gene bank or an individual cosmid was crossed into an *E. coli* or *P. putida trp* mutant, pLAFR1 alone was transferred into that mutant in a separate cross to test for reversion of the mutant.

Three to ten potentially complemented colonies were picked from selection plates and grown overnight in 5 ml of LB broth with tetracycline. Plasmids isolated from these cultures were used to transform *E. coli* HB101 or TC4. The cosmid was conjugated from strain HB101 or TC4 back to the *trp* mutant from which it was isolated to confirm that the cosmid contained a *trp* gene.

Southern hybridization. Restriction digests of cosmids and total genomic DNA were run on 0.7% agarose gels and blotted to nitrocellulose (23). About 3 μg of total genomic DNA or 300 to 600 ng of isolated cosmid DNA was used per lane. Isolated cosmid DNA was labeled with [α-^{32}P]CTP from New England Nuclear Corp. by using a nick translation kit from Amersham Corp. Approximately 800 ng of labeled DNA with a specific activity of 1×10^7 to 4×10^7 cpm/μg of DNA was used to probe each blot (12 by 16 cm) containing up to 16 lanes of DNA. The hybridizations were carried out at 65°C for 18 to 24 h. Blots were washed once for 5 min in 2× SSC (23; 1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate at room temperature followed by three 15-min washes at 65°C in 0.1× SSC and 0.1% sodium dodecyl sulfate. Autoradiographs were prepared by exposing X-Omat AR5 X-ray film from Kodak for 2 to 24 h at −70°C.

RESULTS

Construction of a cosmid library. A cosmid library of *Z. mobilis* genomic DNA was constructed in the broad-host-range vector pLAFR1. Over 2,700 independent cosmids were transferred into *E. coli* HB101 in six separate experiments. Twenty randomly chosen cosmids were isolated, digested with *Eco*RI, and analyzed on 0.7% agarose gels. An average of 22 kb of *Z. mobilis* DNA was inserted into the *Eco*RI site of 80% of the cosmids. A total of 2,000 cosmids with 22-kb inserts would give a greater than 99.9% probability that any particular *Z. mobilis* gene had been cloned (6).

Isolation and characterization of cosmids containing *trp* genes. The cosmid library of *Z. mobilis* genomic DNA was conjugated en masse into *E. coli trpE*, *trpD*, *trpC*, *trpF*, *trpB*, and *trpA* mutants and into *P. putida trpE* and *trpD*

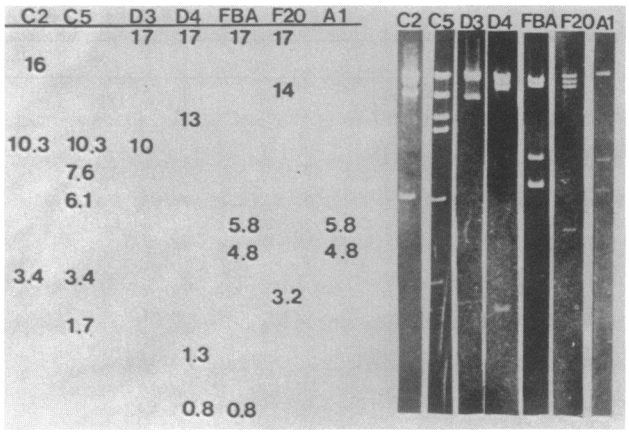

FIG. 1. *Eco*RI fragments of *Z. mobilis* DNA inserted in six isolated cosmids. Data on the left indicate the sizes of the *Eco*RI fragments in kilobases. On the right are strips from ethidium bromide-stained agarose gels of *Eco*RI digests of the cosmids. The top band in each lane is the 21.6-kb vector pLAFR1.

mutants. The Tet^r marker of the cosmid was transferred from the library to each of the *E. coli* mutants at a frequency of $\sim 10^{-4}$ per recipient and to the *P. putida* mutants at about 10^{-6}. Tet^r Trp$^+$ transconjugants of *E. coli trpD*, *trpC*, *trpF*, and *trpA* mutants and *P. putida trpD* mutants arose at 10^3-fold-lower frequencies. The gene bank did not complement *trpB* or *trpE* mutants. Transfer of pLAFR1 to the mutants resulted in Tet^r at frequencies of 10^{-4} for *E. coli* and 10^{-6} for *P. putida*, while Tet^r Trp$^+$ transconjugants appeared at frequencies of less than 10^{-10} in all control crosses in which the vector alone was transferred.

Several cosmids that complemented *E. coli trpC*, *trpF*, and *trpA* mutants and a *P. putida trpD* mutant were chosen for further study (Table 1 and Fig. 1). Cosmids pC2 and pC5 complemented only *trpC* mutants, and pD3 and pD4 complemented only *trpD* mutants. Cosmid pFBA9 complemented *trpF*, *trpB*, and *trpA* mutants, whereas pF20 and pA1 could complement only *trpF* or *trpA* mutants, respectively. None of the isolated cosmids complemented the *E. coli* or *P. putida trpE* mutants.

The ability of the *Z. mobilis trp* genes to complement the *E. coli* and *P. putida trp* mutants varied. Growth of *E. coli* and *P. putida trpF* mutants containing either pFBA9 or pF20 was accelerated only slightly by the addition of tryptophan to minimal-medium plates. At the other extreme, complemented *trpC*, *trpB*, and *trpA* mutants appeared 3 to 5 days later on minimal-medium plates than on minimal-medium plates with tryptophan.

Molecular analysis of isolated cosmids. Restriction digests (Fig. 1) and cross-hybridization (data not shown) of pC2 and pC5 indicated that they shared 10.3- and 3.4-kb *Eco*RI fragments. Both cosmids hybridized to the same restriction fragments in digests of *Z. mobilis* chromosomal DNA as were found in the isolated cosmids (Fig. 2). An *Eco*RI-*Hin*dIII restriction map of the 13.7-kb region likely to contain the *Z. mobilis trpC* gene is shown in Fig. 3A.

Cosmids that complemented *trpD* mutants shared a 17-kb *Eco*RI fragment (Fig. 1 and 3B). The 17-kb *Eco*RI fragments from the cosmids cross-hybridized (data not shown). This *Eco*RI fragment was not cut by digestion with *Hin*dIII but was cleaved into five fragments by *Bam*HI digestion. These are the fragments common to the *Eco*RI-*Bam*HI digestions of pD3 and pD4 in Fig. 2. Both cosmids hybridized to the

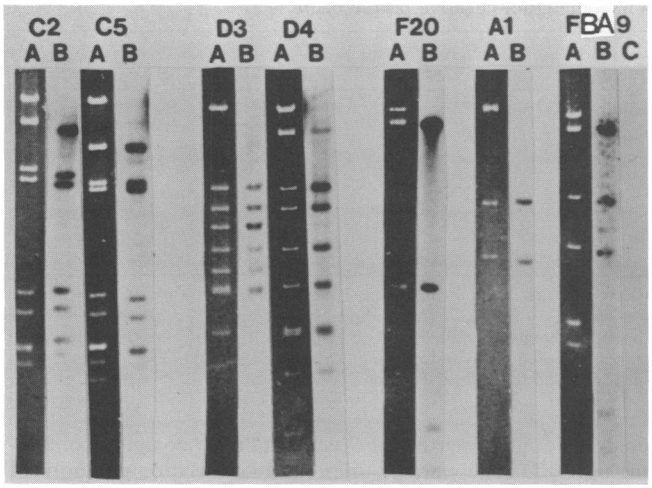

FIG. 2. Restriction digests and hybridization of cosmids to chromosomal DNA. Lanes labeled A are ethidium bromide stains of *Eco*RI-*Hin*dIII double digests of pC2, pC5, pF20, pA1, and pFBA9 and *Eco*RI-*Bam*HI double digests of pD3 and pD4. The top band in each lane is the vector. Lanes labeled B are autoradiographs of *Eco*RI-*Hin*dIII double digests of *Z. mobilis* genomic DNA probed with pC2, pC5, pF20, pA1, or pFBA9 or *Eco*RI-*Bam*HI double digests of *Z. mobilis* genomic DNA probed with pD3 or pD4. Lane C from pFBA9 is an autoradiograph of a *Hin*dIII digest of *E. coli* W3110 DNA probed with pFBA9.

same-size restriction fragments in digests of chromosomal DNA as observed in digests of the cosmids (Fig. 2).

*Eco*RI restriction digests (Fig. 1) and cross-hybridization (data not shown) indicated that pFBA9 shared a 17-kb *Eco*RI fragment with pF20 and 5.8- and 4.8-kb *Eco*RI fragments with pA1. The 17- and 5.8-kb *Eco*RI fragments were subcloned from pFBA9 into pLAFR1 and conjugated into *E. coli trpF*, *trpB*, and *trpA* mutants. The 17-kb subclone complemented only *trpF* mutants. The 5.8-kb subclone complemented only *trpA* mutants. The *trpB* mutant was complemented by only intact pFBA9.

Cosmids pA1 and pF20 hybridized to the same restriction fragments in digests of *Z. mobilis* chromosomal DNA as were observed in digests of the cosmids (Fig. 2). However, hybridization of pFBA9 to chromosomal digests revealed that this cosmid had undergone at least some rearrangement

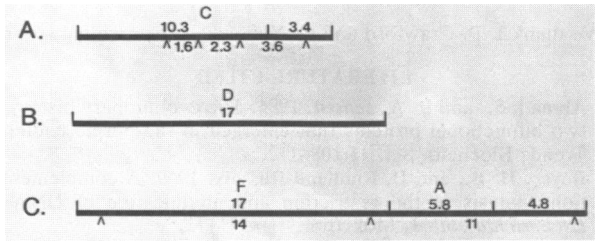

FIG. 3. *Eco*RI (|) and *Hin*dIII (^) restriction maps of regions of *Z. mobilis* DNA that complemented *trp* mutants from *E. coli* and *P. putida*. (A) 13.7-kb region common to pC2 and pC5. The *trpC* gene could be located anywhere within this entire 13.7-kb region. (B) 17-kb *Eco*RI fragment of pD3 and pD4. (C) Fragments likely to contain the *trpF* and *trpA* genes found in pF20, pA1, and pFBA9. The maps were constructed by analyzing partial and complete *Eco*RI and *Hin*dIII restriction digests of the isolated cosmids and of chromosomal DNA probed with the cosmids. The 17-kb fragment common to pD3 and pD4 was not cut by *Hin*dIII digestion.

during cloning. The 2.0- and 2.3-kb *Eco*RI-*Hin*dIII fragments of pFBA9 were not present as such in the *Z. mobilis* chromosome (Fig. 2). On the other hand, pFBA9 had homology to a 1.3-kb *Eco*RI-*Hin*dIII fragment in the chromosome that was not found in digests of the cosmid. The DNA rearrangement was not due to the presence of *E. coli* DNA (Fig. 2, FBA9, lane C). At this stringency, none of these cosmids hybridized to *E. coli* chromosomal DNA (data not shown).

Restriction digests and hybridizations indicated that the 17-kb *Eco*RI fragment containing the *trpF* gene was adjacent to the 5.8-kb *Eco*RI fragment containing the *trpA* gene (Fig. 3C). Cosmid pFBA9 contained an 11-kb *Hin*dIII fragment and hybridized to an 11-kb *Hin*dIII fragment in chromosomal DNA (Fig. 4, lanes 1 and 2). Cosmids pF20, pF17, and pA1 also hybridized to an 11-kb *Hin*dIII fragment in digests of *Z. mobilis* chromosomal DNA (Fig. 4, lanes 3 to 5). The 11-kb *Hin*dIII fragment in pFBA9 and in the chromosome was cut by *Eco*RI. The 4.8-kb *Eco*RI fragment was cut by *Hin*dIII (Fig. 2). The 17.0-, 5.8-, and 4.8-kb *Eco*RI fragments of pF20, pA1, and pFBA9 were arranged as shown in Fig. 3C.

DISCUSSION

The vector pLAFR1 can be transferred to and maintained in a variety of gram-negative bacteria, including *E. coli*, *P. putida*, and *Rhizobium leguminosarum* (15). However, attempts to transfer pLAFR1 or the cosmid library into *Z. mobilis* 29192 or into *trp* mutants derived from this strain were unsuccessful. Therefore, *Z. mobilis trp* genes were identified by complementation of *E. coli* and *P. putida trp* mutants. *E. coli* or *P. putida trp* mutants have been used to clone *trp* genes from a variety of organisms, including species of *Caulobacter* (39), *Agrobacterium* (24), *Leptospira* (42), *Brevibacterium* (13), *Saccharomyces* (26), and *Neurospora* (34).

All attempts to complement *E. coli* or *P. putida trpE* mutants were unsuccessful. The lack of complementation of these *trpE* mutants could have been caused by poor expression of the *Z. mobilis trpE* gene, lack of the *trpE* gene in the genomic library, or instability of the cosmids containing the *trpE* gene.

Cosmid pFBA9 contained some restriction fragments that were not present in the chromosome (Fig. 2). The aberrant restriction fragments may have arisen by duplication and/or deletion of normal sequences while pFBA9 was in the Rec^+ *E. coli trpF* mutant. Cosmid pFBA9 was kept in the *E. coli* mutant for over 1 week before it was isolated and transferred to HB101. Gross rearrangements of the other cosmids were avoided by removing them from the Rec^+ background as soon as complementation was recognized.

The *trpF* and *trpA* genes were clustered on adjacent *Eco*RI fragments (Fig. 2 and 3C). The *trpB* gene and its regulatory sequences may have spanned the *Eco*RI site separating the 17-kb *trpF* fragment from the 5.8-kb *trpA* fragment, or they may have been contained in sequences unique to pFBA9.

In most of the bacteria that have been investigated, the *trpF*, *trpB*, and *trpA* genes are closely linked (Table 2). The *Rhizobium meliloti trpF*, *trpB*, and *trpA* genes were present on an R-prime plasmid that contained a minimum of 5.8 kb of *Rhizobium* DNA (21). The *trp* genes of *P. acidovorans* were mapped by transduction (5). The *trp* genes of *Acinetobacter calcoaceticus* were mapped by transformation (32). Analysis of the DNA sequences of the *trpA* and *trpB* genes from *E. coli*, *Salmonella typhimurium*, *P. aeruginosa*, and *C. crescentus* indicate that these genes overlap or are separated by

TABLE 2. Organization of tryptophan genes in some gram-negative purple bacteria

Species	*trp* gene organization[a]	Superfamily[b]	References
Zymomonas mobilis	D C FBA*	C	This study
Rhizobium spp.	EG DC* FBA*	C	21
Caulobacter crescentus	EDC* FBA	C	39
Pseudomonas acidovorans	E GDC* FBA*	A	5
Acinetobacter calcoaceticus	E GDC FBA*	B	32
Pseudomonas putida	EGDC F BA	B	17
Escherichia coli	E(G)DC(F)BA[c]	B	41

[a] The gene order within linkage groups marked with an asterisk (*) is not known. Since the different organisms have been analyzed by different genetic techniques, linkage has different physical meanings in each organism.

[b] Superfamilies are subdivisions of the gram-negative purple bacteria as defined by rRNA homology (40). The A, B, and C designations are those described by Jensen (20). The placement of *Z. mobilis* (16) and *C. crescentus* (28) in superfamily C is based on hybridization analysis of rRNA and DNA.

[c] Parentheses indicate that the *trpG* gene is fused to the *trpD* gene and the *trpF* gene is fused to the *trpC* gene in *E. coli*.

a few base pairs (12, 18, 30). If *Z. mobilis* is like these other bacteria, then the *trpB* gene should be closely associated with the *trpA* gene.

The *Z. mobilis trpD* and *trpC* genes were not directly associated with the *trpF*, *trpB*, and *trpA* gene cluster. Restriction digests and Southern hybridizations indicated that a 4.8-kb *Eco*RI fragment flanked the 5.8-kb *Eco*RI (*trpA*) fragment on the right as shown in Fig. 3C. Cosmid pF20 contained 14- and 3.2-kb *Eco*RI fragments (Fig. 1) that flanked the 17-kb *trpF* fragment on the left as it is shown in Fig. 3C. Therefore, the *trpD* and *trpC* genes must be a minimum of 4.8 kb away from the *trpA* gene and 17 kb away from the *trpF* gene.

The minimal distance separating the *trpC* and *trpD* genes from each other has not been determined. Although two cosmids containing the *trpC* gene and two cosmids containing the *trpD* gene were examined, nonoverlapping regions of these cosmids were not mapped. The possibility that the *Eco*RI fragments containing the *trpD* and *trpC* genes are adjacent has not been ruled out. However, it seems unlikely that these genes are closely linked, since cosmids containing both the *trpC* and the *trpD* genes were not identified.

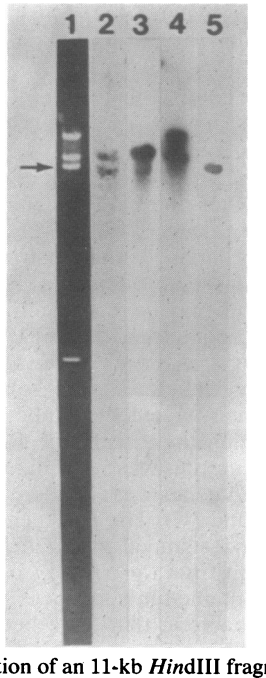

FIG. 4. Identification of an 11-kb *Hin*dIII fragment that overlaps the internal *Eco*RI sites of pFBA9 shown in Fig. 3C. Lanes: 1, ethidium bromide stain of a *Hin*dIII digest of pFBA9; 2 to 5, autoradiographs of *Hin*dIII digests of *Z. mobilis* chromosomal DNA probed with pFBA9 (lane 2), pF17 (lane 3), pF20 (lane 4), and pA1 (lane 5). The arrow indicates the 11-kb fragment. Hybridization of pF20 and pF17 is expected to be weak, as only about 1 kb of the DNA contained in these cosmids should be homologous to the 11-kb *Hin*dIII fragment.

The *trpD* and *trpC* genes are linked or clustered in the gram-negative purple bacteria shown in Table 2. Analysis of *trpD* and *trpC* DNA sequences from *E. coli* (41) and *A. calcoaceticus* (22) showed that these genes are separated by less than 20 base pairs. In other bacteria, these genes were mapped by less precise genetic methods. For example, the *trpD* and *trpC* genes from *Rhizobium leguminosarum* were isolated on an R-prime plasmid containing a minimum of 30 kb of *Rhizobium* DNA. That particular R-prime plasmid did not complement any other *trp* genes (21). The isolation of the *Z. mobilis trpC* and *trpD* genes on different sets of plasmids suggests that they are probably separated by more than a few base pairs. However, they could be within 30 kb of each other.

Comparisons of rRNA cistrons (16) and analysis of phenylalanine biosynthesis (1) suggest that *Z. mobilis* is related to organisms in superfamily C of the purple bacteria, such as *Acetobacter* spp., *Agrobacterium* spp., and *Rhizobium* spp. The organization of the *Z. mobilis trp* genes appears to be similar to the organization observed in *Rhizobium* spp. and other nonenteric gram-negative bacteria in that the *trp* genes are encoded in two or three distinct regions of the chromosome instead of in an operon (Table 2).

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