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Complementation of a *trpE* Deletion in *Escherichia coli* by *Spirochaeta aurantia* DNA Encoding Anthranilate Synthetase Component I Activity

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A 2.7-kilobase *Sau*3A fragment of *Spirochaeta aurantia* DNA cloned in pBR322 complemented a *trpE* deletion in *Escherichia coli*. Deletion analysis and Tn*5* mutagenesis of the resulting plasmid pBG100 defined a 2-kilobase-pair region that was required for both the complementation and the synthesis of 59,000- and 47,000-molecular-weight polypeptides (59K and 47K polypeptides) in maxicells. Both the 59K and the 47K polypeptides appear to be encoded by a single gene. A maxicell analysis of pBG100 ::Tn*5* mutants suggests that the 47K polypeptide is not sufficient for the *trpE* complementation. In vitro and in vivo anthranilate synthetase (AS) assays indicate that the complementing activity encoded by pBG100 was functionally analogous to the AS component I of *E. coli* in that it utilized NH_3 but not glutamine as the amino donor. pBG100 did not encode a glutamine amidotransferase activity, although the AS component I it encoded was capable of interacting with *E. coli* AS component II to catalyze the glutamine-requiring reaction. Expression appeared to depend on a promoter in the cloned *S. aurantia* DNA.

The spirochetes represent a group of bacteria of ancient origin, constituting 1 of about 10 recognized sublines of eubacteria. rRNA oligonucleotide cataloging further indicates that this group exhibits an extremely deep branching, with the two major clusters (the *Spirochaeta-Treponema-Borrelia* group and the *Leptospira* group) being so distantly related that the customary numerical analysis did not reveal the relationship, which, however, was demonstrated by analysis of signature sequences (23). In part because there is as yet no genetic transfer system for these organisms, very little is known about gene organization or genetic regulation in this group of metabolically and ecologically diverse bacteria. Recently, the RNA polymerase of *Spirochaeta aurantia*, a heterotrophic, facultatively anaerobic spirochete isolated from freshwater sediments (6), was characterized (1). Although similar in subunit composition to other eubacterial RNA polymerases, it is resistant to rifampin, and the polypeptide subunit sizes are not of the common type. This led to the speculation that perhaps spirochete promoters are appreciably different from those of other bacteria (1).

To begin an investigation of the molecular genetics of this organism, we used molecular cloning techniques to isolate *S. aurantia* DNA which complemented mutations in the arginine and in the tryptophan biosynthetic pathways of *Escherichia coli* (B. Brahamsha and E. P. Greenberg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, I66, p. 157). The tryptophan biosynthetic pathways of a variety of organisms have been studied and are highly conserved (9, 10). The sequences of enzymatic reactions are identical, although gene organization is variable and is characterized by the presence of different gene fusions and by the variable number of operons in which these genes are found (9, 10, 32). Comparative sequence data are also available for a number of the *trp* genes (2, 11, 21, 22). Thus, the tryptophan pathway provides an ideal context in which to initiate studies of the molecular genetics of spirochetes. Furthermore, a segment of DNA from *Leptospira biflexa* which complements a *trpE* deletion in *E. coli* has recently been described (33, 34). Since leptospiras are representatives of one of the two main clusters of spirochetes, while *S. aurantia* is a member of the other, a comparison of functionally equivalent DNAs should provide further information regarding the evolutionary history of spirochetes. We describe here our investigations of a *trpE*-complementing *S. aurantia* DNA fragment and the proteins this DNA encodes.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. aurantia* M1 (6) was grown in GTY medium as described elsewhere (15). The *E. coli* strains used are listed in Table 1. The complex medium for the growth of *E. coli* was L broth (26) adjusted to pH 7.5 with 10 N NaOH prior to autoclaving. The minimal medium consisted of salts (7) with 0.4% glucose, and this was supplemented with appropriate amino acids at final concentrations of 40 μg/ml. L agar and minimal agar medium contained 1.5% agar. All cultures of *E. coli* were grown at 37°C, with shaking for broth cultures. Antibiotics were used, as appropriate, for the selection or maintenance of plasmids or transposons at the following concentrations (micrograms per milliliter): ampicillin, 100; kanamycin, 50; and tetracycline, 15. Cells for in vitro anthranilate synthetase (AS) assays were grown in Vogel-Bonner salts (28) supplemented with 0.5% glucose, the required amino acids at concentrations of 50 μg/ml, and 5 μg of indole per ml for *E. coli* JA221 (pBR322) and W3110 *trpED23* (pBR322). Cultures for these experiments were grown at 37°C with aeration for 15 h. To detect *trpE* and *trpG* activity in vivo, medium B (pH 6 or pH 8) described by Zalkin and Murphy (36) was used. This medium contains acid-hydrolyzed casein and low levels of ammonium sulfate. In this medium at pH 6, the ammonium salt is NH_4^+ and is not available for tryptophan synthesis. At pH 8, approximately 5% of the ammonium salt is unprotonated and is available for tryptophan synthesis. Thus, strains which rely solely on the NH_3-dependent activity of AS

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TABLE 1. *E. coli* strains

E. coli strain	Relevant genotype	Source (reference)
JA221	*ΔtrpE5 leuB6 hsdR hsdM⁺ recA1*	N. Charon (33)
CSR603	*thr-1 leuB6 proA2 phr-1 argE3 thi-1 uvrA6 ara-14 lacY1 galK2 xyl-5 mtl-1 gyrA98 (nal-1798) rpsL31 tsx-33 λ⁻ supE44*	CGSC[a] (25)
MC4100	*F⁻ araD139 Δ(argF-lac)U169 rpsL150 relA1 flbB5301 deoC*	R. Mortlock (26)
LE392	*F⁻ hsdR514 supE44 supF58 lacY1 galK2 galT22 metB1 trpR55 λ⁻*	R. Zuerner (13)
C600	*r⁻ m⁺ leu thr thi lacY* T1[r] T5[r]	D. Clegg (20)
W3110 *trpA33*	*trpA33*	N. Charon (33)
W3110 *trpB9579*	*trpB9579*	N. Charon (33)
W3110 *trpC670*	*trpC670*	N. Charon (33)
W3110 *trpD562*	*trpD562*	N. Charon (33)
W3110 *trpED23*	*ΔtrpE(G)23 trpR his cysB*	D. Yelton (34)

[a] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

component I are unable to grow at pH 6. Cells for these experiments were cultured overnight in L broth, washed twice in medium B (pH 6), diluted 1:100 in 5 ml of medium B at either pH 6 or pH 8 containing 50 μg of L-cysteine per ml for *trpED23* strains, and incubated with shaking at 37°C. Growth was monitored by measuring the optical density at 660 nm.

Isolation of *S. aurantia* chromosomal DNA. *S. aurantia* cells were washed once and suspended in cold TES buffer (10 mM Tris hydrochloride [pH 8 at 5°C], 0.1 M disodium EDTA, and 0.15 M NaCl). Lysozyme (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 100 μg/ml, and the suspension was incubated at 37°C for 5 min. Sodium dodecyl sulfate (SDS) purchased from Bio-Rad Laboratories, Richmond, Calif., (20% [wt/vol] in deionized water) was added to a final concentration of 0.5%, and the mixture was incubated at 37°C for 5 min. Following the addition of 1 μg of predigested (20) pronase (Sigma) per ml, the mixture was incubated at 37°C for 1 h. The resulting lysate was then extracted with an equal volume of water-saturated phenol (Bethesda Research Laboratories, Gaithersburg, Md.), followed by a chloroform-isoamyl alcohol (24:1) extraction. The DNA was then precipitated with ethanol, treated with DNase-free RNase (20), reextracted with phenol and chloroform-isoamyl alcohol, precipitated with ethanol, suspended in TE buffer (20), and stored at 4°C.

Plasmids, plasmid isolation, and transformations. The plasmids used in this study are derivatives of pBR322 (4), and construction of these plasmids is described below. Large-scale preparations of purified plasmid DNA were by the method of Kahn et al. (18). Minipreparations were by the method of Holmes and Quigley (16). The $CaCl_2$ transformation procedure outlined by Maniatis et al. (20) was used to transform *E. coli*.

Cloning protocol. *S. aurantia* M1 chromosomal DNA was partially digested with *Sau*3A to give an average fragment size of 4 to 7 kilobase pairs (kb). pBR322 was digested with *Bam*HI and then treated with calf intestinal phosphatase. Chromosomal DNA (1 μg) was mixed with 2 μg of pBR322 and ligated with T4 DNA ligase (20). The entire ligation mixture was used to transform *E. coli* JA221. By using this procedure, 1.5×10^4 independent transformants per μg of total DNA were obtained, 98% of which contained inserts. Following the transformation, the cells were grown overnight in L broth at 37°C in the presence of 100 μg of ampicillin per ml before being plated on minimal agar lacking tryptophan.

All of the enzymes used in the cloning procedure were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind., and were used according to the recommendations of the manufacturer.

DNA-DNA hybridizations. For the Southern blot analysis (20), DNA transfer to nitrocellulose (0.45-μm-diameter pore size; Schleicher & Schuell, Inc., Keene, N.H.) was done by the method of Maniatis et al. (20) by using 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) as the transfer buffer. Nick translation of purified plasmid DNA was carried out in the presence of [^{32}P]dCTP (Amersham Corp., Arlington Heights, Ill.) by using a kit from Bethesda Research Laboratories. Specific activities of the probe DNAs ranged from 4×10^7 to 7×10^7 cpm/μg of DNA.

Hybridizations were at 38°C in a buffer containing 50% formamide, 5× SSC, 10× Denhardt solution (0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin), 30 mM Tris (pH 7.5), 0.1% SDS, 1 mM EDTA, and 50 μg of denatured salmon sperm DNA per ml as described by Compton et al. (8). Following a 4-h prehybridization, 4×10^7 cpm of probe DNA was added and hybridization was continued for 24 h. The filters were then washed four times at room temperature with 0.1× SSC–0.1% SDS. This was followed by four 15-min washes at 55°C in the same buffer. Finally, the filters were washed twice at room temperature in 1× SSC, air dried, wrapped in Saran Wrap, and used to expose X-ray film (XRP-5; Eastman Kodak Co., Rochester, N.Y.).

Tn*5* mutagenesis of pBG100. *E. coli* MC4100 was transformed with pBG100 and then mutagenized by using λ467 (*b*221 *c*I857 *O*am29 *P*am80 *rex*::Tn*5*) by the procedure of deBruijn and Lupski (13). Plasmid DNA from pooled Km[r] colonies was used to transform *E. coli* JA221. The resulting Km[r] Ap[r] transformants were tested for their ability to grow on minimal agar lacking tryptophan.

In vitro mutagenesis of pBG100. For inactivation of the *tet* promoter of pBR322, 2.5 μg of pBG100 was cut with a threefold excess of *Hin*dIII. Following ethanol precipitation, the DNA was suspended in 25 μl of TE buffer to which was added 23 μl of deionized water and 6 μl of 10× *Bal* 31 buffer (20). The mixture was then cooled to 20°C, and 0.5 U of *Bal* 31 (Boehringer Mannheim) was added. At 5-s intervals, 10-μl samples were transferred to a tube containing 1 μl of 0.2 M ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*′,*N*′-tetraacetic acid (EGTA) and incubated at 68°C for 10 min. The 10- and 20-s digestions were pooled, ethanol precipitated, and suspended in 20 μl of ligation buffer. T4 DNA ligase (1 U) was added, and the mixture was incubated at 14°C for 18 h, after which it was used to transform *E. coli* JA221. The resulting transformants were screened for their ability to grow in the absence of tryptophan, and plasmids were extracted and tested for the presence of an intact *Hin*dIII restriction site.

Maxicell analysis. The protocol used was that of Sancar et al. (25). Polypeptides were labeled with [^{35}S]methionine (1,108 Ci/mmol; New England Nuclear Corp., Boston, Mass.). SDS-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (19) by using a 4.5% poly-

acrylamide stacking gel and a 12.5% separating gel. The gels were processed for fluorography by using En^{3}Hance (New England Nuclear).

Enzyme assays. AS assays, with either glutamine or NH_3 as the donor, were as described by Zalkin and Kling (35) with the following modifications. The final reaction volume was 3 ml, and for the assays done in the presence of NH_3, 1 mM dithiothreitol was used in place of 2 mM thioglycerol. Rates of anthranilate formation were determined by using a fluorescence spectrophotometer (The Perkin-Elmer Corp., Norwalk, Conn.). The activation wavelength was 325 nm, and emission was 400 nm. Protein concentrations were determined by the Bradford method (5) by using reagents obtained from Bio-Rad.

RESULTS

Formation of an *S. aurantia* genomic library and isolation of Trp$^+$ clones. An *S. aurantia* genomic library was prepared by partial restriction of the chromosome with *Sau*3A and ligation of the DNA fragments into the *Bam*HI site of the *tet* gene of pBR322. We sought to complement the *trpE* deletion in *E. coli* JA221 by transforming this strain with plasmids from our gene bank and selecting for growth on minimal medium lacking tryptophan. One such screening resulted in the appearance of five prototrophic colonies. When plasmids isolated from the colonies were used to transform *E. coli* JA221, they all conferred tryptophan independence. When further analyzed, these plasmids were found to contain an identical 2.7-kb insert. We chose one of these, pBG100, for all subsequent analyses.

Restriction and complementation analysis of pBG100. A restriction map of pBG100 is shown in Fig. 1. Southern blot analysis was used to demonstrate that the DNA inserted in pBR322 originated from *S. aurantia* (Fig. 2). The results of this analysis show that while pBG100 hybridized to itself, to pBR322, and to *Sau*3A and *Bam*HI digests of *S. aurantia* DNA, it did not hybridize to either *Sau*3A or *Bam*HI chromosomal digests of *E. coli* C600, a *trpE*$^+$ strain. This indicates that the cloned complementing DNA was of *S. aurantia* origin.

To determine whether the insert present in pBG100 encoded complementing activities other than *trpE*, we used this plasmid to transform mutants of *E. coli* blocked in other steps of the tryptophan biosynthetic pathway. *trpA*, *trpB*, *trpC*, and *trpD* mutants were not complemented by this plasmid. However, as discussed below, a *trpE(G)* mutant was complemented.

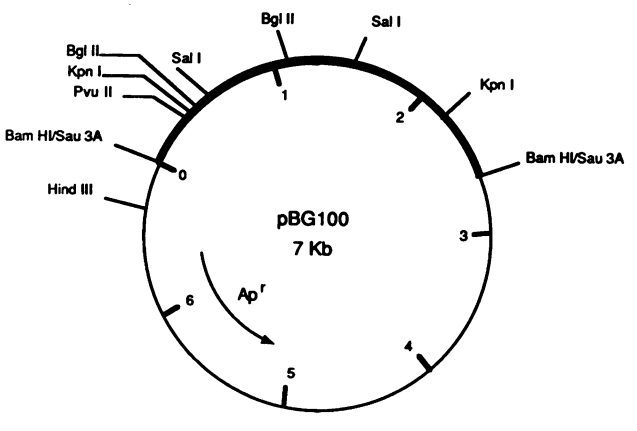

FIG. 1. Restriction map of pBG100 based on analysis of restriction fragments from single and double digests with the enzymes indicated. The thin line represents pBR322, and the thick line is the *S. aurantia* DNA insert.

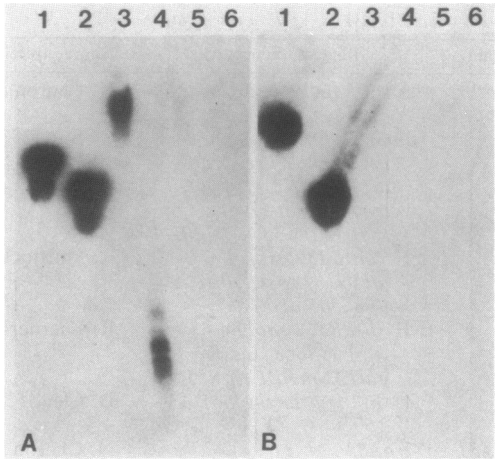

FIG. 2. Southern blot analysis of pBG100. Lanes: 1, pBG100; 2, pBR322; 3, *Bam*HI digest of *S. aurantia* DNA; 4, *Sau*3A digest of *S. aurantia* DNA; 5, *Bam*HI digest of *E. coli* C600 DNA; 6, *Sau*3A digest of *E. coli* C600 DNA. The probes were pBG100 (A) and pBR322 (B).

Tn*5* mutagenesis and deletion analysis of pBG100. To localize the *S. aurantia trpE* gene on the inserted DNA, pBG100 was mutagenized with Tn*5*. The pooled Km^r plasmids were then transformed into *E. coli* JA221, and the Trp phenotype of the resulting transformants was determined. A map showing the locations of seven Tn*5* insertions resulting in tryptophan auxotrophy, as well as that of one not affecting complementation of the *E. coli trpE* deletion, is shown in Fig. 3. On the basis of these results, approximately 1.8 kb of the inserted *S. aurantia* DNA in pBG100 were required for *trpE* complementation.

Furthermore, because Tn*5*::40, the insertion which did not affect the complementation, mapped between the *tet* promoter of pBR322 and the *S. aurantia* DNA required for *trpE*

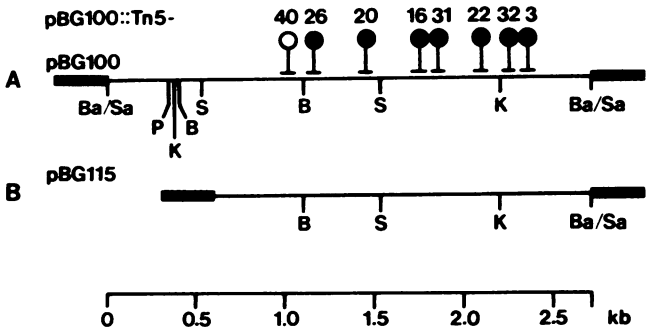

FIG. 3. Tn*5* mutagenesis and deletion analysis of pBG100. (A) Restriction map of cloned *S. aurantia* DNA with locations of Tn*5* insertions. Ba, *Bam*HI; Sa, *Sau*3A; P, *Pvu*II; K, *Kpn*I; B, *Bgl*II; S, *Sal*I. The symbols above the map represent the locations of Tn*5* insertions that were positioned by analyzing *Hin*dIII, *Bgl*II, and *Kpn*I digests of the corresponding plasmids. The positions of the Tn*5* insertions are accurate to ±75 base pairs. The closed symbols indicate insertions giving rise to Trp auxotrophy, while the open symbol indicates the location of an insertion not affecting *trpE*-complementing activity. (B) Restriction map of the *S. aurantia* DNA in pBG115.

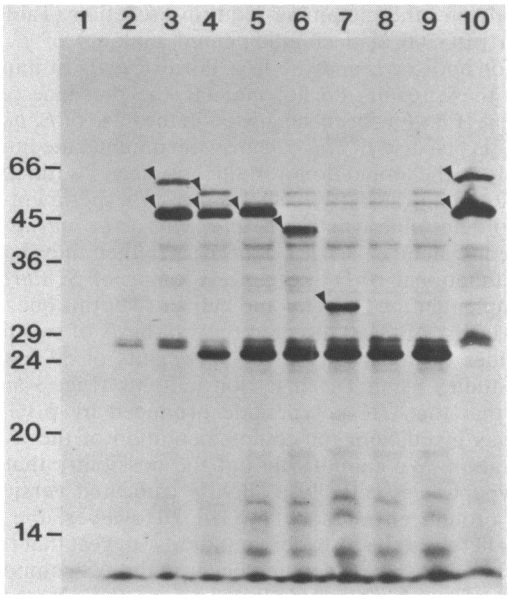

FIG. 4. Maxicell analysis of polypeptides synthesized by *E. coli* CSR603 (lane 1), pBR322 (lane 2), pBG100 (lane 3), pBG100::Tn*5*-40 (lane 4), pBG100::Tn*5*-26 (lane 5), pBG100::Tn*5*-20 (lane 6), pBG100::Tn*5*-16 (lane 7), pBG100::Tn*5*-31 (lane 8), pBR322::Tn*5* (lane 9), and pBG115 (lane 10). Lanes 1, 2, and 9 are control lanes showing the polypeptides synthesized by the maxicell strain, pBR322, and pBR322 carrying a Tn*5* insertion, respectively. The 28,500-molecular-weight polypeptide in lane 2 is the β-lactamase encoded by pBR322. The intense 27,000-molecular-weight band and the faint 52,000- and 48,500-molecular-weight bands in lanes 4 to 9 are encoded by Tn*5*. The locations of polypeptides encoded by *S. aurantia* DNA are indicated by the arrows. The migrations of protein standards are indicated to the left in kilodaltons.

complementation (Fig. 3), it appears that transcription of the *S. aurantia* DNA in pBG100 is independent of the *tet* promoter. It has been reported that modification of the *Hin*dIII site of pBR322 results in a 20-fold reduction in the expression of its *tet* promoter (30). To obtain additional evidence that an intact *tet* promoter is not involved in the expression of the *trpE*-complementing activity, pBG100 was cut with *Hin*dIII, subjected to a brief *Bal* 31 exonuclease digestion, religated, and used to transform *E. coli* JA221. Plasmids extracted from Trp prototrophs were no longer cleaved by *Hin*dIII, and several had undergone sizeable deletions, including one, pBG115, which had lost about 500 base pairs of the inserted DNA extending from the right of the left *Bam-Sau* site (Fig. 3). This result supports the conclusion that the expression of the *trpE*-complementing activity was independent of the *tet* promoter. Furthermore, because there are no pBR322 promoters in the vicinity of the right *Bam-Sau* junction (27), it is likely that transcription proceeds from a sequence in the cloned DNA that is either a native *S. aurantia* promoter or one recognized as a promoter by *E. coli*.

Maxicell analysis of pBG100 and pBG100 mutants. The maxicell technique (25) was used to identify the polypeptides encoded by pBG100. pBG100 directed the synthesis of two novel polypeptides with apparent molecular weights of 59,000 and 47,000 (59K and 47K polypeptides), in addition to the β-lactamase of pBR322 (Fig. 4, lane 3). Both of the insert-encoded polypeptides appeared to be well expressed as judged by band intensity.

To determine which, if not both, of these polypeptides was responsible for the *trpE*-complementing activity and to determine the direction of transcription, the mutant plasmids described above were analyzed by the maxicell technique (Fig. 4). While pBG115 produced both 47K and 59K polypeptides, none of the Tn*5* insertion mutant plasmids directed the synthesis of the 59K polypeptide. pBG100::Tn*5*-3, -32, -22, and -31 directed the synthesis of neither the 59K nor the 47K polypeptide. pBG100::Tn*5*-31, the one of the four with its insertion mapping the furthest into the insert, is shown in Fig. 4, lane 8. Two of the plasmids, pBG100::Tn*5*-16 and -20, directed the synthesis of single truncated polypeptides with molecular weights at 32,500 and 43,000, respectively, while pBG100::Tn*5*-26 directed the synthesis of only a 47K polypeptide. In pBG100::Tn*5*-40, the Tn*5* insertion did not result in a Trp^- phenotype, and this plasmid directed the synthesis of a 53K and a 47K polypeptide. Presumably, the 53K polypeptide is a truncated 59K polypeptide. Thus, these results indicate that synthesis of a protein larger than the 47K polypeptide encoded by pBG100::Tn*5*-26 was required for *trpE* complementation, although the results do not exclude the possibility that both this protein and the 47K polypeptide are needed. Furthermore, the map positions of the Tn*5* insertions (Fig. 3) taken together with the results of the maxicell analysis (Fig. 4) demonstrate that transcription was proceeding from right to left.

The results of Tn*5* mutagenesis and deletion analysis defined a 2-kb region that was required for the expression of the 47K and 59K polypeptides. This represents the coding capacity for a 73,000-dalton polypeptide and therefore appears insufficient for two contiguous or adjacent genes producing polypeptides of the observed sizes (see Discussion). Furthermore, the results of time course and pulse-chase experiments in maxicells (data not shown) indicate that neither the 59K nor the 47K polypeptide was a post-translational product of the other.

AS encoded by pBG100 and complementation of *E. coli trpE(G)* mutants. In the organisms in which it has been studied, AS is a two-component enzyme system (12, 17, 24, 35). Component I is the product of the *trpE* gene in *E. coli*, while component II is the amino-terminal third of the *trpD* gene product in *E. coli* (17) and the product of a separate *trpG* gene in some other bacteria (9). Together, components I and II catalyze the reaction of chorismate with L-glutamine to yield anthranilate, L-glutamate, and pyruvate. To determine whether pBG100 encodes a *trpG* function in addition to the *trpE* function, we transformed a mutant of *E. coli* deleted for both *trpE* and the first third of *trpD*. The *E. coli trpE(G)* mutant, W3110 *trpED23*, acquired the ability to grow on minimal medium without added tryptophan when transformed with pBG100. However, because it was possible that like the *E. coli* AS component I, the *S. aurantia trpE* function alone could also synthesize anthranilate by using NH_3 instead of glutamine (17, 36), in vitro enzyme assays and further growth studies were performed to ascertain whether a *trpG*-like activity was present (Table 2). In extracts of *E. coli* W3110 *trpED23* containing pBG100, AS activity was detected if NH_3 but not glutamine was provided as the amino donor. In extracts of *E. coli* JA221 (pBG100), which presumably contains an intact *trpD* gene, activity was detected with both NH_3 and glutamine, although the activity with glutamine was low. Furthermore, in medium B (36), cultures of *E. coli* JA221 (pBG100) were able to grow at pH 6 and at pH 8. *E. coli* W3110 *trpED23* (pBG100) was capable of growth at pH 8 but not at pH 6 (Table 2). In this medium, strains relying solely on the NH_3-dependent activity of AS component I for tryptophan synthesis were unable to grow

TABLE 2. AS activities in *trpE* and *trpE(G)* deletion strains of *E. coli* carrying pBG100 or pBR322

E. coli strain (plasmid)	Sp act[a] of AS with amino donor:		Growth in medium B[b]	
	Gln	NH_3	pH 6	pH 8
JA221 Δ*trpE5* (pBR322)	<0.005	<0.005		
JA221 Δ*trpE5* (pBG100)	0.061	0.73	0.92	1.9
W3110 Δ*trpED23* (pBR322)	<0.005	<0.005		
W3110 Δ*trpED23* (pBG100)	<0.005	0.15	0.08	1.7

[a] Specific activity is given as nanomoles of anthranilate synthesized per minute per milligram of protein.

[b] Growth was measured as the optical density at 660 nm 24 h after inoculation. Immediately after inoculation, the optical density of each culture was approximately 0.02.

when the concentration of NH_3 was low (pH 6). Presumably, the *E. coli trpE(G)* mutant W3110 *trpED23* containing pBG100 could grow at pH 8 by synthesizing tryptophan from the available NH_3 by using the *S. aurantia* AS component I, but it did not grow at pH 6 when NH_3 was not available. *E. coli* JA221 (pBG100) did grow at pH 6, when, presumably, anthranilate was synthesized from glutamine by using the *S. aurantia* AS component I and the *E. coli* AS component II. The enzyme assay and growth results are consistent with one another and indicate that pBG100 did not encode a *trpG*-like activity even though it permitted growth on minimal medium of *E. coli* strains lacking functional *trpE* and *trpD(G)* genes. Apparently, this is owing to the ability of the *S. aurantia*-encoded polypeptide to catalyze the reaction in *E. coli* by using NH_3 as the donor, a property it shares with component I of the ASs from other organisms (17, 24, 35, 36).

DISCUSSION

This report demonstrates that at least one *S. aurantia trp* gene could be functionally expressed in *E. coli*. A segment of *S. aurantia* DNA was cloned into pBR322, and the resulting plasmid pBG100 complemented a *trpE* deletion in *E. coli*. The results of Tn*5* mutagenesis and maxicell analysis (Fig. 3 and 4) defined an area of the insert, transcribed from right to left, that contains a sequence recognized as a promoter in *E. coli*. Whether this in fact represents a native spirochete promoter remains to be determined. Sequencing of this region as well as the availability of *S. aurantia* RNA polymerase (1) for protection experiments should make further studies of spirochete promoter structure possible. It should be noted that the G+C content of *S. aurantia* DNA is rather high (ca. 60 to 65 mol%) (6). Thus, adenine- and thymine-rich regions characteristic of *E. coli* promoter sequences should not be particularly common.

In *E. coli*, the *trpE* gene codes for component I of AS, a two-component enzyme system which catalyzes the first reaction of the tryptophan biosynthetic pathway. Component II, a glutamine amidotransferase, is the amino-terminal third of the product of the *trpD* gene in *E. coli* (17) and the product of a separate gene, *trpG*, in some other bacteria (9). The results of growth studies and enzyme assays demonstrate that the complementing activity encoded by pBG100 allowed utilization of NH_3 but not of glutamine for the synthesis of anthranilate from chorismate, thus making it equivalent in function to the AS component I of *E. coli* (Table 2). Furthermore, it appears that in *E. coli* JA221 containing pBG100, the spirochete equivalent of AS component I is capable of combining with the *E. coli* AS component II in catalyzing the glutamine-requiring reaction (Table 2). Although pBG100 also complemented mutants of *E. coli* deleted for both *trpE* and the first third of *trpD* in minimal medium, these results do not indicate the presence of AS component II, as has been suggested in the case of *L. biflexa* (34). That is because *E. coli* can grow on minimal medium by using NH_3 as the amino donor in the absence of a functional glutamine amidotransferase. Thus, further experiments are needed to determine whether *S. aurantia* or *L. biflexa* spp. has the equivalent of a *trpG* gene or whether they have a fused, bifunctional *trpD*-like gene. Cloning of *S. aurantia trpD*-complementing DNA should help answer this question.

In maxicells, pBG100 directed the synthesis of two novel polypeptides of apparent molecular weights of 59,000 and 47,000. Studies using Tn*5* insertion mutants (Fig. 3 and 4) indicate that the 47K polypeptide produced by pBG100::Tn*5*-26 was insufficient for complementation of the *E. coli trpE* mutation. We cannot rule out the possibility that this 47K polypeptide is actually a slightly truncated version of the 47K protein encoded by pBG100. However, the map distances between the insertion mutations suggest that this is not the case. For this reason and because the AS component I of both *E. coli* (22) and *Bacillus subtilis* (2) are ca. 59-kilodalton polypeptides and that of *L. biflexa* is a ca. 54-kilodalton polypeptide (34), the 59K polypeptide encoded by pBG100 may represent AS component I. The mutagenesis results do not exclude the possibility that the 47K polypeptide was also required for complementation. Also, pBG100::Tn*5*-40 directed the synthesis of a 53K polypeptide rather than a 59K polypeptide. Because this plasmid still conferred Trp prototrophy, this indicates that the carboxy-terminal 6,000 daltons of the protein were not required for its activity, if indeed the 59K polypeptide is AS component I.

The deletion analysis and Tn*5* mutagenesis of pBG100 defined a 2-kb region that was required both for *trpE* complementation and for the synthesis of the two polypeptides seen in maxicells. This does not represent sufficient coding capacity for two contiguous genes producing polypeptides of the size observed. Although overlap of genes in the *trp* operons of other bacteria is not uncommon, with the *B. subtilis trpD* and *trpE* genes overlapping by 29 base pairs (2) and the *trpE* and *trpG* genes of *Pseudomonas aeruginosa* overlapping by 23 base pairs (11), we do not feel that this is a likely explanation in the present case. First, the overlap would have to be much more extensive, with one gene almost completely contained in the other. Second, the results of the maxicell analysis of Tn*5* insertion mutants (Fig. 4) revealed the presence of just one truncated polypeptide which elongated roughly in agreement with the distance between the insertions instead of the expected two truncated polypeptides that should be present if there were two overlapping genes coding for two separate polypeptides. Rather, we think that pBG100 probably encodes just the 59K polypeptide in *S. aurantia* but that the spirochete DNA may contain a sequence which *E. coli* CSR603 often, but not always, recognizes as either a transcription termination or a translational stop signal. That this is occurring at either the transcriptional or the translational level is supported by the fact that time course and pulse-chase experiments indicate that neither the 47K nor the 59K polypeptide was a post-translational product of the other.

There are precedents for leaky translational terminators (3, 29). For example, in the coat protein cistron of coliphage Qβ, there is a leaky UGA nonsense codon which is read part of the time, resulting in the synthesis of two polypeptides, with the larger one being a readthrough product (29). There

are also exceptions to the universal genetic code involving translational stop sequences; *Mycoplasma capricolum* reads UGA as tryptophan (31), and of course, mitochondria provided the first example of such exceptions to the code (14). Thus, it is possible that codon usage in *S. aurantia* is slightly different from that in *E. coli*. Further studies, including sequencing of the *S. aurantia trpE* gene, should help clarify the matter.

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