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Characterization of *dapB*, a Gene Required by *Pseudomonas syringae* pv. tabaci BR2.024 for Lysine and Tabtoxinine–β-Lactam Biosynthesis

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The *dapB* gene, which encodes L-2,3-dihydrodipicolinate reductase, the second enzyme of the lysine branch of the aspartic amino acid family, was cloned and sequenced from a tabtoxin-producing bacterium, *Pseudomonas syringae* pv. tabaci BR2.024. The deduced amino acid sequence shared 60 to 90% identity to known *dapB* gene products from gram-negative bacteria and 19 to 21% identity to the *dapB* products from gram-positive bacteria. The consensus sequence for the NAD(P)H binding site [(V/I)(A/G)(V/I)XGXXGXXG)] and the proposed substrate binding site (HHRHK) were conserved in the polypeptide. A BR2.024 *dapB* mutant is a diaminopimelate auxotroph and tabtoxin negative. The addition of a mixture of L-,L-, D,D-, and *meso*-diaminopimelate to defined media restored growth but not tabtoxin production. Cloned DNA fragments containing the parental *dapB* gene restored the ability to grow in defined media and tabtoxin production to the *dapB* mutant. These results indicate that the *dapB* gene is required for both lysine and tabtoxin biosynthesis, thus providing the first genetic evidence that the biosynthesis of tabtoxin proceeds in part along the lysine biosynthetic pathway. These data also suggest that L-2,3,4,5-tetrahydrodipicolinate is a common intermediate for both lysine and tabtoxin biosynthesis.

Pseudomonas syringae pv. tabaci BR2.024, a plasmid-free derivative of strain BR2, is a tabtoxin-producing bacterium that causes wildfire disease on green beans (27, 31). Tabtoxin, a dipeptide, is not biologically active, but hydrolysis by peptidases produces tabtoxinine–β-lactam (TβL), the active toxin (22). Feeding studies with labeled precursors (26, 34, 40) indicated that carbons 1 to 6 of TβL (Fig. 1) are derived from aspartate and pyruvate, and labelling patterns led to speculation that the TβL and lysine biosynthetic pathways may have common initial steps. Although lysine and diaminopimelic acid (DAP) are structurally related to TβL (Fig. 1), label from these amino acids is not effectively incorporated into TβL. The methyl group of methionine can provide the β-lactam carbonyl carbon. Unkefer et al. (40) suggested that the two pathways might diverge after L-dihydrodipicolinate (DHDPA). Reduction to L-2,3,4,5-tetrahydrodipicolinate (THDPA) by DapB (5) would lead to DAP, while hydration would place a hydroxyl group on the carbon that becomes carbon 5 of TβL.

All of the genes required for TβL biosynthesis and resistance are located in a region of about 32 kb of the BR2 chromosome (19). Approximately 3 kb of that region has been sequenced, and two genes (*tabA* and *tblA*), essential for TβL production but with unknown functions, have been identified. The deduced *tabA* product has an amino acid sequence homologous to *meso*-DAP decarboxylase (the *lysA* gene product) characterized in other bacteria (9, 32); however, *tabA* is unable to complement an *Escherichia coli lysA* mutant (9). The second gene, *tblA*, encodes a product with an amino acid sequence unrelated to those of known polypeptides (2). The transcription of *tblA* is positively regulated by the product of the *lemA* gene, which is located outside the TβL biosynthetic region (2). LemA plus the product of a second gene, *gacA*, constitute a two-component regulatory system and are involved in the regulation of lesion formation and toxin production in *P. syringae* pv. syringae (3, 13, 44).

In prokaryotes, lysine is considered a member of the aspartic acid family of amino acids, although there is limited direct biochemical and genetic evidence to support this prediction. *E. coli* has served as the paradigm, and in that organism, the consecutive action of the first two enzymes, aspartokinase and aspartic acid semialdehyde dehydrogenase, leads to the formation of aspartic acid semialdehyde, a precursor of all members of the aspartic acid family (39). The first step unique to lysine biosynthesis, the condensation of aspartic acid semialdehyde with pyruvate, is catalyzed by L-2,3-DHDPA synthase, the product of the *dapA* gene (33, 45). The reduction of DHDPA to THDPA by NAD(P)H is catalyzed by DHDPA reductase, the *dapB* gene product. THDPA can be converted to DAP, a key component of bacterial peptidoglycans, by three different pathways (37). *meso*-DAP is converted to L-lysine by *meso*-DAP decarboxylase, the product of the *lysA* gene (37). Little is known about the genetics of lysine biosynthesis in pseudomonads. Only *lysA* has been isolated from *Pseudomonas aeruginosa* and characterized (25), and a cloned fragment of *P. aeruginosa* DNA contains a partial open reading frame (ORF) with a deduced protein product showing homology to the carboxyl terminus of the *E. coli* DapB (20).

This work describes studies of the DAP biosynthetic pathway in BR2.024 and the relationship between that pathway and TβL biosynthesis. *dapB* mutants were generated by Tn*5* mutagenesis, and the *dapB* gene was isolated from a BR2.024 genomic library by complementing *E. coli dapB* mutant AT999 (6). *dapB* was shown to be required for both DAP and TβL biosyntheses.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and culture conditions. The bacterial strains and plasmids used for this study are listed in Table 1. *E. coli* and *P. syringae* strains were grown in LB medium (21) at 37 and 30°C, respectively. A mixture of L,L-, D,D-, and *meso*-DAP (90 μg/ml) and L-lysine (50 μg/ml) was added when necessary. *P. syringae* pv. tabaci BR2.024 was also grown in King's B

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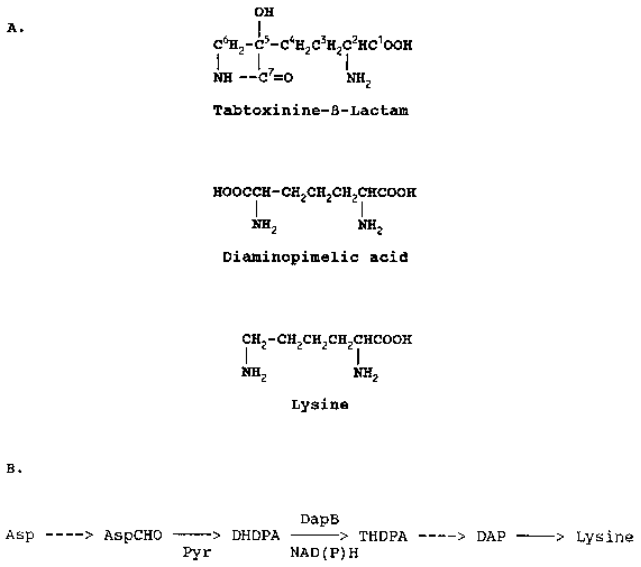

FIG. 1. (A) Structures of TβL, DAP, and lysine. (B) Abbreviated pathway of lysine biosynthesis in *E. coli*. Asp, aspartic acid; AspCHO, aspartic acid semialdehyde; Pyr, pyruvate. Dashed arrows indicate multiple enzymatic steps.

(18) or VB (41) medium. For *P. syringae* pv. tabaci strains, antibiotic concentrations were as follows: ampicillin, 100 μg/ml; tetracycline, 12.5 μg/ml; nalidixic acid, 200 μg/ml; and kanamycin, 15 μg/ml. For *E. coli* strains, antibiotic concentrations were as follows: ampicillin, 100 μg/ml; tetracycline, 12.5 μg/ml; kanamycin, 50 μg/ml; and chloramphenicol, 25 μg/ml.

Tabtoxin assays. Tabtoxin was detected by inhibition of *Salmonella typhimurium* essentially as described by Piwowarski and Shaw (30), except that DAP and lysine were added to certain assay plates.

DNA manipulations. Plasmid DNA was isolated by the method of Chen et al. (7). Chromosomal DNA was isolated from 5-ml overnight cultures of *P. syringae* strains (19). Restriction digestions, ligations, and transformations were performed according to standard procedures (35).

Electroporation. Competent cells of *E. coli dapB* mutant AT999 were prepared as described in the Bio-Rad bacterial electro-transformation and pulse controller (model no. 1652096 and 1652098) instruction manual. One microliter (approximately 0.5 μg of DNA/μl) of ligation mixture was used for each transformation. Electro-transformation was carried out at the following settings: 25 μF, 2.5 kV, and 200 Ω for 4 to 5 ms. After electroporation, cells were grown in SOC medium (35) containing DAP for 1 h at 37°C. Cells were collected by centrifugation at 12,000 rpm in an Eppendorf centrifuge for 1 min, washed once with 1 ml of LB medium, and spread onto LB plates containing either ampicillin or both DAP and ampicillin.

Tn*5* mutagenesis. For Tn*5* mutagenesis, the donor was *E. coli* WA803, containing plasmid pGS9 (38), and the recipients were BR2.024 and BR11.000. Donor and recipient strains were grown overnight in 5 ml of LB or King's B medium with appropriate antibiotics, and the donor strain (300 μl) and recipient strain (1,200 μl) were mixed and collected by centrifugation at 12,000 rpm for 1 min. The cells were washed once with 1 ml of King's B medium and finally suspended in 100 μl of King's B medium containing DAP. The cell suspensions were spotted onto King's B plates containing DAP. After incubation at 30°C for 24 h, quarters of each mating spot were transferred onto LB plates containing kanamycin, nalidixic acid, DAP, and lysine. The mating mixtures were suspended in 50 μl of sterile distilled water and spread over the plates. Colonies appeared after 2 days of incubation at 30°C. The colonies were replicated to VB plates containing kanamycin and lysine to identify DAP auxotrophs.

Isolation of deletion mutants deficient in both tabtoxin production and tabtoxin resistance. Dilutions of an overnight culture of BR2.024 grown in LB medium were plated onto VB medium, and colonies were patched onto tabtoxin assay plates to detect spontaneous mutant colonies that failed to produce a zone of inhibition. Such mutants were plated as a lawn on VB medium, and a tabtoxin-producing strain was patched onto the lawn. A clear zone of inhibition surrounding the tabtoxin-producing strain identified spontaneous mutants inhibited by

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Characteristics[a]	Source or reference
Strains		
P. syringae pv. tabaci		
BR2.024	Tox^+ Tox^r Nal^r Rif^r plasmid-free strain of BR2	27
BR11.000	Tox^- Tox^s Nal^r spontaneous deletion mutant of BR2.024	This study
PSb1	Km^r Sm^r Nal^r Tox^- *dapB*::Tn*5* mutant of BR2.024	This study
PSDb1	Km^r Sm^r Nal^r Tox^- Tox^s *dapB*::Tn*5* mutant of BR11.000	This study
PSDb2	Km^r Sm^r Nal^r Tox^- Tox^s *dapB*::Tn*5* mutant of BR11.000	This study
PSDb3	Km^r Sm^r Nal^r Tox^- Tox^s *dap* Tn*5* mutant of BR11.000	This study
E. coli		
DH5α	F^- ϕ80d*lacZ* M15 *recA1 endA1 gyrA96 thi-1 hsdR17* (r_k^- m_k^+) *supE44 relA1 deoR* (*lacZYA-argF*)U169	Promega
HB101	F^- *thi-1 hsdS20* (r_B^- m_B) *supE44 recA13 ara-14 leuB6 proA2 lacY1 rpsL20* (Str^r) *xyl-5 mtl-1*	Promega
WA803	*Met thi* r_k^- m_k^-	38
AT999 (CGSC4549)	*dapB*::*Mu* λ^- *relA1 spoT1 thi-1*	6
S. typhimurium TA1975	*hsd* Rif^r	J. B. Johnston
Plasmids		
pGEM11-zf(+)	Amp^r	Promega
pSELECTTM-1	Tet^r	Promega
pRK415	Tet^r	17
pGS9	Cm^r Nm^r Tra+ (N-type) p15A replicon (Tn*5*)	38
pGESb3	Amp^r, 3.0-kb DNA fragment from BR2.024 chromosome in pGEM-11zf(+)	This study
pRKb3	Tet^r, 3.0-kb DNA fragment in pRK415	This study
pGESb4	Amp^r, 1.9-kb DNA fragment from BR2024 in pGEM-11zf(+)	This study
pRKb4	Tet^r, 1.9-kb DNA fragment in pRK415	This study
pRK2013	Km^r	11
pGES816-2	Amp^r Km^r, 4.2-kb *Sal*I fragment contains part of *dapB* and Tn*5* from PSDb2 chromosome in pGEM-11zf(+)	This study
pGEN810	Amp^r, 2.8-kb *Nsi*I fragment from the 22-kb *Eco*RI fragment (9) of BR2.024 chromosome	This study
pAS820	Tet^r Km^r, 4.2-kb *Sal*I fragment contains part of *dapB* and Tn*5* from PSb1 chromosome in pSELECT-1	This study

[a] Abbreviations: Nal, nalidixic acid; Rif, rifampin; Sm, streptomycin; Nm, neomycin; Amp, ampicillin; Km, kanamycin; Tet, tetracycline; Tox+, TβL production; Tox^-, TβL nonproduction; Tox^r, TβL resistant; Tox^s, TβL sensitive.

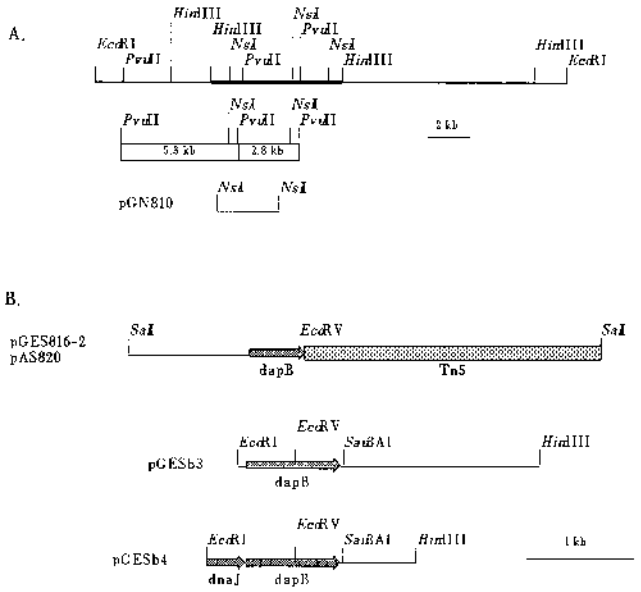

FIG. 2. Schematic representation of DNA segments used for Southern hybridization and DNA sequencing. (A) Twenty-two-kilobase *Eco*RI fragment containing a portion of the TβL biosynthetic region (9, 19). The broad line indicates the 6-kb *Hin*dIII fragment. The boxed area contains the *Pvu*II fragments present in all tabtoxin-producing *P. syringae* strains (19). (B) DNA fragments containing *dapB* obtained separately from Tn*5* insertion mutants and prototrophic strains. Plasmids pGES816-2 and pAS820 contain only a portion of *dapB*; pGESb3 and pGESb4 contain the complete gene.

tabtoxin. Genomic DNA of mutants deficient in tabtoxin production and sensitive to tabtoxin was analyzed by Southern hybridization to identify the desired deletion mutants.

Southern hybridization analysis. Fragments used as probes were cut from 0.8 or 1.2% agarose gels and purified with GENECLEAN II (Midwest Scientific) according to the procedure recommended by the supplier. DNA probes were prepared with the Bionick labelling kit from GIBCO BRL. DNA was transferred to PhotoGene nylon membranes with Blotter (Stratagene), and hybridizations were done in 50% formamide at 42°C. The blots were washed in 0.1× SSC (15 mM sodium chloride, 1.5 mM sodium citrate) containing 1% sodium dodecyl sulfate at 50°C.

DNA sequencing. Double-stranded DNA was used as a template. DNA sequencing was accomplished manually (14) or by automatic sequencing (Genetic Engineering Facility of University of Illinois at Urbana-Champaign).

Sequence analyses. Nucleotide sequences were compared to those in the GenBank database by the Blastx search program (1, 42), and deduced amino acid sequences were determined with DNA Strider version 1.1 (24). Sequence comparisons were analyzed for similarity to known *dapB* gene products by the Clustal V program (12).

Complementation tests. DNA regions of interest were cloned into shuttle vector pRK415 (17). The resulting pRK415 derivatives were introduced into recipient strains by triparental mating (43) with plasmid pRK2013 (11) as a helper. The resulting transconjugants were selected with the appropriate antibiotics.

GenBank accession number. The DNA sequence data reported in this paper have been deposited in GenBank with the accession number U47017.

RESULTS

Isolation of deletion mutants deficient in both tabtoxin production and tabtoxin resistance. To obtain spontaneous mutants lacking most of the tabtoxin biosynthetic region, individual colonies of BR2.024 were screened for tabtoxin production and resistance. Two mutants with the desired phenotype were identified, and one, designated BR11.000, was selected for further characterization. To confirm deletion of the tabtoxin production and resistance region, the *Nsi*I fragment (Fig. 2A), present in the contiguous 5.3- and 2.8-kb *Pvu*II fragments conserved in all tabtoxin-producing strains (9, 19), was cloned into pGEM-11zf(+) to produce the plasmid designated pGN810. This construct failed to hybridize to DNA fragments obtained by *Eco*RI or *Hin*dIII digestion of genomic DNA from the mutant and separated by agarose gel electrophoresis (see Fig. 5).

Generation and characterization of Tn*5* mutants. To identify genes involved in both tabtoxin and lysine biosynthesis, we mutagenized the genome of BR2.024 and BR11.000 with transposon Tn*5*. A single Tn*5* mutant of BR2.024, designated PSb1, requiring DAP and lysine and three DAP- and lysine-requiring Tn*5* mutants of BR11.000, designated PSDb1, PSDb2, and PSDb3, were obtained by screening approximately 200 and 1,000 kanamycin-resistant mutants, respectively. Restriction digestion and Southern hybridization (data not shown) suggested that PSb1, PSDb1, and PSDb2 had Tn*5* inserts in the same or nearby sites, while the Tn*5* insertion in PSDb3 was at different site but in the same *Sal*I fragment.

To determine the sites of Tn*5* insertion in PSb1 and PSDb2, chromosomal DNA was extracted from DAP-requiring Tn*5* mutants of strains BR11.000 (PSDb2) and BR2.024 (PSb1). The DNA from each mutant was completely digested with *Sal*I, and the fragments were ligated to *Sal*I-digested pGEM11-zf (+) (for PSDb2 digests) or to pSELECT-1 (for PSb1 digests). The products of ligation were transformed into *E. coli* DH5α, and the cells were spread onto LB plates containing the appropriate antibiotics. Tn*5* contains a single *Sal*I site, the left portion of which contains the kanamycin phosphotransferase gene (16); therefore, only transformants with plasmids that contain the left portion of Tn*5* and the region flanking the transposon were capable of growing on plates containing kanamycin. A single colony of each transformant was selected, and plasmids, designated as pGES816-2 from PSDb2 and pAS820 from PSb1 (Fig. 2B) were isolated from cultures of each. The DNA sequences of about 300 and 700 bp of the regions flanking Tn*5* in pAS820 and pGES816-2 (Fig. 2B), respectively, were determined with a Tn*5* primer. The amino acid sequence of the deduced product of these partial DNA sequences had significant homology to the *dapB* gene product of *E. coli* (data not shown), suggesting that Tn*5* had inserted into the PTBR2.024 *dapB* gene.

The Tn*5*-containing insert in plasmid pGES816-2 was also used to probe *Sal*I- or *Eco*RI-digested chromosomal DNA of PSb1. As shown in Fig. 3, this DNA hybridized to a 10.4-kb *Eco*RI fragment in digests of DNA from PSb1. This fragment

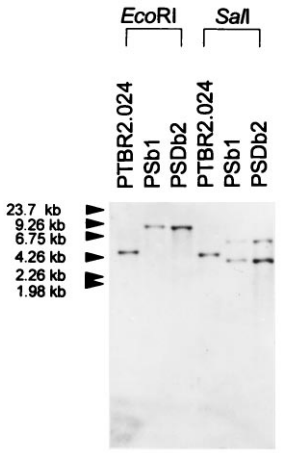

FIG. 3. Southern hybridization of *Eco*RI or *Sal*I digests of DNA from *P. syringae* pv. tabaci BR2.024 (PTBR2.024) and Tn*5* insertion mutants PSb1 and PSDb2 probed with the insert from plasmid pGES816-2.

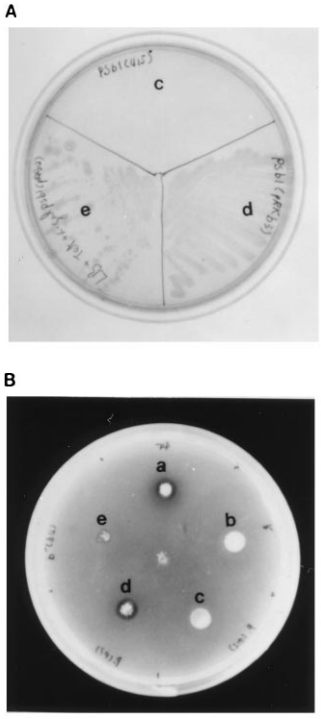

FIG. 4. Effect of various constructs on growth of mutant PSb1 and its ability to produce tabtoxin in defined media. (A) Growth. PSb1 containing pRKb3 (d) or pRKb4 (e) is able to grow on minimal media. The vector alone (c) does not complement the mutant. (B) Tabtoxin production. Clear zones of inhibition indicative of tabtoxin production are present surrounding colonies of BR2.024 (a) and PSb1 containing pRKb3 (d) or pRKb4 (e) but not PSb1 (b) or PSb1 containing the vector (c). DAP was applied to the plate at the sites of inoculation with PSb1 and PSb1 containing the vector to allow growth of the mutant.

is approximately 5.8 kb larger than the *Eco*RI band (4.6 kb) that hybridized to the probe in digests of DNA from BR2.024. The probe hybridized to 6.8- and 4.2-kb *Sal*I fragments in digests of mutant PSb1 DNA and to a 4.5-kb *Sal*I fragment in BR2.024 digests. The additional *Sal*I band present in digests of genomic DNA from the Tn*5* mutant is a result of a *Sal*I site located in the transposon (16). These results confirm that the mutant strain contains a single Tn*5* insertion. Disruption of the *dapB* gene of BR2.024 resulted in a requirement for DAP and lysine and the inability to produce TβL (Fig. 4). The addition of a mixture of L,L-, D,D-, and *meso*-DAP to defined medium restored growth to *dapB* mutants PSb1 and PSDb2. However, these supplements did not restore TβL production to PSb1 (Fig. 4B). To ensure that the lack of tabtoxin production was not due to a spontaneous deletion of the TβL biosynthetic region (19), *Hin*dIII-digested chromosomal DNA from PSb1 was probed with the 2.8-kb *Nsi*I fragment (Fig. 2A) contained on plasmid pGNP810. As shown in Fig. 5, both the Tn*5* mutant, PSb1, and parent strain, BR2.024, yielded a 6.0-kb *Hin*dIII fragment, which includes the conserved 2.8-kb *Nsi*I fragment (Fig. 2A), while the spontaneous TβL-deficient mutant, BR11.000, lacked the fragment.

Isolation and characterization of the BR2.024 *dapB* gene. To isolate the *dapB* gene, we partially digested BR2.024 genomic DNA with *Sau*3A1, ligated the fragments to *Bam*HI-digested pGEM-11zf(+), and transformed the recombinant plasmids into *E. coli dapB* mutant strain AT999 by electroporation. Ampicillin-resistant transformants were screened for their ability to grow on LB medium. Three such strains were isolated, and their respective plasmids were designated as pGESb2, pGESb3, and pGESb4. *Hin*dIII and *Eco*RI restriction endonuclease digests of plasmids pGESb2, pGESb3, and pGESb4 yielded DNA fragments with sizes of 3.5, 3.0, and 1.9 kb, respectively. Plasmids pGESb3 and pGESb4 were chosen for further characterization.

Both strands of a 1.0-kb region contained within the 3.0-kb insert from pGESb3 and a 1.3-kb region contained within the 1.9-kb insert from pGESb4 were sequenced (Fig. 2B). The DNA sequence of pGESb3 was colinear to the sequenced region of the 1.3-kb fragment (Fig. 6). The 1.3-kb region contained one ORF, designated ORF2, and one partial ORF, designated ORF1 (Fig. 6). No ORF was found by partial sequencing of the remaining 540 bp of the insert of pGESb4. In addition, a comparison of DNA sequences downstream from the *Sau*3A1 site (Fig. 2B) of the two plasmids showed that these regions were different, suggesting that the separate inserts were derived from noncontiguous *Sau*3A1 chromosomal fragments. Comparison of the ORF2 sequence to the sequenced regions of the pGES816-2 and pAS820 inserts showed that they were identical and defined the sites of Tn*5* insertion into ORF2 (Fig. 6).

Computer-aided alignment showed that the predicted polypeptide of truncated ORF1 shared 47% identity to the C terminus of the *dnaJ* gene product of *E. coli* (data not shown). An alignment of the BR2.024 ORF2 product with *dapB* products from other gram-negative bacteria is shown in Fig. 7. The deduced amino acid sequence of the ORF2 product was approximately 89% identical to the deduced amino acid sequence of a presumed DapB of *P. aeruginosa* (20). The predicted amino acid sequence of the ORF2 product also had significant homology (62% identity) to DapB of *E. coli* (5), *Klebsiella pneumoniae* (62% identity) (4), and *Haemophilus influenzae* (61% identity) (accession number U32756). It showed less homology (19 to 24% identity) to DapB of the gram-positive bacteria *Bacillus subtilis* (accession number L38424), *Mycobacterium bovis* (8), *Brevibacterium lactofermentum* (29), and *Corynebacterium glutamicum* (accession number X67737) (data not shown). As shown in Fig. 7, the (V/I)(A/G)(V/I)XGXXGXXG consensus sequence for NAD(P)H binding, which is highly conserved in NAD(P)H-dependent dehydrogenases and in DHDPA reductase (29, 36), is present in the deduced ORF2 product. The proposed substrate binding site, HHRHK, of the DHDPA reductase in *E. coli* (36) was also found in the predicted ORF2 protein product. We therefore designated ORF2 as *dapB*.

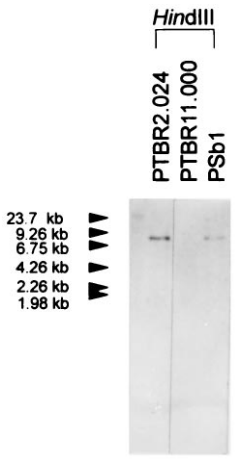

FIG. 5. Southern hybridization of *Hin*dIII digests of *P. syringae* pv. tabaci BR2.024 (PTBR2.024), Tn*5* mutant PSb1, and deletion mutant BR11.000 with a fragment from the TβL biosynthetic region.

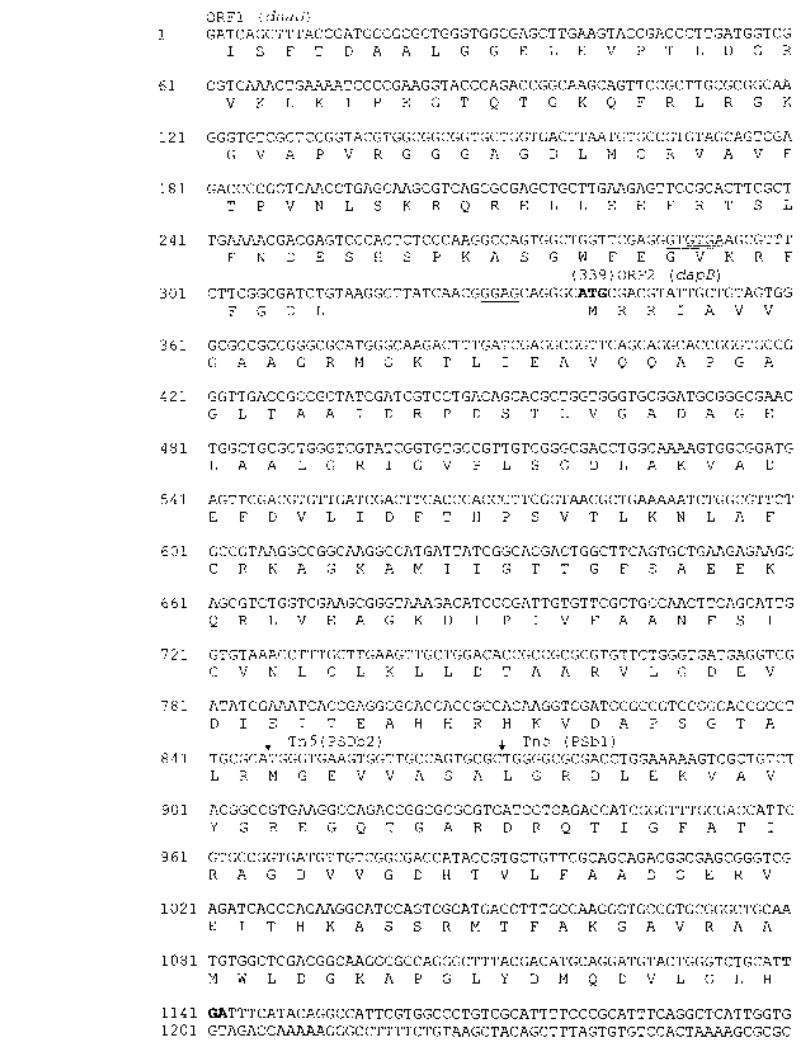

FIG. 6. Nucleotide sequence of a 1.3-kb region of the pGESb4 insert. The predicted amino acid sequence is shown. Predicted start and stop codons are in boldface. A possible ribosome binding site is underlined, and a possible promoter region is double underlined. The sites of Tn*5* insertion in PSb1 and PSDb2 are indicated by arrows.

Complementation tests. As shown in Fig. 4A, PSb1 containing pRKb3 or pRKb4 was able to grow on VB medium, while the vector alone failed to complement the mutant phenotype. Plasmid pRKb3 or pRKb4, but not the vector, also restored tabtoxin production (Fig. 4B); however, PSb1 containing pRKb3 or pRKb4 grew more slowly and produced less tabtoxin than the parent strain. Reduced tabtoxin production was most noticeable in PSb1 with pRKb4, which consistently gave smaller hazy zones. This may be caused by the instability of the pRK415 derivatives in cells growing in media lacking tetracycline, which could not be used in the tabtoxin bioassay. No zones of inhibition were observed when glutamine, which prevents the action of tabtoxin (22) was added to the medium (data not shown).

DISCUSSION

It was possible to generate DAP auxotrophic mutants of BR2.024 strains because, unlike *P. aeruginosa* (25), the mutants are capable of taking up DAP in amounts sufficient to sustain growth. A mutant lacking the region responsible for TβL biosynthesis and TβL resistance was examined initially because of evidence (9, 26, 34, 40) suggesting a possible redundancy in genes encoding products that function in either TβL or lysine biosynthesis. A region of more than 25 kb starting from about 10 kb upstream from the left *Eco*RI site and extending 15 kb downstream of that *Eco*RI site (Fig. 2A) is deleted spontaneously from the chromosome of BR2 strains of *P. syringae* pv. tabaci at frequencies of about 2.1×10^{-3}/CFU (19). The gene(s) that confers resistance to TβL lies in the unmapped 10-kb region. Hybridization experiments (Fig. 5) show that the simultaneous loss of tabtoxin production and resistance by BR11.000 is the result of such a deletion. DAP mutants, however, were isolated from both the parental strain and the mutant in which the TβL biosynthesis locus had deleted, and restriction digestion and Southern hybridization analysis indicated that Tn*5* had inserted into the same locus in the mutant and the parent. DNA sequence analysis indicated that the transposon had inserted into the *dapB* gene of BR2.024, and this conclusion was confirmed by demonstrating that a parental DNA fragment could complement an *E. coli dapB* mutant. The observed identity of the parental and Tn*5*-containing sequences led us to conclude that under our culture conditions, BR2.024 contains a single functional copy of *dapB*, that the gene is not located in the TβL biosynthetic region, and that this region does not contain a gene that encodes a protein with DHDPA reductase activity.

In addition to requiring DAP and lysine for growth, PSb1 also failed to produce TβL. The addition of a mixture of L,L-, D,D-, and *meso*-DAP and lysine to minimal media allowed growth but did not restore tabtoxin production. These results are consistent with those of other studies (34, 40) indicating that neither DAP nor lysine is a tabtoxin precursor. The presence of the 6.0-kb *Hin*dIII fragment, which contains the conserved 2.8-kb *Pvu*II region (Fig. 2A), in digests of PSb1 DNA (and its absence in control BR11.000 digests) indicates that the

FIG. 7. Sequence comparison of BR2.024 DapB to homologous proteins from other gram-negative bacteria. Identical amino acids are in boldface. Abbreviations are as follows, with accession numbers in parentheses: Pt, BR2.024 (U47017); Pa, *P. aeruginosa* (U04992); Kp, *K. pneumoniae* (X79817); Ec, *E. coli* (M10611); Hi, *H. influenzae* (U32756). Conserved NAD(P)H and proposed substrate binding sites are underlined.

loss of tabtoxin production was not the result of a deletion of the TβL biosynthesis region.

The isolation of the *dapB* gene was accomplished by transformation of cloned *Sau*3A1-digested BR2.024 genomic DNA fragments into *E. coli dapB* mutant AT999. DNA sequence analysis of the cloned fragments isolated from two of three colonies that grew in the absence of DAP and lysine identified an ORF that could encode a product with significant amino acid sequence homology to the *dapB* products of other prokaryotes (Fig. 7). Putative NAD(P)H (29, 36) and substrate binding sites (36) were present at the predicted locations in the deduced ORF product. These results indicated that the ORF was the BR2.024 *dapB* gene. The presence of a possible promoter region (TGTAAG and GGGTGT in Fig. 6) similar to the −10 and −35 regions (TATAAG and TTGTGT) of an *argF* promoter identified in *P. aeruginosa* (15) suggests that the *dapB* gene is transcribed independently from upstream genes.

The deduced product of a partial ORF in the pRKb4 insert has sequence homology to the C terminus of the *dnaJ* gene product from *E. coli* (28). DnaJ is a heat shock protein that functions as part of complex also containing DnaK and GrpE (23). There is no evidence for an involvement of a possible product of the *dnaJ* homolog in DAP or TβL biosynthesis.

The mutant strain PSb1, containing plasmid pRKb3 or pRKb4, was able to grow in media without DAP, and tabtoxin production was restored. The absence of inhibition zones in assays done in the presence of glutamine confirmed that inhibition was due to tabtoxin. Because pRKb4 contains only a single complete ORF (*dapB*), it is unlikely that this plasmid can restore TβL production as well as prototrophy if the tabtoxin-negative phenotype were to be the result of a polar mutation. These results, coupled with the observed phenotype of Tn*5* mutant PSb1, lead to the prediction that DapB is an enzyme required for both DAP and TβL biosynthesis. An alternative, but less likely, explanation is that loss of TβL production is the result of a spontaneous mutation in a gene having the function of *dapB* and that overproduction of DapB by the plasmid-borne gene compensates for the mutation. The results also indicate that THDPA is a common intermediate in their respective biosynthetic pathways. This prediction differs from the hypothesis (10) that the first step unique to the TβL pathway is the hydration of DHDPA to 5-hydroxy-2,3,4,5-tetrahydrodipicolinate. It is possible, however, that DHDPA is modified (e.g., by oxidation at C-4) to produce a compound that can serve as a substrate for DapB and that this THDPA derivative is converted to TβL. The inability of a mixture of DAP isomers to restore TβL production to mutant PSb1 supports the proposal (34) that L,L-DAP is not a TβL precursor. Our results thus define the most likely branch point of the lysine and TβL biosynthesis pathways at a site after THDPA synthesis and before L,L-DAP formation.

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