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A Possible Role for Acetylated Intermediates in Diaminopimelate and Tabtoxinine–β-Lactam Biosynthesis in *Pseudomonas syringae* pv. tabaci BR2.024

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The deduced product of an open reading frame (ORF3) located in the tabtoxinine–β-lactam (TβL) biosynthetic region of *Pseudomonas syringae* pv. tabaci BR2.024 (BR2.024) has significant sequence homology to the *dapD* products of other bacteria. *dapD* encodes L-2,3,4,5-tetrahydrodipicolinate succinyl coenzyme A succinyltransferase (THDPA-ST), an enzyme in the diaminopimelate (DAP) and lysine biosynthetic pathway. Complementation studies, in vitro transcription-translation experiments, and enzymatic assays indicated that ORF3 encodes a product with THDPA-ST activity in *Escherichia coli dapD* mutant β274. However, a BR2.024 mutant with an insert in ORF3 was prototrophic, and only basal THDPA-ST activity was detected in extracts of both parent and mutant. This finding suggested that ORF3 was not required for DAP biosynthesis and that it did not encode a product with THDPA-ST activity. The results of enzymatic studies, indicating that BR2.024 uses acetylated intermediates for DAP biosynthesis, are consistent with the hypothesis that BR2.024 does not need THDPA-ST for DAP biosynthesis. The ORF3 mutant produced reduced levels of tabtoxin, indicating that ORF3 may have a role in TβL biosynthesis. We have named the gene *tabB* and have proposed a possible function for the gene product.

In bacteria, lysine is a member of the aspartic acid family of amino acids (reference 34 and references therein). The first two reactions in the pathway are the ATP-linked phosphorylation of aspartic acid to L-4-aspartyl phosphate and the pyridine nucleotide reduction of that compound to aspartic acid-4-semialdehyde. At this point the pathways for the biosynthesis of the aspartic acid family diverge, and the first step unique to lysine biosynthesis is the DapA-catalyzed condensation of the semialdehyde with pyruvate to form L-2,3-dihydrodipicolinate (DHDPA). The next step, the pyridine nucleotide-linked reduction of DHDPA to L-2,3,4,5-tetrahydrodipicolinate (THDPA), is catalyzed by DapB. Three pathways for the conversion of THDPA to *meso*-diaminopimelate (*meso*-DAP) have been identified in bacteria. In *Escherichia coli*, THDPA is acylated by THDPA succinyl coenzyme A (CoA) succinyltransferase (THDPA-ST), the *dapD* product, to form *N*-succinyl-2-amino-6-oxo-L-pimelate. Transamination, catalyzed by DapC, followed by removal of the succinyl group by DapE yields L,L-diaminopimelate. That compound is converted to *meso*-DAP by an epimerase (DapF). A similar pathway is used by *Bacillus megaterium* (36) and by *Bacillus subtilis* (6) except that THDPA is acetylated in a reaction catalyzed by an acetyltransferase (THDPA-AT), and subsequent intermediates are acetyl rather than succinyl derivatives. *Bacillus sphaericus* bypasses acylated intermediates by the direct reduction of THDPA and ammonium to *meso*-DAP by NAD(P)H in a reaction catalyzed by *meso*-DAP dehydrogenase (DDH) (42). Some bacteria have more than one pathway for *meso*-DAP biosynthesis. *Bacillus macerans*, for example, has both the acetyl and the dehydrogenase pathways (2), while *Corynebacterium glutamicum* has the succinyl and dehydrogenase pathways (34). The conversion of *meso*-DAP to L-lysine in the three pathways is catalyzed by *meso*-DAP decarboxylase (LysA). All of the *E. coli* genes involved in lysine biosynthesis except *dapC* (3–5, 12, 27–29, 35) and *ddH* from *C. glutamicum* (14) have been identified and sequenced, but genes of the acetyl pathway have not been characterized physically. Among other gram-negative bacteria, the only lysine biosynthetic genes that, to our knowledge, have been characterized are *lysA* (22) from *Pseudomonas aeruginosa* and *dapB* from *Pseudomonas syringae* pv. tabaci (21).

P. syringae pv. tabaci BR2.024 (BR2.024) (24), a plasmid-free strain of *P. syringae* pv. tabaci BR2 (26), produces tabtoxin, which can be hydrolyzed to form the active toxin, tabtoxinine–β-lactam (TβL) (37). TβL is structurally related to lysine, and labeling studies (23, 31, 39) indicated some commonality in the biosynthetic pathways of those two compounds. Genetic studies (21) showed that DapB is essential for both pathways and that THDPA may be an intermediate in both pathways. Two other genes, *tblA* (1) and *tabA* (8), required for TβL biosynthesis are located within a 31-kb region that contains all of the genes needed for TβL biosynthesis and tabtoxin resistance (17). There is no obvious relationship between TblA and known polypeptides, but TabA has significant sequence homology to LysA from *E. coli* and *P. aeruginosa*. *tabA*, however, is unable to complement an *E. coli lysA* mutant (8). A partial open reading frame (ORF3) with a deduced product having sequence homology to DapD from *E. coli* was found immediately downstream from *tabA*. The proximity of ORF3 and *tabA* in the TβL biosynthetic gene cluster and the sequence similarities of their putative products to lysine biosynthetic enzymes suggested that ORF3 might also have a role in TβL biosynthesis. This report describes experiments to characterize and determine a function for ORF3 and to identify the pathway used by BR2.024 for lysine biosynthesis. Our results

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Characteristics[a]	Reference or source
Strains		
P. syringae pv. tabaci		
BR2.024	Tox^+ Tox^r Nal^r Rif^r plasmid-free strain of BR2	23
Pstabb1	Nal^r Km^r *tabB*::Omegon-Km mutant of BR2.024	This study
E. coli		
DH5α	F^- ϕ80d*lacZ*ΔM15 *recA1 endA1 gyrA96 thi-1 hsdR17* (r_K^- m_K^+) *supE44 relA1 deoR* Δ(*lacZYA-argF*)*U169*	Promega
β274	Cm^r *thi-1 dapD*::Cm	C. Richaud
S. typhimurium TA1975	*hsd* Rif^r	J. B. Johnston
C. glutamicum ATCC 13032		L. Eggeling
B. subtilis 1085	*his met trp*	G. Ordal
Plasmids		
pGEM-11zf (+)	Amp^r	Promega
pRK415	Tet^r	15
pRK2013	Km^r	11
pSELECT-1	Tet^r	Promega
pJFF350	Km^r	10
pRE702A	Tet^r, 22-kb *Eco*RI fragment from pWE702 (8) in pRK415	This study
pRB805	Tet^r, 10.5-kb *Eco*RI-*Bam*HI fragment from pRE702A in pRK415	This study
pGN810	Amp^r, 2.8-kb *Nsi*I fragment from pRE702 in pGEM-11zf (+)	21
pRN810	Tet^r, above 2.8-kb *Nis*I fragment in pRK415	This study
pRN810 (Km)	Tet^r Km^r, pRN810 with Km cassette in *Xho*I site	This study
pGNP812	Amp^r, pGN810 with 1.5-kb *Pst*I fragment deleted	This study
pRK807	Tet^r	This study
pRK812	Tet^r, insert from pGNP812 in pRK415	This study
pGNX813	Amp^r, 0.8-kb *Nsi*I-*Xho*I fragment from pGNP810 in pGEM-11zf (+)	This study

[a] Abbreviations: Nal, nalidixic acid; Rif, rifampin; Amp, ampicillin; Km, kanamycin; Tet, tetracycline; Cm, chloramphenicol; Tox^+, tabtoxin production; Tox^-, tabtoxin nonproduction; Tox^r, tabtoxin resistant.

implicate ORF3 in TβL biosynthesis and suggest that BR2.024 utilizes acetylated intermediates for lysine biosynthesis.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and culture conditions. Bacterial strains and plasmids used for this study are listed in Table 1. *E. coli* and *P. syringae* pv. tabaci strains were grown in LB (19) at 37 and 30°C, respectively. *P. syringae* strains were also grown in King's B (16) or Vogel-Bonner (40) medium. Woolley's minimal medium (43) containing $ZnSO_4$ (10 μM) was used for TβL production (20). 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.

DNA manipulation. DNA isolation, restriction digestion, ligation reactions, transformation, and hybridization were carried out as previously described (21). The dideoxy-chain termination method (33) and Deaza sequencing kit (Pharmacia) were used for double-stranded sequencing. Sequencing primers, 15-mers, were synthesized by the University of Illinois Genetic Engineering Laboratory.

Construction of plasmids pRE702A, pGN810, pRN810, pRN810(Km), pGN812, pGNX813, pRB805, and pRK807. The 22-kb *Eco*RI fragment in plasmid pWE702 (8) was excised and inserted into plasmid pRK415 to give plasmid pRE702A (Fig. 1). pGN810 was constructed by subcloning a 2.8-kb *Nsi*I fragment from the 22-kb *Eco*RI fragment of pRE702A into the *Nsi*I site of vector pGEM-11zf(+) and transforming that construct into *E. coli* DH5α. The insert was excised from the pGN810 polylinker by *Hin*dIII and *Xba*I digestion and ligated into *Hin*dIII/*Xba*I-digested vector pRK415 to produce pRN810. Plasmid pRN810 was digested with *Xho*I, and the ends were filled in with the Klenow fragment of DNA polymerase (GIBCO, BRL). Omegon-Km (3.8 kb) was released from plasmid pJFF350 (10) by *Eco*RI digestion, and the ends were filled in as described above. The two linear fragments were ligated by using T4 DNA ligase to give pRN810(Km). Plasmids pGNP812 and pGNX813 were constructed by deletion of the *Pst*I and *Xho*I/*Nsi*I fragments, respectively, from the insert in plasmid pGN810. The *Eco*RI/*Bam*HI fragment and the *Xba*I/*Bam*H1 fragment were excised from pWE702 and inserted into pRK415 to give pRB805 and pRK807, respectively.

Marker exchange mutagenesis. Plasmid pRN810(Km) was introduced into strain BR2.024 by triparental mating with *E. coli* HB101 containing plasmid pRK2013 as the mobilizing helper (11). Colonies resistant to kanamycin and sensitive to tetracycline were selected, and the sites of insertion were confirmed by Southern hybridization analysis. Chromosomal DNA was isolated from six kanamycin-resistant (Kan^r) and tetracycline-sensitive (Tet^s) transconjugants, the DNA was digested with *Eco*RI or *Hin*dIII, and the fragments were separated by agarose gel electrophoresis. The Omegon-Km fragment from pJFF350 and the internal 684-bp *Pvu*II-*Pst*I fragment of ORF3 (Fig. 1) were used as hybridization probes.

In vitro transcription and translation. An *E. coli* S30 extract (Promega) was used for the coupled in vitro transcription and translation of DNA templates. [^{35}S]methionine (Amersham) was used to detect translation product in sodium dodecyl sulfate-polyacrylamide gel (12%) electrophoretograms. Gels were vacuum dried and used for autoradiography (32).

TβL isolation, purification, and assay. TβL was isolated and purified from 1-liter cultures as described by Thomas et al. (38) except that Dowex-50X8 (H^+ phase) was used instead of Amberlite IRC-50 (H^+ phase) in the first step. To determine TβL production, triplicate 1-ml samples of cultures were taken at three different time points when tabtoxin levels are near the maximum. The cells were removed by centrifugation at 14,000 rpm for 5 min, and supernatant solutions were stored at −20°C. Samples (100 μl) were applied to assay discs (10 mm), and the discs were placed onto plates seeded with *Salmonella typhimurium* (25). Diameters of inhibition zones were recorded after incubation of the plates for 24 h at 37°C, and the concentration of TβL in each sample was determined by using standard curves prepared by plotting diameters of inhibition zones versus the TβL concentration in serial dilutions of purified TβL. Reported values are the averages of the values given by a single time point at which maximum production was observed.

Enzymatic assays. BR2.024 was grown in Woolley's medium; other strains were grown in LB medium. Cells from 100-ml overnight cultures were collected by centrifugation at 5,000 rpm for 15 min. The cells were washed once with an equal volume of 20 mM Tris-HCl buffer (pH 8.0) (for acetylase and succinylase assays) or 50 mM phosphate buffer (pH 7.5) (for *meso*-DAP dehydrogenase assays). The cells were suspended in those same wash buffers (3 ml) and sonicated (Branson Sonifier 450) at 4°C, using at a microtip output of 6. *E. coli* and *P. syringae* pv. tabaci strains were sonicated for 40 s in 10-s pulses. Sonication of

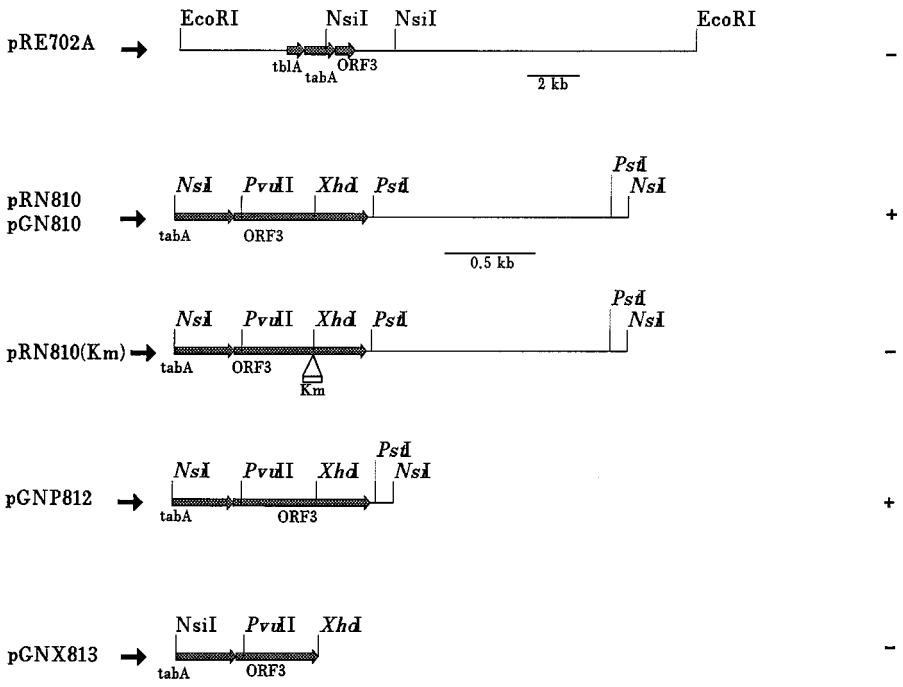

FIG. 1. Schematic representation of cloned fragments containing *tabB*. + and −, constructs that did and did not complement *E. coli dapD* mutant β274. Arrows indicate the orientation of *lac* promoters on the vector. Only relevant restriction sites are shown.

C. glutamicum and *B. subtilis* required 200 s in 10-s pulses. Cell debris was removed by centrifugation at 14,000 rpm for 30 min at 4°C, and extracts were used immediately for enzymatic assays.

meso-DAP dehydrogenase was assayed at room temperature by measuring the reduction of NADP at 340 nm in the presence of a mixture of L,L-, D,D-, and *meso*-DAP as described by Yeh et al. (44). One unit of activity is defined as formation of 1 nmol of NADPH/min, and specific activity is defined as units/minute/milligram of protein. Protein concentrations were determined by using the Bio-Rad assay with bovine serum albumin (U.S. Biochemical) as a standard.

The potassium salt of THDPA was synthesized from *N*-toluenesulfonyl-*cis*-2,6-piperidinedicarboxylate as described by Chrystal et al. (7). L-2,3,4,5-THDPA *N*-succinyl (acetyl)transferase assays were done as described by Schrumpf et al. (34). Enzyme activity was determined by measuring an increase in absorbance at 412 nm due to the reaction of free CoA with Ellman's reagent [5,5′-dithiobis(2-nitrobenzoic acid)]. The ε_{412} for the product used in calculating activity was 14,150 cm^2/mol (30), and 1 U is defined as formation of 1 nmol of Ellman's reagent-CoA complex/min. Specific activity is defined as units of activity/minute/milligram of protein.

GenBank accession number. The DNA sequence reported in this paper has been deposited in the GenBank database as revised accession no. M88485.

RESULTS

Characterization of ORF3. The complete sequence of ORF3 is shown in Fig. 2. Potential translation start sites, located at positions 61 (ATG), 76 (TTG), and 136 (GTG), could produce polypeptides with 276, 271, and 251 amino acids, respectively. Possible ribosome binding sites are located 10 bp upstream from the TTG codon and 12 bp upstream from the GTG codon. A possible promoter region, differing in two nucleotides from the −10 (GATAAA) and −35 (TTGACA) regions of the *E. coli dapD* gene promoter (28), was identified. No obvious ribosome binding site was detected upstream from the ATG codon, but GAG, the proposed ribosome binding site in the *E. coli dapD* gene (28), was present 10 nucleotides upstream from the TTG codon, and a possible site, AAGAA, was found 12 nucleotides upstream from the GTG codon. Two copies of the hexad repeat motif, $[(I,V,L)GXXXX]_5(I,V,L)$, proposed (41) as a consensus sequence characteristic of acetyltransferases were identified in ORF3. Computer-aided analysis using the Clustal V program (13) (Fig. 3) confirmed previous results (8) predicting that the deduced amino acid sequence of the ORF3 product had significant homology to the predicted *dapD* products of *E. coli* (28), *Haemophilus influenzae* (accession no. 45284), and *Actinobacillus pleuropneumoniae* (18). The ORF3 product, for example, had 53% homology (66% considering conserved replacements) to DapD from *E. coli*, and the sequences of the four polypeptides are 45% identical.

To determine the function of ORF3, a mutation was introduced into BR2.024 by marker exchange using plasmid pRN810 (Km) (Fig. 1). Homogenotization was confirmed by Southern hybridization using Omegon-Km (10) and the 684-bp *Pvu*II-*Pst*I fragment, which contains most of ORF3 (Fig. 1), as probes (data not shown). One of the mutants, Pstabb1, was selected for further study. Both BR2.024 and Pstabb1 grew on Vogel-Bonner medium with a generation time of about 3 h. The addition of a mixture of L,L-, D,D-, and *meso*-DAP to the medium decreased the generation times of both the mutant and the parent to about 1.4 h. TβL production, however, was reduced in Pstabb1 to 13 ± 1 μg/ml, compared to 24 ± 2 μg/ml produced by BR2.024. TβL production was increased to 23 ± 2 μg/ml in cultures of Pstabb1 containing plasmid pRB805 (Fig. 4), but production was not restored by plasmid pRN810, pRK812, or pRK807. Because of the apparent role of ORF3 in TβL production, we have named the putative gene *tabB*.

Because of the homology of TabB to DapD in *E. coli*, we transferred recombinant plasmids containing *tabB* into *E. coli dapD* mutant β274. Although plasmid pRE702A failed to complement the mutant, plasmids pRN810 and pGNP812 (Fig. 1), which contain *tabB* downstream from a *lac* promoter in the vector, were able to complement the mutant phenotype of *E. coli* β274. Plasmid pRN810(Km), in which the Omegon-Km cassette has been inserted into *tabB*, and pGNX813, which has a 326-bp deletion in the 3′ end of the gene, failed to complement the mutant.

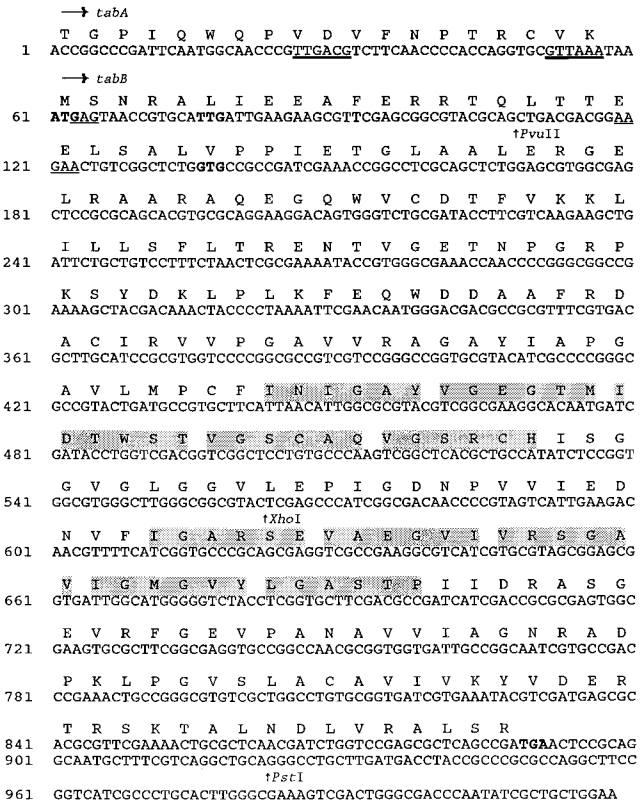

FIG. 2. Nucleotide and deduced amino acid sequences of ORF3. Potential ribosome binding sites are underlined, and possible promoter regions are double underlined. Putative translation start and stop codons are shown in boldface, and hexad repeats characteristic of acetyltransferases (41) are shadowed.

To confirm the expression of *tabB* in *E. coli*, plasmids pGN812 and pGNX813 (Fig. 1) were used in coupled in vitro transcription-translation assays using an *E. coli* extract. As shown in Fig. 5, lane 2, an additional polypeptide with an apparent molecular mass of 29 kDa was present when plasmid pGNP812 was used as a template. In contrast, a band corresponding to a 19.5-kDa polypeptide (lane 1) was observed when plasmid pGNX813 was used as a template. The size of the *tabB* product was in close agreement with the size of the polypeptide deduced from the nucleotide sequence if the ATG at position 61 (29,307 Da) or the TTG at position 76 (28,765 Da) was used as a translation start codon. If the GTG at position 136 were used as a translation start codon, the predicted polypeptide would have a molecular mass of 26,449 Da. The polypeptide produced by the truncated *tabB* was significantly larger than the predicted polypeptides produced from either the ATG (approximately 17.9 kDa) or the TTG (approximately 16.7 kDa). Further examination of plasmid pGNX813 revealed that extension of the partial *tabB* translation product into the polylinker added 14 additional amino acids (approximately 1.7 kDa) to the partial *tabB* product.

The foregoing results indicated that *tabB* was expressed in *E. coli* β274. To determine if that gene encoded a product with THDPA-ST activity, extracts of *E. coli* strain β274 and that mutant containing plasmid pGNP812 (β274/pGNP812) were assayed for THDPA-ST activity. The transferase activity of strain β274/pGNP812 extracts was 22-fold higher than that of strain β274 (Table 2) and about double the activity of *E. coli* DH5α, used as a positive control.

FIG. 3. Amino acid sequence alignment of ORF3 of BR2.024 (Ps) and DapD from *E. coli* (Ec), *H. influenzae* (Hi), and *A. pleuropneumoniae* (Ap). Identical amino acids are indicated by asterisks; conserved amino acids are indicated by dots.

Assays for DAP biosynthetic enzymes. The growth of Pstabb1 on a minimal medium indicated that *tabB* was not required for DAP biosynthesis. To determine whether BR2.024 has a gene that encodes a product with THDPA-ST activity or if it uses an alternative pathway for DAP biosynthesis, BR2.024 extracts were examined for the activities of THDPA-ST, THDPA-AT, and DDH. The data in Table 2 show that only basal levels of THDPA-ST activity, equivalent to the activity in extracts of *E. coli dapD* mutant β274, were found in BR2.024 and Pstabb1 extracts. In contrast, THDPA-AT activity was detected in extracts of both BR2.024 and Pstabb1. The activity in extracts of these two strains was essentially the same as that found in extracts of *B. subtilis*, an organism that utilizes the acetyl pathway for DAP biosynthesis (6). DDH activity was high in extracts of *C. glutamicum*, an organism in which DDH plays a significant role in DAP biosynthesis (34). In contrast, the DDH activity in BR2.024 and Pstabb1 extracts was about the background level found in extracts of *E. coli*, an organism that lacks this enzyme (34).

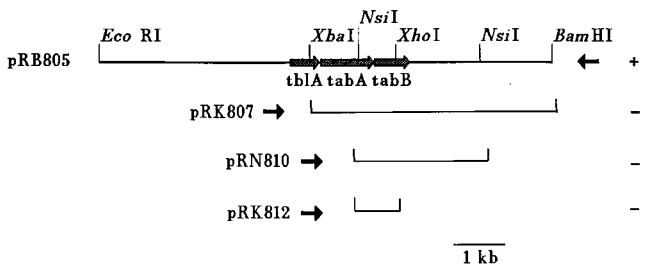

FIG. 4. Schematic representation of cloned fragments tested for complemation of Pstabb1. + and −, constructs that did and did not restore tabtoxin production. Arrows indicate the orientation of *lac* promoters on the vector.

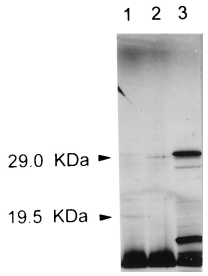

FIG. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of coupled in vitro transcription-translation products. Constructs are shown in Fig. 1. Lane 1, pGNX813; lane 2, pGNP812; lane 3, pGEM-11zf(+). Size standards are shown at the left. Products of complete and partial ORF3 translation products are indicated by arrowheads.

DISCUSSION

TabB has significant sequence homology to THDPA-ST from *E. coli* (28) (Fig. 3), and when cloned into a vector such that it is under the control of a *lac* promoter, *tabB* complemented an *E. coli dapD* mutant. The failure of pRE702A to complement the mutant might be because the *lac* promoter in the vector is about 6 kb upstream from *tabB*, and the possible promoter region upstream from *tabB* (Fig. 2) is not recognized by the *E. coli* RNA polymerases. When under the control of a *lac* promoter, *tabB* was transcribed and translated in vitro by *E. coli* extracts to produce a polypeptide of a size predicted from the DNA sequence (Fig. 5) if either ATG or TTG is the translation start codon. Furthermore, high levels of THDPA-ST activity were detected in extracts of the *E. coli* mutant containing pGNP812, while only basal activity was present in extracts of the mutant. The higher THDPA-ST activity in extracts of *E. coli* containing pGNP812 than in extracts of *E. coli* DH5α could be due to the high copy number of the plasmid or possibly to strain differences. Our interpretation of these results is that *tabB* is expressed in *E. coli* and that the gene product is an enzyme with THDPA-ST activity.

In contrast, a *tabB* mutant of BR2.024, Pstabb1, did not require DAP or lysine for growth, and there was no detectable THDPA-ST activity in extracts of either the parent or the mutant strains (Table 2). We considered three possible explanations for these observations. (i) BR2.024 contains a second gene encoding a protein with THDPA-ST activity; however, attempts to detect such a gene by complementing *E. coli dapD* mutant β274 with DNA from a genomic library of BR11.000, a mutant in which the region containing *tabB* had been deleted (21), were unsuccessful (data not shown). (ii) *tabB* is expressed at low levels in BR2.024 under our growth conditions. This is possible but unlikely, as levels of THDPA-ST activity are essentially the same in extracts of parent, mutant, and *E. coli dapD* mutant β274 (Table 2). (iii) *tabB* has no role in DAP biosynthesis and either is not expressed or does not encode a product with THDPA-ST activity in BR2.024. The detection of THDPA-AT activity in extracts of both BR2.024 and Pstabb1 implicate an acetyl pathway for DAP biosynthesis, thus eliminating a DapD requirement and lending support for the this last possibility.

TABLE 2. Activities of THDPA acylases and *meso*-DAP dehydrogenase

Strain[a]	Sp act[b] (nmol/min/mg of protein)		
	THDPA-ST	THDPA-AT	DDH
P. syringae pv. tabaci			
BR2.024	1.2	3.2	2.0
Pstabb1	1.6	3.7	2.2
B. subtilis	ND[c]	3.3	ND
C. glutamicum	ND	ND	82.7
E. coli			
DH5α	11.3	0.4	1.7
β274	1.0	ND	ND
β274/pGNP812	22.0	ND	ND

[a] *P. syringae* strains were grown in 100 ml of Woolley's medium (43). Other bacteria were grown in 100 ml of LB medium (19).
[b] Average of three determinations. The maximum variation in each set of measurements was ±17%.
[c] ND, not determined.

Although *tabB* apparently has no function in DAP biosynthesis, a 50% reduction in TβL by Pstabb1 indicates that the gene product has a role in TβL biosynthesis. The ability to restore TβL production to parental levels by pRB805 indicates that the reduced TβL production was a result of the mutation. The inability to restore production by pRK807, which lacks the upstream *Eco*RI-*Xba*I fragment present in pRB805, indicates that neither the *lac* promoter in the vector nor the *E. coli*-like promoter region upstream from *tabB* functions in *tabB* transcription. It also suggests that a positive regulatory element upstream from the *Xba*I site is required for *tabB* expression. A possible promoter element and a *tblA* (Fig. 4) transcription start site have been proposed (1), but there is no evidence that *tblA* is part of an operon. The simplest explanation of our results is that *tabB* encodes a product that is involved in but not essential for TβL biosynthesis. A role for additional genes downstream from *tabB* cannot be ruled out, however, because the Omegon-Km insert has transcription and translation terminators at both ends (10) and so is capable of causing polar mutations.

In summary, our results indicate that *tabB* has the following properties. It can replace a defective *dapD* gene in *E. coli*, but it is not required for DAP biosynthesis in BR2.024. TabB has THDPA-ST activity in *E. coli*, but if *tabB* is expressed in BR2.024, the product has neither THDPA-ST nor THDPA-AT activity in BR2.024. An insertion in *tabB* causes reduced TβL production. A possible explanation for these apparent discrepancies is that *tabB* is not required for either DAP or TβL biosynthesis but that the insert in *tabB* causes a polar mutation in a downstream gene involved in TβL biosynthesis. This appears unlikely, however, because complementation studies implicate regions upstream but not downstream from *tabB* in TβL production. A possible alternative explanation is the use of different translation start codons by *E. coli* and BR2.024. In vitro transcription-translation experiments suggest that *E. coli* initiates translation from either AUG or UUG, leading to a functional THDPA-ST. If translation in BR2.024 began at a GUG codon, as has been suggested for *tblA* (1) and *tabA* (8), this would result in the loss of the 25 amino acids at the amino terminus of the polypeptide. As this region is reasonably well conserved among the THDPA-ST transferases (Fig. 3), this could lead to loss of that enzymatic activity.

We suggest that this truncated TabB is an acetyltransferase that converts an unknown compound to an acetyl derivative that is further metabolized to TβL. The presence of two copies of the tandem hexad repeat motif, $[(I,L,V)GXXX]_5(I,V,L)$, found in enzymes with acetyltransferase activity but not in *E. coli* DapD (41) supports a function for DapB as an acetylase rather than a succinylase. This is consistent with the proposal of Feistner et al. (9) that acetylated intermediates are involved

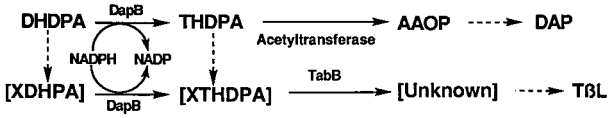

FIG. 6. Hypothetical biosynthetic pathways for DAP and TβL in BR2.024. AAOP, *N*-acetyl-2-amino-6-oxo-L-pimelate. XDHPA and XTHDPA are hypothetical derivatives of DHPA and THDPA.

in tabtoxin biosynthesis. We also suggest that the THDPA-AT in the DAP pathway can compensate partially for a mutation in *tabB* so that such mutants are able to produce TβL although at reduced levels. A model to explain our results is shown in Fig. 6. We reported previously (21) that DapB is required for both DAP and TβL biosynthesis. Thus, either THDPA is an intermediate in both pathways or DapB is able to reduce both DHDPA and a hypothetical derivative of that compound (XDHPA) to precursors of DAP (THDPA) and TβL (XTHDPA).

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