JOURNAL OF BACTERIOLOGY, July 2000, p. 3626–3631 Vol. 182, No. 13
0021-9193/00/$04.00+0

Characterization of a *Bordetella pertussis* Diaminopimelate (DAP) Biosynthesis Locus Identifies *dapC*, a Novel Gene Coding for an *N*-Succinyl-L,L-DAP Aminotransferase

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Received 25 February 1999/Accepted 3 April 2000

The functional complementation of two *Escherichia coli* strains defective in the succinylase pathway of *meso*-diaminopimelate (*meso*-DAP) biosynthesis with a *Bordetella pertussis* gene library resulted in the isolation of a putative *dap* operon containing three open reading frames (ORFs). In line with the successful complementation of the *E. coli dapD* and *dapE* mutants, the deduced amino acid sequences of two ORFs revealed significant sequence similarities with the DapD and DapE proteins of *E. coli* and many other bacteria which exhibit tetrahydrodipicolinate succinylase and *N*-succinyl-L,L-DAP desuccinylase activity, respectively. The first ORF within the operon showed significant sequence similarities with transaminases and contains the characteristic pyridoxal-5′-phosphate binding motif. Enzymatic studies revealed that this ORF encodes a protein with *N*-succinyl-L,L-DAP aminotransferase activity converting *N*-succinyl-2-amino-6-ketopimelate, the product of the succinylase DapD, to *N*-succinyl-L,L-DAP, the substrate of the desuccinylase DapE. Therefore, this gene appears to encode the DapC protein of *B. pertussis*. Apart from the pyridoxal-5′-phosphate binding motif, the DapC protein does not show further amino acid sequence similarities with the only other known enzyme with *N*-succinyl-L,L-DAP aminotransferase activity, ArgD of *E. coli*.

D,L-Diaminopimelate (D,L-DAP) is the direct precursor of L-lysine and moreover an important constituent of the cell wall peptidoglycan of many bacteria (39). There are three alternative pathways in bacteria leading to the synthesis of D,L-DAP (29): (i) the dehydrogenase variant in which the intermediate tetrahydrodipicolinate (THDP) common to all three pathways is converted in a single step to DAP, (ii) the succinylase variant involving two succinylated intermediates, and (iii) the acetylase variant using the acetyl residue instead of succinyl as the blocking group (Fig. 1).

In the succinylase pathway, THDP is converted by the succinyltransferase (DapD) to *N*-succinyl-2-amino-6-ketopimelate, which is the substrate of the aminotransferase DapC. Its product, *N*-succinyl-L,L-DAP, is converted by DapE, a desuccinylase, to the common product of both the succinylase and acetylase pathways, L,L-DAP (27). The acetylase and/or dehydrogenase pathways are found among members of the genus *Bacillus* (38), while the succinylase pathway is present in *Escherichia coli* (18). In *Corynebacterium glutamicum*, both the succinylase and dehydrogenase pathways can operate in D,L-DAP and L-lysine biosynthesis (30). This high variability and flexibility of DAP pathways might ensure the availability of a sufficient amount of *meso*-DAP for cell wall synthesis under different environmental conditions (37). In addition to the vital role of DAP in the cross-linking of the glycan backbones in the bacterial cell wall and in providing lysine for protein biosynthesis, DAP is a central constituent in the *Bordetella pertussis* tracheal cytotoxin, which is an important virulence factor that causes several pathological effects in epithelial cells (7, 21).

Since DAP is neither produced nor required by humans, many efforts have been made to study DAP biosynthetic enzymes (8, 24, 28), and DAP analogs are evaluated for their potential to inhibit bacterial growth. Furthermore, the use of DAP auxotrophic mutants of *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG, *Salmonella* subspecies, or *Helicobacter pylori* as attenuated vaccine strains or for the maintenance of cloning vectors expressing foreign antigens in such attenuated strains has been proposed (9, 17, 23).

Although the biochemistry of the DAP-lysine pathway is very well understood, the genes encoding enzymes involved in this pathway have not been completely characterized. Indeed, only three out of the four genes required for the succinyl pathway of *E. coli*, *dapD*, *dapE*, and *dapF*, encoding THDP succinylase, *N*-succinyl-L,L-DAP desuccinylase, and DAP epimerase, respectively, were known (2). Surprisingly, despite the availability of the entire genomic sequence of *E. coli* and other bacteria, a gene encoding the *N*-succinyl-L,L-DAP aminotransferase had not been identified in any organism. Only recently, ArgD of *E. coli* was shown to exhibit both *N*-acetylornithine and DAP aminotransferase activity, which indicates its participation not only in the arginine but also in the DAP-lysine biosynthesis pathways (20). In the present paper we describe a novel gene locus of *B. pertussis* containing the *dapD* and *dapE* genes as well as a third gene that was characterized as *dapC*, encoding a novel *N*-succinyl-L,L-DAP aminotransferase. The identification of this gene will contribute to our understanding of the DAP biosynthesis pathways and possibly to the development of novel antimicrobials targeting these essential anabolic pathways.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. Strains and plasmids used are listed in Table 1. *E. coli* strains were grown in Luria-Bertani medium (Gibco), and *B. pertussis* was grown on Bordet-Gengos (BG) agar plates supplemented with 15% sheep blood (3), in Stainer-Scholte broth (33), or in Stainer-Scholte broth with

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or relevant characteristic(s)	Reference or source
Strains		
E. coli		
DH5α	F⁻ *recA1 endA1 gyrA96 thi-1 hsdR17*(r_K^- m_K^+) *sup44 relA1* λ⁻ ϕ80d*lacZ*Δ*M15*(*lacZYA-argF*)*U169*	Gibco BRL
DH5αMCR	F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) ϕ80d*lacZ*Δ*M15* Δ(*lacZYA-argF*)*U169 deoR recA1 endA1 supE44* λ⁻ *thi-1 gyrA96 relA1*	14
RDE51	*araD139 thi* Δ(*lac*)*U168 strA dapE*::MuCts	25
AT982	*rel-1 thi-1 dapD4*	7
AT997	*rel-1 thi-1 dapC15*	7
SM10	RP4-2-Tc::Mu *thi thr leu* su_{III} Kan^r; mobilizing donor strain	31
B. pertussis		
Tohama I (TI)	Wild type	Rino Rappuoli
TIΔ*dapC*	Allelic exchange mutant of TI with a 228-bp deletion in *dapC*	This study
Plasmids		
pBluescript SK	High-copy cloning vector	Stratagene
pSS1129	*Bordetella* suicide vector	34
pSK50	5.0-kb *Eco*RI fragment from TI in pSK including the *dap* operon of *B. pertussis* (Fig. 2)	This study
pSK-dapC	2.9-kb *Apa*I fragment from pSK50 containing *dapC* and truncated *dapD* (Fig. 2)	This study
pSK-ΔdapC	Derivative of pSK-dapC with a 228-bp *Nco*I deletion	This study
pSS-ΔdapC	*Eco*RI-*Bam*HI fragment from pSK-ΔdapC joined to pSS1129	This study

Casamino Acids in place of defined amino acid solutions (13). When appropriate, ampicillin (100 μg/ml), streptomycin (100 μg/ml), gentamicin (10 μg/ml), nalidixic acid (20 μg/ml), DAP (40 μg/ml), or lysine (50 μg/ml) was added. Strains were grown aerobically at 37°C with the exception of *E. coli* RDE51, which was cultivated at 30°C. The preparation of competent cells, transformations, plasmid preparations, and DNA manipulations were performed according to standard protocols (26).

Complementation of *E. coli* mutants auxotrophic for DAP biosynthesis. High-molecular-weight chromosomal DNA of the Tohama I wild-type strain was isolated as described previously (12) and digested with *Eco*RI. Fragments with an average size of 1 to 8 kb were ligated into the *Eco*RI-cleaved and calf intestinal alkaline phosphatase-dephosphorylated pBluescript SK vector and cloned in *E. coli* DH5α (Stratagene, San Diego, Calif.). For complementation analyses, two DAP auxotrophic *E. coli* strains, RDE51 and AT982, lacking functional *dapE* and *dapD* loci, respectively, which are able to grow only in the presence of 50 μg of diaminopimelic acid per ml (a mixture of the three DAP isomers; Sigma Chemical Co., St. Louis, Mo.), were used. Competent cells of the RDE51 and AT982 strains were transformed with the pBluescript SK gene library from *B. pertussis* and selection was carried out on Luria-Bertani agar containing ampicillin (50 μg/ml) but no diaminopimelic acid. Plasmid DNA was isolated from colonies grown overnight or after 2 days of incubation.

Construction of a deletion in the *dapC* gene. The plasmid pSK-dapC was digested with *Nco*I and religated, resulting in a 228-bp in-frame deletion within the *dapC* gene (pSK-ΔdapC) (see Fig. 2). An *Eco*RI-*Bam*HI fragment was cloned into the vector pSS1129, resulting in the construct pSS-ΔdapC, which was then transformed in the *E. coli* strain SM10 (31). Plasmid pSS-ΔdapC was then conjugated into *B. pertussis* Tohama I, plating the bacteria on BG agar plates containing DAP and lysine. Selection for allelic exchange was carried out as described elsewhere (6, 34). The presence of the deletion in the *dapC* gene in the respective mutants was verified by Southern blot analysis and by PCR with specific oligonucleotides (26).

DNA sequence analysis. DNA fragments derived from *B. pertussis* and complementing the *E. coli dapD* and *dapE* mutants were sequenced using the Applied Biosystems Prism sequencing kit from Perkin-Elmer and the automated sequencer ABI Prism 310. Sequence data for both strands were obtained by subcloning and primer walking. Analysis of the nucleotide sequences was performed using the Genetics Computer Group program package (10). Protein homology searches were conducted in the SwissProt database using the FASTA and TFASTA programs and in the Prosite database using the MOTIFS program and were further elaborated using the PILEUP program.

Determination of transaminase activity. *E. coli* was grown on minimal medium consisting of (per liter) 7 g of KH_2PO_4, 3 g of K_2HPO_4, 1 g of $(NH_4)_2SO_4$, 246 mg of $MgSO_4 \cdot 7H_2O$, 1 mg of $CaCl_2 \cdot 2H_2O$, 0.5 mg of $FeSO_4 \cdot 7H_2O$, 0.5 mg of $MnSO_4 \cdot 4H_2O$, 0.5 mg of $ZnSO_4 \cdot H_2O$, 0.1 mg of $CuSO_4 \cdot 5H_2O$, 0.05 mg of thiamine, and 5.5 g of glucose · H_2O. Cells were harvested after overnight incubation at 37°C, washed with 0.9% NaCl, resuspended in 20 mM Tris-HCl (pH 8.0), and disrupted with a microtip-equipped sonifier. The homogenate was centrifuged for 20 min at 20,000 × *g*, and the resulting extract was applied to a PD-10 column (Amersham-Pharmacia). Determination of the *N*-acyl-L,L-DAP

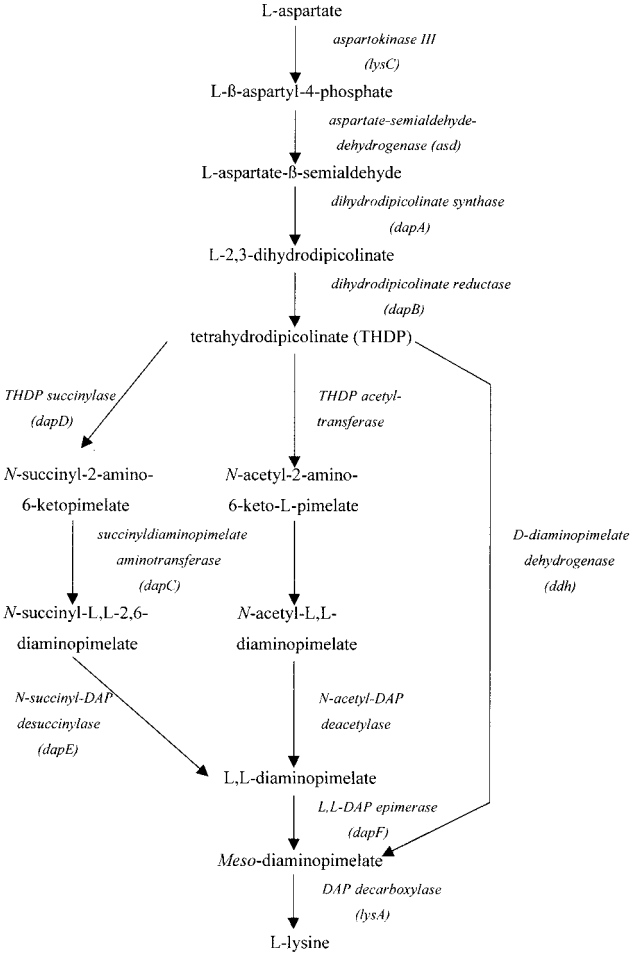

FIG. 1. The split pathway for the synthesis of DAP and lysine in prokaryotes. On the left is shown the succinylase branch, and on the right is shown the dehydrogenase branch. The acetylase variant in the middle is comparable to the succinylase variant but uses acetyl groups instead of succinyl groups. The symbols for genes are given below the enzymes.

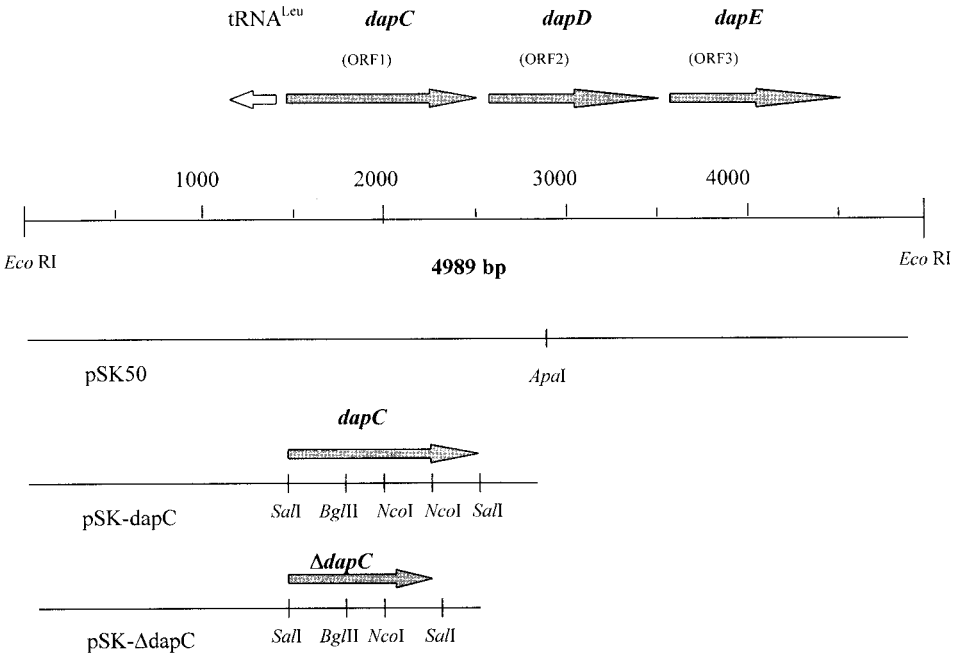

FIG. 2. Schematic representation of the *dapCDE* gene locus of *B. pertussis*. The arrows below the ORFs indicate their transcriptional polarity. Below the *dapCDE* operon the different subclones used in this study are indicated.

aminotransferase activity (EC 2.6.1.17) was based on the succinyl-DAP (or acetyl-DAP-)-dependent formation of glutamate from α-ketoglutarate. The assay system consisted of 200 mM Tris-HCl (pH 8.0), 0.25 mM pyridoxal-5′-phosphate, 4 mM α-ketoglutarate, 8 mM acyl-2,6-DAP, 1 mM EDTA, and gel-filtered extract. Assay mixtures were incubated at 37°C. Samples (30 μl) were taken at different time intervals, and reactions were stopped by addition of 30 μl of stop reagent (0.75 M $HClO_4$ in 7 M ethanol), neutralized with 20 μl of neutralizing solution (0.1 M K_2CO_3, 20 mM Tris-HCl [pH 8.0]), and used for glutamate quantification. This was done by automated precolumn derivatization with *o*-phthaldialdehyde, followed by separation by reversed phase chromatography (LC ChemStation HP 1900) with fluorometric detection (15). Protein concentration was determined after precipitation of the protein (1). All experiments were carried out at least three times.

Nucleotide sequence accession number. The nucleotide sequence of the 5.0-kb *Eco*RI fragment containing the *dapCDE* genes of *B. pertussis* has been deposited in the EMBL data bank under accession number AJ009834.

RESULTS

Cloning and characterization of the *dap* locus of *B. pertussis*. A partial gene bank from *B. pertussis* Tohama I DNA digested with *Eco*RI was established in the high-copy-number vector pBluescript SK and was used to identify six plasmids conferring a stable DAP prototrophy to the *E. coli dapE* mutant RDE51. All six plasmids contained a 5.0-kb *Eco*RI fragment (pSK50) that was also able to complement the *E. coli dapD* mutant AT982, which is blocked in the succinylase step of DAP biosynthesis (Fig. 1). The successful complementation of both *E. coli* strains, RDE51 and AT982, indicated a close linkage of the *dapD* and *dapE* genes in *B. pertussis*.

The complete nucleotide sequence of the 5.0-kb *Eco*RI fragment of pSK50 was determined using a primer walking strategy. The DNA fragment contains three open reading frames (ORFs) consisting of 1,191, 819, and 1,137 bp, encoding putative proteins of 397, 273, and 379 amino acids (Fig. 2), respectively. A tRNA gene coding for the rare $tRNA^{Leu_W}$ was found immediately upstream of the start codon of ORF1. In all three ORFs only one codon specific for this tRNA is present, located in ORF1 at nucleotide position 58 to 60 (see Fig. 4). ORFs 1 and 2 are separated by 26 nucleotides. ORF2 and ORF3 overlap by one codon, indicating translational coupling of the two genes, suggesting that the three ORFs are organized in an operon.

Search for sequence similarities of the putative ORFs. The 5.0-kb *Eco*RI fragment derived from *B. pertussis* complements auxotrophic *E. coli* mutants with defective *dapD* and *dapE* genes on media lacking DAP. Indeed, the growth characteristics of the complemented mutants are very similar to that of the *E. coli* wild-type strain (data not shown). Consequently, comparison of the amino acid sequences revealed high similarities between the putative protein encoded by ORF2 and the DapD proteins from *E. coli* (25), *Haemophilus influenzae* (11), and *Actinobacillus pleuropneumoniae* (19) and between the protein deduced from the DNA sequence of ORF3 and the DapE proteins from *E. coli* (4), *H. influenzae* (11), and *H. pylori* (36). Similarities shown in Fig. 3 range from 79 to 82% (DapD) and from 58 to 77% (DapE). These data demonstrate that ORF2 and ORF3 do indeed code for the *Bordetella* counterparts of the THDP succinylase DapD and the *N*-succinyl-L,L-DAP desuccinylase DapE, respectively.

ORF1 encodes a hypothetical protein with an overall amino acid similarity of about 40% to several putative proteins from *H. pylori* (36), *Bacillus subtilis* (32), *Synechocystis* sp. (16) and other bacteria (Fig. 4). The motif search revealed that the amino acid sequences of ORF1 and the homologous proteins contain a pyridoxal-5′-phosphate attachment site (Fig. 4), which is a characteristic feature of transaminases (35). Accordingly, at least one of the proteins with significant sequence similarities to *B. pertussis* ORF1, AspB from *B. subtilis*, was shown to exhibit aminotransferase activity (32).

Characterization of ORF1 and elucidation of its function as a transaminase involved in DAP biosynthesis. The sequence analysis and the close linkage of ORF1 to the THDP succinylase- and *N*-succinyl-L,L-DAP desuccinylase-encoding genes *dapD* and *dapE* suggested that it might encode the *N*-succinyl-L,L-DAP aminotransferase converting the product of the succinylase to the substrate of the desuccinylase. We therefore

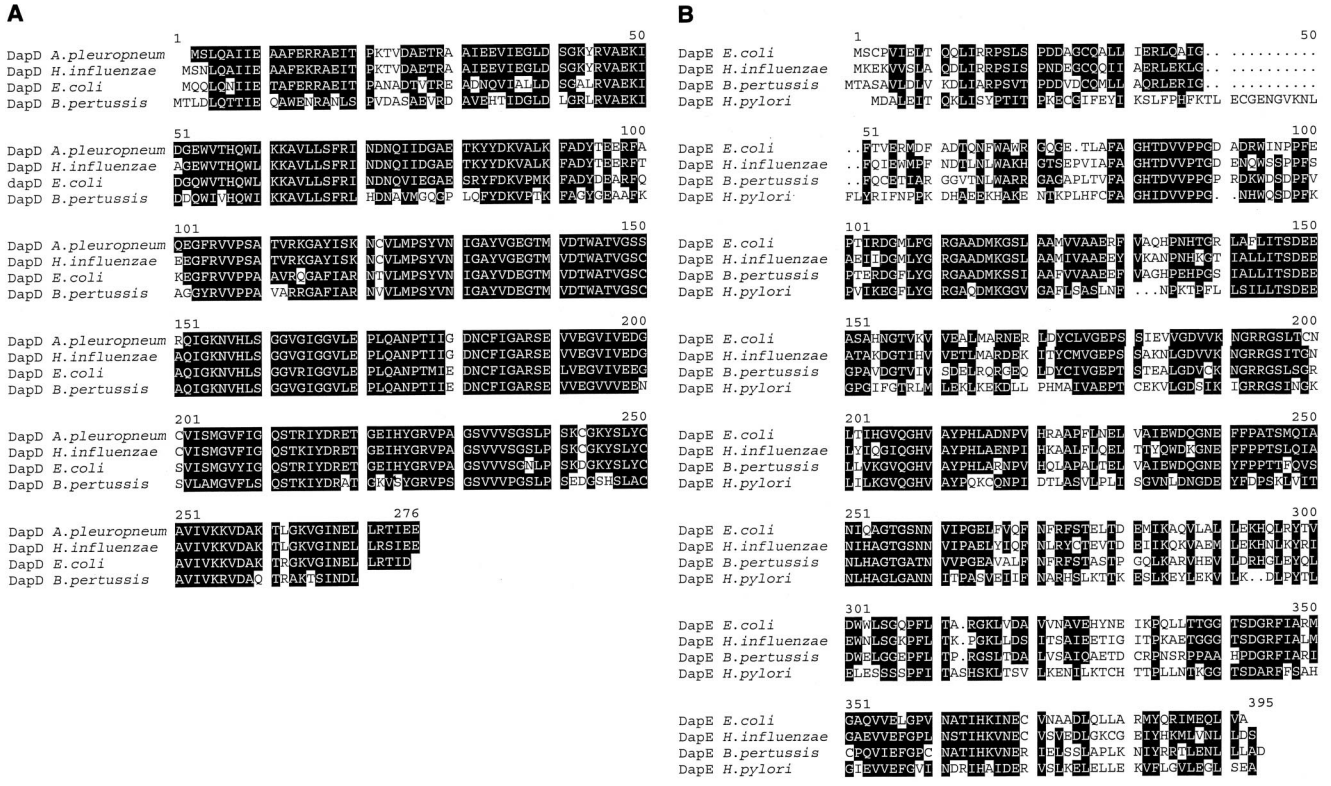

FIG. 3. Sequence homologies between the DapD and DapE proteins of several bacteria. Shown are multiple sequence alignments of DapD of *A. pleuropneumoniae* (GenBank accession number P41396), *H. influenzae* (GenBank accession number P45284), and *E. coli* (GenBank accession number K02970) to the product of ORF2 (DapD *B. pertussis*) (A) and of DapE of *E. coli* (GenBank accession number X57403), *H. influenzae* (GenBank accession number P444514), and *H. pylori* (36) (HP0212) (B) to the product of ORF3 (DapE *B. pertussis*). Amino acids identical or similar in at least three proteins are shaded. Groups of similar amino acids are as follows: D, E, Q, and N; A, S, G, P, and T; F, Y, and W; K, H, and R; V, L, I, and M; and C.

functionally characterized the gene product of ORF1 by directly assaying for transaminase activity. For this purpose, the *E. coli* strain DH5αMCR was transformed with pSK50 containing the 5.0-kb *Eco*RI fragment with the entire operon consisting of ORF1, *dapD*, and *dapE*, with plasmid pSK-dapC, a derivative of pSK50 containing only ORF1 and a truncated *dapD*, or, as controls, with plasmid pSK-ΔdapC or the vector alone (Fig. 2). When the extracts of the two control strains were incubated with succinyl-DAP and α-ketoglutarate, formation of L-glutamate was obtained only due to the chromosomally encoded transaminase activity of *E. coli* (Fig. 5A). However, when the extract of *E. coli* pSK50 or pSK-dapC was used in the enzyme assay, a significant increase in succinyl-DAP-dependent L-glutamate accumulation was observed (Fig. 5A). Based on the amount of protein present in the respective assay of recombinant *E. coli* strains, the following specific activities (in micromoles $minute^{-1}$ milligram of $protein^{-1}$) were determined by subtracting the basal activity and by calculation of the amount of protein present in the assay: with pSK50, 0.014 ± 0.006, and with pSK-dapC, 0.013 ± 0.005. Therefore, ORF1 exhibits transaminase activity and was accordingly designated *dapC*. In addition to succinyl-DAP, acetyl-DAP was also applied as a substrate for the transaminase, since the various bacteria analyzed so far possess succinyl-DAP- or acetyl-DAP-specific enzyme activities when assayed in crude extracts (30, 38). Using acetyl-DAP as an alternative substrate, a significant activity with extracts of *E. coli* pSK50 and pSK-dapC was detected (Fig. 5B), whereas extracts of *E. coli* pSK and pSK-ΔdapC showed almost no transaminase activity. The specific activity for extracts of *E. coli* pSK50 or pSK-dapC with acetyl-DAP as the substrate was calculated (see above) to be 0.0047 ± 0.001 μmol min^{-1} mg of $protein^{-1}$. The low background activity is consistent with the inability of the *E. coli* enzyme to use acetyl-DAP as a substrate when assayed in extracts.

Characterization of a *B. pertussis dapC* mutant. To further substantiate the participation of ORF1 in the DAP-lysine biosynthesis pathway, we tried to introduce by allelic exchange the *dapC* allele containing the deletion of its pyridoxal phosphate binding motif into the chromosome of *B. pertussis*. However, despite the addition of DAP and lysine to the selection medium, after several independent attempts we were able to identify only a single mutant out of several hundred clones screened that carried the deletion within the *dapC* gene (data not shown). The only obvious phenotype of this mutant was a significantly reduced generation time compared with that of the wild type (7.05 ± 0.60 h versus 5.57 ± 0.30 h). The addition of DAP or lysine to the medium had no obvious effect and did not influence the generation time of this mutant.

DISCUSSION

The investigation of a chromosomal locus of *B. pertussis* able to complement *E. coli dapD* and *dapE* mutants led to the identification of a new gene, *dapC*. Due to the close linkage of these three genes they appear to constitute an operon with *dapC* being the first gene in the order of transcription. The *dapD* and *dapE* genes encode enzymes with THDP succinylase and *N*-succinyl-L,L-DAP desuccinylase activity, respectively, and their amino acid sequences show extensive similarities with

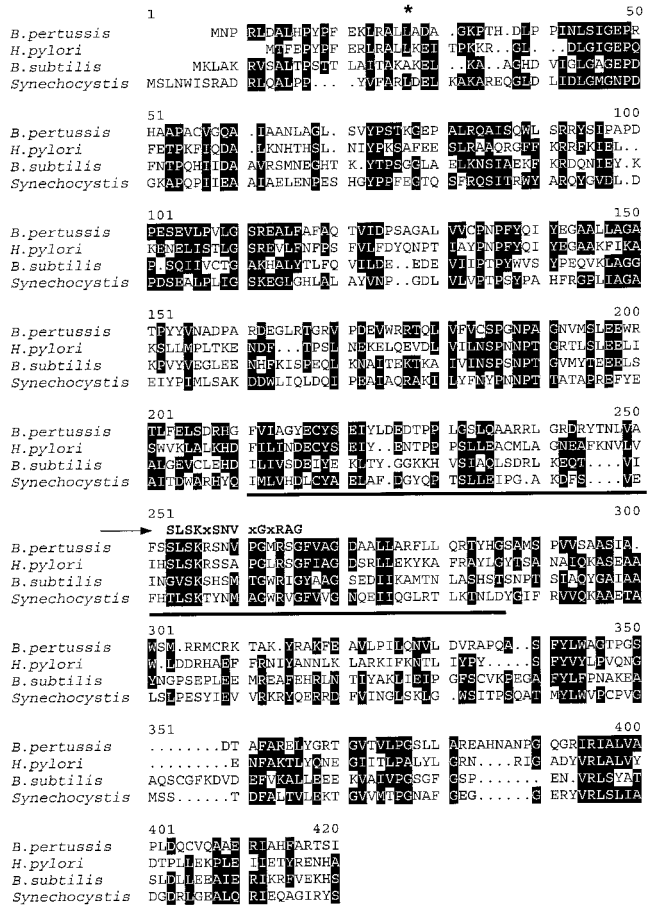

FIG. 4. Sequence homologies between DapC and several putative aminotransferases. Shown is a multiple sequence alignment of DapC from *B. pertussis* and the product of three ORFs from *H. pylori* (36) (HP0624), *B. subtilis* (GenBank accession number P53001), and *Synechocystis* sp. (GenBank accession number D64000). Amino acids identical or similar in at least three positions are shaded. Groups of similar amino acids are as given in the legend to Fig. 3. The arrow indicates the consensus sequence of the pyridoxal-5′-phosphate binding site common to several aminotransferases. The active site lysyl residue that binds pyridoxal phosphate through Schiff base linkage is found within this consensus sequence at position 256. The bar below the sequence marks 76 amino acids which were deleted in pSK-ΔdapC. The only specific codon for the rare $tRNA^{LeuW}$ encoded by a gene immediately upstream of ORF1 is marked by an asterisk.

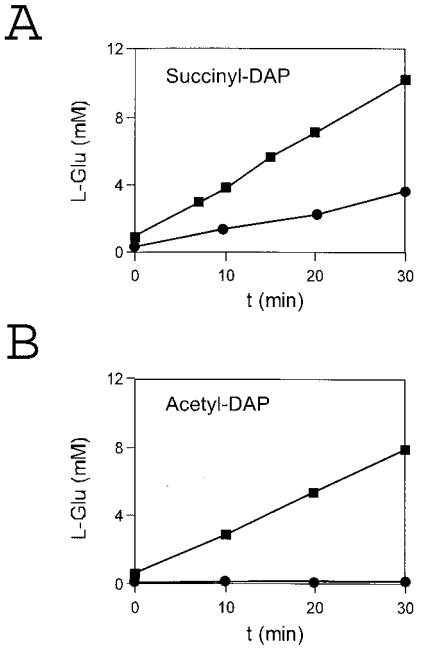

FIG. 5. Transaminase activity in crude extracts of *E. coli* strains harboring plasmids containing different parts of the *B. pertussis dapCDE* locus, as quantified by L-glutamate accumulation, with either pSK50 (■) or pSK (●) and *N*-succinyl-L,L-DAP as substrate (A) or with either pSK-dapC (■) or pSK (●) and *N*-acetyl-L,L-DAP as substrate (B). The protein amount in the respective assay was 37 μg with pSK50 and 22 μg with pSK (Fig. 5A) and 114 μg with pSK50 and 44 μg with pSK (Fig. 5B), respectively.

their *E. coli* counterparts (Fig. 3). The *dapC* gene codes for a protein sharing significant sequence similarities with several aminotransferases. The presence of the *dapC* gene within the putative DAP operon prompted us to investigate its possible involvement in DAP biosynthesis. In fact, a transaminase is required to convert the product of DapD, *N*-succinyl-2-amino-6-ketopimelate, to the substrate of DapE, *N*-succinyl-L,L-2,6-DAP. Accordingly, transaminase activity could be detected in crude lysates of an *E. coli* strain transformed with the *B. pertussis dapC* gene using *N*-succinyl-DAP as a substrate. Interestingly, *N*-acetyl-DAP was also accepted as a substrate, although with about half of the activity observed for *N*-succinyl-DAP. We conclude that *dapC* encodes the transaminase involved in DAP biosynthesis of *B. pertussis* and that its most likely substrate in vivo is *N*-succinyl-L,L-DAP.

In early experiments with extracts of *E. coli*, Weinberger and Gilvarg (38) demonstrated a quite strict requirement of the *E. coli N*-succinyl-L,L-DAP aminotransferase, which accepted *N*-succinyl-L,L-DAP but not *N*-succinyl-*meso*-DAP as a substrate. More recently, similar experiments using the purified enzyme contradicted these findings and showed that the purified *E. coli* transaminase accepted compounds with rather large structural alterations at the position of the succinyl group (8). These data are in agreement with our results regarding the substrate specificity of the *B. pertussis* enzyme. In fact, many transaminases are known to exhibit quite relaxed substrate specificities (30). The apparently much less pronounced sequence conservation among DapC homologs of different bacteria as compared to the strong sequence conservation of DapD or DapE proteins of different organisms (Fig. 3 and 4) could represent the structural counterpart of this characteristic feature.

In the literature confusion still exists regarding the gene symbol-enzyme relationships of the *dapC* and *dapD* genes (22), and in several database annotations *dapD* genes are still incorrectly reported to encode the *N*-succinyl-L,L-DAP aminotransferases instead of the THDP succinylases (2). Moreover, in none of the bacterial genomes sequenced so far could a candidate gene encoding a DAP-specific aminotransferase be identified (20). In this respect, it is worth mentioning that the *B. pertussis dapCDE* locus was not able to functionally complement the only available *E. coli* strain (AT997) (data not shown) that was isolated as a *dapC* mutant (5) but later did not prove to have a clear phenotype. In fact, it was impossible to reconcile the originally reported map position of the mutation and the genome sequence of *E. coli*.

A major breakthrough in our understanding of these puzzling findings was recently achieved in a study which shows that in *E. coli* the *argD*-encoded *N*-acetylornithine aminotransferase also exhibits *N*-succinyl-L,L-DAP aminotransferase activity and appears to be engaged in both pathways (20). This finding is of particular importance because it may provide an explanation for the unusual difficulties we experienced with the

genetic inactivation of the *dapC* gene of *B. pertussis*. Assuming that the *dapC* gene product is participating in additional biosynthetic pathways, its deletion may require suppressor mutations in other aminotransferases, which generally show quite low substrate specificities, to supply the lost function(s). In fact, the only *dapC* mutant of *B. pertussis* that was obtained exhibits a general growth defect which appears to be independent of the presence of DAP or lysine in the culture medium. The involvement of the *B. pertussis* DapC protein in additional pathways will be the subject of future investigations.

It is interesting that, apart from the pyridoxal-5′-phosphate binding motif, the DapC protein of *B. pertussis* and ArgD of *E. coli* do not show further amino acid sequence similarities, and accordingly, BLAST searches with DapC in the *E. coli* genome sequence or vice versa with ArgD in the *B. pertussis* genome sequence resulted in the alignment with several other putative aminotransferases with high significance values, but not with each other (data not shown). The identification of ArgD of *E. coli* and of DapC of *B. pertussis* as enzymes with *N*-succinyl-L,L-DAP aminotransferase activities finally solves a long-lasting debate and closes an important gap in our knowledge about this crucial biosynthetic pathway specific to eubacteria. Moreover, the involvement of proteins with entirely different primary structures in identical biosynthetic steps poses interesting evolutionary questions and has important consequences for the design of antimicrobial drugs directed against such enzymes.

ACKNOWLEDGMENTS

We thank Degussa AG for the synthesis of succinyl-DAP and acetyl-DAP, Klaus Hantke (Tübingen) for the DAP auxotroph *E. coli* mutants, and Carol Gibbs and Dagmar Beier for critical reading of the manuscript.

This work was supported by a postdoctoral fellowship of the Deutsche Forschungsgemeinschaft to T.M.F., by grant SFB479/A2 of the Deutsche Forschungsgemeinschaft, and by the Fonds der Chemischen Industrie to R.G.

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