

# PduL Is an Evolutionarily Distinct Phosphotransacylase Involved in B<sub>12</sub>-Dependent 1,2-Propanediol Degradation by *Salmonella enterica* Serovar Typhimurium LT2<sup>∇</sup>

Yu Liu,<sup>1</sup> Nicole A. Leal,<sup>2</sup> Edith M. Sampson,<sup>3</sup> Celeste L. V. Johnson,<sup>4</sup>  
Gregory D. Havemann,<sup>5</sup> and Thomas A. Bobik<sup>1\*</sup>

Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, Iowa 50011<sup>1</sup>; Foundation for Applied Molecular Evolution, Gainesville, Florida 32601<sup>2</sup>; Department of Pediatrics, University of Florida, Gainesville, Florida 32611<sup>3</sup>; Department of Pediatrics, Section of Oncology and Hematology, Baylor College of Medicine, Houston, Texas 77030<sup>4</sup>; and Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611<sup>5</sup>

Received 28 July 2006/Accepted 3 December 2006

*Salmonella enterica* degrades 1,2-propanediol (1,2-PD) in a coenzyme B<sub>12</sub>-dependent manner. Previous enzymatic assays of crude cell extracts indicated that a phosphotransacylase (PTAC) was needed for this process, but the enzyme involved was not identified. Here, we show that the *pduL* gene encodes an evolutionarily distinct PTAC used for 1,2-PD degradation. Growth tests showed that *pduL* mutants were unable to ferment 1,2-PD and were also impaired for aerobic growth on this compound. Enzyme assays showed that cell extracts from a *pduL* mutant lacked measurable PTAC activity in a background that also carried a *pta* mutation (the *pta* gene was previously shown to encode a PTAC enzyme). Ectopic expression of *pduL* corrected the growth defects of a *pta* mutant. PduL fused to eight C-terminal histidine residues (PduL-His<sub>8</sub>) was purified, and its kinetic constants were determined: the  $V_{\max}$  was  $51.7 \pm 7.6 \mu\text{mol min}^{-1} \text{mg}^{-1}$ , and the  $K_m$  values for propionyl-PO<sub>4</sub><sup>2-</sup> and acetyl-PO<sub>4</sub><sup>2-</sup> were 0.61 and 0.97 mM, respectively. Sequence analyses showed that PduL is unrelated in amino acid sequence to known PTAC enzymes and that PduL homologues are distributed among at least 49 bacterial species but are absent from the *Archaea* and *Eukarya*.

A number of bacterial genera including *Salmonella*, *Klebsiella*, *Shigella*, *Yersinia*, *Listeria*, *Lactobacillus*, and *Lactococcus* include members that grow on 1,2-propanediol (1,2-PD) in a coenzyme B<sub>12</sub>-dependent fashion. 1,2-PD is a major product of the fermentation of rhamnose and fucose, which are common sugars in plant cell walls, bacterial exopolysaccharides, and the glycoconjugates of intestinal epithelial cells. Accordingly, the ability to degrade 1,2-PD is thought to provide a selective advantage in anaerobic environments such as the large intestines of higher animals, sediments, and the depths of soils. Recent studies with *Salmonella enterica* have shown that 1,2-PD degradation is one of the most complex metabolic processes known (6, 14, 15, 18, 19, 31). The degradation of this small molecule requires catabolic enzymes, a system for recycling inactive cobalamins to coenzyme B<sub>12</sub>, and an unusual polyhedral body-microcompartment that is composed of metabolic enzymes encased within a multiprotein shell.

A pathway for 1,2-PD degradation by *S. enterica* has been proposed based on enzymatic studies of crude cell extracts and genetic analyses (25, 37). In the first step of the pathway, 1,2-PD is converted to propionaldehyde via coenzyme B<sub>12</sub>-dependent diol dehydratase (1). Propionaldehyde is then converted to 1-propanol and propionic acid by a reaction series thought to involve 1-propanol dehydrogenase, coenzyme A

(CoA)-dependent propionaldehyde dehydrogenase, phosphotransacylase (PTAC), and propionate kinase (25, 37). This pathway generates one ATP, an electron sink, and a three-carbon intermediate (propionyl-CoA), which feeds into central metabolism via the methylcitrate pathway (16).

The genes specifically required for 1,2-PD utilization (*pdu*) by *S. enterica* form a single contiguous cluster, the *pdu* locus. DNA sequence analyses indicate that this locus includes 23 genes (6, 7, 10). Based on experimental and/or bioinformatic analyses, six *pdu* genes are thought to encode enzymes needed for the 1,2-PD degradative pathway (6), two are involved in transport and regulation (5, 10), two are probably involved in diol dehydratase reactivation (6), two are used for the conversion of vitamin B<sub>12</sub> to coenzyme B<sub>12</sub> (18, 31), four are of unknown function, and seven share similarity to genes involved in the formation of carboxysomes, a polyhedral body found in certain cyanobacteria and chemoautotrophs (6, 10). Although the methylcitrate pathway is required for the growth of *S. enterica* on 1,2-PD as a sole carbon source, the genes for this pathway map outside the *pdu* locus (16).

It was recently shown that *S. enterica* forms polyhedral bodies (PhBs) during growth on 1,2-PD (6, 15, 36). These bodies are extremely large macromolecular complexes that are 100 to 150 nm in cross section and consist of metabolic enzymes encased within a protein shell. Purification of these structures showed that they are composed of at least 14 different polypeptides (PduABB'CDEGHJKOPTU) (14). This includes four enzymes and as many as seven shell proteins, all of which are encoded by the *pdu* locus. The first two steps of 1,2-PD degradation are thought to occur in the lumen of the PhBs, and

\* Corresponding author. Mailing address: Iowa State University Department of Biochemistry, Biophysics, and Molecular Biology, Ames, IA 50011. Phone: (515) 294-4165. Fax: (515) 294-0453. E-mail: bobik@iastate.edu.

<sup>∇</sup> Published ahead of print on 8 December 2006.

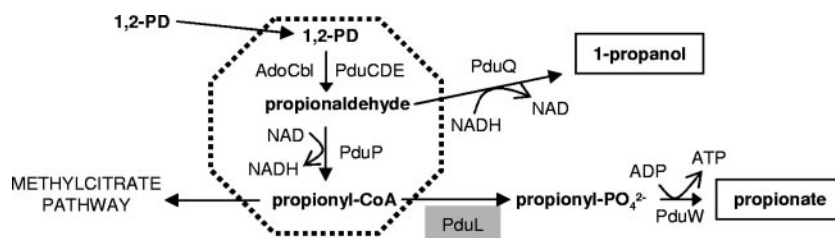


FIG. 1. Model for 1,2-propanediol degradation by *S. enterica*. Coenzyme B<sub>12</sub> (AdoCbl), coenzyme B<sub>12</sub>-dependent diol dehydratase (PduCDE), propionaldehyde dehydrogenase (PduP), phosphotransacylase (PduL), propionate kinase (PduW), and 1-propanol dehydrogenase (PduQ) are depicted. The first two steps of 1,2-PD degradation (conversion of 1,2-PD to propionyl-CoA) are proposed to occur within a polyhedral body-microcompartment. The dashed line indicates the shell of the polyhedral body, which is composed of up to seven different polypeptides. ATP is generated during the conversion of propionyl-PO<sub>4</sub><sup>2-</sup> to propionate. Given a suitable terminal electron acceptor such as O<sub>2</sub>, propionyl-CoA is degraded via the methylcitrate pathway, where biosynthetic precursors and additional energy are produced. Two additional enzymes associated with the polyhedral bodies (but not shown in the figure) are a putative diol dehydratase reactivase (PduGH) and an ATP:cob(I)alamin adenosyltransferase (PduO) involved in B<sub>12</sub> recycling.

the remaining steps are thought to occur in the cytoplasm of the cell (Fig. 1) (14). The function of PhBs is uncertain. Previous studies suggested that they act as microcompartments that sequester propionaldehyde (an intermediate of 1,2-PD degradation) in order to minimize toxicity and/or prevent carbon loss (9, 14, 15, 28). Interestingly, recent genomic analyses tentatively indicated that there are seven functionally distinct polyhedral bodies distributed among over 40 genera of bacteria (4). Thus, polyhedral bodies may be a general mechanism of metabolic organization in the *Bacteria*.

As described above, previous enzymatic analysis of crude cell extracts indicated that a PTAC is used for 1,2-PD degradation (25, 37). However, the enzyme involved was not identified. Here, we show that the *pduL* gene encodes a conserved, evolutionarily distinct PTAC that functions in 1,2-PD degradation by *S. enterica*.

#### MATERIALS AND METHODS

**Chemicals and reagents.** Antibiotics, vitamin B<sub>12</sub>, and acetyl-PO<sub>4</sub><sup>2-</sup> were obtained from Sigma Chemical Company (St. Louis, MO). Propionyl-PO<sub>4</sub><sup>2-</sup> was synthesized as described previously (27). Isopropyl-β-D-thiogalactopyranoside

(IPTG) was obtained from Diagnostic Chemicals Limited (Charlotteville, PEI, Canada). Restriction enzymes and T4 DNA ligase were obtained from New England Biolabs (Beverly, MA). Coomassie brilliant blue R-250, EDTA, ethidium bromide, 2-mercaptoethanol, and sodium dodecyl sulfate (SDS) were obtained from Bio-Rad (Hercules, CA). Other chemicals were obtained from Fisher Scientific (Pittsburgh, PA).

**Bacterial strains, media, and growth conditions.** The bacterial strains used in this study are listed in Table 1. The rich medium used was Luria-Bertani/Lennox (LB) medium (Difco, Detroit, MI) (23). The minimal medium used was no-carbon-E (NCE) medium containing supplements indicated in the text and figure legends (3, 38). MacConkey-1,2-PD indicator medium contained MacConkey agar base (Difco), 1% 1,2-PD, and 200 ng ml<sup>-1</sup> of vitamin B<sub>12</sub>.

**General molecular methods.** Agarose gel electrophoresis was performed as described previously (30). Plasmid DNA was purified by the alkaline lysis procedure (30) or by using QIAGEN (Chatsworth, CA) products according to the manufacturer's instructions. Following restriction digestion or PCR amplification, DNA was purified using Promega Wizard PCR Preps (Madison, WI) or QIAGEN gel extraction kits. Restriction digests were carried out using standard protocols (30). For ligation of DNA fragments, T4 DNA ligase was used according to the manufacturer's directions. Electroporation was carried out as previously described (6).

**Protein methods.** Polyacrylamide gel electrophoresis (PAGE) was performed using Bio-Rad Redigels and Bio-Rad Mini-Protein II electrophoresis cells according to the manufacturer's instructions. Following gel electrophoresis, Coomassie brilliant blue R-250 was used to stain proteins. The

TABLE 1. Bacterial strains used in this study

Species	Strain	Genotype
<i>S. enterica</i> serovar Typhimurium LT2	BE47	<i>thr-480::Tn10dCam</i>
	BE188	<i>ΔpduL670</i>
	BE281	<i>pta-406::Tn10</i>
	BE284	<i>ΔpduL670/pLAC22-pduL (Amp<sup>r</sup>)</i>
	BE285	<i>ΔpduL670/pLAC22-no insert (Amp<sup>r</sup>)</i>
	BE286	<i>pLAC22-pduL (Amp<sup>r</sup>)</i>
	BE287	<i>pLAC22-no insert (Amp<sup>r</sup>)</i>
	BE291	<i>ΔpduL670 pta-209::Tn10</i>
	BE527	<i>pta-209::Tn10</i>
	BE529	<i>pta-209::Tn10/pLAC22-no insert (Amp<sup>r</sup>)</i>
	BE530	<i>pta-209::Tn10/pLAC22-pduL (Amp<sup>r</sup>)</i>
	BE548	<i>pta-209::Tn10/pBE522-no insert (Kan<sup>r</sup>)</i>
	BE549	<i>pta-209::Tn10/pBE522-pta (Kan<sup>r</sup>)</i>
	<i>E. coli</i>	BL21(DE3)RIL
BE554		BL21(DE3)RIL/pTA925- <i>pduL</i> -His <sub>8</sub> (T7 expression vector containing a minimal clone for production of PduL-his <sub>8</sub> , Kan <sup>r</sup> )
BE119		BL21(DE3)RIL/pTA925-no insert (Kan <sup>r</sup> )
S17.1λpir		<i>recA</i> (RP4-2-Tc::Mu) λpir

protein concentration of solutions was determined using Bio-Rad protein assay reagent (Bio-Rad).

**P22 transduction.** Transductional crosses were performed using P22 HT105/1 *int*-210 (12), a mutant phage that has high transducing ability (32), as described previously. Transductants were tested for phage contamination and sensitivity by streaking on green plates against P22 H5.

**Construction of plasmids for production of PduL and PduL-His<sub>8</sub>.** PCR was used to amplify the *pduL* coding sequence from template pMG52 (15). The primers used for amplification were 5'-GCCGCCAGATCTATGGATAAAGA GCTTCTGCAATCA-3' and 5'-GCCGCCAAGCTTATTATCGCGGGCCTAC CAGCCG-3'. These PCR primers introduced BglIII and HindIII restriction sites that were used for cloning into vector pLAC22 (39). Following ligation, clones were introduced into *Escherichia coli* DH5 $\alpha$  by electroporation, and transformants were selected by plating onto LB agar supplemented with 100  $\mu\text{g ml}^{-1}$  ampicillin (Amp) (18). Pure cultures were prepared from selected transformants. The presence of insert DNA was verified by restriction analysis or PCR, and the DNA sequence of selected *pduL* clones was determined. Clones with the expected DNA sequence were used for further study.

A similar procedure was used to clone PduL fused to eight C-terminal histidine residues (PduL-His<sub>8</sub>) with the following differences. The primers used for PCR amplification were 5'-GCCGCCAGATCTATGGATAAAGAGCTTCTG CAATCA-3' and 5'-GCCGCCAAGCTTATTAATGATGATGATGATGATGATG ATGATGTCGCGGGCCTACCAAGCCG-3'. The PCR product was ligated into T7 expression vector pTA925 (18). Transformation was done by electroporation, and LB plates supplemented with 25  $\mu\text{g ml}^{-1}$  kanamycin (Kan) were used to select for transformants.

**Growth of the PduL-His<sub>8</sub> production strain.** A T7 expression plasmid (pTA925) was used for the production of PduL-His<sub>8</sub>. The host used for protein expression was *E. coli* BL21(DE3)RIL (Stratagene). This strain expresses T7 RNA polymerase as well as rare tRNAs for arginine, isoleucine, and leucine. Expression and control strains (BE554 and BE119) were grown in 400 ml LB broth containing 25  $\mu\text{g/ml}$  Kan and 10  $\mu\text{g/ml}$  chloramphenicol. Cultures were incubated at 15°C with shaking at 275 rpm in a 1-liter baffled Erlenmeyer flask. Cells were grown to an optical density at 600 nm of 0.6 to 0.8, and protein expression was induced by the addition of 1 mM IPTG. Cells were incubated for an additional 16 h and harvested by centrifugation at 8,000  $\times g$  for 10 min and at 4°C using a Beckman JA-10 rotor and an Avanti J-25 centrifuge.

**Purification of PduL-His<sub>8</sub>.** The PduL-His<sub>8</sub> production strain (BE554) was grown as described above. One gram of cells (wet weight) was suspended in 3 ml of buffer containing 50 mM Tris-HCl (pH 7.2), 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and 0.4 mM AEBF [4-(2-aminoethyl)benzenesulfonylfluoride-HCl]. Cells were broken using a French pressure cell at 20,000 lb/in<sup>2</sup>, and the resulting cell extract was centrifuged at 20,000  $\times g$  using a Beckman JA-17 rotor. The supernatant fraction was filtered through a 0.45- $\mu\text{m}$  syringe filter. A chromatography column containing 1 ml of Ni-nitrilotriacetic acid resin (QIAGEN) was equilibrated with 5 ml of buffer A [50 mM Tris-HCl (pH 7.7), 25 mM KCl, 300 mM NaCl, 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 10 mM imidazole, and 5 mM 2-mercaptoethanol]. Filtered cell extract (2.5 ml, about 50 mg protein) was applied to the column. The column was washed with 15 ml of buffer A supplemented with 10% glycerol and 80 mM imidazole. The column was eluted with 10 ml of buffer A (in two of 5-ml steps) supplemented with 10% glycerol and 400 mM imidazole.

**Preparation of cell extracts of *S. enterica*.** Cells were grown under conditions that induce the *pdu* operon (5). Cell paste was suspended in 25 mM Tris-HCl (pH 7.2) containing 0.4 mM protease inhibitor AEBF (3 ml of buffer per gram cells [wet weight]). Cells were broken using a French pressure cell (Thermo Electron Corp., Waltham, MA) at 20,000 lb/in<sup>2</sup>.

**PTAC assays.** PTAC assays measured the conversion of acyl-PO<sub>4</sub><sup>2-</sup> plus coenzyme A (HS-CoA) to acyl-CoA and were performed as described previously (21). Standard assay mixtures contained 50 mM Tris-HCl (pH 7.2), 20 mM KCl, 0.2 mM HS-CoA, and 1 mM acyl-PO<sub>4</sub><sup>2-</sup> in a total volume of 1 ml. For kinetic studies, the concentrations of certain assay components varied as indicated in the text. Activity was determined by monitoring the absorbance at 232 nm over time and by using an  $E_{232}$  of 5.5  $\text{mM}^{-1} \text{cm}^{-1}$  for calculations.

**Construction of a nonpolar PduL deletion.** Bases 22 to 612 of the *pduL* coding sequence were deleted via a PCR-based method (24). The deletion was designed to leave all predicted translational start and stop signals of *pdu* genes intact. The following primers were used for PCR amplification of the flanking regions of the *pduL* gene: 5'-GCTCTAGAGCCGAAATCAGCCTAATCGATGGCG-3' (primer 1), 5'-CGTTCATCGCGGGCCTACAGCCGATCCATTACGCTTACCTCG C-3' (primer 2), 5'-CGGCTGGTAGGCCGCGATGAACG-3' (primer 3), and 5'-CGAGCTCGCAGATGCATGATTTACTC-3' (primer 4). Primers 1 and 2 were used to amplify a 498-bp region upstream of the *pduL* gene, and primers 3 and 4 were used to amplify 527 bases downstream of the *pduL* gene. The

upstream and downstream amplification products were purified and then fused by a PCR that included 1 ng/ $\mu\text{l}$  of each product and primers 1 and 4. The fused product was digested with XbaI and SacI (these sites were designed into primers 1 and 4, respectively) and ligated into suicide vector pCVD442 that had been similarly digested. The ligation mixture was used to transform *E. coli* S17.1 by electroporation, and transformants were selected on LB medium supplemented with Amp (100  $\mu\text{g/ml}$ ). Six transformants were screened by restriction analysis, and all transformants released an insert of the expected size (1,058 bp). One of these transformants was used to introduce the *pduL* deletion into the *S. enterica* chromosome using the procedure described previously by Miller and Mekalanos (24), with the following modifications. For the conjugation step, strain BE47 was used as the recipient, and exconjugants were selected by plating onto LB agar supplemented with Amp (100  $\mu\text{g/ml}$ ) and chloramphenicol (20  $\mu\text{g/ml}$ ). Deletion of the *pduL* coding sequence was verified by PCR using chromosomal DNA as a template. Finally, the *thr-480* dCAM insertion used for selection of exconjugants was "crossed off" by P22 transduction using a phage lysate prepared with the wild-type strain and by selecting for prototrophy on NCE glucose minimal medium.

**Aerobic growth curves.** Growth media are described in the figure legends. For strains carrying pLAC22, media were also supplemented with 100  $\mu\text{g/ml}$  Amp and 0.2 mM IPTG. To prepare the inoculum, LB cultures (2 ml) were incubated overnight at 37°C, and cells were then collected by centrifugation and resuspended in growth curve medium. Media were inoculated to a density of 0.15 absorbance units, and growth was monitored by measuring the optical density at 600 nm using a BioTek Synergy microplate reader with 48-well flat-bottom plates (Falcon), 0.5 ml of growth medium per well at 37°C, and shaking set at level 4. A relatively low volume of growth medium (0.5 ml) in a 48-well microplate was necessary for adequate aeration, and controls showed that growth under these conditions was similar to growth in shake flasks (data not shown).

**Anaerobic growth curves.** A procedure similar to that for aerobic growth curves was used to measure the fermentation of 1,2-PD but with the following differences. Cultures were inoculated to an initial density of 0.1 absorbance units. Microplates were sealed with polyolefin membranes (Fisher Scientific) inside an anaerobic growth chamber (Coy Laboratory Products, Grass Lake, MI). To monitor whether anaerobic conditions were maintained within the microplate, NCE glycerol minimal medium was added to several wells and inoculated with wild-type *S. enterica*. *S. enterica* cannot ferment glycerol; therefore, growth on glycerol would indicate the presence of oxygen. For the experiments described in this study, no growth in wells containing glycerol minimal medium was observed, indicating that the headspace contained minimal amounts of oxygen (data not shown).

**DNA sequencing and analysis.** DNA sequencing was carried out at the Iowa State University (ISU) DNA Facility using automated sequencing equipment from Applied Biosystems Inc. The template for DNA sequencing was plasmid DNA purified using QIAGEN 100 tips or QIAGEN Mini-Prep kits. Blast software was used for sequence similarity searching (2).

**High-pressure liquid chromatography (HPLC).** A Microsorb C<sub>18</sub> column (150 by 4.6 mm) was used with a Varian ProStar system that included a model 230 solvent delivery module, a model 430 autosampler, and a model 325 UV-Vis detector (Varian, Palo Alto, CA). Buffers A and B contained 10 mM NH<sub>4</sub> formate (pH 4.6) and 10% or 90% methanol, respectively. The flow rate was 1 ml min<sup>-1</sup>, and the buffer composition varied as follows (minute:percent buffer): 0:0, 5:0, 17:100, 22:100, 23:0, and 28:0.

**HPLC electrospray ionization mass spectrometry (HPLC-ESI-MS).** An Agilent (Palo Alto, CA) ion trap model 1100 mass spectrometer was operated in the positive mode. The ion source parameters were optimized for the formation of [M + H]<sup>+</sup> ions with a source temperature of 310°C, a capillary voltage of 3.2 kV, and a cone voltage of 25 V. Nitrogen was used as the nebulizing gas and as the drying gas at flow rates of 15 and 400 liters h<sup>-1</sup>, respectively.

## RESULTS

**The *pta* gene is nonessential for 1,2-PD degradation.** Prior biochemical studies indicated that PTAC was needed for 1,2-PD degradation by *S. enterica* (25, 37). In the proposed biochemical pathway, this enzyme catalyzed the conversion of propionyl-CoA to propionyl-phosphate (transfer of a three-carbon acyl group) (Fig. 1). An enzyme with homology to known PTAC enzymes is not encoded by the *pdu* locus (6). However, *S. enterica* produces a phosphotransacylase (Pta)

encoded by the *pta* gene, which maps outside the *pdu* operon at centisome 50 (20). Pta is used for growth on acetate and inositol and plays a key role in the interconversion of acetyl-CoA and acetyl- $\text{PO}_4^{2-}$  (transfer of a two-carbon acyl group) (20). In vitro, Pta not only mediates the interconversion of acetyl-CoA and acetyl phosphate, it also catalyzes the interconversion of propionyl-CoA and propionyl- $\text{PO}_4^{2-}$  (20, 35). This raised the question of whether *pta* plays a role in 1,2-PD degradation. Two independent *pta* mutants (BE281 and BE527) were examined for growth on 1,2-PD. Aerobically, both grew slightly faster than wild-type *S. enterica*. The doubling times for the wild type were typically 7.5 to 8.5 h, and those for the *pta* mutants were 6 to 7 h. Both *pta* mutants were reconstructed via P22 transduction to ensure that they were isogenic with the wild type and that both still exhibited a small increase in the growth rate on 1,2-PD (not shown). These results confirmed previous studies that showed that *pta* is not essential for aerobic growth of *S. enterica* on 1,2-PD minimal medium (26).

Similarly, *pta* was unnecessary for the fermentation of 1,2-PD. Strains with *pta* mutations and wild-type *S. enterica* both had doubling times of 5 to 6 h during 1,2-PD fermentation. Phenotypic test showed that the *pta* mutants used in the above-mentioned studies were impaired for growth on acetate or inositol minimal medium and lacked detectable *pta* activity in cell extracts, as is expected for *pta* mutants (20). In addition, the growth defects on acetate and inositol were corrected by a *pta* minimal clone, and the chromosomal location of both *pta* insertions was verified by PCR (data not shown). Hence, we conclude that the *pta* gene is not essential for  $\text{B}_{12}$ -dependent 1,2-PD degradation under either aerobic or anaerobic growth conditions.

**Strains with *pduL* mutations produce less propionic acid on MacConkey-1,2-propanediol indicator medium.** The finding that the *pta* gene was unnecessary for 1,2-PD degradation suggested that *S. enterica* expresses an additional PTAC that is sufficient to mediate this process. As part of ongoing studies of 1,2-PD degradation by *S. enterica*, we constructed a series of precise *pdu* deletion mutations using PCR-based methods (11, 24). Tests with MacConkey-1,2-PD medium (17) indicated that three independent *pduL* deletion mutants each produced less propionic acid from 1,2-PD than did wild-type *S. enterica*. The enzymes that convert 1,2-PD to propionic acid were proposed to include coenzyme  $\text{B}_{12}$ -dependent diol dehydratase, propionaldehyde dehydrogenase, propionate kinase, and PTAC (Fig. 1) (25, 37). Of these, only the gene for PTAC was not identified (7, 19, 26). Hence, these results tentatively suggested that the *pduL* gene encodes a PTAC enzyme.

**Enzyme assays indicate that *pduL* encodes a PTAC enzyme.** Wild-type *S. enterica* as well as *pduL* and *pta* null mutants were grown under conditions that induce the *pdu* operon (5). Cell extracts were prepared and PTAC activity was measured using an assay that monitors the conversion of acyl-phosphate plus HS-CoA to acyl-CoA and inorganic phosphate (the reverse reaction with respect to 1,2-PD degradation). Both propionyl- $\text{PO}_4^{2-}$  and acetyl- $\text{PO}_4^{2-}$  were used as substrates. Cell extract from the wild-type strain had  $8.9 \mu\text{mol min}^{-1} \text{mg}^{-1}$  PTAC activity with propionyl- $\text{PO}_4^{2-}$  (Table 2). Extracts from *pta* or *pduL* mutants had partial activity ( $6.0$  and  $2.6 \mu\text{mol min}^{-1} \text{mg}^{-1}$ , respectively) (Table 2). PTAC activity was undetectable

TABLE 2. Phosphotransacylase activity in cell extracts from selected strains of *S. enterica*<sup>a</sup>

Strain (relevant genotype)	PTAC activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ ) <sup>b</sup>		
	Propionyl- $\text{PO}_4^{2-}$	Acetyl- $\text{PO}_4^{2-}$	Propionyl- $\text{PO}_4^{2-}$ preference <sup>c</sup>
Wild type (PduL <sup>+</sup> Pta <sup>+</sup> )	8.9	3.5	2.5
BE188 (PduL <sup>-</sup> Pta <sup>+</sup> )	2.6	3.2	0.8
BE527 (PduL <sup>+</sup> Pta <sup>-</sup> )	6.0	0.4	15.0
BE291 (PduL <sup>-</sup> Pta <sup>-</sup> )	ND	ND	

<sup>a</sup> Cells were grown on NCE succinate minimal medium supplemented with 1,2-PD to ensure induction of the *pdu* operon.

<sup>b</sup> PTAC assay mixtures contained 0.2 mM HS-CoA and 1 mM propionyl- $\text{PO}_4^{2-}$  or acetyl- $\text{PO}_4^{2-}$  and assay buffer. Activity was determined by monitoring the absorbance of reaction mixtures at 232 nm. ND, not detected (the lower detection limit of the assay is estimated to be  $0.03 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ).

<sup>c</sup> Propionyl- $\text{PO}_4^{2-}$  preference = (activity with propionyl- $\text{PO}_4^{2-}$ )/(activity with acetyl- $\text{PO}_4^{2-}$ ).

in cell extracts from the double mutant (*pta pduL*). The simplest interpretation of these results is that *pduL* and *pta* each encode PTAC enzymes, which was previously shown for *pta* (20). Furthermore, the *pta pduL* double mutant lacked detectable PTAC activity, demonstrating that the activity in Pta<sup>-</sup> PduL<sup>+</sup> cell extracts required PduL.

Enzyme assays also showed that the PTAC in Pta<sup>-</sup> PduL<sup>+</sup> cell extracts was 15-fold more active with propionyl- $\text{PO}_4^{2-}$  than with acetyl- $\text{PO}_4^{2-}$  ( $6 \mu\text{mol min}^{-1} \text{mg}^{-1}$  compared to  $0.4 \mu\text{mol min}^{-1} \text{mg}^{-1}$ ) (Table 2). This is consistent with a role for PduL in 1,2-PD degradation. On the other hand, the PTAC activity in Pta<sup>+</sup> PduL<sup>-</sup> cell extracts was 1.6-fold more active with acetyl- $\text{PO}_4^{2-}$ , consistent with the role of Pta in acetyl group metabolism (20).

For the above-described enzyme assays, controls showed that detectable PTAC activity required acyl-phosphate and HS-CoA, and in all cases, PTAC activity was linear with the enzyme concentration (data not shown).

**PduL mutants are impaired for aerobic growth on 1,2-PD.** Compared to wild-type *S. enterica*, a *pduL* mutant (BE188) was impaired for aerobic growth on 1,2-PD (Fig. 2A). The doubling time for *S. enterica* was  $7.9 \pm 0.5$  h, and that of a *pduL* mutant was  $12.6 \pm 0.6$  h. Further impairment of growth was not seen in the *pduL pta* double mutant even though this mutant lacked measurable PTAC activity (data not shown). These findings are consistent with the idea that PduL is a PTAC involved in 1,2-PD degradation. Aerobically, growth on 1,2-PD is expected to proceed in the absence of PTAC, since propionyl-CoA (which is formed prior to the PTAC reaction) can be metabolized via the methylcitrate pathway (Fig. 1) (16). The observed growth impairment of the *pduL* mutant was likely due to reduced ATP synthesis resulting from a block in the oxidative branch of the 1,2-PD degradative pathway, although other explanations cannot be ruled out.

***pduL* mutants are unable to obtain energy from 1,2-PD under fermentative conditions.** In the absence of an exogenous electron acceptor, the methylcitrate pathway is inoperative, and *S. enterica* is unable to grow on 1,2-PD as a sole carbon source (29). Under these conditions, *S. enterica* converts 1,2-PD to 1-propanol and propionate (Fig. 1). This stimulates growth on minimal medium supplemented with a small

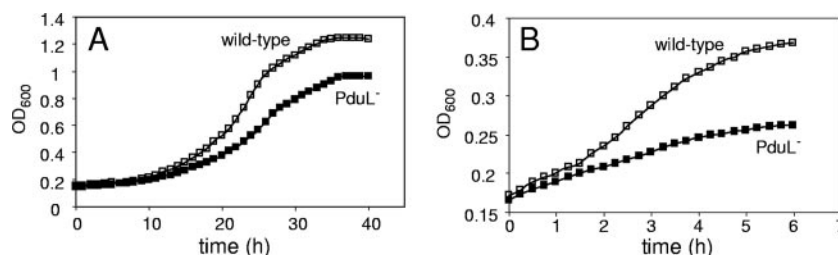


FIG. 2. Growth of a *pduL* null mutant on 1,2-PD. (A) Aerobic growth of wild-type *S. enterica* and a *pduL* mutant (BE188) on 1,2-PD minimal medium. (B) Fermentation of 1,2-PD. BE188 carries a precise deletion of the *pduL* gene made by a PCR-based method. The observed growth defects of BE188 were corrected by a *pduL* minimal clone. Each growth curve was performed three times in quadruplicate. Representative curves are shown. Log-phase doubling times with standard deviations are given in the text. The aerobic growth medium was NCE supplemented with 52 mM 1,2-PD, 200 ng ml<sup>-1</sup> vitamin B<sub>12</sub>, and 0.3 mM valine, isoleucine, leucine, and threonine. The fermentation medium was NCE supplemented with 52 mM 1,2-PD, 200 ng ml<sup>-1</sup> vitamin B<sub>12</sub>, and 0.2% yeast extract. The inoculation and incubation procedures are described in Materials and Methods. OD<sub>600</sub>, optical density at 600 nm.

amount of yeast extract by providing a source of ATP (29). The production of ATP via the conversion of 1,2-PD to propionate is expected to require PTAC (Fig. 1). Therefore, we tested the effect of a *pduL* mutation on this process. The doubling times of wild-type *S. enterica* in the presence and absence of 1,2-PD under fermentative conditions were  $5.5 \pm 0.35$  h and  $10.7 \pm 0.29$  h, respectively (Fig. 2B); the fermentation of 1,2-PD stimulated the growth rate about twofold, which is consistent with previous studies (29). The doubling times of the *pduL* mutant (BE188) under fermentative conditions in the presence and absence of 1,2-PD were  $13.1 \pm 1.0$  and  $13.3 \pm 0.95$  h, respectively. This indicated that energy production via 1,2-PD fermentation was completely eliminated by the *pduL* mutation. This is consistent with a role for PduL in 1,2-PD degradation.

The *pduL* mutant used in the above-described studies contained a wild-type *pta* gene, indicating that Pta did not substitute for PduL under the conditions used to measure 1,2-PD fermentation. Furthermore, a *pta* null mutation (alone or in combination with a *pduL* null mutation) did not affect the fermentation of 1,2-PD (data not shown). These results indicate that the Pta enzyme is insufficient to support the fermentation of 1,2-PD and that PduL is required.

**The observed phenotypes of a *pduL* mutant are complemented by a *pduL* minimal clone.** Growth tests showed that the aerobic and anaerobic growth phenotypes described above for a *pduL* mutant (BE188) were fully corrected by the expression of a *pduL* minimal clone using a tightly regulated expression vector, pLAC22. Under aerobic conditions, the doubling times for BE287 (wild-type *S. enterica*/pLAC22-no insert) and BE286 (wild-type *S. enterica*/pLAC22-*pduL*) were  $5.4 \pm 0.12$  h and  $6.0 \pm 0.32$  h, respectively. The doubling times for strains for BE285 (*pduL*/pLAC22-no insert) and BE284 (*pduL*/pLAC22-*pduL*) were  $8.2 \pm 0.71$  and  $5.5 \pm 0.25$  h, respectively. Under anaerobic conditions, the doubling times for *S. enterica*/pLAC22-no insert, *S. enterica*/pLAC22-*pduL*, *pduL*/pLAC22-no insert, and *pduL*/pLAC22-*pduL* were  $5.0 \pm 0.22$ ,  $5.2 \pm 0.37$ ,  $11.1 \pm 0.19$ , and  $5.3 \pm 0.40$  h, respectively. Thus, under aerobic and anaerobic conditions, a *pduL* minimal clone complemented the growth defect of the *pduL* mutation. On the other hand, vectors without an insert did not correct the observed growth defects of the *pduL* mutant. These results show that the observed phenotypes of the *pduL* mutant (BE188) resulted

from the deletion of the *pduL* gene but not from polarity or a mutation acquired during strain construction.

#### PduL substitutes for Pta in vivo during acetate utilization.

Growth tests were performed to determine whether PduL could substitute for Pta in vivo. Previous studies showed that *pta* mutants grow very slowly on minimal medium with >30 mM acetate (20). Here, we show that ectopic expression of PduL corrects this defect (Fig. 3). The doubling times of the Pta<sup>+</sup> strain and the *pta* mutant on acetate minimal medium were  $4.4 \pm 0.41$  and  $28.6 \pm 1.4$  h, respectively. The doubling time of a *pta* mutant producing PduL from expression vector pLAC22 was  $3.7 \pm 0.1$  h, which was slightly faster than the control ( $4.4 \pm 0.41$  h). Hence, ectopic expression of *pduL* fully corrected the growth defect of the *pta* mutant on acetate minimal medium. Since Pta is a well-studied PTAC enzyme, this finding indicates that PduL also has PTAC activity.

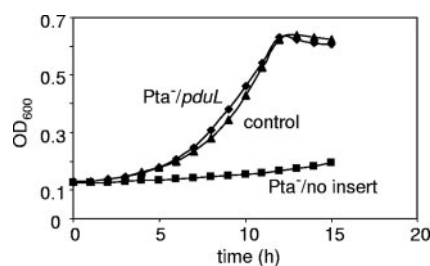


FIG. 3. Ectopic expression of a *pduL* minimal clone corrects the growth defect of a *pta* mutant on acetate minimal medium. Data for *S. enterica* harboring expression vector pLAC22 without an insert (control), a *pta* mutant carrying pLAC22 without an insert (Pta<sup>-</sup>/no insert), and a *pta* mutant harboring a *pduL* minimal clone in pLAC22 (Pta<sup>-</sup>/*pduL*) are shown. The relevant strains are BE287, BE529, and BE530 (Table 1). Controls showed that pLAC22 with or without the *pduL* insert did not observably affect the growth of the wild-type strain on acetate minimal medium (data not shown). In addition, the growth defect of the *pta* mutant was fully corrected by a *pta* minimal clone, showing that the effects of this mutation were not due to polarity (data not shown). The growth medium was NCE supplemented with 0.2% Na acetate and 0.2 mM IPTG. Each growth study was performed twice in quadruplicate. Representative curves are shown. Log-phase doubling times with standard deviations are given in the text. OD<sub>600</sub>, optical density at 600 nm.

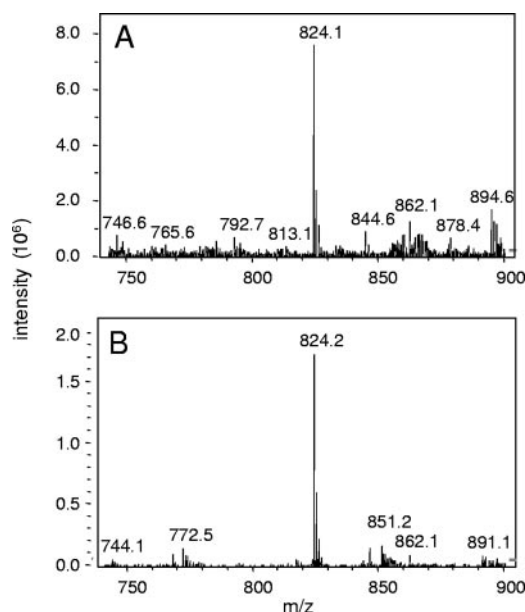


FIG. 4. Propionyl-CoA is produced by the PduL reaction. (A) HPLC-ESI-MS of propionyl-CoA standard. (B) HPLC-ESI-MS of a PduL assay after 10 min of incubation at 30°C. The assay mixture initially contained 10  $\mu\text{g}$  cell extract from BE527 ( $\text{Pta}^- \text{PduL}^+$ ), 0.4 mM HS-CoA, 1 mM propionyl- $\text{PO}_4^{2-}$ , and buffer.

**Propionyl-CoA is a product of the PduL reaction.** The finding that HS-CoA was required for PduL activity (see above) indicated that an acyl-CoA was formed in assay mixtures. Moreover, the PTAC assay used to measure PduL activity monitored the absorbance at 232 nm, which monitors the formation of thioester bonds (13). To confirm that acyl-CoA was formed in PduL enzyme assays, HPLC and HPLC-mass spectrometry were performed. Assay mixtures contained cell extract from BE527 ( $\text{Pta}^- \text{PduL}^+$ ) or BE291 ( $\text{Pta}^- \text{PduL}^-$ ), HS-CoA, propionyl- $\text{PO}_4^{2-}$ , and standard components; therefore, propionyl-CoA was the expected acyl-CoA product. Reverse-phase HPLC with UV detection at 260 nm was used to identify CoA compounds present in reaction mixtures. A single major compound with a retention time of 10.9 min was detected when  $\text{Pta}^- \text{PduL}^+$  cell extracts were used. This compound coeluted with authentic propionyl-CoA following coinjection and produced a mass spectrum characteristic of propionyl-CoA via HPLC-ESI-MS (Fig. 4). The major peaks for authentic propionyl-CoA and the PduL reaction product that eluted at 10.9 min were at  $m/z$  824.1 or 824.2, respectively. These peaks correspond to  $[\text{M} + \text{H}]^+$  for propionyl-CoA. In contrast, propionyl-CoA was undetectable by HPLC in assay mixtures containing cell extract from BE291 ( $\text{Pta}^- \text{PduL}^-$ ), and the major HPLC peak observed corresponded to HS-CoA (retention time, 4.5 min). Our interpretation of the above-described results is that propionyl-CoA is a product of the PduL reaction.

**Production of PduL-His<sub>8</sub> protein.** *E. coli* strain BE554 was constructed to produce high levels of recombinant PduL fused to eight C-terminal histidine residues (PduL-His<sub>8</sub>). Protein expression by BE554 and a control strain (BE119) was examined by SDS-PAGE (not shown) and enzyme assays. Relatively

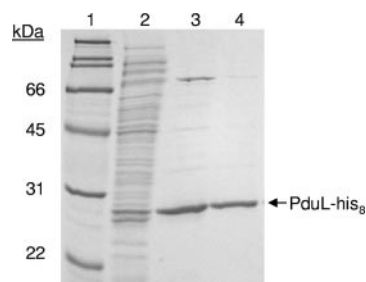


FIG. 5. SDS-PAGE analysis of PduL-His<sub>8</sub> purification. Lane 1, molecular mass markers; lane 2, soluble crude extracts from the PduL-His<sub>8</sub> production strain (BE554) (10  $\mu\text{g}$  protein); lane 3, fraction obtained from the first elution step using buffer with 400 mM imidazole (2  $\mu\text{g}$  protein); lane 4, fraction obtained from the second elution step using buffer with 400 mM imidazole (2  $\mu\text{g}$  protein). A 12% acrylamide gel was stained with Coomassie brilliant blue R-250.

high amounts of a protein with a molecular mass of nearly 27 kDa were present in the inclusion body fraction from the *pduL* expression strain, which is near the predicted molecular mass for PduL-His<sub>8</sub> (24 kDa). However, a modest amount of soluble protein near this mass was produced.

To better assess the production of PduL-His<sub>8</sub>, cell extracts were tested for PTAC activity. Soluble extracts from the PduL production strain typically contained about 50  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  activity, whereas the control strain produced only 2  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  PTAC activity. Hence, a reasonable amount of PduL-His<sub>8</sub> appeared to be soluble and active under the production conditions used. On the other hand, although the inclusion body fraction from the expression strain contained high amounts of PduL-His<sub>8</sub> protein, the PTAC activity of this fraction was typically about twofold lower than that of the soluble fraction, indicating that most of the PduL in this fraction was inactive. No PTAC activity was detected in the inclusion body fraction from the control strain. The PTAC assay used measured the conversion of propionyl- $\text{PO}_4^{2-}$  and HS-CoA to propionyl-CoA and  $\text{HPO}_4^{2-}$ . No activity could be detected when enzyme, HS-CoA, or propionyl- $\text{PO}_4^{2-}$  was omitted from the assay mixture. In each case, PTAC activity was linear with regard to the enzyme concentration (data not shown).

**Purification and kinetic characterization of PduL-His<sub>8</sub>.** PduL-His<sub>8</sub> was purified from cell extracts of strain BE554 by nickel affinity chromatography (Fig. 5). Purified PduL-His<sub>8</sub> appeared to be homogenous following SDS-PAGE and Coomassie blue staining (Fig. 5). Kinetic analysis showed that purified PduL-His<sub>8</sub> was 3.8-fold more active with propionyl- $\text{PO}_4^{2-}$  than with acetyl- $\text{PO}_4^{2-}$  ( $V_{\text{max}}$  of 51.7 and 13.5  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ , respectively) (Table 3). It also had a lower  $K_m$  for propionyl- $\text{PO}_4^{2-}$  than for acetyl- $\text{PO}_4^{2-}$  ( $0.61 \pm 0.06$  versus  $0.97 \pm 0.26$  mM). No activity was detected when PduL-His<sub>8</sub>, HS-CoA, or propionyl- $\text{PO}_4^{2-}$  (or acetyl- $\text{PO}_4^{2-}$ ) was omitted from the assay mixture. In each case, the PduL-His<sub>8</sub> activity was linear with the enzyme concentration (data not shown).

For PduL-His<sub>8</sub>, crude cell extracts typically had PTAC activity of about 50  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ . Nickel affinity chromatography yielded purified enzyme with specific activities of about 45 to 55  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ . Visual inspection of SDS-PAGE gels indicated that PduL-His<sub>8</sub> comprised about 2 to 5%

TABLE 3. Kinetic constants for purified recombinant PduL-His<sub>8</sub><sup>a</sup>

Substrate	$K_m$ (mM)	$V_{max}$ ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	$k_{cat}$ ( $\text{s}^{-1}$ )	$k_{cat}/K_m$ ( $\text{mM s}^{-1}$ )
Propionyl- $\text{PO}_4^{2-}$	$0.61 \pm 0.06$	$51.7 \pm 7.6$	20.7	33.9
Acetyl- $\text{PO}_4^{2-}$	$0.97 \pm 0.26$	$13.4 \pm 1.8$	5.4	5.6

<sup>a</sup> PTAC assays were performed by monitoring the absorbance of reaction mixtures at 232 nm as described in Materials and Methods. The kinetic constants for propionyl- $\text{PO}_4^{2-}$  and acetyl- $\text{PO}_4^{2-}$  were determined using an excess of HS-CoA (0.4 mM).

of the total cell protein (Fig. 5); hence, these results suggest that PduL-His<sub>8</sub> lost activity during the course of purification. Indeed, PduL-His<sub>8</sub> was unstable in the purified form and lost about 50% of its activity 48 h after purification. The reported kinetic constants are based on assays done as soon as possible after purification. As the activity of PduL-His<sub>8</sub> decreased, its  $K_m$  for HS-CoA and propionyl- $\text{PO}_4^{2-}$  remained unchanged within experimental error. Its activity with propionyl- $\text{PO}_4^{2-}$  and acetyl- $\text{PO}_4^{2-}$  decreased in direct proportion. We infer that the  $K_m$  values reported here are representative but that the  $V_{max}$  values are probably underestimated.

## DISCUSSION

Here, we presented genetic and biochemical evidence that the *pduL* gene encodes a PTAC involved in 1,2-PD degradation. Growth tests showed that a *pduL* mutant was impaired for 1,2-PD degradation under aerobic and anaerobic conditions, indicating a role for *pduL* in 1,2-PD degradation. Growth studies also showed that ectopic expression of PduL corrected the growth defect of a *pta* mutant on acetate minimal medium, indicating that PduL has phosphotransacetylase activity in vivo. Enzyme assays of crude cell extracts demonstrated that *pduL* was necessary for the production of detectable PTAC activity in a genetic background that contained a *pta* mutation. HPLC-ESI-MS showed that propionyl-CoA was produced from propionyl- $\text{PO}_4^{2-}$  and HS-CoA in PduL enzyme assays. PduL-His<sub>8</sub> was purified and found to have a specific activity of  $57.4 \mu\text{mol min}^{-1} \text{mg}^{-1}$  with propionyl- $\text{PO}_4^{2-}$  as a substrate, indicating the PduL is sufficient for PTAC activity. Thus, genetic and biochemical studies indicate that PduL is a PTAC involved in 1,2-PD degradation.

BLAST and PSI-BLAST analyses (five iterations) showed that PduL lacks significant similarity to known PTAC enzymes. Thus, PduL is evolutionarily distinct. It either evolved independently of known PTAC enzymes or became highly divergent from them over time. Sequence analyses also showed that PduL homologues (expect  $<7 \times 10^{-30}$ ) are found in 49/337 (14.5%) complete microbial genomes present in the GenBank database. No homologues of PduL were found among the *Archaea* or *Eukarya*. Hence, PduL is apparently a conserved protein specific to the *Bacteria*. In addition, BLAST analyses identified four PduL homologues that are fused to sequences with homology to acetate kinases. These may be dual-function enzymes that convert acyl-CoA to its corresponding organic acid with the synthesis of ATP.

Including PduL, there are three known classes of phosphotransacylases. The *S. enterica* PduL, Pta, and EutD enzymes are representatives of these classes. PduL is comprised of 210

amino acids, while Pta and EutD are 714 and 338 amino acids in length, respectively (8). EutD and the C-terminal region of Pta are homologous. PduL lacks significant similarity to EutD and Pta. The N-terminal region of Pta contains BioD-like and "DRTGG" domains. These two domains are absent from EutD and PduL. The physiological role of these domains is uncertain. In enteric bacteria, acetyl group metabolism is linked to two-component signal transduction systems and DNA damage and repair in a complex manner (22, 34, 40). The N-terminal domains of Pta may be needed to accommodate expanded physiological roles.

Kinetic studies with purified PduL-His<sub>8</sub> were performed (Table 3). The  $V_{max}$  of PduL with propionyl- $\text{PO}_4^{2-}$  as a substrate was  $51.7 \pm 7.6 \mu\text{mol min}^{-1} \text{mg}^{-1}$ . Previously purified Pta enzymes were reported to have specific activities between 32 and  $9006 \mu\text{mol min}^{-1} \text{mg}^{-1}$  when acyl- $\text{PO}_4^{2-}$  was used as a substrate (33). The activity of PduL-His<sub>8</sub> is at the low end of this range but may be underestimated due to its instability (see Results).

The  $K_m$  values of PduL-His<sub>8</sub> for HS-CoA and propionyl- $\text{PO}_4^{2-}$  were determined to be  $0.61 \pm 0.06$  and  $0.032 \pm 0.06$  mM, respectively. Reported  $K_m$  values of Pta homologues for HS-CoA range from 0.03 to 1.7 mM, and those for acetyl- $\text{PO}_4^{2-}$  range from 0.024 to 4.7 mM (33). Thus,  $K_m$  values of PduL for HS-CoA and propionyl- $\text{PO}_4^{2-}$  appear to be within a physiologically meaningful range. Kinetic studies found that the  $k_{cat}/K_m$  ratio of Pdu-His<sub>8</sub> was about sixfold higher for propionyl- $\text{PO}_4^{2-}$  than for acetyl- $\text{PO}_4^{2-}$ . The selectivity of PduL for propionyl- $\text{PO}_4^{2-}$  is consistent with a role in 1,2-PD degradation. Furthermore, its relatively low activity with acetyl- $\text{PO}_4^{2-}$  may be important to minimize the perturbation of acetyl group metabolism (22, 40).

Two findings in this report were surprising to us: (i) the observation that *pduL* mutants were significantly impaired for growth on 1,2-PD minimal medium (Fig. 2) and (ii) the finding that a *pduL pta* double mutant was not further impaired for growth on 1,2-PD. Because Pta is known to be active with propionyl- $\text{PO}_4^{2-}$ , we expected that Pta would partly substitute for PduL during growth of *S. enterica* on 1,2-PD. However, in the studies reported here, it did not. A simple explanation is that PduL is more efficient at propionyl group metabolism in vivo. Two explanations that we think are more interesting are the differential regulation of *pta* and *pduL* as well as the possibility that PduL is specifically adapted to function in concert with the polyhedral body-microcompartment involved in 1,2-PD degradation.

## ACKNOWLEDGMENTS

We thank the ISU DNA Sequencing and Synthesis Facility for assistance with DNA analyses. We thank Ann Perera and the ISU Metabolomics Laboratory for help with mass spectrometry.

We thank the ISU Office of Biotechnology for financial support.

## REFERENCES

1. Abeles, R. H., and H. A. Lee. 1961. Intramolecular oxidation-reduction requiring a cobamide coenzyme. *J. Biol. Chem.* **236**:2347–2350.
2. Altschul, S. F., T. L. Madden, A. A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**:3389–3402.
3. Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. Roth, and B. N. Ames. 1968. Procedure for identifying nonsense mutations. *J. Bacteriol.* **96**:215–220.

4. **Bobik, T. A.** 2006. Polyhedral organelles compartmenting bacterial metabolic processes. *Appl. Microbiol. Biotechnol.* **70**:517–525.
5. **Bobik, T. A., M. Ailion, and J. R. Roth.** 1992. A single regulatory gene integrates control of vitamin B<sub>12</sub> synthesis and propanediol degradation. *J. Bacteriol.* **174**:2253–2266.
6. **Bobik, T. A., G. D. Havemann, R. J. Busch, D. S. Williams, and H. C. Aldrich.** 1999. The propanediol utilization (*pdu*) operon of *Salmonella enterica* serovar Typhimurium LT2 includes genes necessary for formation of polyhedral organelles involved in coenzyme B<sub>12</sub>-dependent 1,2-propanediol degradation. *J. Bacteriol.* **181**:5967–5975.
7. **Bobik, T. A., Y. Xu, R. M. Jeter, K. E. Otto, and J. R. Roth.** 1997. Propanediol utilization genes (*pdu*) of *Salmonella typhimurium*: three genes for the propanediol dehydratase. *J. Bacteriol.* **179**:6633–6639.
8. **Brinsmade, S. R., and J. C. Escalante-Semerena.** 2004. The *eutD* gene of *Salmonella enterica* encodes a protein with phosphotransacetylase enzyme activity. *J. Bacteriol.* **186**:1890–1892.
9. **Brinsmade, S. R., T. Paldon, and J. C. Escalante-Semerena.** 2005. Minimal functions and physiological conditions required for growth of *Salmonella enterica* on ethanolamine in the absence of the metabolosome. *J. Bacteriol.* **187**:8039–8046.
10. **Chen, P., D. I. Andersson, and J. R. Roth.** 1994. The control region of the *pdu/cob* regulon in *Salmonella typhimurium*. *J. Bacteriol.* **176**:5474–5482.
11. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* **97**:6640–6645.
12. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics: a manual for genetic engineering. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
13. **Dawson, R. M. C., D. C. Elliott, W. H. Elliott, and K. M. Jones (ed.).** 1969. Data for biochemical research, 2nd ed. Oxford University Press, Oxford, United Kingdom.
14. **Havemann, G. D., and T. A. Bobik.** 2003. Protein content of polyhedral organelles involved in coenzyme B<sub>12</sub>-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. *J. Bacteriol.* **185**:5086–5095.
15. **Havemann, G. D., E. M. Sampson, and T. A. Bobik.** 2002. PduA is a shell protein of polyhedral organelles involved in coenzyme B<sub>12</sub>-dependent degradation of 1,2-propanediol in *Salmonella enterica* serovar Typhimurium LT2. *J. Bacteriol.* **184**:1253–1261.
16. **Horswill, A., and J. Escalante-Semerena.** 1997. Propionate catabolism in *Salmonella typhimurium* LT2: two divergently transcribed units comprise the *ppp* locus at 8.5 centisomes, *pppR* encodes a member of the  $\sigma^{54}$  family of activators, and the *pppBCDE* genes constitute an operon. *J. Bacteriol.* **179**:928–940.
17. **Jeter, R. M.** 1990. Cobalamin-dependent 1,2-propanediol utilization by *Salmonella typhimurium*. *J. Gen. Microbiol.* **136**:887–986.
18. **Johnson, C. L., E. Pecheonick, S. D. Park, G. D. Havemann, N. A. Leal, and T. A. Bobik.** 2001. Functional genomic, biochemical, and genetic characterization of the *Salmonella pduO* gene, an ATP:cob(I)alamin adenosyltransferase gene. *J. Bacteriol.* **183**:1577–1584.
19. **Leal, N. A., G. D. Havemann, and T. A. Bobik.** 2003. PduP is a coenzyme A-acylating propionaldehyde dehydrogenase associated with the polyhedral bodies involved in B<sub>12</sub>-dependent 1,2-propanediol degradation by *Salmonella enterica* serovar Typhimurium LT2. *Arch. Microbiol.* **180**:353–361.
20. **LeVine, S. M., F. Ardeshir, and G. F. Ames.** 1980. Isolation and characterization of acetate kinase and phosphotransacetylase mutants of *Escherichia coli* and *Salmonella typhimurium*. *J. Bacteriol.* **143**:1081–1085.
21. **Lundie, L. L., Jr., and J. G. Ferry.** 1989. Activation of acetate by *Methanosarcina thermophila*. *J. Biol. Chem.* **264**:18392–18396.
22. **McCleary, W. R., J. B. Stock, and A. J. Ninfa.** 1993. Is acetyl phosphate a global signal in *Escherichia coli*? *J. Bacteriol.* **175**:2793–2798.
23. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
24. **Miller, V. L., and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. *J. Bacteriol.* **170**:2575–2583.
25. **Obradors, N., J. Badia, L. Baldoma, and J. Aguilar.** 1988. Anaerobic metabolism of the L-rhamnose fermentation product 1,2-propanediol in *Salmonella typhimurium*. *J. Bacteriol.* **170**:2159–2162.
26. **Palacios, S., V. J. Starai, and J. C. Escalante-Semerena.** 2003. Propionyl coenzyme A is a common intermediate in the 1,2-propanediol and propionate catabolic pathways needed for expression of the *pppBCDE* operon during growth of *Salmonella enterica* on 1,2-propanediol. *J. Bacteriol.* **185**:2802–2810.
27. **Paoli, P., P. Cirri, L. Camici, G. Manao, G. Cappugi, G. Moneti, G. Pieraccini, G. Camici, and G. Ramponi.** 1997. Common-type acylphosphatase: steady-state kinetics and leaving-group dependence. *Biochem. J.* **327**:177–184.
28. **Penrod, J. T., and J. R. Roth.** 2006. Conserving a volatile metabolite: a role for carboxysome-like organelles in *Salmonella enterica*. *J. Bacteriol.* **188**:2865–2874.
29. **Price-Carter, M., J. Tingey, T. A. Bobik, and J. R. Roth.** 2001. The alternative electron acceptor tetrathionate supports B<sub>12</sub>-dependent anaerobic growth of *Salmonella enterica* serovar Typhimurium on ethanolamine or 1,2-propanediol. *J. Bacteriol.* **183**:2463–2475.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
31. **Sampson, E. M., C. L. Johnson, and T. A. Bobik.** 2005. Biochemical evidence that the *pduS* gene encodes a bifunctional cobalamin reductase. *Microbiology* **151**:1169–1177.
32. **Schmieger, H.** 1971. A method for detection of phage mutants with altered transducing ability. *Mol. Gen. Genet.* **110**:378–381.
33. **Schomburg, I., A. Chang, C. Ebeling, M. Gremse, C. Heldt, G. Huhn, and D. Schomburg.** 2004. BRENDA, the enzyme database: updates and major new developments. *Nucleic Acids Res.* **32**:D431–433.
34. **Shi, I. Y., J. Stansbury, and A. Kuzminov.** 2005. A defect in the acetyl coenzyme A $\leftrightarrow$ acetate pathway poisons recombinational repair-deficient mutants of *Escherichia coli*. *J. Bacteriol.* **187**:1266–1275.
35. **Shimizu, M., T. Suzuki, K. Y. Kameda, and Y. Abiko.** 1969. Phosphotransacetylase of *Escherichia coli* B, purification and properties. *Biochim. Biophys. Acta* **191**:550–558.
36. **Shively, J. M., C. E. Bradburne, H. C. Aldrich, T. A. Bobik, J. L. Mehlman, S. Jin, and S. H. Baker.** 1998. Sequence homologs of the carboxysomal polypeptide CsoS1 of the thiobacilli are present in cyanobacteria and enteric bacteria that form carboxysomes-polyhedral bodies. *Can. J. Bot.* **76**:906–916.
37. **Toraya, T., S. Honda, and S. Fukui.** 1979. Fermentation of 1,2-propanediol and 1,2-ethanediol by some genera of *Enterobacteriaceae*, involving coenzyme B<sub>12</sub>-dependent diol dehydratase. *J. Bacteriol.* **139**:39–47.
38. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. *J. Biol. Chem.* **218**:97–106.
39. **Warren, J. W., J. R. Walker, J. R. Roth, and E. Altman.** 2000. Construction and characterization of a highly regulable expression vector, pLAC11, and its multipurpose derivatives, pLAC22 and pLAC33. *Plasmid* **44**:138–151.
40. **Wolfe, A. J., D. E. Chang, J. D. Walker, J. E. Seitz-Partridge, M. D. Vidaurri, C. F. Lange, B. M. Pruss, M. C. Henk, J. C. Larkin, and T. Conway.** 2003. Evidence that acetyl phosphate functions as a global signal during biofilm development. *Mol. Microbiol.* **48**:977–988.