APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2008, p. 7073–7079 Vol. 74, No. 22
0099-2240/08/$08.00+0 doi:10.1128/AEM.01074-08

Exogenous or L-Rhamnose-Derived 1,2-Propanediol Is Metabolized via a *pduD*-Dependent Pathway in *Listeria innocua*▽

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Received 13 May 2008/Accepted 16 September 2008

1,2-Propanediol (1,2-PD) added exogenously to cultures or produced endogenously from L-rhamnose is metabolized to *n*-propanol and propionate in *Listeria innocua* Lin11. The *pduD* gene, which encodes a diol dehydratase ß subunit homolog, is required for 1,2-PD catabolism. *pduD* and 16 other genes within the *pduA*-to-*pduF* region of a large gene cluster are induced in medium containing 1,2-PD.

1,2-Propanediol (1,2-PD) is a three-carbon glycol that is produced during the catabolism of rhamnose and fucose, which are abundant sugars in plant matter and mammalian glycoconjugates (1, 3). 1,2-PD metabolism has been investigated extensively in *Salmonella* species that can use 1,2-PD as a carbon source and in which its metabolism appears to be important for virulence (3). More recently, operons consisting of genes for 1,2-PD utilization (*pdu*) have been identified in *Listeria monocytogenes* EGDe and *L. innocua* CLIP11262 by bioinformatic analyses of genome sequences (7). It has been proposed that, as in *Salmonella*, these genes may be important for virulence in *L. monocytogenes* (7). So far, only a limited number of studies indicate that *pdu* genes may be functional in *Listeria*. For example, it has been shown for *L. monocytogenes* that the transcription of some *pdu* genes increases when glucose is depleted from culture media (22), and several *pdu* genes are switched on during intracellular growth in mammalian cells (17). Experiments showing that *pdu* genes actually serve in 1,2-PD catabolism in *Listeria* have not yet been performed.

In *Salmonella*, 1,2-PD catabolism begins with the conversion of 1,2-PD to propionaldehyde by a hexameric vitamin B_{12}-dependent diol dehydratase encoded by the *pduCDE* genes (11) (Fig. 1). Under fermentation conditions, two molecules of propionaldehyde are subsequently converted to *n*-propanol and propionate (3, 15, 20). These steps produce ATP and regenerate NAD^+ but do not provide a source of carbon for biosynthesis. Nonetheless, it has been shown that 1,2-PD catabolism augments anaerobic growth in the presence of poor-quality carbon sources (25). Under aerobic conditions, and anaerobic respiration with tetrathionate as the electron acceptor, *Salmonella* can use 1,2-PD as both a carbon and an energy source (25). The propionyl coenzyme A (CoA) produced in the 1,2-PD pathway is converted into the biosynthesis precursor pyruvate by the 2-methylcitric acid cycle (16, 23) (Fig. 1). ATP is produced by respiration, using reducing equivalents derived from 2-methylcitric acid cycle reactions. *Salmonella* assembles polyhedral bodies wherein 1,2-PD is formed and converted into propionyl CoA (15, 28). Polyhedral bodies are proteinaceous shells that are formed from proteins encoded by the *pduA*, *-B*, *-J*, *-K*, *-N*, *-T*, and *-U* genes. These structures serve as microcompartments wherein propionaldehyde is formed from 1,2-PD and is sequestered to protect the cell from its toxic effects (28).

In *Salmonella*, all 23 *pdu* genes are clustered together and located adjacent to a gene cluster (*cbi*/*cob*) carrying genes needed for vitamin B_{12} biosynthesis (7, 12, 33). Both the *pdu* and the *cbi*/*cob* genes are coordinately regulated by the *pocR* gene, which is located between the two gene clusters (2, 26). The PocR transcription factor is activated by 1,2-PD and stimulates transcription by binding to control sites upstream of these operons (10, 27). Possibly due to the sensitivity of *cob* gene products to O_2 inactivation, vitamin B_{12} can be synthesized de novo only under anaerobic conditions, and it or the precursor cobinamide must be imported into cells for growth on 1,2-PD under aerobic conditions (26).

In *Listeria*, *pdu* genes reside within a large cluster that is interspersed with *cbi*, *cob*, and ethanolamine utilization (*eut*) genes (3, 7, 18). The genes appear to be organized into several operons, and a *pocR* ortholog also is located within the cluster (Fig. 2). As an initial step in the analysis of the *pdu* gene function in *Listeria*, we studied the requirement for diol dehydratase in 1,2-PD catabolism and the regulation of the diol dehydratase-encoding portion of the *pdu* genes by 1,2-PD under aerobic growth conditions in *L. innocua*.

Growth of *L. innocua* Lin11 in 1,2-PD, L-rhamnose, and L- and D-fucose media. To test for growth on 1,2-PD and other carbon sources, cultures of the wild-type *L. innocua* Lin11 strain (36) were grown aerobically at 30°C in synthetic liquid minimal medium (HTM) (32) supplemented with 20 nM vitamin B_{12}. Growth was observed in HTM when 0.2% L-rhamnose or 0.2% D-glucose was added as the carbon source but did not occur in medium using 0.2% L-fucose or 52 mM 1,2-PD as the sole carbon source. Additional experiments performed with an API 50 CHB/E carbohydrate fermentation test system (bioMérieux, France) confirmed that the strain can grow on L-rhamnose but not on L- or D-fucose medium.

As discussed above, *Salmonella* metabolizes propionyl

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▽ Published ahead of print on 19 September 2008.

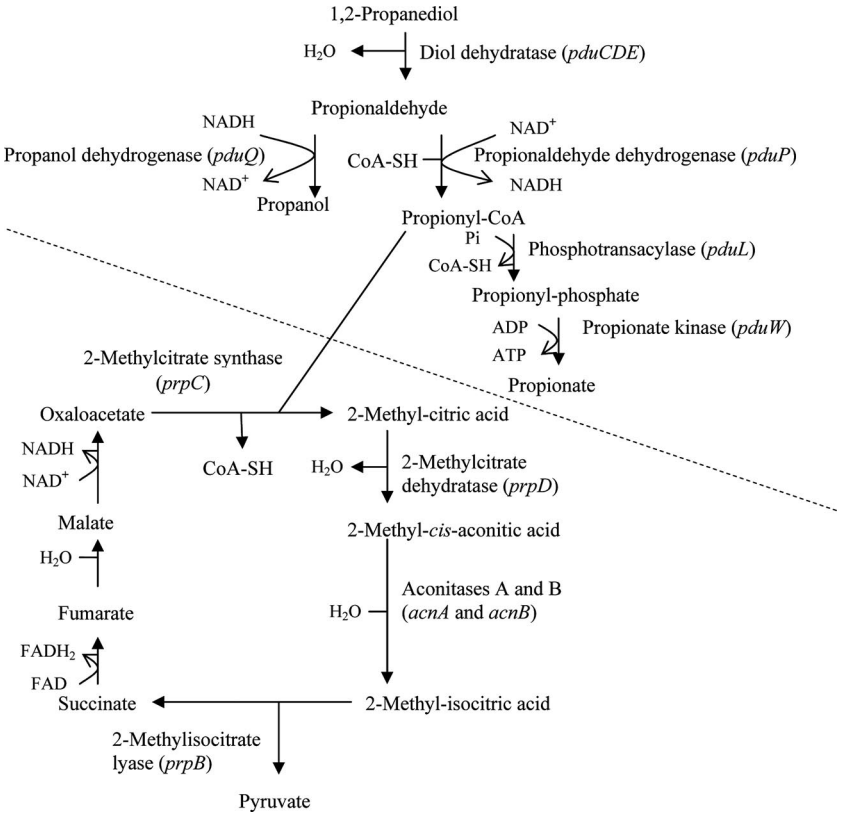


FIG. 1. Pathways of 1,2-PD catabolism in *Salmonella* and the putative pathway of 1,2-PD catabolism in *L. innocua* Lin11. The *pdu* pathway steps in the upper part of the diagram (above the dashed line) appear to occur in both species. The 2-methylcitric acid cycle reactions shown in the lower part of the diagram do not appear to take place in *Listeria*. The figure has been adapted from work published in references 3, 4, and 23.

CoA produced from 1,2-PD catabolism via the 2-methylcitric acid cycle to obtain energy and a source of carbon for growth under aerobic conditions (Fig. 1). While BLAST-p searches conducted at the ListiList website identified *L. innocua* CLIP11262 and *L. monocytogenes* EGDe homologs of *Salmonella* genes encoding the 2-methylcitric acid cycle enzymes PrpC (CitZ), AcnA (CitB), AcnB (LeuC), and PrpB (Lmo0075/Lin0067), a PrpD homolog could not be identified (Fig. 1). Searches also revealed that *prp* homologs are scattered throughout the *Listeria* genomes, whereas they reside in one operon (*prpBCDE*) in *Salmonella* (16). We also searched for homologs of AcnD (2-methylcitrate dehydratase) and its PrpF accessory factor that, together, have been shown to substitute for PrpD in *Shewanella oneidensis* and *Vibrio cholerae* (14). Although *L. innocua* appears to contain homologs of AcnD (CitB and LeuC), no homolog of PrpF was found. Because AcnD activity alone cannot convert 2-methyL-*cis*-aconitic acid, it is

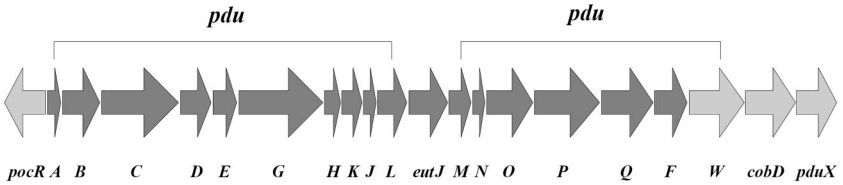


FIG. 2. Genetic organization of the *pdu* gene cluster in *L. innocua* (7). Genes induced by 1,2-PD are shown in dark gray. The putative function of each gene is as follows (2, 3, 6, 7, 13, 15, 20, 28; http://genolist.pasteur.fr/ListiList). *pocR* (lin1114), a transcriptional activator of the *pdu* operon; *pduA* (lin1115), polyhedral body component; *pduB* (lin1116), polyhedral body component; *pduC* (lin1117), diol dehydratase large (α) subunit; *pduD* (lin1118), diol dehydratase medium (β) subunit; *pduE* (lin1119), diol dehydratase small (γ) subunit; *pduG* (lin1120), diol dehydratase-reactivating factor large subunit; *pduH* (lin1121), diol dehydratase-reactivating factor small subunit; *pduK* (lin1122), polyhedral body component; *pduJ* (lin1123), polyhedral body component; *pduL* (lin1124), phosphotransacylase; *eutJ* (lin1125), unknown; *pduM* (lin1126), unknown; *pduN* (lin1127), polyhedral body component; *pduO* (lin1128), adenosyltransferase; *pduP* (lin1129), propionaldehyde dehydrogenase; *pduQ* (lin1130), propanol dehydrogenase; *pduF* (*glpF*, lin1131), propanediol diffusion facilitator; *pduW* (lin1132), propionate kinase; *cobD* (lin1133), L-threonine-*O*-3-phosphate decarboxylase; *pduX* (lin1134), L-threonine kinase. Gene names and numbers used at the ListiList server are provided in parentheses to facilitate searches at that website.

TABLE 1. Primers used for construction of the *pduD* deletion and complementation mutant strains[a]

Primer	Site	Sequence
Deletion mutants		
lin1118-A-EcoRI	F	CGGAATTC TGCCAGAACGTAAC ATGG
lin1118-B	R	GCGTTTTTACCTTGAACAACTACT TCTGAGATAATTCCG
lin1118-C	F	GTTGTTCAAGGTAAAAACGC
lin1118-D-BamHI	R	CGGGATCCCTAAAATACGCTCAT CTGG
Complementation mutants		
tetLP-F-XbaI	F	GCTCTAGAACGGGCCATATTGTT GTATAAG
tetLP-R	R	ATTTCACCCTCCAATAATGAGG
lin1118-F2	F	CCTCATTATTGGAGGGTGAAATA TGGTTGAAATTAACGAAAA AGTG
lin1118-R2-SalI	R	ACGCGTCGACTTAATTTACTTGTA GTTCGACTGC

[a] F, forward primer; R, reverse primer. The restriction sites incorporated into primers for cloning purposes are underlined. The overhangs complementary to the lin1118-C primer and to the tetLP-R primer (both used for splice-by-overlap extension PCR) are underlined in the primers lin1118-B and lin1118-F2, respectively. Primers were designed using the *L. innocua* CLIP11262 genome sequence available at ListiList (http://genolist.pasteur.fr/ListiList).

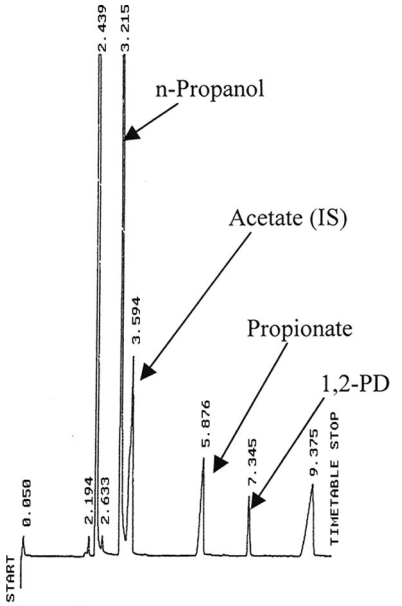
proposed that *L. innocua* Lin11 may not be able to utilize 1,2-PD for aerobic growth due to the lack of a complete 2-methylcitric acid cycle pathway.

The *pduD* gene encodes a diol dehydratase that participates in 1,2-PD catabolism in *L. innocua* Lin11. Two approaches were used to determine if *L. innocua* Lin11 can nonetheless degrade 1,2-PD in a *pdu*-dependent manner. These involved (i) quantitation of *n*-propanol and propionate production in growth medium containing 1,2-PD, and (ii) measurement of diol dehydratase activities in cell extracts. Analyses were conducted with the wild-type *L. innocua* Lin11 strain, a mutant with an in-frame deletion in *pduD*, which encodes a homolog of the ß subunit of diol dehydratase (Fig. 1), and the deletion strain complemented with the *pduD* gene cloned in plasmid pAM401 (35). Note that the deletion strains retain intact copies of the *pduC* and *pduE* genes, which encode homologs of the α and γ subunits of diol dehydratase.

The deletion mutant was constructed by replacing the wild-type *pduD* gene (lin1118) that encodes the 219-amino-acid PduD polypeptide with a truncated *pduD* gene that encodes only the N-terminal 16 amino acids and the C-terminal 13 amino acids of PduD. The deletion gene was constructed by splice-by-overlap extension PCR in plasmid pKSV7 and introduced into Lin11 by homologous recombination (9, 37). The plasmid used in complementation experiments (pJX1118) was constructed by separately amplifying the promoter sequence of the *tetL* gene present in plasmid pLTV3 (8) and the complete *L. innocua* Lin11 *pduD* coding sequence and fusing them together by splice-by-overlap extension PCR. Subsequently, the *tetL-pduD* hybrid gene was cloned into the shuttle vector pAM401 and transformed into the deletion mutant (24). The *tetL* promoter sequence was fused to *pduD* because this gene resides at an internal location in the *pdu* cluster and appears to lack its own promoter. The PCR primers used to construct the deletion mutant and pJX1118 are listed in Table 1. The sequences of all constructs were verified by DNA sequencing.

Cell cultures used in the analysis of *n*-propanol and propionate production were grown aerobically for 48 h at 30°C in HTM liquid medium supplemented with 0.2% yeast extract, 20 nM vitamin B_{12}, and 52 mM 1,2-PD or 0.2% L-rhamnose. A medium lacking glucose was selected because glucose represses *pdu* transcription in *Salmonella* (2) and possibly in *L. monocytogenes* (22 and see discussion below). A poor carbon source (0.2% [wt/vol] yeast extract) was added so that the strains would grow in 1,2-PD medium and to see if growth is improved in the presence of 1,2-PD (25).

1,2-PD, *n*-propanol, and propionate concentrations in culture media were determined using a gas-liquid chromatography method that offers the advantage of simultaneously deter-



	Lin11	Lin11Δ1118	Lin11Δ1118/ pJX1118
1,2-PD	1.4 ± 1.1	50 ± 11	4.9 ± 2.6
Propanol	1.4 ± 0.6	ND	0.9 ± 0.1
Propionate	0.9 ± 0.4	ND	0.5 ± 0.3

FIG. 3. Gas-liquid chromatography analysis of 1,2-PD metabolites in culture media. A representative chromatogram of medium composition obtained from the wild-type Lin11 strain grown for 48 h in HTM, 0.2% yeast extract, 20 nM vitamin B_{12}, 52 mM 1,2-PD medium is shown. Peaks were separated using an Equity-1 capillary column, identified by using commercially available standards, and quantified by using 1 mg of acetate as the internal standard (IS). Numbers above each peak are on-column retention times in minutes. Note that while peak areas are proportional to metabolite concentrations, the detector response factors differ for each compound, and thus, the relative peak heights do not mirror the relative concentrations listed in the table. Values for metabolite concentrations in culture media are expressed as mM for the three strains and are reported as means ± standard deviations ($n = 3$) in the table. In all cases, metabolite concentration values for the deletion and complementation strains differed significantly from those in Lin11 ($P < 0.01$). The peak eluting at 2.439 min is ethanol, which is derived from the solvent used for the addition of lipoic acid to HTM medium. ND, not detected, indicating that concentrations are at least 50-fold lower than that observed for the Lin11 strain.

TABLE 2. Concentrations of 1,2-PD metabolites produced by *L. innocua* strains grown in L-rhamnose medium[a]

Carbon source	Avg concn (mM) ± SD of metabolite produced[b]			*P* value[c]
	Lin11 (significant difference value)	Lin11Δ1118 (significant difference value)	Lin11Δ1118/pJX1118 (significant difference value)	
1,2-PD	0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.01	0.67
n-Propanol	0.06 ± 0.02 (2)	ND (3)	0.28 ± 0.03 (1)	0.001
Propionate	0.25 ± 0.07 (1)	ND (3)	0.09 ± 0.02 (2)	<0.001

[a] Values are expressed as average mM concentrations ± standard deviations (SD) ($n = 6$). ND, not detected, i.e., concentrations are at least 50-fold lower than that observed for the Lin11 strain.

[b] Metabolite concentration values that are significantly different between strains are indicated by numbers in parentheses (1, 2, or 3), with 1 assigned to the highest concentration and 3 assigned to the lowest concentration.

[c] *P* values were calculated to determine if the concentration of each metabolite differed for the three strains.

mining the concentrations of the three compounds (21, 34). Prior to analysis, media samples were centrifuged and filtered to remove cells, and proteins were precipitated using metaphosphoric acid at a final concentration of 25% (wt/vol). Subsequently, 1-μl samples of processed culture media were analyzed using an HP 5890-II model gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with an Equity-1 capillary column (30-m by 0.53-mm inside diameter by 1.50-μm film thickness; Supelco, Bellefonte, PA) and flame ionization detector. Injector and detector temperatures were set at 225°C and 275°C, respectively. Oven temperature was initiated at 50°C and then after 5 min was increased to 225°C at a rate of 8°C/min. Ultra-high-purity hydrogen was the carrier gas. The retention times of 1,2-PD, *n*-propanol, and propionate were determined using commercially available standards (Sigma, St. Louis, MO). The identities of 1,2-PD, *n*-propanol, and propionate peaks in medium chromatograms were further confirmed by spiking samples with each standard and verifying that endogenous and exogenous compounds coeluted as single peaks. Peak areas were integrated using an HP 3396-II integrator and quantified using 1 mg of acetate or 1 mg of 2-ethylbutyrate as the internal standard (5). Data represent the averages of three to six separate experiments and were analyzed by analysis of variance using GLM software of SAS (SAS Institute, Cary, NC).

All three strains grew poorly in 1,2-PD medium, reaching a final optical density at 600 nm (OD_{600}) range of only 0.2 to 0.3. In addition, 1,2-PD supplementation did not improve the growth of any of the strains compared to the control medium lacking 1,2-PD. Nonetheless, 1,2-PD was depleted, and *n*-propanol and propionate were produced in the medium used to grow the wild-type Lin11 strain (Fig. 3). In marked contrast, the level of 1,2-PD was not reduced compared to that in the unincubated medium, and *n*-propanol and propionate were not produced in the medium used to grow the deletion strain Lin11Δ1118. Finally, the expression of *pduD* from pJX1118 in the complementation strain (Lin11Δ1118/pJX1118) restored 1,2-PD degradation and synthesis of *n*-propanol and propionate. The results observed for the complementation strain eliminate the possibility that 1,2-PD is not catabolized in the deletion mutant due to a polar effect on the transcription of genes located downstream of the *pduD* deletion site.

The growth of all three strains in L-rhamnose medium was better, with strains reaching final OD_{600} nm values of 0.4 to 0.6. While the strains all produced 1,2-PD from L-rhamnose metabolism, only the wild-type and complementation strains converted 1,2-PD to *n*-propanol and propionate (Table 2). Note that the relative levels of *n*-propanol and propionate differed in the culture media obtained from the wild-type and complementation strain when strains are grown in L-rhamnose medium (Table 2), but not in 1,2-PD medium (Fig. 3). This suggests that the fluxes through the two branches of the 1,2-PD pathway (upper part of Fig. 1) may possibly be regulated differently in L-rhamnose and 1,2-PD media.

We also examined the ability of *L. innocua* to convert 1,2-PD to propionaldehyde in a *pduD*-dependent manner by measuring diol dehydratase activities in cell-free protein extracts obtained from the wild type, the deletion mutant, and the complementation strain. The cultures used in these assays were grown aerobically at 30°C in Luria-Bertani (LB) medium with or without 20 nM vitamin B_{12} and 52 mM 1,2-PD, until early stationary phase. Cells were broken by glass bead disruption using a FastPrep-24 apparatus (MP Biomedicals, Solon, OH), and cell-free protein extracts were prepared following an established procedure (29). Diol dehydratase activity was measured by the 3-methyl-2-benzothiazolinone hydrazone method (31) and was verified to be dependent on the vitamin B_{12}-derived cofactor adenosylcobalamin, in vitro.

The results of the assay (Table 3) are consistent with the data obtained by measurement of the levels of *n*-propanol and propionate in culture media. Diol dehydratase activity was relatively high in the wild-type strain, very low in the deletion mutant, and partially restored in the complementation strain. Although clearly present in the wild-type strain, activity was

TABLE 3. Diol dehydratase activities of cell-free protein extracts of strains grown in media with and without 1,2-PD[a]

Strain	Diol dehydratase activity (avg units/mg total cell protein ± SD)[c]		*P* value[b]
	+1,2-PD	−1,2-PD	
Lin11	$6.7 \times 10^{-2} \pm 2.1 \times 10^{-2}$	$4.0 \times 10^{-3} \pm 0$	<0.01
Lin11Δ1118	$3.0 \times 10^{-3} \pm 0$	$4.0 \times 10^{-3} \pm 1.0 \times 10^{-3}$	>0.05
Lin11Δ1118/pJX1118	$1.3 \times 10^{-2} \pm 3.0 \times 10^{-3}$	$7.0 \times 10^{-3} \pm 3.0 \times 10^{-3}$	<0.05

[a] Values are expressed as average units of diol dehydratase activity per mg of total cellular protein ± standard deviations (SD) in medium with (+) and without (−) 1,2-PD ($n = 3$; $n = 4$ for Lin11 in medium +1,2-PD). One unit of diol dehydratase activity is equivalent to the amount of enzyme that converts 1 μmol/min of 1,2-PD to propionaldehyde.

[b] *P* values were calculated to determine if enzyme activities differed significantly due to the addition of 1,2-PD to the medium.

[c] *P* values were calculated to determine if enzyme activities differed significantly between strains; with 1,2-PD, the *P* value in a comparison of strains Lin11, Lin11Δ1118, and Lin11Δ1118/pJX1118 was <0.01, and without 1,2-PD, the *P* value among these strains was >0.05.

considerably lower than that reported for *Salmonella* (15). Importantly, the activity levels observed for the wild-type and complementation strains were induced by the addition of vitamin B_{12} and 1,2-PD to the growth medium. We speculate that the residual activity in the deletion mutant may arise from a second dehydratase, such as vitamin B_{12}-dependent glycerol dehydratase, which can use 1,2-PD as substrate, because no activity was observed when adenosylcobalamin was deleted from the assay buffer used to analyze the deletion strain extract (data not shown). Glycerol dehydratases are present along with diol dehydratases in species such as *Klebsiella pneumoniae* and other members of the family *Enterobacteriaceae* (11, 30). However, a BLAST-p search of the *L. innocua* CLIP11262 genome, using the *K. pneumoniae* DhaBCE glycerol dehydratase sequences, matched only PduCDE. Thus, the source of the residual activity in the deletion strain remains unknown.

Taken together, the data show that the *pduD* gene product is a functional diol dehydratase that participates in the catabolism of 1,2-PD to *n*-propanol and propionate in *L. innocua* Lin11. Interestingly, the amounts of catabolites detected in culture medium from the wild-type *L. innocua* Lin11 strain grown in 1,2-PD are smaller than would be expected for *Salmonella* (23, 28). This implies that *Listeria* deals more efficiently with propionaldehyde and further metabolizes propionate, although probably not via the 2-methylcitric acid cycle, as discussed above. We speculate that propionate is converted to another end product, which may serve to decrease its toxicity to *Listeria* (19). Last, the less efficient conversion of 1,2-PD to *n*-propanol and propionate observed for the complementation strain appears to be due to the relatively low level of diol dehydratase activity produced in this strain (Table 3).

Stimulation of *pduA-pduF* transcription in *L. innocua* Lin11 by 1,2-PD. To determine whether transcription of *pduD* and other genes is positively regulated by 1,2-PD, RNA was isolated from wild-type *L. innocua* Lin11 grown in medium with and without 1,2-PD and analyzed by real-time reverse transcription-PCR (RT-PCR) using primers complementary to *pdu* genes. Cells were grown aerobically at 30°C in LB medium with or without 20 nM vitamin B_{12} and 52 mM 1,2-PD for ~4 h from a starting OD_{600} of 0.03 to 0.5 for RNA isolation. No additional carbon sources were added. The mRNA quantitation method has been described previously (36), and primers complementary to *pdu* genes and a 16S rRNA endogenous control gene are listed in Table 4. REST statistics software was used to compare mRNA levels (36).

As shown in Table 5, transcripts for all genes in the *pduA*-to-*pduF* region were significantly induced (range, 84- to 3.2-fold; $P < 0.01$) by 1,2-PD. The mRNA level for the *pduD* gene increased by 12-fold due to the addition of 1,2-PD to the medium ($P < 0.01$). Transcript levels for *pduW*, *cobD*, and *pduX*, which are downstream of *pduF*, were unchanged, indicating that these genes are not induced by 1,2-PD, at least under the conditions studied. In addition, the mRNA of the divergently transcribed *pocR* gene was induced 3.1-fold ($P < 0.05$) by 1,2-PD. Taken together, the data indicate that the gene encoding the putative 1,2-PD transporter (*pduF*) and all but one of the *pdu* genes (*pduW*) that may serve in the putative pathway by which 1,2-PD is converted to *n*-propanol and propionate are induced by 1,2-PD (Fig. 1). The finding that *L. innocua pduW* is not induced by 1,2-PD varies from observations with *Salmonella*, where *pduW* appears to be part of a longer *pdu* operon (13, 28). Perhaps in *L. innocua*, the basal activity of PduW (propionate kinase) is sufficient to keep pace with flux from propionaldehyde to propionate so that *pduW* induction is not required for 1,2-PD metabolism.

Interestingly, we observed that transcript levels for genes in

TABLE 4. Primers used for real-time RT-PCR analysis of *pdu* gene transcript levels[a]

Primer	Sequence
pocR (lin1114)	
F	AAATGTCGCTCGCAAAAGAT
R	TAGCTCGTTTGGGTGAATCC
pduA (lin1115)	
F	GAGGGGATGTAGGTGCAGTT
R	ATGCGGACGAGGAATAACAT
pduB (lin1116)	
F	GGCCTAGTGATTGCGAATGT
R	CACCAGTACGGGCTCCTAAA
pduC (lin1117)	
F	ACAATATGTTTGCGGGTTCC
R	ACTGGCGTTAAACCACCATC
pduD (lin1118)	
F	CTTACCGGGCAATTGGTAAA
R	AATTTTGGACGAGCCATTTG
pduE (lin1119)	
F	AAAATGCAAGCGCAAGTAGC
R	TACGCTCATCTGGAACAACG
pduG (lin1120)	
F	AGCGCGTTTTTGTAACGAAT
R	GGCTGATCCTCCGACAATAA
pduH (lin1121)	
F	TTTGGTTGGAATTACGCTCA
R	TGCGCAAAAACGATATTCTG
pduK (lin1122)	
F	ACAAACGGCAATAGCCTCAG
R	GGCACGATTTCTTCTTTTGG
pduJ (lin1123)	
F	TGATGTTGGCGCAGTAAAAG
R	CATTGTGTGGACGTGGGATA
pduL (lin1124)	
F	TAAAGCAACCTGGCGAATTT
R	AAAATTTTCGAAGCGGACCT
eutJ (lin1125)	
F	TACCGCGAGTCCGTAGAAGT
R	CCAGGTGGAATAGCTCCAGA
pduM (lin1126)	
F	ATTCGTGCCGCAAATAAAAC
R	CGAACTGTCCCACTTTCCAT
pduN (lin1127)	
F	GAACATGTGCGCTCAGAAGA
R	CTGCAAATACGCTCCTAGCC
pduO (lin1128)	
F	TCAGGTGCAGGTTGTCTCAG
R	AGCGTGTTCCATTCGGTAAT
pduP (lin1129)	
F	CGACAAGAGCAGTTGCAGAG
R	ATCTTCTACCCCTGGCGTTT
pduQ (lin1130)	
F	CAAATGCCACGAACATTGAC
R	CAAGCAAGCGTCTTTCAGTG
pduF (lin1131)	
F	TTGGCGGAATTTATTGGAAC
R	CCCAGTTTGCTCCTTGTGAT
pduW (lin1132)	
F	CGGATGTTTACTGGCCAAAT
R	CACCAACTCCAGCCGTAAAT
cobD (lin1133)	
F	CGTATTGCCTCGTTTCATCA
R	TTTGCGCCTTGGTTACTTTT
pdu X (lin1134)	
F	AAAAGGGTCCGTGATTGAAA
R	TTCTTGCCGATATGTTGCAG
16S rRNA	
F	AAGCAACGCGAAGAACCTTA
R	TGCACCACCTGTCACTTTGT

[a] F, forward primer; R, reverse primer. Primers were designed using the *L. innocua* CLIP11262 genome sequence available at the ListiList server (http://genolist.pasteur.fr/ListiList).

TABLE 5. Relative levels of *pdu* mRNAs in *L. innocua* Lin11[a]

Gene	Relative fold induction of mRNA (range [lowest-highest])	
	Lin11 in LB medium − 1,2-PD	Lin11 in LB medium + 1,2-PD[b]
pocR (lin1114)	1.0 (0.6–1.6)	3.1 (2.1–4.5)*
pduA (lin1115)	1.0 (0.8–1.2)	84 (67–110)*
pduB (lin1116)	1.0 (0.7–1.4)	75 (51–100)*
pduC (lin1117)	1.0 (0.8–1.2)	43 (35–54)*
pduD (lin1118)	1.0 (0.6–1.8)	12 (9.2–17)*
pduE (lin1119)	1.0 (0.9–1.1)	13 (11–15)*
pduG (lin1120)	1.0 (0.8–1.3)	15 (13–19)*
pduH (lin1121)	1.0 (0.7–1.3)	12 (9.4–16)*
pduK (lin1122)	1.0 (0.8–1.2)	11 (8.8–13)*
pduJ (lin1123)	1.0 (0.9–1.1)	7.6 (6.6–8.9)*
pduL (lin1124)	1.0 (0.8–1.2)	7.6 (6.0–9.8)*
eutJ (lin1125)	1.0 (0.8–1.3)	7.8 (6.4–9.6)*
pduM (lin1126)	1.0 (0.8–1.2)	5.2 (4.5–6.0)*
pduN (lin1127)	1.0 (0.8–1.2)	5.5 (4.7–6.6)*
pduO (lin1128)	1.0 (0.9–1.2)	3.2 (2.8–3.8)*
pduP (lin1129)	1.0 (0.5–1.8)	5.0 (3.7–6.7)*
pduQ (lin1130)	1.0 (0.9–1.2)	4.2 (3.5–5.1)*
pduF (lin1131)	1.0 (0.6–1.7)	3.2 (1.4–7.0)*
pduW (lin1132)	1.0 (0.8–1.3)	1.1 (0.7–1.9)
cobD (lin1133)	1.0 (0.6–1.5)	0.9 (0.7–1.1)
pduX (lin1134)	1.0 (0.7–1.5)	0.9 (0.7–1.2)

[a] The values shown are averages of relative levels of *pdu* mRNAs in *L. innocua* Lin11 grown aerobically in LB/vitamin B_{12} medium with (+) or without (−) 52 mM 1,2-PD, obtained using three independent RNA preparations. Transcript levels were measured in triplicate for each RNA preparation. The cycle threshold values for PCR products were compared to those measured in strain lin11 grown in LB medium without 1,2-PD to determine differences. Values in parentheses signify the lowest and highest values, respectively, measured for each mRNA.

[b] Asterisks indicate values that are significantly different between the two media ($P < 0.01$).

the *pdu* cluster decreased across the *pduA*-to-*pduF* series of genes (Table 5). We speculate that the reduction in mRNA levels could be caused by stability differences for the coding regions within a long *pduA*-to-*pduF* polycistronic transcript. Alternatively, the data could be explained by the presence of internal promoter or terminator sequences within the gene cluster. However, we did not find evidence by sequence analysis for strong consensus σ^{70} promoter sequences or ρ-independent terminator sequences, even immediately after *pduF*, within this gene cluster.

Last, we determined, by real-time RT-PCR analysis, that the *pduA*-to-*pduF* genes are not induced by 1,2-PD in brain heart infusion medium, which contains 0.2% glucose (data not shown). This is consistent with findings in *Salmonella*, where it has been demonstrated that glucose inhibits the *pdu* operon expression via repression of *pocR* transcription (2).

Conclusions. It has been demonstrated for the first time that an *L. innocua* strain can degrade 1,2-PD to *n*-propanol and propionate via a *pduD*-dependent pathway and that, based on comparative studies with *Salmonella*, degradation may proceed as shown in Fig. 1. We also determined that *pduD* and all but one other gene (*pduW*) needed for 1,2-PD transport and conversion to *n*-propanol and propionate by the conventional pathway are coordinately upregulated by 1,2-PD, possibly via a PocR-dependent mechanism. Because *pdu* genes are highly conserved between *L. innocua* and *L. monocytogenes*, it seems possible that the pathogen also may be able to catabolize 1,2-PD to *n*-propanol and propionate. Although *L. innocua* Lin11 did not grow aerobically on 1,2-PD medium, we propose that 1,2-PD catabolism nonetheless could be important for growth in poor-quality medium containing L-rhamnose. In this regard, dihydroxyacetone phosphate and 1,2-PD are both formed during L-rhamnose catabolism (25, 33). While metabolism of dihydroxyacetone phosphate probably supplies most of the energy needed for growth, the breakdown of 1,2-PD to *n*-propanol and propionate could contribute to energy production.

We thank Thomas A. Bobik for helpful discussions.
We thank The University of Wyoming for financial support.

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