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Cloning and Sequencing of a 2,5-Dichlorohydroquinone Reductive Dehalogenase Gene Whose Product Is Involved in Degradation of γ-Hexachlorocyclohexane by *Sphingomonas paucimobilis*

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***Sphingomonas* (formerly *Pseudomonas*) *paucimobilis* UT26 utilizes γ-hexachlorocyclohexane (γ-HCH), a halogenated organic insecticide, as a sole carbon and energy source. In a previous study, we showed that γ-HCH is degraded to 2,5-dichlorohydroquinone (2,5-DCHQ) (Y. Nagata, R. Ohtomo, K. Miyauchi, M. Fukuda, K. Yano, and M. Takagi, J. Bacteriol. 176:3117–3125, 1994). In the present study, we cloned and characterized a gene, designated *linD*, directly involved in the degradation of 2,5-DCHQ. The *linD* gene encodes a peptide of 343 amino acids and has a low level of similarity to proteins which belong to the glutathione *S*-transferase family. When LinD was overproduced in *Escherichia coli*, a 40-kDa protein was found after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Northern blot analysis revealed that expression of the *linD* gene was induced by 2,5-DCHQ in *S. paucimobilis* UT26. Thin-layer chromatography and gas chromatography-mass spectrometry analyses with the LinD-overexpressing *E. coli* cells revealed that LinD converts 2,5-DCHQ rapidly to chlorohydroquinone (CHQ) and also converts CHQ slowly to hydroquinone. LinD activity in crude cell extracts was increased 3.7-fold by the addition of glutathione. All three of the Tn*5*-induced mutants of UT26, which lack 2,5-DCHQ dehalogenase activity, had rearrangements or a deletion in the *linD* region. These results indicate that LinD is a glutathione-dependent reductive dehalogenase involved in the degradation of γ-HCH by *S. paucimobilis* UT26.**

γ-Hexachlorocyclohexane (γ-HCH [also called γ-BHC and lindane]) is a halogenated organic insecticide which has been used worldwide. Because of its toxicity and long persistence in soil, most countries have prohibited its use; however, many contaminated sites remain throughout the world. Moreover, some countries are presently using γ-HCH for economic reasons, and new sites are continually being contaminated.

Sphingomonas (formerly *Pseudomonas*) *paucimobilis* UT26 utilizes γ-HCH as a sole source of carbon and energy (8). UT26 degrades γ-HCH through the pathway shown in Fig. 1 (14, 16, 17). γ-HCH is likely converted by two steps of dehydrochlorination via γ-pentachlorocyclohexene (γ-PCCH) to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN). This is productively metabolized to 2,5-dichloro-3,5-cyclohexadiene-1,4-diol (2,5-DDOL) by two steps of hydrolytic dehalogenation. Then, 2,5-DDOL is further degraded to 2,5-dichlorohydroquinone (2,5-DCHQ), and finally 2,5-DCHQ is mineralized. Two dead-end products, 1,2,4-trichlorobenzene (1,2,4-TCB) and 2,5-dichlorophenol (2,5-DCP), have also been found in culture supernatants.

In previous studies, we cloned and sequenced three genes involved in the early steps of γ-HCH degradation in UT26 (7, 16, 17). The *linA* gene encodes γ-HCH dehydrochlorinase (LinA), which converts γ-HCH to 1,2,4-TCB via γ-PCCH. LinA shows no homology to known proteins (7). The *linB* gene encodes 1,4-TCDN chlorohydrolase (LinB), which converts 1,4-TCDN to 2,5-DDOL via 2,4,5-trichloro-2,5-cyclohexadiene-1-ol (2,4,5-DNOL). LinB shows significant similarity to hydrolytic dehalogenase, DhlA, from *Xanthobacter autotrophicus* (9). The *linC* gene encodes 2,5-DDOL dehydrogenase, which converts 2,5-DDOL to 2,5-DCHQ (17). LinC shows homology to the members of the short-chain alcohol dehydrogenase family (19).

In this study, we describe the isolation and characterization of a gene directly involved in the degradation of 2,5-DCHQ and show that its product is a glutathione-dependent reductive dechlorinase which converts 2,5-DCHQ to hydroquinone (HQ) via chlorohydroquinone (CHQ).

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *Sphingomonas* strains, *Pseudomonas* strains, and *Escherichia coli* were grown on Luria broth (12) or W minimal medium (8). Cultures were incubated at 30°C for *Sphingomonas* and *Pseudomonas* strains and at 37°C for *E. coli* strains. Antibiotics were used at final concentrations of 50 μg/ml for ampicillin and kanamycin, 25 μg/ml for nalidixic acid, and 20 μg/ml for tetracycline.

Preparation of CHQ. CHQ was prepared as follows. A solution of 2-chloro-1,4-benzoquinone (Aldrich, Milwaukee, Wis.) solution (174 mM in ethylacetate) was mixed with the same amount of ascorbic acid solution (1.1 M in 100 mM phosphate buffer [pH 7.0]) and vortexed. After centrifugation, the upper phase was diluted 10 times by ethylacetate. The resultant solution was used as a CHQ stock solution, which was diluted 1,000 times just before the assay.

Isolation of DNA. Plasmid DNA of *E. coli* was isolated by the alkaline lysis method of Maniatis et al. (12) and, if needed, purified by cesium chloride-ethidium bromide density gradient centrifugation. Total DNA from *Sphingomonas* strains was isolated as described previously (15).

Construction of a gene library. A gene library of *S. paucimobilis* in *Pseudomonas putida* PpY101 or *E. coli* HB101 was constructed by using a broad-host-range cosmid vector, pKS13 (11), as described previously (16).

Assay for 2,5-DCHQ dehalogenase activity. A small quantity of each colony was picked and suspended in 100 μl of the assay solution (20 mM phosphate buffer [pH 7.0] containing 2,5-DCHQ at 1 μg/ml). The solution was incubated for 12 to 18 h at 30°C for *P. putida* and at 37°C for *E. coli*. Then, 100 μl of

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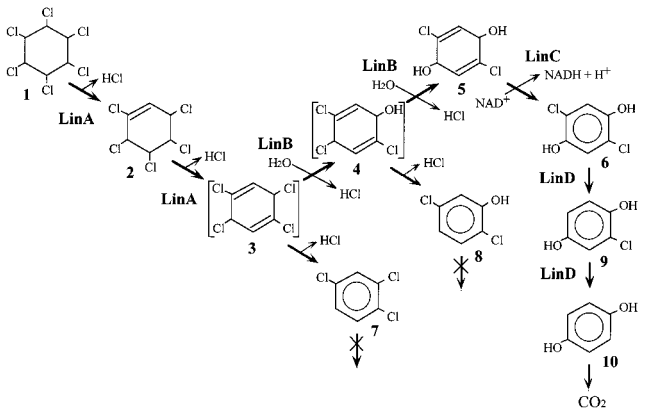

FIG. 1. Proposed assimilation pathway of γ-HCH in *S. paucimobilis* UT26. Compounds: 1, γ-HCH/γ-BHC; 2, γ-PCCH; 3, 1,4-TCDN; 4, 2,4,5-DNOL; 5, 2,5-DDOL; 6, 2,5-DCHQ; 7, 1,2,4-TCB; 8, 2,5-DCP; 9, CHQ; 10, HQ.

ethylacetate was added and the mixture was vortexed for 1 min. After centrifugation (15,000 × *g*, for 5 min), the ethylacetate layer was recovered. One microliter of this extract was analyzed by gas chromatography (GC) with an electron capture detector (ECD).

GC and GC-MS analyses. GC analysis with an ECD was performed under the same conditions as described previously (16). GC-mass spectrometry (MS) analysis was performed with a GC-MS QP5000 spectrometer (Shimadzu, Kyoto, Japan) and a DB-1 capillary column (0.25 mm thick by 30 m long; J&W Scientific, Folsom, Calif.). The column temperature was increased from 80 to 160°C at a rate of 5°C/min and then from 160 to 260°C at a rate of 10°C/min. The carrier gas flow rate was 20 ml/min.

Nucleotide sequence determination. Nucleotide sequences were determined by the dideoxy-chain termination method with the Applied Biosystems model 373A DNA sequencing system (Applied Biosystems, Foster City, Calif.).

Southern blot analysis. Southern blot analysis was performed at 56°C as described previously (16). The ECL gene detection system (Amersham, Arlington Heights, Ill.) was used according to the provided protocol.

Northern blot analysis. *S. paucimobilis* UT26 was grown on W medium, and 2,5-DCHQ was added during the exponential phase (optical density at 660 nm, 0.3 to 0.4). After 1 h of incubation, total RNA was isolated by the method described by Hopwood et al. (6). As a control, total RNA was isolated from cells incubated without 2,5-DCHQ. Hybridization and detection were performed by using digoxigenin-labeled DNA with the CSPD system (Boehringer, Mannheim, Germany), according to the provided protocol.

Analysis of the *linD* gene product. For overexpression of the *linD* gene product, plasmid pMYLD2 was constructed from pAQN (23). Plasmid pAQN was digested with *Eco*RI and *Hin*dIII to replace the 1.8-kb aqualysin-coding fragment with the 1.2-kb *Eco*RI-*Hin*dIII fragment including the *linD* gene from pKM2 for pMYLD2. In pMYLD2, the *linD* gene is expressed under the control of the *tac* promoter. Expression is repressed tightly by the *lacI*^{q} gene product, which is produced from the same plasmid when IPTG (isopropyl-β-D-thiogalactopyranoside) is not added. Overexpression of LinD was achieved as described previously (7) by using *E. coli* MV1190 containing pMYLD2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was done as described previously (16).

TLC analysis. To detect the metabolites resulting from 2,5-DCHQ by LinD, we used thin-layer chromatography (TLC). TLC plates (HPTLC Precoated Silica Gel 60) were purchased from Merck (Darmstadt, Germany). The substrate, 2,5-DCHQ (50 μg/ml [final concentration]), was added to resting cells of *E. coli* MV1190 (50 mg [wet weight] per ml in 20 mM phosphate buffer [pH 7.0]) overexpressing LinD or LinB as a control (16). After incubation for various time periods, 500 μl of the culture was extracted with ethylacetate. After evaporation, the reaction mixture was resuspended in a small amount of ethylacetate and the suspension was spotted onto the TLC plate. The solvent system for the separation of metabolites consisted of benzene-ethylacetate (85:15, vol/vol). After development, the TLC plate was dried and exposed to iodine vapor.

LinD activity assay using crude cell extracts. To investigate the effect of glutathione on LinD activity, we used cell extracts prepared from *E. coli* MV1190

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Source or reference
S. paucimobilis strains		
UT26	Growth on γ-HCH; Nal^r	8
UT102	Nal^r Km^r	18
UT103	Nal^r Km^r	18
UT116	Nal^r Km^r	18
P. putida strain		
PpY101	*met* Nal^r	11
E. coli strains		
HB101	F$^-$ *hsdS recA ara proA lacY galK rpsL xyl mtl supE*	12
MV1190	Δ*lac-proAB thi supE* Δ*srl-recA306*::Tn*10* F′ *traD36 proAB lacI*^{q}*Z*ΔM15	17
Plasmids		
pAQN	pMB9 replicon; *lacI*^{q} *aqn*^{a} Ap^r	23
pKM1	pUC18 carrying 3-kb *Sac*I-*Hin*dIII fragment; direction of *linD* is identical to that of *lac* promoter of pUC18	This study
pKM1R	pUC18 carrying same fragment as pKM1 with opposite direction	This study
pKM2	pUC18 carrying 1.2-kb *Eco*RI-*Mlu*I fragment of pKM170	This study
pKM2R	pUC18 carrying same fragment as pKM2 with opposite direction	This study
pKM170	pUC18 carrying 2.0-kb fragment of pKM1; deletion plasmid with deletion about 900 bp from 5′ end of fragment inserted in pKM1	This study
pKM9372	pUC18 carrying 8-kb *Eco*RI fragment of pMM9372	This study
pKS13	RK2 replicon; *cos* Mob$^+$ Tc^r	11
pKSM937	pKS13 with 20 kb of UT26 DNA containing *linD*	This study
pMFY42	RSF1010 replicon; Km^r Tc^r	4
pMM9372	pMFY42 carrying 8-kb *Eco*RI fragment of pKSM937	This study
pMYLB1	pAQN carrying *linB* in place of *aqn*^{a}	16
pMYLD2	pAQN carrying *linD* in place of *aqn*^{a}	This study
pRK2013	ColE1::RK2 Tra$^+$ Km^r	3
pUC18	pMB9 replicon of pKM9372; Ap^r	26

[a] *aqn*, aqualysin I gene of *Thermus aquaticus.*

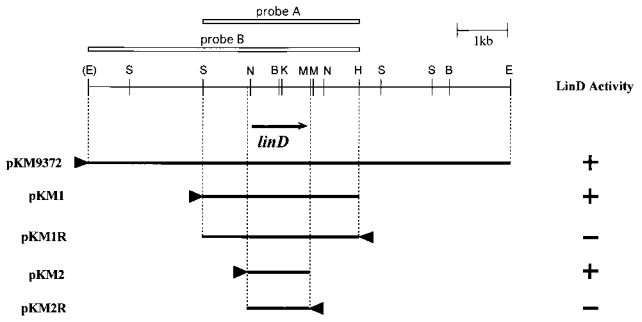

FIG. 2. Restriction map of pKM9372 and deletion analysis of pKM1. The directions of transcription by the *lac* promoter are indicated by arrowheads. The procedure for the measurement of LinD activity is described in Materials and Methods. The deduced location and direction of the *linD* gene is indicated by the arrow under the restriction map. The white bars above the map indicate the DNA fragments used as probes in Southern blot analyses. B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; M, *Mlu*I; N, *Nae*I; S, *Sac*I.

overexpressing LinD. Cells were suspended in 20 mM Tris-HCl buffer (pH 7.5) and sonicated. After centrifugation (17,000 × *g*) at 4°C for 10 min, the supernatant was used as the crude cell extract. Ninety microliters of 2,5-DCHQ solution (100 μM in 20 mM potassium phosphate buffer [pH 7.0] including 200 μM ascorbic acid) was mixed with 10 μl of crude extract with and without 2 mM glutathione. After incubation for 10 min at 30°C, the reaction mixture was extracted with ethylacetate and analyzed by GC-MS. One unit of LinD activity was defined as the amount of enzyme that transformed 1 μmol of 2,5-DCHQ per min. Crude cell extract of *E. coli* MV1190 not overexpressing LinD was used as a control. Protein concentrations of crude extracts were measured by a protein assay kit (Bio-Rad, Hercules, Calif.).

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper has been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession no. D89733.

RESULTS

Screening of clones with 2,5-DCHQ degradation activity. The gene library of UT26 was constructed in *P. putida* PpY101, and clones which showed 2,5-DCHQ degradation activity (we designated it LinD activity) were identified upon analysis of biotransformation products by GC provided with an ECD. Of 1,000 recombinants tested, six clones showed LinD activity. The positive clones completely transformed 1 ppm of 2,5-DCHQ within 12 h. The activity was not detected in *E. coli* HB101, which had the same positive cosmids. This suggested either that the gene responsible for the degradation of 2,5-DCHQ has a promoter which is functional in *P. putida* but not in *E. coli* HB101 or that only *P. putida* has some factor(s) necessary for LinD activity. None of the positive clones had LinA, LinB, or LinC activity, suggesting that the *linD* gene is not linked to the *linA*, *linB*, and *linC* genes, which are responsible for the upstream pathway of γ-HCH degradation in *S. paucimobilis* UT26.

Subcloning and restriction enzyme mapping of the *linD* gene. *Eco*RI digestion of one of the positive cosmids, pKSM937, produced three fragments (2, 7, and 8 kb) suitable for subcloning. Each of these fragments was subcloned into pMFY42, and the recombinant plasmids were introduced into *P. putida* PpY101. All clones which showed LinD activity had the 8-kb fragment. The resulting plasmid was called pMM9372. When the 8-kb *Eco*RI fragment was ligated to pUC18 and introduced into *E. coli* MV1190, the transformant also showed LinD activity. This may be due to the higher copy number of pUC18 than that of pKS13. A summary of the restriction enzyme mapping of this 8-kb fragment is shown in Fig. 2. Later investigation indicated that the *Eco*RI site, which was located at the 5′ end of pKM9732, was derived from the cloning site of the cosmid vector, pKS13. Southern blot analysis was performed with the cloned 3.0-kb *Sac*I-*Hin*dIII fragment as a probe (probe A [Fig. 2]). Total DNA of UT26, digested with *Sac*I, *Eco*RI, and *Bam*HI, was separated on a 0.6% (wt/vol) agarose gel, blotted, and hybridized with the ECL-labeled probe. Only one signal was detected, indicating that the cloned fragment originated from UT26 and that there was no other sequence highly homologous to the *linD* gene in UT26 (data not shown).

Deletion and sequence analysis of the *linD* gene. Deletion analysis of the 3-kb *Sac*I-*Hin*dIII fragment revealed that the 1.2-kb region was necessary for LinD activity (Fig. 2). Sequence analysis of this region revealed that there was only one open reading frame of reasonable size (1,038 bp) in this region. As this open reading frame was preceded by a putative Shine-Dalgarno sequence (22), we designated it *linD*. The *linD* gene likely encodes a polypeptide of 346 amino acids, and its deduced molecular mass is 38.7 kDa. A sequence with high homology to the promoter of *E. coli* or which was expected to form a stem-loop structure to function as a terminator was not found around the *linD* gene. The G+C content of the *linD* gene is 60.8%, which is close to the total G+C contents of the type strain of *S. paucimobilis* (65%) (20), *linB* (64.3%), and *linC* (62.5%) (16, 17). Although a computer search revealed no nucleotide sequence with significant similarity to the whole *linD* gene, the LinD protein sequence showed a low level of similarity with some proteins which belong to the theta class of the glutathione *S*-transferase (GST) family, e.g., GST of *E. coli* (Swiss-Prot P39100) and *Arabidopsis thaliana* (Swiss-Prot P42769). The sequence with the most significant similarity was PcpC (Swiss-Prot Q03520) (20% identity, 31% similarity), the tetrachlorohydroquinone dechlorinase of *Flavobacterium* sp. strain ATCC 39723, which catalyzes the dechlorination of tetrachlorohydroquinone to 2,6-dichlorohydroquinone by two steps. The alignment of LinD with PcpC is shown in Fig. 3.

Overexpression of *linD* in *E. coli* and identification of the gene product. To identify the *linD* gene product, we constructed plasmid pMYLD2, in which the *linD* gene was under the control of the *tac* promoter. *E. coli* MV1190 transformed with this plasmid was incubated with or without IPTG, and total proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. An overproduced protein band corresponding to about 40 kDa was observed in the IPTG-treated cells (data not shown). The molecular mass of this protein was almost equal to that deduced from the nucleotide sequence of the *linD* gene. Thus, it was confirmed that the product of *linD* is a protein with the molecular mass of about 40 kDa.

Expression of the *linD* gene in UT26. We previously demonstrated that LinD activity was inducibly expressed in UT26 in the presence of 2,5-DCHQ (18). Northern blot analysis was performed with the total RNA of UT26 incubated with or without 2,5-DCHQ during the log phase. The result indicated that the *linD* gene was inducibly expressed by 2,5-DCHQ (data not shown).

Southern blot analysis of *linD* in LinD-less mutants. We previously isolated UT102, UT103, and UT116, Tn*5*-induced mutants defective in 2,5-DCHQ dehalogenase activity which showed phenotypes different from each other (18). UT102 is a mutant which constitutively expresses faint LinD activity, while UT103 and UT116 have no LinD activity at all. Southern blot analysis with the 5.0-kb *Eco*RI-*Hin*dIII fragment containing *linD* (probe B [Fig. 2]) as a probe to the total DNAs of the mutants digested with *Eco*RI and *Hin*dIII indicated that UT116 lacked the whole *linD* gene, while UT102 and UT103 had rearrangements in the *linD* gene or in its flanking regions

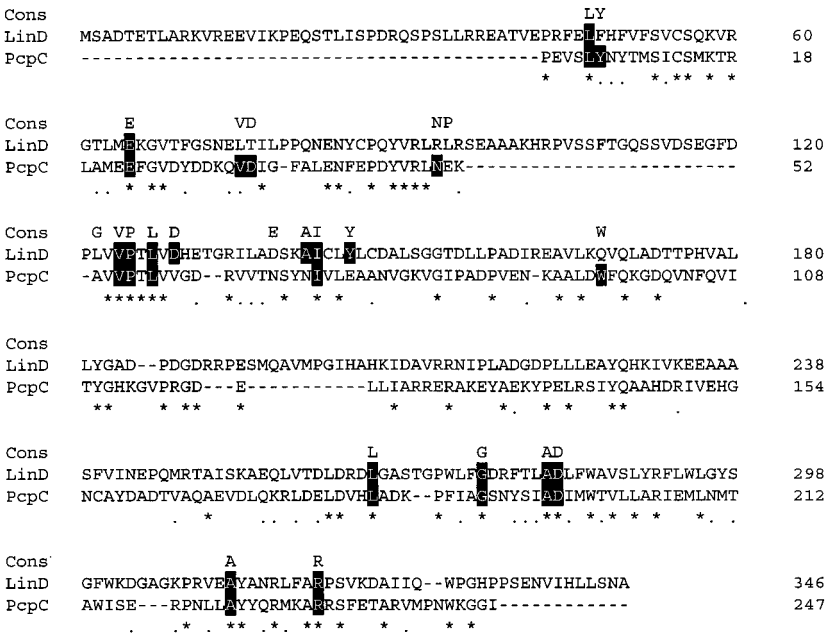

FIG. 3. Alignment between LinD and PcpC. Identical and similar residues between LinD and PcpC are indicated by asterisks and dots, respectively. "Cons" indicates residues conserved in at least 10 of 13 bacterial GST sequences in the review by Vuilleumier (27). The amino acid residues with black backgrounds are conserved between LinD or PcpC and those in the top row (Cons). The alignment was generated by using the ClustalW sequence alignment program.

(Fig. 4). This result supported the hypothesis that *linD* is directly involved in 2,5-DCHQ degradation activity in UT26.

Identification of metabolites of 2,5-DCHQ by the LinD protein. To detect the metabolites of 2,5-DCHQ catalyzed by LinD, we used TLC. 2,5-DCHQ (spot 1) was changed rapidly to spot 2, which was slowly converted to spot 3 by *E. coli* cells overexpressing LinD (Fig. 5A). In contrast, no change in 2,5-DCHQ was observed when *E. coli* cells overexpressing LinB were used as a control (Fig. 5B).

Each spot was extracted by ethylacetate and analyzed by GC-MS. It was found that the retention times and mass spectra of spot 2 and spot 3 were identical to those of CHQ and HQ, respectively (data not shown). Furthermore, the mobilities of the spots corresponding to authentic compounds of 2,5-DCHQ, CHQ, and HQ were the same as spot 1, spot 2, and spot 3, respectively (Fig. 5A). To investigate whether CHQ was a substrate of LinD, CHQ was incubated with *E. coli* cells overexpressing LinD or LinB. The former cells slowly converted CHQ to HQ, whereas the latter cells did not (data not shown). This indicated that CHQ is also a substrate of LinD, although it seems to be a poor substrate compared with 2,5-DCHQ.

LinD activity is increased by the addition of glutathione. Because LinD has homology to GSTs, we investigated the effect of glutathione on LinD activity. Crude cell extracts of *E. coli* MV1190 overexpressing LinD were prepared. When glutathione was added to a reaction mixture, LinD activity increased from 1.57 U/mg of protein to 5.85 U/mg of protein (a 3.7-fold increase). This result strongly suggested that LinD activity is glutathione dependent.

DISCUSSION

We have cloned the *linD* genes as a candidate for direct involvement in the degradation of 2,5-DCHQ, the intermediate of γ-HCH degradation by *S. paucimobilis* UT26. The fact that *E. coli* cells overproducing LinD converted 2,5-DCHQ to HQ and that the Tn*5*-induced mutants which show deficiencies in LinD activity have a deletion or rearrangements in the *linD* gene support the hypothesis that the *linD* gene is directly involved in 2,5-DCHQ degradation in UT26. Recently, we revealed that rearrangements occurred in the *linD* gene in UT103, which has no LinD activity (13).

Northern blot analysis revealed that *linD* mRNA is expressed upon induction with 2,5-DCHQ, whereas *linA*, *linB*, and *linC* are constitutively expressed. This suggested the existence of a regulatory system for *linD* gene expression.

TLC analysis revealed that LinD rapidly converts 2,5-DCHQ to CHQ and slowly converts CHQ to HQ. This indicates that LinD catalyzes the reductive dehalogenation of 2,5-DCHQ. However, the conversion of CHQ to HQ may not be essential for the degradation pathway of γ-HCH in *S. paucimobilis* UT26, because the conversion rate of CHQ to HQ by LinD is much lower than the rate of CHQ degradation by resting UT26 cells (13). Another degradation pathway of CHQ may exist in *S. paucimobilis* UT26.

D. B. Janssen classified the dehalogenation reaction of ha-

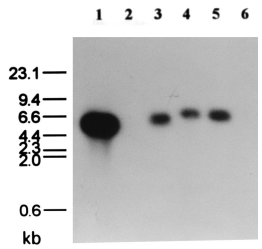

FIG. 4. Southern blot analysis of UT26 and its Tn*5*-induced mutants probed with the *linD* gene. Lanes: 1, 5-kb *Eco*RI-*Hin*dIII fragment containing the *linD* gene; 2, λ DNA digested with *Hin*dIII; 3 to 6, total DNA of UT26, UT102, UT103, and UT116, respectively, digested with *Eco*RI and *Hin*dIII. The conditions of the experiment are described in Materials and Methods.

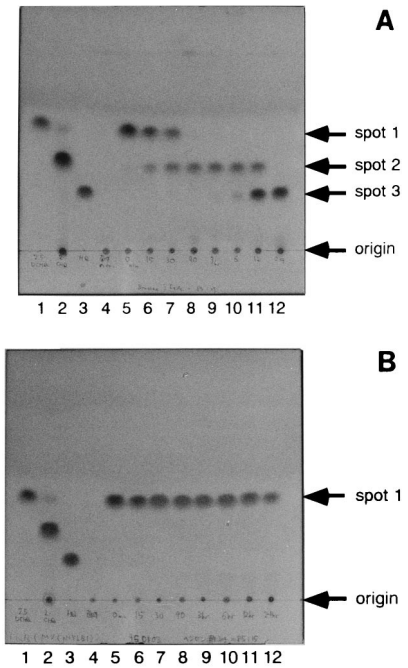

FIG. 5. TLC analysis of the 2,5-DCHQ degradation activity of *E. coli* overproducing the LinD protein (A) and *E. coli* overproducing the LinB protein (negative control) (B). Lanes: 1, authentic 2,5-DCHQ (spot 1); 2, authentic CHQ (spot 2); 3, authentic HQ (spot 3); 4, extraction from *E. coli* MV1190 cells overproducing LinD or LinB; 5 to 12, extraction from *E. coli* MV1190 cells overproducing LinD or LinB incubated with 50 ppm of 2,5-DCHQ for 0 min, 15 min, 60 min, 90 min, 3 h, 6 h, 12 h, and 24 h, respectively. The procedure for TLC analysis was described in Materials and Methods.

logenated compounds into different groups according to their reaction mechanisms (10), and the reaction by LinD can be categorized into the reductive dehalogenation group. There are a few examples of enzymes from aerobic microorganisms belonging to this group, which is further categorized into two subgroups based on the presence of a reaction catalyzed by GSTs and on the presence of a reaction not catalyzed by GSTs (1, 2, 5, 21, 24, 25). To our knowledge, there is no report of the cloning of genes encoding enzymes catalyzing the latter type of reaction.

We found that LinD activity was increased by the addition of glutathione. The amino acid sequence of LinD has similarity to some known GSTs, although its molecular mass is larger than other GSTs. Bacterial GSTs were reviewed recently (27) and were placed in the theta class. Actually, LinD has similarity to GSTs of this class such as PcpC of *Flavobacterium*, the theta class GST of a plant (*A. thaliana*), and the GST of *E. coli*. Furthermore, LinD has 15 of 23 amino acids which are conserved in 10 of the 13 bacterial GST sequences aligned in the review article (Fig. 3). These results suggest the possibility that LinD is a novel member of the bacterial GSTs.

The purification of chlorophenol 4-monooxygenase in *Burkholderia cepacia* AC1100, which degrades 2,4,5-trichlorophenoxyacetic acid via 2,5-DCHQ, was reported previously (28). This enzyme converts 2,4,5-trichlorophenol to 2,5-DCHQ and then to 5-chloro-1,2,4-trihydroxybenzene in an NADH-dependent manner. Although *B. cepacia* AC1100 converts the same substrate as LinD, 2,5-DCHQ, its degradation pathway seems to be different from that of UT26.

From previous work and this study, we show that *S. paucimobilis* UT26 completely dechlorinates γ-HCH to HQ by three different types of dehalogenases, LinA, LinB, and LinD (Fig. 1). Each enzyme removes two chlorines which are located symmetrically at the γ-HCH ring.

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