Identification and Characterization of Genes Involved in the Downstream Degradation Pathway of γ -Hexachlorocyclohexane in *Sphingomonas paucimobilis* UT26

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Sphingomonas paucimobilis UT26 utilizes γ -hexachlorocyclohexane (γ -HCH) as a sole source of carbon and energy. In our previous study, we cloned and characterized genes that are involved in the conversion of γ -HCH to maleylacetate (MA) via chlorohydroquinone (CHQ) in UT26. In this study, we identified and characterized an MA reductase gene, designated *linF*, that is essential for the utilization of γ -HCH in UT26. A gene named *linEb*, whose deduced product showed significant identity to LinE (53%), was located close to *linF*. LinE is a novel type of ring cleavage dioxygenase that catalyzes the conversion of CHQ to MA. LinEb expressed in *Escherichia coli* transformed CHQ and 2,6-dichlorohydroquinone to MA and 2-chloromaleylacetate, respectively. Our previous and present results indicate that UT26 (i) has two gene clusters for degradation of chlorinated aromatic compounds via hydroquinone-type intermediates and (ii) uses at least parts of both clusters for γ -HCH utilization.

Sphingomonas paucimobilis UT26 utilizes the halogenated organic insecticide γ -hexachlorocyclohexane (γ -HCH; also called γ -BHC and lindane) as a sole source of carbon and energy under aerobic conditions (7). UT26 degrades γ -HCH through the pathway shown in Fig. 1 (25). γ -HCH is converted to 2,5-dichlorohydroquinone (2,5-DCHQ) by sequential reactions catalyzed by LinA (γ -HCH dehydrochlorinase) (8, 20, 26, 37), LinB (1,3,4,6-tetrachloro-1,4-cyclohexadiene chlorohydrolyase) (21, 24), and LinC (2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase) (22). 2,5-DCHQ is then dechlorinated to chlorohydroquinone (CHQ) by LinD (2,5-DCHQ dechlorinase) (17), and CHQ is further transformed to maleylacetate (MA) by LinE (CHQ 1,2-dioxygenase) (18). LinE is the first reported enzyme that prefers hydroquinone (HQ)-type compounds, i.e., aromatic compounds with two hydroxyl groups at the *para* position, as its substrate to catechol (18). The *linA*, *linB*, and *linC* genes for the upstream pathway are dispersed on the UT26 genome and are constitutively expressed (25). On the other hand, the *linD* and *linE* genes form an operon and their expression is induced by a LysR-type transcriptional regulator (LTTR), LinR, in the presence of HQ-type compounds, such as CHQ, HQ, and 2,5-DCHQ (19).

By a series of reactions catalyzed by LinA to LinE, γ -HCH is converted to MA (Fig. 1). MA is known as one of the major intermediates in the degradation of aromatic compounds, and it is usually converted to β -keto adipate by MA reductase. β -Keto adipate is metabolized by the β -keto adipate degrada-

tion pathway widely distributed in soil bacteria and fungi (5, 9, 13, 33, 34). In this study, we identified and characterized a gene, designated *linF*, encoding MA reductase, which was necessary for the assimilation of γ -HCH in UT26. We further characterized a gene encoding a LinE homologue that was located close to *linF*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *Escherichia coli* cells were grown at 37°C in Luria broth (LB) (15), and *S. paucimobilis* cells were grown at 30°C in 1/3 LB (3.3 g of Bacto Tryptone per liter, 1.7 g of yeast extract per liter, 5 g of sodium chloride per liter) or W minimal medium containing an appropriate carbon source (7). Ampicillin and kanamycin were used at a final concentration of 50 μ g/ml, and tetracycline was used at a final concentration of 20 μ g/ml.

DNA manipulation and DNA sequence analysis. Established methods were used for the preparation of genomic and plasmid DNAs, digestion of DNA with restriction endonucleases, ligation, agarose gel electrophoresis, and transformation of *E. coli* cells (15). The nucleotide sequences were determined with an ABI PRISM 310 sequencer (Applied Biosystems, Foster City, Calif.) and an ABI Prism Big Dye Terminator kit (Applied Biosystems). Southern hybridization analysis was carried out by the conventional protocols (29) with a DIG system (Roche Diagnostics, Mannheim, Germany). Nucleotide sequences were analyzed with the Genetyx program, version 12 (SDC Inc., Tokyo, Japan). Homology searches were performed with BLAST programs available at the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Transposon mutagenesis. Random mutagenesis of *S. paucimobilis* UT26 was accomplished with pTnMod-OKm, which carries pMB1-based replication machinery in a Tn5-derived minitransposon (2). Transformation of UT26 cells by pTnMod-OKm was performed as described previously (12). Km^r transformants were streaked on W minimal medium agar containing 0.5 g of γ -HCH per liter, and mutants deficient in γ -HCH utilization were selected. The γ -HCH degradation activities of the mutants were also monitored by Shimadzu GC-17A gas chromatography (GC) with an electron capture detector (ECD) and an Rtx-1 capillary column (30 m by 0.25 mm by 0.25 μ m; Restek, www.restekcorp.com) as described previously (21). The column temperature was increased from 100 to 260°C at a rate of 20°C/min, and the gas flow rate was 30 ml/min.

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FIG. 1. Proposed pathways of γ -HCH and 2,6-DCHQ degradation by *S. paucimobilis* UT26. PcpA and PcpE are the enzymes involved in PCP degradation by *S. chlorophenolicum* ATCC 39723 (1). Compounds: 1, γ -HCH; 2, pentachlorocyclohexene; 3, 1,3,4,6-tetrachloro-1,4-cyclohexadiene; 4, 1,2,4-trichlorobenzene; 5, 2,4,5-trichloro-2,5-cyclohexadiene-1-ol; 6, 2,5-dichlorophenol; 7, 2,5-dichloro-2,5-cyclohexadiene-1,4-diol; 8, 2,5-DCHQ; 9, CHQ; 10, HQ; 11, acylchloride; 12, γ -hydroxymuconic semialdehyde; 13, MA; 14, β -ketoadipate; 15, 2,6-DCHQ; 16, 2-CMA. GSH, glutathione, (reduced form); GS-SG, glutathione (oxidized form).

Characterization of transposon insertion sites. Genomic DNA prepared from a mutant into which TnMod-OKm had been inserted was digested with BamHI or NcoI. The digested DNA was self-ligated, and the self-replicating Km^r plasmids were recovered by transformation of *E. coli* DH5 α cells. The plasmids thus recovered were used to determine the sequences of flanking regions of the TnMod-OKm insertion sites with primers 5'-GCTGGCCTTTGCTCAC-3' and 5'-TTGAGACACAACGTGGC-3', which anneal the regions close to both ends of TnMod-OKm.

Allelic-exchange mutagenesis. A BamHI-flanked Tc^r gene cassette derived from pEX18Tc (6) was inserted into the BamHI site of p1023Bam (Table 1). The resultant plasmid, p1023BamTc, was introduced into UT26 by electroporation, and Km^r but Tc^r transformants were selected. The double-crossover-mediated homologous recombination of one of these transformants (UT1023d) was confirmed by Southern blot analysis. To disrupt *linEb* on the UT26 genome, we used PCR to amplify an internal fragment of *linEb* lacking both its 5' and 3' ends with primers orf3midFB1 (5'-CCGGATCCTGGAATGGTGGATCGGCC-3') and orf3midRP1 (5'-GCCCTGCAGCGAGAAGTCGGTGAAGCCG-3') and cloned it into Km^r-encoding vector pHS299. The resultant plasmid, pHS299, was introduced into UT26 to obtain Km^r transformants. Southern blot analysis indicated that one such mutant (UT26orf3d) was formed by homology-dependent single-crossover-mediated integration of the plasmid into *linEb* so that the two *linEb*-derived sequences on the genome were both truncated.

Construction of plasmids for analyses of the *linF* and *linEb* products. The *linF* gene was amplified by PCR with the UT26 genome as the template and primers linFFB1 (5'-GCCGGATCCTCTGCCCGGATATTC-3') and linFRH1 (5'-GC CAAGCTTGGCTTATGCGGTGATCG-3'). The PCR-amplified fragment was inserted into pAQNM (Table 1) to obtain pMYLF1, in which the *linF* gene was under the control of the *tac* promoter. The primers used for amplification of *linEb* were orf3FB1 (5'-GCCGGATCCAAGAGCGCGATTTC-3') and orf3RH1 (5'-GCCAAGCTTCCAGGACTTCAAGGTTCC-3'). Insertion of the amplified fragment into pAQNM generated pRE3.

Assay of MA reductase activity. *E. coli* DH5 α (pMYLF1) cells were suspended in 50 mM potassium phosphate buffer (pH 7.5) containing 10% glycerol and disrupted by sonication. After centrifugation (17,000 $\times$ g) at 4°C for 10 min, the supernatant was used as a crude cell extract. MA reductase activity was measured spectrophotometrically by monitoring NADH oxidation at 340 nm (31). One unit of enzyme activity was defined as the amount that consumes 1 μ mol of NADH per min for MA reduction. MA was prepared on the day of its use by alkaline hydrolysis of *cis*-dienelactone (3).

GC-MS analysis. *E. coli* cells expressing LinF and/or LinEb were suspended in 100 mM Tris-HCl buffer (pH 7.5) containing 10 μ M substrates and then incubated at 37°C for 3 h. The mixtures were extracted with ethyl acetate after acidification by adding 10 μ l of HCl and then analyzed by GC-mass spectrometry (MS) with a GP2010 spectrometer (Shimadzu, Kyoto, Japan) and a DB-17 capillary column (30 m by 0.25 mm by 0.25 μ m; J&W Scientific). The column temperature was increased from 100 to 260°C at a rate of 10°C/min and then held at 260°C. The carrier gas flow was 14.0 ml/min. To analyze trimethylsilylated samples, we increased the column temperature from 100 to 200°C at a rate of 40°C/min and then from 200 to 280°C at a rate of 15°C/min. The carrier gas flow was 23.7 ml/min.

Nucleotide sequence accession number. The nucleotide sequence determined in this study has been deposited in the DDBJ/EMBL/GenBank databases under accession number AB177985.

RESULTS

Isolation of a UT26 mutant deficient in γ -HCH utilization.

To analyze the γ -HCH degradation pathway of UT26 in greater detail, we carried out random transposon mutagenesis of UT26 with pTnMod-OKm and screened 1,600 Km^r trans-

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics	Source or reference
<i>S. paucimobilis</i>		
UT26	HCH ⁺ ^a ; NaI ^r	7
UT1023	UT26 <i>linF</i> ::TnMod-OKm; Km ^r	This study
UT1023d	UT26 <i>linF</i> ::TnMod-OKm; knockout mutant by use of p1023BamTc	This study
UT26orf3d	UT26 <i>linEb</i> disruptant via single crossover by use of pHSGorf3d; Km ^r	This study
UT116	UT26 Δ <i>linRED</i>	23
<i>E. coli</i> DH5 α	F ⁻ ϕ 80d <i>lacZ</i> Δ M15 Δ (<i>lacZYA-argF</i>)U169 <i>deoR recA1 endA1 hsdR17 phoA supE44 thi-1 gyrA96 relA1</i>	29
Plasmids		
pUC18	pMB9 replicon; Ap ^r	38
pUC1918	pMB9 replicon; Ap ^r	32
pHSG299	pMB9 replicon; Km ^r	38
pAQN	pMB9 replicon; <i>lacI</i> ^q <i>aqn</i> ^b Ap ^r ; <i>tac</i> promoter	36
pAQNM	pAQN derivative whose EcoRI-HindIII-flanked <i>aqn</i> ^b gene was replaced with the multiple cloning site from pHSG299	This study
pTnMod-OKm	pMB1 replicon, Tn5 inverted repeat; Km ^r	2
pKS13	RK2 replicon; <i>cos</i> Mob ⁺ Tc ^r	11
p1023Bam	TnMod-OKm-containing plasmid recovered by self-ligation of the BamHI-treated genome of UT1023; Km ^r	This study
p1023Nco	TnMod-OKm-containing plasmid recovered by self-ligation of the NcoI-treated genome of UT1023; Km ^r	This study
pKSF1	pKS13 carrying <i>linF</i> , Pu promoter ^c	This study
pRE1	pUC18 carrying <i>linF</i>	This study
pRE2	pUC18 carrying <i>linEb</i>	This study
pMYLF1	pAQNM carrying <i>linF</i>	This study
pRE3	pAQNM carrying <i>linEb</i>	This study
pEX18Tc	Tc ^r <i>sacB oriT</i> ; suicide vector for gene replacement carrying the pUC18-derived multiple cloning sites	6
pTc1918	pUC1918 carrying Tc ^r cassette amplified by PCR from pEX18Tc	This study
p1023BamTc	p1023Bam derivative with BamHI fragment containing Tc ^r gene from p1918Tc	This study
pHSGorf3d	pHSG299 derivative carrying the BamHI-PstI fragment of <i>linEb</i> that was amplified by PCR	This study

^a HCH⁺, grown on γ -HCH.^b *aqn*, aqualysin I gene of *Thermus aquaticus*.^c Pu promoter, necessary for constitutive expression of the *linA* gene in *S. paucimobilis* UT26 (25).

formants to find mutants able to grow on W minimal medium agar containing succinate but not on the same agar containing γ -HCH as the sole carbon source. One of these mutants, UT1023, had no detectable defects in the LinA-to-LinE activities assayed by GC with an ECD. A single copy of the TnMod-OKm insertion in the UT1023 genome was confirmed by Southern blot analysis (data not shown).

Identification of the *linF* gene involved in γ -HCH degradation. The UT1023-derived DNA fragments containing the TnMod-OKm insertion site were recovered as plasmids by digestion of the genomic DNA with BamHI or NcoI, self-ligation, and transformation of *E. coli*. The plasmids obtained with BamHI and NcoI were named p1023Bam and p1023Nco, respectively. Sequencing analysis revealed that TnMod-OKm was inserted into a 1,056-bp open reading frame (ORF) encoding a protein homologous to MA reductases from *Sphingobium chlorophenolicum* ATCC 39723 (PcpE; 92% identity) and other bacterial strains (approximately 55% identity). Amino acid residues considered to be important for Fe(II) binding-type alcohol dehydrogenases (33) are conserved in the deduced amino acid sequence of the ORF (His237, His241, Gly244, Gly245, and His251), suggesting that this ORF en-

codes MA reductase. Since MA is produced from γ -HCH by a series of reactions catalyzed by LinA to LinE (Fig. 1), the product of this ORF was predicted to catalyze the next step. Therefore, we designated the ORF the *linF* gene. Southern blot analysis revealed that UT26 has one copy of *linF* (data not shown). To confirm the function of *linF* in UT26, we disrupted the wild-type *linF* gene by allelic-exchange mutagenesis as described in Materials and Methods. The resultant *linF* disruptant, UT1023d, lost the ability to grow on W minimal medium agar containing γ -HCH, and this growth defect was complemented by the introduction of pKSF1, in which *linF* is located downstream of the Pu promoter (25) so as to allow the constitutive expression of *linF* in UT26. These results clearly indicated that *linF* is directly involved in γ -HCH utilization by UT26.

Expression and characterization of the *linF* gene product. To express and characterize the *linF* gene product in *E. coli*, we constructed pMYLF1. *E. coli* DH5 α (pMYLF1) cells were incubated with or without isopropyl- β -D-thiogalactopyranoside (IPTG), and their crude extracts were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. An approximately 38-kDa protein band was observed in the extract of the

FIG. 2. Mass spectra of the trimethylsilylated products of MA, CHQ, and 2,6-DCHQ incubated with *E. coli*-expressing LinF and/or LinEb. Mass spectra of the trimethylsilylated product of 2,6-DCHQ was identical with that of trimethylsilylated 2-CMA (27).

IPTG-treated cells. The molecular mass of this protein was close to that deduced from the nucleotide sequence of *linF* (36.3 kDa). The MA reductase activity of the cell extracts of DH5 α (pMYLF1) were assayed spectrophotometrically by NADH consumption (31). The extract of the IPTG-treated cells showed a level of activity (4.9×10^2 U/mg of protein) 40-fold higher than that of IPTG-untreated cells (17 U/mg of protein). To identify a product of MA, we incubated an MA solution with *E. coli* cells expressing LinF and extracted the metabolites with ethyl acetate, trimethylsilylated them, and analyzed them by GC-MS. The mass spectrum of the peak that was specifically detected by incubation of MA with the LinF-expressing cells was equivalent to that of trimethylsilylated

authentic β -ketoadipate (Fig. 2). These results demonstrated that the *linF* gene encodes MA reductase to produce β -ketoadipate from MA.

Organization of the *linF*-containing gene cluster. We determined the nucleotide sequence of the 9,888-bp region containing the *linF* gene. This region contained, in addition to the *linF* gene, six complete ORFs and one partial ORF (Fig. 3). The putative gene products of all eight ORFs showed a relatively high degree of similarity to those of the corresponding ORFs in one of the gene clusters for pentachlorophenol (PCP) degradation by ATCC 39723 (Fig. 3). The enzymes for the PCP degradation pathway of ATCC 39723 are encoded by at least two gene clusters (1). One gene cluster contains the *pcpB*,

Name	Number of nucleotide (bp)	Homologous Protein of ATCC39723 ^a (aa ^b Identity)	Putative function
<i>orf7</i>	627	Orf7 (74%)	Glutathione <i>S</i> -transferase
<i>orf1</i>	615	Orf9 (86%)	Unknown
<i>linF</i>	1056	PcpE (92%)	Maleylacetate reductase
<i>orf2</i>	987	PcpM (92%)	LysR-type transcriptional regulator
<i>linEb</i>	960	PcpA (96%)	2,6-Dichloro- <i>p</i> -hydroquinone 1,2-dioxygenase
<i>orf4</i>	1128	Orf15 (84%)	Na ⁺ /H ⁺ antiporter Arylsulfatase precursor
<i>orf5</i>	978	Orf16 (81%)	Arylsulfatase precursor
<i>orf6*</i>	533	Orf19 (89%)	Glutathione <i>S</i> -transferase

* Orf6 is not terminated in the sequenced region.

^a *S. chlorophenolicum* ATCC39723

^b Amino acid

FIG. 3. Comparison of the *linF*-containing region in *S. paucimobilis* UT26 and the *pcp* gene cluster in *S. chlorophenolicum* ATCC 39723. Arrows indicate the sizes and orientations of the ORFs. Homologues are distinguished by filled patterns. A flag represents the TnMod-OKm insertion site. The leftward direction of the flag represents the orientation of the Km^r-encoding gene on TnMod-OKm.

pcpD, and *pcpR* genes encoding PCP hydrolase, tetrachlorobenzoquinone reductase, and a PCP-responsive LTTR, respectively. The other cluster contains the *pcpE*, *pcpM*, *pcpA*, and *pcpC* genes encoding MA reductase, LTTR, 2,6-dichloro-*p*-hydroquinone (2,6-DCHQ) 1,2-dioxygenase, and tetrachloro-*p*-hydroquinone reductive dehalogenase, respectively. The organization of the *linF*-containing region was similar to that of the latter gene cluster of ATCC 39723, although the latter one contains additional ORFs having no counterparts in the *linF*-containing region (Fig. 3). The upstream regions of *linF* (−343 to −328 from the start codon) and *orf3* (−1471 to −134) had the consensus palindromic TN₁₁A sequences for LTTR binding (30) (5′-ATTCGCCCCATGAAT-3′ and 5′-CTTCGCTTTTCGAAG-3′, respectively [the initial T and final A nucleotides are underlined]).

Analyses of *orf3* (*linEb*). Because the putative *orf3* gene product showed significant similarity to the novel type of ring cleavage dioxygenases, PcpA and LinE (96 and 53% amino acid identity, respectively), we renamed *orf3* *linEb*. Substrates of PcpA and LinE are HQ-type compounds, i.e., aromatic compounds with two hydroxyl groups at the *para* position (18, 27, 40). This type of dioxygenase shows a low degree of similarity to *meta* cleavage dioxygenases, such as 2,3-dihydroxybiphenyl 1,2-dioxygenases (BphC) (18). All of the residues proposed to be important for the Fe(II)-binding and enzyme activities of BphC (4, 35) are conserved in the deduced amino

acid sequence of LinEb (His159, His227, and Glu276), suggesting that LinEb is functional. To characterize the function of LinEb, we expressed it in *E. coli* and analyzed the CHQ and 2,6-DCHQ transformation abilities by GC-MS. CHQ and 2,6-DCHQ were transformed by LinEb to MA and 2-chloromaleylacetate (2-CMA), respectively (Fig. 2). To characterize the function of *linEb* in UT26, we constructed a *linEb* disruptant of UT26 (UT26orf3d) as described in Materials and Methods. UT26orf3d was able to grow on W minimal medium agar containing γ -HCH, and it degraded 2,5-DCHQ and CHQ at a level comparable to that of UT26 (Fig. 4a and b). However, UT26orf3d degraded 2,6-DCHQ at a much lower rate than did UT26 (Fig. 4c). On the other hand, a spontaneous *linRED* deletion mutant, UT116, that is unable to utilize γ -HCH (23) showed an obvious defect in 2,5-DCHQ and CHQ degradation (Fig. 4a and b) but not in 2,6-DCHQ degradation (Fig. 4c). These results indicated that *linEb* has an important role in 2,6-DCHQ degradation but not in γ -HCH utilization by UT26. The 2,6-DCHQ degradation activity induced in UT26orf3d after 60 min of incubation (Fig. 4c) was considered to be derived from LinE because LinE showed degradation activity toward 2,6-DCHQ (K. Miyauchi et al., unpublished data).

Substrate specificity of LinF. Usually, MA reductases for degradation of chlorinated aromatic compounds convert not only MA but also CMA (1, 10, 39). The LinEb-expressing DH5 α (pRE3) cells converted 2,6-DCHQ to 2-CMA as described

FIG. 4. Degradation of CHQ (a), 2,5-DCHQ (b), and 2,6-DCHQ (c) by *S. paucimobilis* UT26 (circles) and its mutants UTorF3d (squares) and UT116 (triangles). All of the cells were grown in 1/3 LB medium. The assay solution containing each substrate at 20 μM and 1 mM ascorbic acid in W medium was incubated with 10 mg of each cell type per ml at 30°C. The concentration of the remaining substrates was measured by GC with an ECD.

above. When 2,6-DCHQ was incubated with LinEb-expressing DH5 α (pRE3) and LinF-expressing DH5 α (pMYLF1) cells, β -ketoadipate was detected as a product (Fig. 2). This result indicated that LinF converts 2-CMA to β -ketoadipate.

DISCUSSION

We have cloned genes for the conversion of γ -HCH to β -ketoadipate in UT26. β -Ketoadipate is usually metabolized by a chromosomally encoded convergent pathway for the degradation of aromatic compounds, and this pathway is widely distributed in soil bacteria and fungi (5). The presence of this

pathway in UT26 is the most plausible, since the utilization of γ -HCH by UT26 requires LinF, which converts MA to β -ketoadipate. We believe that all of the specialized genes involved in γ -HCH degradation in UT26 have been identified. The three genes for the upstream pathway, *linA*, *linB*, and *linC*, exist separately from one another on the genome and are expressed constitutively (25). On the other hand, the genes for the downstream pathway, *linD* and *linE*, can be induced under the control of LinR. In this study, we demonstrated that the *linF* gene is a part of another gene cluster. The genes for γ -HCH degradation in UT26 are not sophisticated and look like a patchwork, suggesting that the pathway might have evolved recently.

It was suggested that the *linF*-containing gene cluster is functional for 2,6-DCHQ degradation by UT26. In our previous study, we demonstrated that the *linRED* operon for 2,5-DCHQ degradation is necessary for γ -HCH utilization by UT26. A spontaneous *linRED* deletion mutant, UT116, is unable to utilize γ -HCH (23), and it had defects in the degradation of 2,5-DCHQ and CHQ (Fig. 4a and b). These results suggested that UT26 uses LinE and LinEb for the utilization of γ -HCH (2,5-DCHQ) and 2,6-DCHQ, respectively. However, we cannot exclude the possibility that LinEb is also involved in γ -HCH utilization because (i) LinEb expressed in *E. coli* converted CHQ to MA (Fig. 2) and (ii) UT26orf3d degraded CHQ at a slightly lower rate than did UT26 (Fig. 4a). Both LinEb and LinE are dioxygenases that cleave aromatic rings with two hydroxyl groups at the *para* position. We designated the pathway that includes this type of ring cleavage step an HQ pathway (18, 19) although the number of reports describing this pathway is still limited. In this study, we demonstrated that UT26 has two gene clusters for the HQ pathway, one for 2,5-DCHQ degradation and the other for 2,6-DCHQ degradation. It is interesting that UT26 uses parts of the two HQ pathways for γ -HCH utilization. In other words, the downstream pathway for γ -HCH utilization by UT26 is formed by a combination of the two gene clusters.

Regulation of the expression of *linF* and *linEb* in UT26 is under investigation. Several LTTRs are involved in the degradation pathways for aromatic compounds such as catechol (28), naphthalene (41), and chlorocatechol (14, 16). The consensus sequence for the binding of LTTRs is known as a palindromic TN₁₁A sequence (30). Such consensus sequences were found at the upstream regions of *linF* and *linEb*, as described above. A similar consensus sequence exists in the upstream region of the *linE* gene (19), and the expression of *linE* is upregulated by LinR, a member of the LTTR family, in the presence of 2,5-DCHQ, CHQ, or HQ in UT26 (19). It has also been reported that similar consensus sequences exist in the upstream regions of the *pcpB*, *pcpA*, and *pcpE* genes in ATCC 39723, and the expression of these genes is upregulated by an LTTR, PcpR, in the presence of PCP in ATCC 39723 (1). These results suggest that the expression of *linF* and *linEb* is also upregulated by a member of LTTRs in the presence of some chlorinated aromatic compounds.

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