5 Aerobic Degradation of Chloroaromatics

D. H. Pieper[1] · *B. González*[2] · *B. Cámara*[1] · *D. Pérez-Pantoja*[2] · *W. Reineke*[3]
[1]Biodegradation Research Group, Division of Microbial Pathogenesis, HZI – Helmholtz Research Centre for Biotechnology, Braunschweig, Germany
dpi@helmholtz-hzi.de
[2]Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, NM-EMBA, CASEB, P. Universidad Católica de Chile, Santiago, Chile
[3]Chemical Microbiology, Bergische Universität Wuppertal, Wuppertal, Germany

K. N. Timmis (ed.), *Handbook of Hydrocarbon and Lipid Microbiology*, DOI 10.1007/978-3-540-77587-4_61,

Abstract: Microorganisms are key players in the global carbon cycle. In addition, it appears that most xenobiotic industrial chemicals can be degraded by microorganisms, either by a combination of cometabolic steps or by serving as growth substrate, leading to the mineralization of at least part of the molecule. Here, we present the principles of the microbial aerobic degradation of chloroaromatic compounds. The so-called peripheral sequences of the oxidative degradation of chloroaromatic compounds in aerobic bacteria yield central intermediates with a diphenolic structure such as catechols or hydroxybenzoquinols. These compounds are subsequently cleaved by enzymes that use molecular oxygen and further metabolized by central pathway sequences. The broad variety of mechanisms resulting in dechlorination that occur in these peripheral or central sequences is specifically discussed.

1 Introduction

Chlorinated hydrocarbons comprise a large spectrum of compounds that are or have been of enormous industrial and economic importance. The introduction of chlorine atom(s) into a hydrocarbon significantly influences its physicochemical and biochemical properties and the tendency for bioaccumulation and environmental persistence. Acting in combination with possible (eco)toxicological effects, these properties have pushed the chlorochemistry into the focus of considerable debate and governmental regulatory action.

For decades, it is known that microorganisms have the capability to mineralize various chlorinated hydrocarbons. Under anaerobic conditions, chlorinated hydrocarbons can function as alternative electron acceptors in a process termed dehalorespiration. Under aerobic conditions, chlorinated hydrocarbons can function as carbon and energy source, which necessitates dechlorination.

This chapter deals with the aerobic degradation and the use of the chloroaromatics as carbon and energy source involving elimination of chloride from the substrate or metabolites.

2 Metabolism of Chloroaromatics

Aerobic microorganisms usually initiate degradation by activation of the aromatic nucleus through oxygenation reactions. A few central intermediates such as catechols, protocatechuate, gentisate, and hydroxybenzoquinols are produced by the introduction of hydroxyl groups, usually in *ortho*- or *para*-position to one another (peripheral reactions). These intermediates are subject to oxygenolytic ring cleavage followed by the channeling of the ring-cleavage products into the central metabolism (See Chapter 4, Vol. 2, Part 2). Despite the fact that various specific dehalogenating enzymes have been identified, microorganisms capable of mineralizing chloroaromatics often employ peripheral reactions, which have their function in the degradation of naturally occurring aromatics such as benzoate, salicylate, or biphenyl (See Chapter 26, Vol. 2, Part 5) (Reineke, 2001). These enzyme systems are predominantly of relaxed-substrate specificity and tolerate lower chlorinated substrate analogs.

2.1 Peripheral Reactions

A broad set of peripheral pathways for chloroaromatic degradation has been described, mainly responsible for transforming chloroaromatics to central catechol or benzoquinol intermediates. *Figure 1* gives an overview on reactions leading to (chloro) catechols.

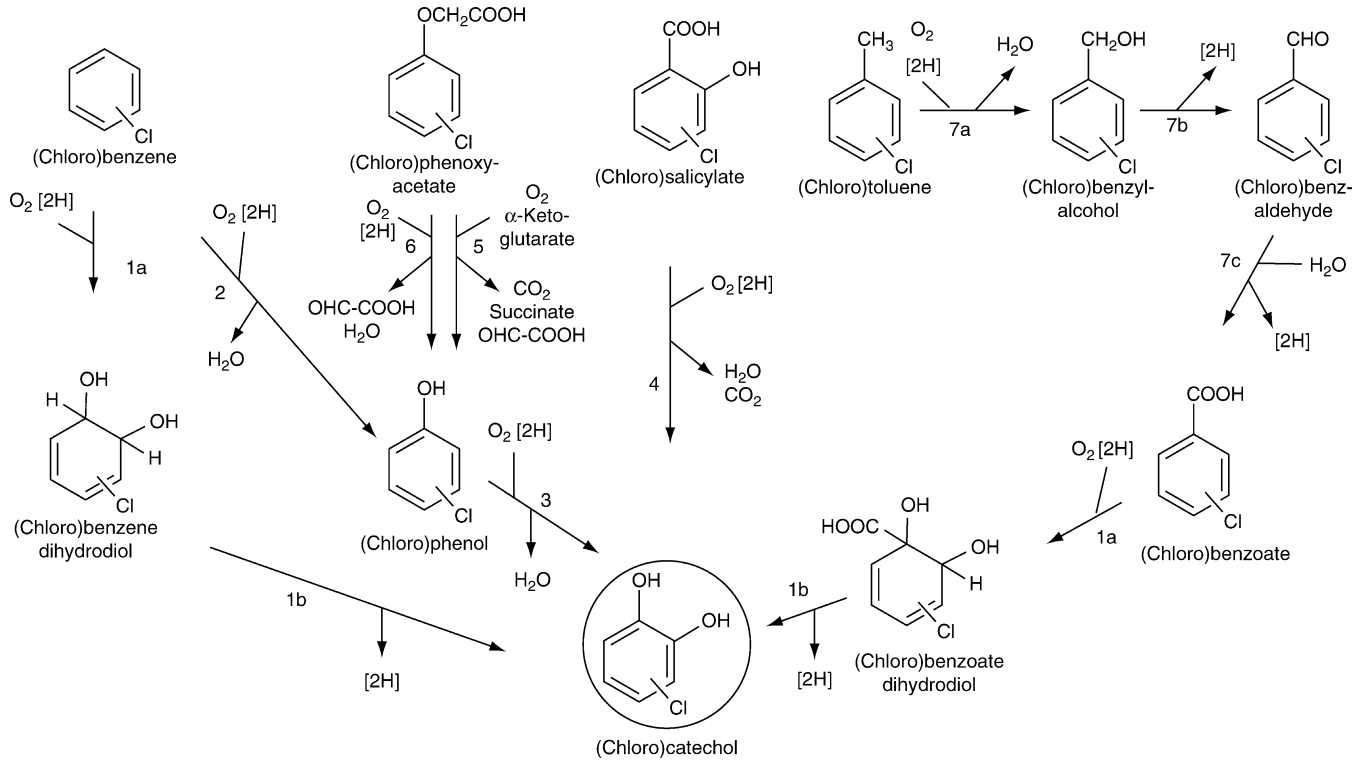

Figure 1

Overview of peripheral reactions in the degradation of chloroaromatics channeling to catechols. (1) Rieske non-heme iron oxygenases (1a) and dehydrogenases (1b) involved in (chloro)benzene and (chloro)benzoate metabolism; (2) aromatic monooxygenases (soluble diiron monooxygenases); (3) phenol hydroxylases (soluble diiron monooxygenases or single component flavin monooxygenases); (4) salicylate 1-hydroxylases (single component flavin monooxygenases); (5) α-ketoglutarate-dependent dioxygenases; (6) 2,4,5-trichloro- and 2,4-dichlorophenoxyacetate monooxygenases; (7) oxidation of side chains (xylene monooxygenases, 7a; benzylalcohol dehydrogenases, 7b; benzaldehyde dehydrogenases, 7c).

2.1.1 Rieske Non-Heme Iron Oxygenases

The so called Rieske non-heme iron oxygenases are one of the key families of enzymes important for aerobic activation and thus degradation of aromatics such as benzoate, benzene, toluene, phthalate, naphthalene, or biphenyl (Gibson and Parales, 2000) (see *Fig. 1*, reaction 1). These multicomponent enzyme complexes, composed of a terminal oxygenase component and different electron transport proteins, usually catalyze the incorporation of two oxygen atoms into the aromatic ring to form arene-*cis*-dihydrodiols, a reaction that is followed by a dehydrogenation catalyzed by *cis*-dihydrodiol dehydrogenases to give (substituted) catechols. Comparison of the amino acid sequences of the terminal oxygenase α-subunits revealed that they form a family of diverse but evolutionarily related sequences. Although none of the enzymes is completely specific, a broad correlation between the grouping in toluene/biphenyl, naphthalene, benzoate, or phthalate families, and the native substrates oxidized by the family members can be observed (Gibson and Parales, 2000). Enzyme engineering studies of biphenyl, benzene, chlorobenzene, and naphthalene dioxygenases showed that the α-subunit of the terminal oxygenase determines substrate specificity and that only slight differences in the amino acid sequence can be associated with dramatic changes in substrate specificity or regioselectivity (Beil et al., 1998; Furukawa et al., 2004). However, as a general rule Rieske non-heme iron oxygenases can transform lower chlorinated substrate analogs and dioxygenation is usually directed to carbon atoms proximal to substituents.

2.1.2 Oxygenolytic Dehalogenations by Rieske Non-Heme Iron Oxygenases

Different bacteria capable of degrading 2-chlorobenzoate have been described. All these organisms catalyze a 1,2-dioxygenation such that one of the vic-hydroxyl groups in the *cis*-dihydrodiol is bound to the same carbon as the chloro-substituent. From such an unstable vic-dihydrodiol, the chloro-substituent is spontaneously eliminated to form catechols (*Fig. 2*).

Two distinct 2-chlorobenzoate-degrading dioxygenase systems have been described. The two-component 2-halobenzoate 1,2-dioxygenases (oxygenase consisting of α- and β-subunits and a reductase as the one from strain *Burkholderia cepacia* 2CBS (Fetzner et al., 1992)), are similar to two-component toluate and benzoate 1,2-dioxygenases of the Rieske non-heme iron oxygenases. They are characterized by their high activity against 2-halosubstituted benzoates but have negligible activity with 4-chloro-, or 2,5-dichlorobenzoate. In contrast, the broad

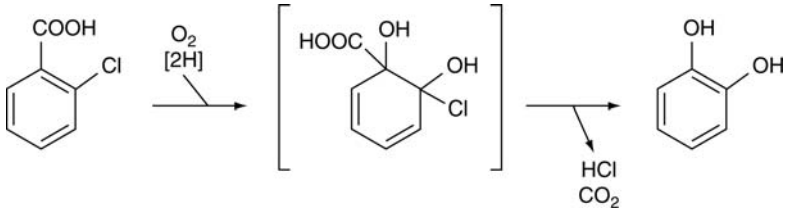

Figure 2
Oxygenolytic dehalogenation of 2-chlorobenzoate by Rieske non-heme iron oxygenases.

specificity 2-chlorobenzoate dioxygenase of *P. aeruginosa* strain 142 is a three-component dioxygenase system (oxygenase consisting of α- and β-subunits, ferredoxin and reductase) (Romanov and Hausinger, 1994), the α-subunit of which exhibits only 22% sequence identity with that of strain 2CBS, but a significant similarity (42%) to salicylate 5-hydroxylase NagG from *Pseudomonas* sp. strain U2. Thus, 2-chlorobenzoate dioxygenases are functionally similar, but represent two different lineages with distinct activities.

Oxidative dehalogenation is not restricted to 2-halobenzoate 1,2-dioxygenases but has also been described for tetrachlorobenzene dioxygenase TecA of *Ralstonia* sp. PS12 (Beil et al., 1998), biphenyl 2,3-dioxygenase BphA of *Burkholderia xenovorans* LB400 (Seeger et al., 1995), or 3-chlorobenzoate 4,5-dioxygenase CbaA from *Comamonas testosteroni* BR60 (Nakatsu and Wyndham, 1993). Dehalogenation always involves dioxygenolytic attack on a chlorosubstituted carbon atom and its unsubstituted neighbor to give unstable intermediates, which spontaneously rearrange with elimination of chloride. However, in all cases mentioned here, only higher chlorinated substrate analogs are dechlorinated. CbaA catalyzes the 4,5-dioxygenation of 3,4-dichlorobenzoate resulting in an unstable dihydrodiol, which spontaneously eliminates chloride to form 5-chloroprotocatechuate, whereas 4,5-dioxygenation of 3-chlorobenzoate yields 5-chloroprotocatechuate after dehydrogenation. TecA catalyzes the dehalogenation of 1,2,4,5-tetrachlorobenzene to form 3,4,6-trichlorocatechol, whereas lower chlorinated benzenes were transformed to the corresponding dihydrodiols. BphA catalyzes the dehalogenation of 2,2′- or 2,4′-dichlorobiphenyl among others (See Chapter 26, Vol. 2, Part 5). For both TecA and BphA, amino acid residues crucial for dehalogenation were identified (Beil et al., 1998; Furukawa et al., 2004).

2.1.3 Soluble Diiron Monooxygenases

Enzymes attacking the nonactivated benzene nucleus by monooxygenation belong to an evolutionary-related family of soluble diiron monooxygenases (Leahy et al., 2003) that are enzyme complexes consisting of an electron transport system comprising a reductase (and in some cases a ferredoxin), a catalytic effector protein, and a terminal hydroxylase with a $(\alpha\beta\gamma)_2$ quaternary structure and a diiron center contained in each α-subunit. The monooxygenases are classified according to their α-subunits, which are assumed to be the site of substrate hydroxylation, into four different phylogenetic groups, comprising the (multicomponent) phenol hydroxylases, and the four-component alkene/aromatic monooxygenases (Leahy et al., 2003).

The multicomponent phenol hydroxylases (*Fig. 1*, reaction 3) such as the phenol hydroxylase of *Pseudomonas* sp. CF600 share the capability to hydroxylate phenol and methylsubstituted derivatives (Shingler et al., 1992), and a few enzymes of this group, such as phenol hydroxylase of *P. stutzeri* OX1, also have been shown to hydroxylate the nonactivated benzene nucleus and thus can catalyze two sequential hydroxylations (Cafaro et al., 2004). The available information on the transformation of chlorophenols by multicomponent phenol hydroxylases is insufficient; however, preliminary data indicate that such a capability is spread among this group of enzymes (Teramoto et al., 1999).

The four-component alkene/aromatic monooxygenases comprise enzymes that oxidize nonhydroxylated compounds (Leahy et al., 2003) (*Fig. 1*, reaction 2). Toluene 4-monooxygenase of *P. mendocina* KR1 also transforms chlorobenzene with 4-chlorophenol as product (Fishman et al., 2005). Regioselectivity of attack is mainly controlled by the α-subunit of the enzyme, but

also by the effector protein (Mitchell et al., 2002), in such a way that the enzyme could be modulated also to produce 2-chloro- and 3-chlorophenol. Tbc2 toluene 2-monooxygenase of strain *Burkholderia cepacia* JS150 was shown to transform chlorobenzene into 2-chlorophenol (Kahng et al., 2001).

2.1.4 Single Component Flavoprotein Monooxygenases

The oxidation of various phenolic aromatic compounds is catalyzed by single-component flavoprotein monooxygenases (class A flavin monooxygenases (van Berkel et al., 2006)). These enzymes catalyze hydroxylation in *ortho*- or *para*-position to the preexisting hydroxyl-group (See Chapter 4, Vol. 2, Part 2) and usually display a narrow substrate specificity (van Berkel et al., 2006). Only a few of these enzymes have been analyzed for their capability to transform chlorinated substrate analogues.

Single-component salicylate 1-hydroxylases (*Fig. 3*) have been reported to catalyze the transformation of salicylate to catechol (*Fig. 1*, reaction 4), and enzymes analyzed in this aspect were capable of transforming chlorinated salicylates into the respective chlorocatechols with high activity (Bosch et al., 1999; Cámara et al., 2007). In accordance with these properties, mineralization of chlorosalicylates by natural or engineered organisms involving salicylate 1-hydroxylase has been reported (Cámara et al., 2007; Lehrbach et al., 1984).

Another group of single component flavin monooxygenases, termed phenol hydroxylases (*Fig. 1*, reaction 3), has been described from phenol degrading *Pseudomonas* strains, among them PheA from *Pseudomonas* sp. strain EST1001, which transforms phenol and 3-methylphenol (Nurk et al., 1991). This substrate specificity significantly differs from that described for a group of enzymes termed 2,4-dichlorophenol hydroxylases and involved in the degradation of 2,4-D or 2,4-dichlorophenol via 3,5-dichlorocatechol, with which phenol hydroxylases share $\leq$50% sequence identity (*Fig. 3*).

In contrast to the substrate specificity of phenol hydroxylases, all the five characterized dichlorophenol hydroxylases, TfdB of *Burkholderia cepacia* 2a (Beadle and Smith, 1982), ClpB from *Defluvibacter lusatiensis* S1 (Makdessi and Lechner, 1997), TfdBa from *Bradyrhizobium* sp. RD5-C2 (Huong et al., 2007), and both TfdBI and TfdBII dichlorophenol hydroxylases from *C. necator* JMP134 (Ledger et al., 2006), are highly active with 2,4-dichloro-, 4-chloro-2-methylphenol and 4-chlorophenol, but activity with phenol and 3-chlorophenol was either low or absent.

Despite the obvious similarity in substrate specificity, these dichlorophenol hydroxylases are just distantly related. Various 2,4-D degraders contain dichlorophenol hydroxylases sequences highly related to that of *B. cepacia* 2a or TfdBII from strain JMP134 (e.g., *A. denitrificans* EST4002, *D. acidovorans* P4a, *Burkholderia* sp. RASC or *V. paradoxus* TV1). In contrast, the closest homolog of TfdBI from strain JMP134 is the enzyme of *D. lusatiensis* (only 76% identity). However, partial amino acid sequences indicate that enzymes highly similar to TfdBI are present in other 2,4-D degrading β-proteobacterial strains (Vallaeys et al., 1999). In addition to ClpB from *D. lusatiensis* and TfdBa from *Bradyrhizobium* sp., a third lineage of dichlorophenol hydroxylases represented by TfdB of *S. herbicidovorans* MH (Müller et al., 2004) has been observed in α-proteobacteria. All these three lineages have only approximately 60% amino acid identity (*Fig. 3*) and similar identity to TfdB proteins from β-proteobacteria, indicating an ancient divergence and evolution without recent horizontal gene transfer in α-proteobacteria.

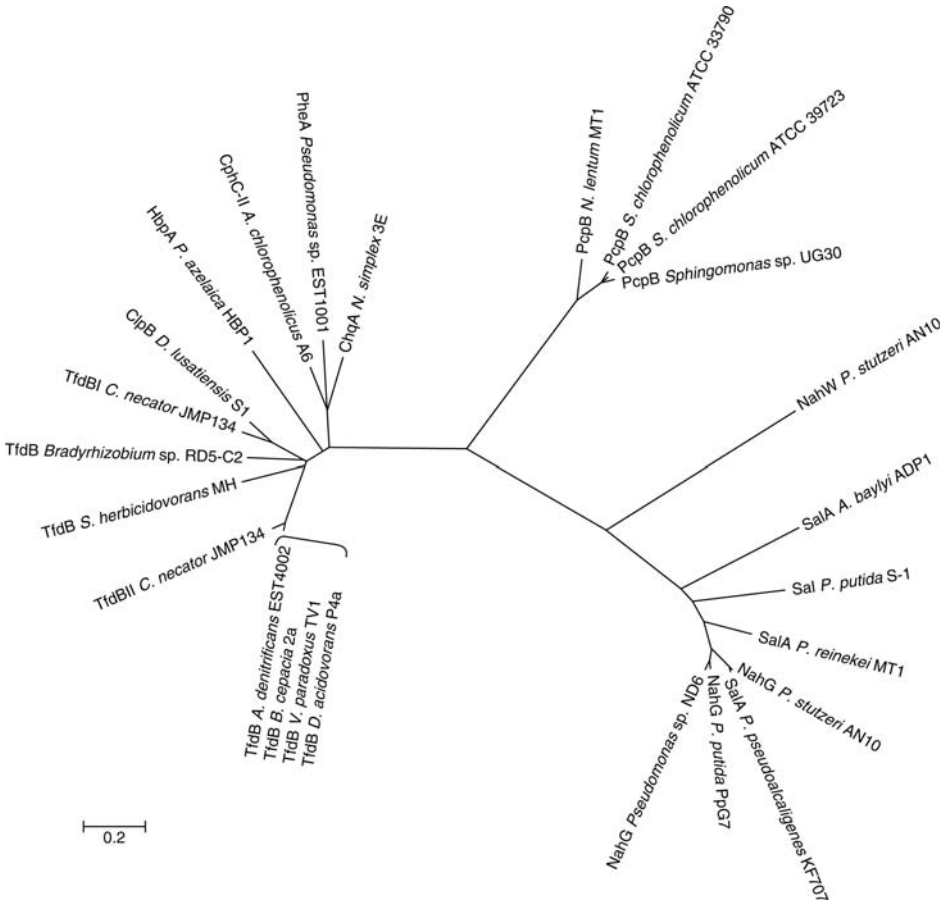

Figure 3

Dendrogram showing the relatedness of single component flavin monooxygenases (2,4-dichlorophenol hydroxylase, TfdB, ClpB; phenol hydroxylase, PheA; salicylate 1-hydroxylase, SalA, NahG, NahW; pentachlorophenol monooxygenase, PcpB; 2-hydroxybiphenyl 3-monooxygenase, HbpA; chlorobenzoquinol monooxygenase, ChqA; unknown function, CphC-II).

2.1.5 Initial Side-Chain Processing

2.1.5.1 Oxidation of Methylgroups

Aromatic compounds that bear alkyl substituents on the aromatic ring may undergo oxidation of the side chain before ring activation. The most intensely described example is the TOL plasmid encoded degradation pathway of toluene and xylenes by *Pseudomonas putida* mt-2, which proceeds via benzoate or the respective methylbenzoates, and catechol or methylcatechols as intermediates (Worsey and Williams, 1975) (*Fig. 1*, reactions 7). The respective enzymes are encoded by the *xyl* genes, which are organized in two functional units localized on

the TOL plasmid pWW0 (Greated et al., 2002). The upper pathway encodes three enzymes, xylene monooxygenase, benzylalcohol dehydrogenase, and benzaldehyde dehydrogenase, that oxidize toluene and xylenes to benzoate and toluates, respectively. The degree of transformation of chlorotoluenes by xylene monooxygenase depends on the position of the chlorine substituent. The substrate analogs 3-chloro- and 4-chlorotoluene are transformed at high rates, while no or only low activity has been found with other chlorotoluenes. Substituents in *ortho* position impaired substrate binding (Brinkmann and Reineke, 1992). Benzylalcohol and benzaldehyde dehydrogenases show broader specificities. But again, a chlorine substituent in *ortho* position leads to a drastic decrease in the substrate conversion. Transfer of the TOL plasmid into strains capable of mineralizing chlorocatechols (see below) allowed the isolation of 3-chloro- and 4-chlorotoluene-degrading organisms (Brinkmann and Reineke, 1992).

2.1.5.2 α-Ketoglutarate-Dependent Dioxygenases

Bacterial metabolism of 2,4-D and MCPA is initiated by the cleavage of the side chain, resulting in the formation of 2,4-dichloro- and 4-chloro-2-methylphenol, respectively (*Fig. 1*, reaction 5). In *C. necator* JMP134 (pJP4), the degradation of 2,4-D is catalyzed by 2,4-dichlorophenoxyacetate/α-ketoglutarate dioxygenase, TfdA (Fukumori and Hausinger, 1993). TfdA belongs to the large superfamily of α-ketoacid-dependent, mononuclear non-heme iron oxygen activating enzymes that catalyze the oxidation of aliphatic C–H bonds (Schofield and Zhang, 1999). During 2,4-D transformation, one oxygen atom is involved in the oxidative decarboxylation of α-ketoglutarate, whereas the other oxygen atom is introduced into the β-carbon of the aliphatic side chain to produce an unstable hemiacetal intermediate that decomposes to glyoxylate, and 2,4-DCP (Fukumori and Hausinger, 1993). TfdA is able to hydroxylate several phenoxyacetates, including 2,4,5-trichlorophenoxyacetate (2,4,5-T) and MCPA; however, 2,4-D is the preferred substrate.

Representatives of *tfdA* genes have been described thus far from α, β, and γ subgroups of the proteobacteria (McGowan et al., 1998). Sequence alignment among β-proteobacterial representatives reveals the presence of three distinct classes of *tfdA* gene sequences with slight variations in each class (*Fig. 4*); however, all share >80% sequence identity and all are capable of transforming several phenoxyacetate compounds. *tfdA*-like genes were also observed in 2,4-D degrading α-proteobacteria belonging to the *Bradyrhizobium-Agromonas-Nitrobacter-Afipia* cluster (Itoh et al., 2002), and these *tfdAα* sequences show 56–60% identity to the β-proteobacterial counterparts. *tfdA*-like genes were also detected in 2,4-D degrading sphingomonads showing 46–51% of nucleotide sequence identity with either *tfdA* or *tfdAα*-genes (Itoh et al., 2004) (*Fig. 4*).

Dichlorprop ((*RS*)-2-(2,4-dichlorophenoxy)propionate) and mecoprop ((*RS*)-2-(4-chloro-2-methylphenoxy)propionate) are chiral molecules that are poor substrates for the TfdA enzymes already mentioned. Experiments with pure enantiomers of mecoprop revealed that *S. herbicidovorans* MH and *D. acidovorans* MC1 can degrade both enantiomers. In both organisms, enantioselective α-ketoglutarate-dependent dioxygenases termed SdpA and RdpA were observed (Müller et al., 2006; Westendorf et al., 2003) with the SdpA proteins (64% sequence identity) being highly specific in transforming only the (*S*)-enantiomers of mecoprop and dichlorprop and the (identical) RdpA proteins active only against the (*R*)-enantiomers. Like TfdA, SdpA and RdpA are α-ketoglutarate-dependent dioxygenases; however SdpA and RdpA share only 25% of protein sequence identity and only less than 35% of identity with previously characterized TfdA proteins (*Fig. 4*).

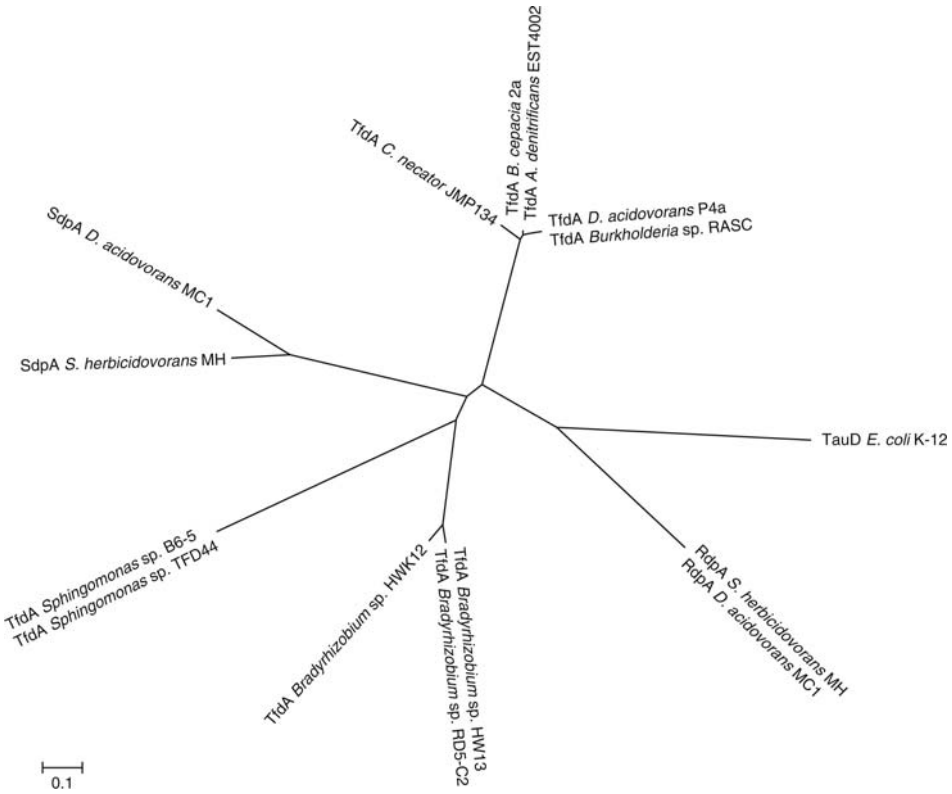

Figure 4

Dendrogram showing the relatedness of 2,4-D/α-ketoglutarate dioxygenases (TfdA), (S)-mecoprop/and (R)-mecoprop/α-ketoglutarate dioxygenases (SdpA and RdpA, respectively). TauD taurine α-ketoglutarate dioxygenase from *E. coli* is shown as outgroup.

2.1.6 Hydrolytic Dehalogenation of 4-Chlorobenzoate

Various *Arthrobacter*, *Pseudomonas* and *Alcaligenes* strains degrading 4-chlorobenzoate (4CB) by a pathway involving an initial dehalogenation event have been described.

The 4-chlorobenzoate dehalogenase system consists of three distinct enzymes, a 4CB-coenzyme A (CoA) ligase, 4CB-CoA dehalogenase, and 4-hydroxybenzoate (4HB)-CoA thioesterase (*Fig. 5*) encoded by the *fcbA*, *fcbB* and *fcbC* genes, respectively. In 4CB degrading actinobacteria, a *fcbABC* gene order is conserved (Schmitz et al., 1992), whereas 4CB degrading proteobacterial strains exhibit a *fcbBAC* gene order (Savard et al., 1992). In some cases, the gene cluster also encodes putative transport proteins between the *fcbB* and *fcbA* genes. In addition to differences in the gene order, significant differences in protein sequence were observed. Actinobacterial 4CB-CoA ligases are highly similar (>95% sequence identity), but differ significantly (38–44% identity) from proteobacterial 4CB-CoA ligases; however, all ligases share significant sequence similarity with proteins catalyzing similar chemistry in the β-oxidation pathway (Babbitt et al., 1992). The following dehalogenation by 4CB-CoA dehalogenase forms 4-hydroxybenzoyl-CoA in a hydrolytic substitution reaction (Liang et al., 1993).

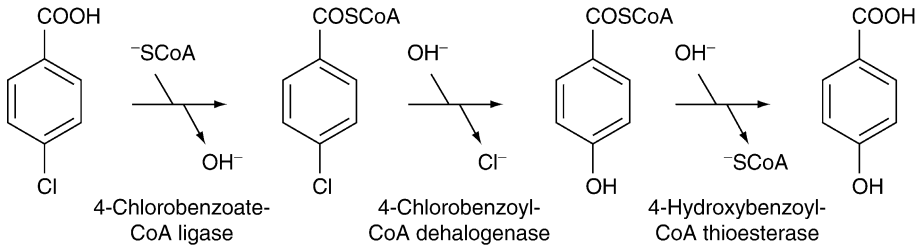

Figure 5

Degradation of 4-chlorobenzoate involving hydrolytic dehalogenation.

This group of enzymes utilizes a unique form of catalysis in which an active site carboxylate bonds to C-4 of the benzoyl ring of the bound substrate to form a Meisenheimer-like complex. Expulsion of the chloride from the Meisenheimer complex with concomitant rearomatization of the benzoyl ring generates an arylated enzyme as the second reaction intermediate (Dong et al., 2002). Hydrolysis of the arylated enzyme occurs by addition of a water molecule to the acyl carbonyl carbon to form a tetrahedral intermediate, which expels the hydroxylbenzoyl group to generate the catalytic carboxylate residue and form 4-hydroxybenzoyl-CoA. As observed for the ligases, the actinobacterial dehalogenases are highly similar (>98% sequence identity) but differ significantly (approximately 50% sequence identity) from proteobacterial dehalogenases. The last reaction step to form 4HB is carried out by the 4HB-CoA thioesterase. The absence of serine, cysteine, or histidine catalytic residues in the *Pseudomonas* sp. strain CBS-3 enzyme had distinguished this protein from previously characterized thioesterases. However, crystallographic investigation (Benning et al., 1998) revealed a high similarity of its three-dimensional structure to that of β-hydroxydecanoyl thiol ester dehydrase from *E. coli*. Both enzymes contained the so-called Hotdog fold, which now was found to be shared by numerous thioesterases and dehydrase proteins (Dillon and Bateman, 2004). An active site aspartate was suggested to participate in nucleophilic catalysis (Zhuang et al., 2002). Other proteobacterial 4HB-CoA thioesterases belong to the same subfamily of Hotdog fold proteins, whereas the respective thioesterases from *Arthrobacter* strains do not share significant sequence similarity with the proteobacterial counterparts. However, they also belong to the same superfamily and an active site glutamate was suggested as catalytic nucleophile (Dillon and Bateman, 2004).

2.1.7 Reactions Leading to (Chloro)Benzoquinols and (Chloro) Hydroxybenzoquinols

The degradation of various, specially higher chlorinated aromatic pollutants, proceeds via (chloro)benzoquinols or (chloro)hydroxybenzoquinols as central intermediates (*Fig. 6*).

2.1.7.1 Degradation of Lindane

One of the most important xenobiotic compounds metabolized through (chloro)benzoquinol is lindane or γ-hexachlorocyclohexane (γ-HCH), the biodegradation of which has been mainly studied in *Sphingobium japonicum* UT26 (Endo et al., 2005) (*Fig. 6*). However, other γ-HCH degrading strains have recently been found to possess nearly identical genes for degradation (Nagata et al., 2007). LinA γ-HCH dehydrochlorinase catalyzes two steps of dehydrochlorination to 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) via

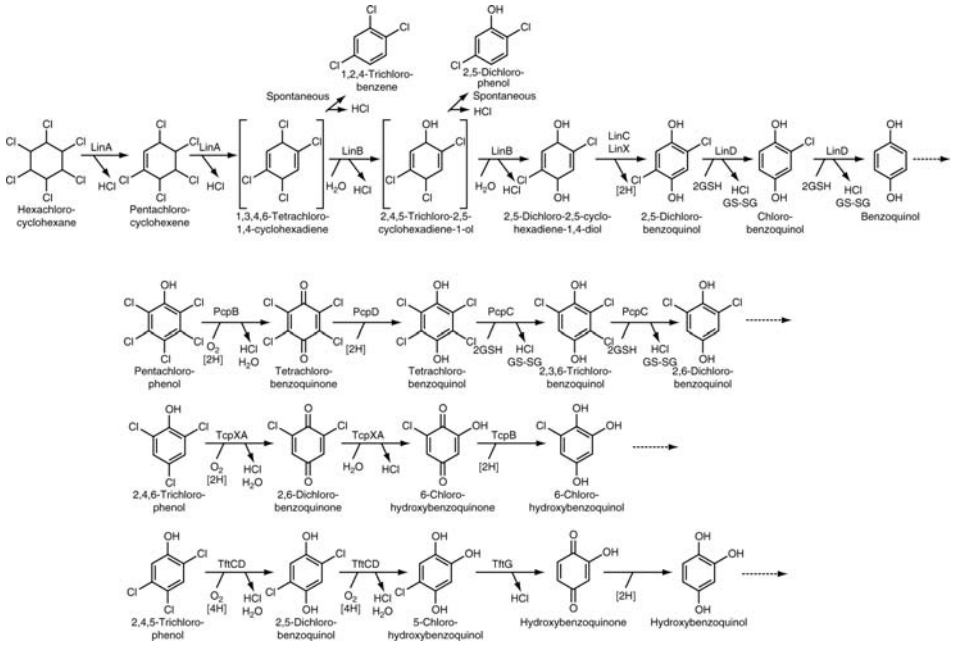

Figure 6

Catabolic pathways leading to (chloro)benzoquinols and (chloro)hydroxybenzoquinols as central intermediates. The metabolism of γ-hexachlorocyclohexane by *S. japonicum* UT26, of pentachlorophenol by *S. chlorophenolicum* ATCC39723, of 2,4,6-trichlorophenol by *C. necator* JMP134 and of 2,4,5-trichlorophenol by *B. phenoliruptrix* AC1100 are shown. Unstable intermediates are enclosed in brackets. Broken arrows indicate reactions of the lower pathway detailed in *Fig. 10*.

γ-pentachlorocyclohexene (Nagata et al., 2007). 1,4-TCDN is further transformed by LinB 1,4-TCDN chlorohydrolase: a reaction competing with spontaneous rearrangement to the dead-end 1,2,4-trichlorobenzene product. Unlike LinA, which seems to be a unique dehydrochlorinase, LinB belongs to the haloalkane dehalogenase family and catalyzes two successive hydrolytic dehalogenations to form 2,5-dichloro-2,5-cyclohexadiene-1,4-diol via 2,4,5-trichloro-2,5-cyclohexadiene-1-ol (Nagata et al., 2007). Further metabolism of 2,5-dichloro-2,5-cyclohexadiene-1,4-diol is achieved by LinC or LinX 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase, members of the short-chain alcohol dehydrogenase superfamily (Nagata et al., 1994), forming 2,5-dichloro-*p*-benzoquinol, which is subject to a reductive dechlorination by 2,5-dichloro-*p*-benzoquinol dechlorinase (LinD). LinD belongs to the glutathione transferases family, and catalyzes a rapid dechlorination to chlorobenzoquinol and a subsequent slow conversion to further dechlorinate and forms benzoquinol (Miyauchi et al., 1998).

2.1.7.2 Transformation of Chlorinated Phenols and Phenoxyacetates to (Chloro)Benzoquinols and (Chloro)Hydroxybenzoquinols

Like HCH, pentachlorophenol (PCP) is degraded through (chloro)benzoquinol and the pathway has been elucidated in detail in *Sphingobium chlorophenolicum* ATCC 39723 (Crawford et al., 2007) (*Fig. 6*). The oxidation of PCP is catalyzed by PCP 4-monooxygenase

(PcpB), a flavin monooxygenase with functional domains similar to those of other bacterial flavoprotein monooxygenases specific to phenolic substrates (see Fig. 3). PcpB can catalyze attack on a broad range of substituted phenols, hydroxylating the *para* position and removing halogen, nitro, amino, and cyano groups (Xun et al., 1992a). The generated tetrachloro-*p*-benzoquinone is reduced to tetrachloro-*p*-benzoquinol by a NADPH-dependent tetrachlorobenzoquinone reductase (PcpD) (Dai et al., 2003). The following TeCH reductive dehalogenase (PcpC)-catalyzed reactions, reductive dehalogenation to 2,3,6-trichloro-*p*-benzoquinol and then to 2,6-dichloro-*p*-benzoquinol (DiCH) (Xun et al., 1992b) resemble those catalyzed by LinD of the HCH degradation pathway. However, in contrast, two chlorine atoms remain on the aromatic ring before ring-cleavage.

The broadly used herbicide 2,4,5-T is also metabolized through the (chloro)hydroxybenzoquinol pathway, which has been well studied in *Burkholderia phenoliruptrix* AC1100 (Hübner et al., 1998). The transformation to 2,4,5-trichlorophenol (2,4,5-TCP) is performed by a two-component monooxygenase (TftAB) (Danganan et al., 1995), which exhibits strong homology to terminal oxygenase subunits of benzoate and toluate dioxygenases and thus contrasts the degradation of 2,4-D, which is typically catalyzed by 2,4-D/α-ketoglutarate dioxygenases (see Section 2.1.5.2). However, 2,4-D monooxygenase genes (*cadAB*) with significant identity (approximately 45% on the amino acid level) with TftAB of *B. phenoliruptrix* AC1100 were observed in *Bradyrhizobium* sp. strain HW13 capable of degrading 2,4-D (Kitagawa et al., 2002). The substrate profiles of the 2,4,5-T oxygenase and 2,4-D oxygenase are similar (Danganan et al., 1995; Kitagawa et al., 2002) with high activity against 2,4-D and 2,4,5-T. Additional experiments indicated the presence of respective genes in various 2,4-D degrading *Sphingomonas* (B6–10, TFD26, TFD44) and *Bradyrhizobium* (HWK12, BTH) strains (Kitagawa et al., 2002).

Further metabolism of 2,4,5-TCP (Fig. 6) is achieved by TftCD 2,4,5-TCP monooxygenase, a class D monooxygenase (van Berkel et al., 2006) comprising a $FADH_2$-utilizing monooxygenase (TfdD) and a NADH:FAD oxidoreductase (TfdC). TfdC supplies $FADH_2$ to TftD (Gisi and Xun, 2003) converting 2,4,5-TCP via 2,5-dichlorobenzoquinol to 5-chloro-2-hydroxybenzoquinol. 5-Chloro-2-hydroxybenzoquinol is dechlorinated to hydroxybenzoquinone by a dechlorinase (TftG) and then reduced to hydroxybenzoquinol by a quinone reductase the gene of which is presently unknown.

A similar pathway has been reported for the degradation of 2,4,6-trichlorophenol (2,4,6-TCP) in *C. necator* JMP134 (Fig. 6). In this bacterium, 2,4,6-TCP monooxygenase (TcpA with 65% sequence identity to TftD) catalyzes the oxidative conversion of 2,4,6-TCP to 2,6-dichlorobenzoquinone, followed by hydrolytic dechlorination to produce 6-chloro-2-hydroxybenzoquinone (Xun and Webster, 2004). Like TftC, TcpX NADH:FAD oxidoreductase provides $FADH_2$ for TcpA catalysis (Matus et al., 2003). 6-Chloro-2-hydroxybenzoquinone is reduced to 6-chlorohydroxybenzoquinol by a quinone reductase (TcpB) (Belchik and Xun, 2008). Recently, evidence was accumulated that also 2,4-dichlorophenol (Ferraroni et al., 2005) and 4-chlorophenol (Nordin et al., 2005) can be degraded via (chloro)hydroxybenzoquinols as intermediate. The downstream catabolism of (chloro)hydroxybenzoquinols is described in Section 2.3.

2.2 Degradation of Chlorocatechols and Chloroprotocatechuates

2.2.1 Metabolism of Chlorocatechols via the 3-Oxoadipate Pathway

The chromosomally encoded 3-oxoadipate pathway is widely distributed in soil bacteria, specifically proteobacteria and actinobacteria (See Chapter 39, Vol. 2, Part 6). The function

of the catechol branch of this pathway is to transform catechol to Krebs cycle intermediates (See Chapter 4, Vol. 2, Part 2) (*Fig. 7*).

From phylogenetic analyses, proteobacterial catechol 1,2-dioxygenases (C12O) and actinobacterial C12Os form separate branches with low sequence identity to one another; however, all those enzymes seem to be characterized by similar kinetic properties and out of the chlorocatechols, only 4-chlorocatechol is transformed at a reasonable rate (Dorn and Knackmuss, 1978; Matsumura et al., 2004). Also proteobacterial and actinobacterial muconate cycloisomerases (MCI) constitute separate evolutionary branches with low sequence identity to one another (*Fig. 8*).

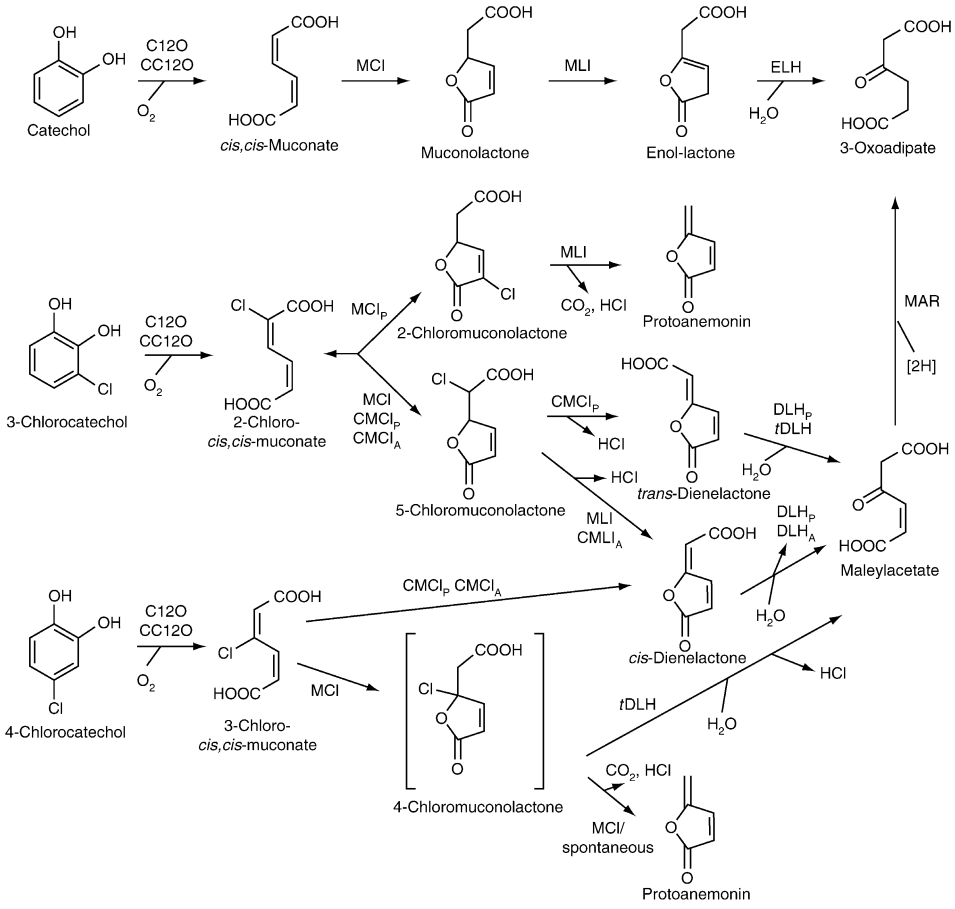

Figure 7

Diversity of pathways for the degradation/transformation of chlorocatechols. C12O, catechol 1,2-dioxygenase; CC12O, chlorocatechol 1,2-dioxygenase; MCI, muconate cycloisomerase; MCI_P, proteobacterial muconate cycloisomerase; $CMCI_P$, proteobacterial chloromuconate cycloisomerase; $CMCI_A$, actinobacteral chloromuconate cycloisomerase; MLI, muconolactone isomerase; $CMLI_A$, actinobacterial chloromuconolactone isomerase; DLH_P, proteobacterial dienelactone hydrolase, DLH_A, actinobacterial dienelactone hydrolase; *t*DLH, *trans*-dienelactone hydrolase; MAR, maleylacetate reductase. The unstable intermediate is enclosed in brackets.

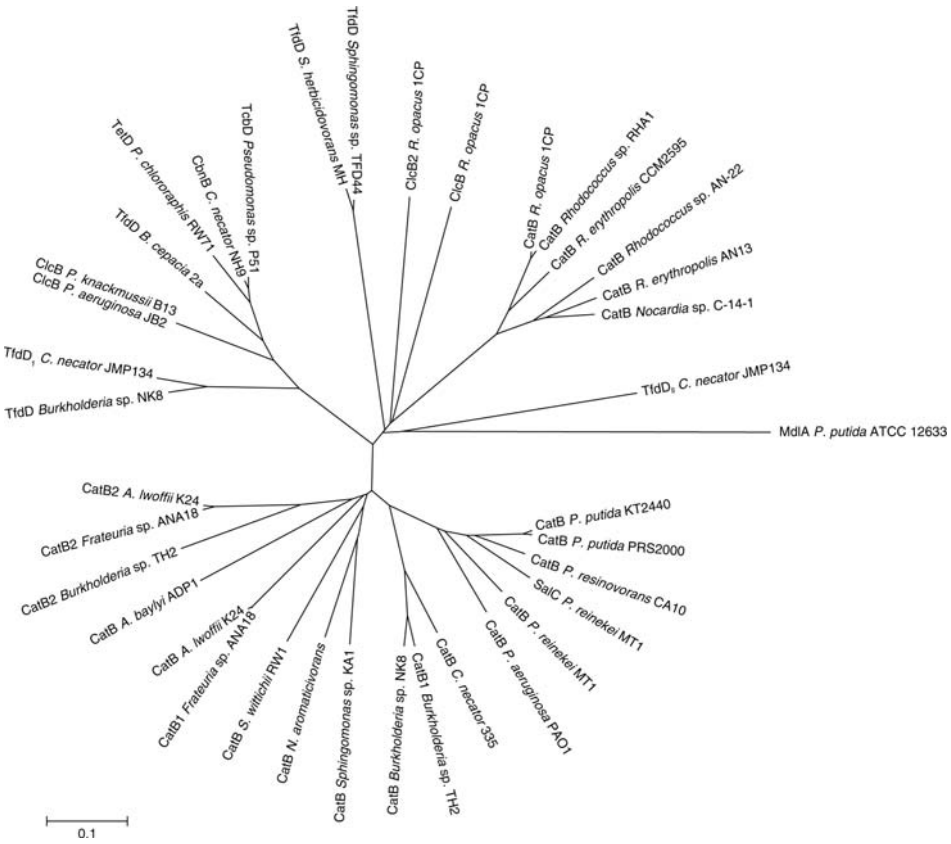

Figure 8

Dendrogram showing the relatedness of muconate cycloisomerases (CatB) and chloromuconate cycloisomerases (ClcB, TfdD, TcbD, TetD). Mandelate racemase MdlA of *P. putida* is shown as outgroup.

Proteobacterial MCIs catalyze the cycloisomerization of 3-chloromuconate at reasonable rates (Cámara et al., 2007). This reaction is assumed to proceed via 4-chloromuconolactone as intermediate (*Fig. 7*), which either spontaneously or enzyme-catalyzed rearranges with concomitant dehalogenation and decarboxylation to form protoanemonin (Blasco et al., 1995; Nikodem et al., 2003), a compound of high toxicity. The cycloisomerization product of 3-chloromuconate by actinobacterial MCIs has not yet been identified. In the case of 2-chloromuconate turnover, proteobacterial MCIs catalyze cycloisomerization only, to form both 2-chloro- and 5-chloromuconolactone as stable products (Vollmer et al., 1994) (*Fig. 7*). Even though only few studies are available on actinobacterial MCIs, some studies suggested that they differ in catalytic properties from their proteobacterial counterparts and form exclusively 5-chloromuconolactone (Solyanikova et al., 1995). Both 5-chloro- and 2-chloromuconolactone are substrates of muconolactone isomerases of the 3-oxoadipate pathway. 5-Chloromuconolactone is transformed predominantly to *cis*-dienelactone probably via abstraction of the C4 proton followed by spontaneous chloride elimination (Prucha et al., 1996)

and 2-chloromuconolactone into protoanemonin, probably by the elimination of CO_2 and chloride from chlorosubstituted 3-oxoadipate enol-lactone (Skiba et al., 2002) (Fig. 7). Mineralization of chlorocatechols via the 3-oxoadipate pathway is thus prevented by (1) restricted substrate specificity of the enzymes, (2) formation of protoanemonin as toxic dead-end product, or (3) formation of *cis*-dienelactone as dead-end product.

2.2.2 The Chlorocatechol Pathway

In most organisms isolated thus far, chlorocatechols are degraded by a chlorocatechol *ortho*-cleavage pathway (Reineke, 2001). This pathway is initiated by a broad specificity chlorocatechol 1,2-dioxygenase with consumption of molecular oxygen to produce the corresponding chloro-*cis*,*cis*-muconates (Dorn and Knackmuss, 1978). This reaction is identical to the one of the 3-oxoadipate pathway, and the main difference between the enzymes is their substrate specificity.

A key enzyme in the pathway is chloromuconate cycloisomerase (CMCI), which differs in various aspects from muconate cycloisomerase. Proteobacterial CMCIs catalyze a specific cycloisomerization of 2-chloro-*cis,cis*-muconate (the metabolite of 3-chlorocatechol degradation) to form preferentially 5-chloromuconolactone, which is dehalogenated to *trans*-dienelactone (Vollmer and Schlömann, 1995). Thus, proteobacterial CMCIs are specific dehalogenases. The formation of *trans*-dienelactone is due to the fact that, after cycloisomerization, the lactone ring has to rotate in the catalytic center to achieve proton abstraction and thus dehalogenation (Schell et al., 1999) (Fig. 7). In contrast, chloride from the 3-position of 3-chloro-*cis,cis*-muconate (the metabolite of 4-chlorocatechol degradation) seems to be directly eliminated during cycloisomerization to form *cis*-dienelactone (Kaulmann et al., 2001) (Fig. 7). Similarly, 2,4-dichloro-*cis,cis*-muconate, the metabolite of 3,5-dichlorocatechol degradation, is converted to 2-chloro-*cis*-dienelactone (Pieper et al., 1991).

The dienelactones are converted into the respective maleylacetates by dienelactone hydrolases (DLH) (Schmidt and Knackmuss, 1980). Evidently, for the degradation of various chloroaromatics, an important prerequisite is that the dienelactone hydrolase is of relaxed substrate specificity and accepts both *cis*- and *trans*-isomers as substrates.

The following enzyme, maleylacetate reductase, plays a major role in the degradation of chloroaromatic compounds either in the *ortho*-cleavage chlorocatechol pathway (Kaschabek and Reineke, 1992) or as part of the benzoquinol pathway. The original function is the reduction of the double bond to channel maleylacetate into the 3-oxoadipate pathway. Maleylacetates with chlorine substituents in the 2-position are subject to reductive dechlorination, e.g., 2-chloromaleylacetate, the intermediate in 3,5-dichlorocatechol degradation, is initially transformed to maleylacetate followed by reduction to 3-oxoadipate (Kaschabek and Reineke, 1992). Obviously, enzymatic attack on the C2-carbon results in an intermediate, which spontaneously eliminates chloride. Probably, dehalogenation of 2-chloromaleylacetate is a general capability of maleylacetate reductases. The *ortho*-cleavage chlorocatechol pathway described tolerates substitution at the aromatic ring of up to four chlorine atoms. Two dechlorination steps have been described up to now.

The genes encoding the chlorocatechol pathway usually form clusters and the structures of the corresponding operons of chlorobenzoate degraders such as *P. knackmussii* B13 or *C. necator* NH9 or chlorobenzene degraders such as *Pseudomonas* sp. P51 or *P. chlororaphis* RW71 are nearly identical, in spite of the geographically distinct origins of the bacteria or the

difference in their phylogenetic position (Pieper, 2005). Interestingly, the genetic organization of microorganisms isolated based on their capability to degrade 2,4-D usually differs substantially from that of chlorobenzoate and chlorobenzene degraders already described and in most strains the module comprises a gene encoding a phenol hydroxylase (*tfdB*), a protein probably involved in 2,4-D transport (*tfdK*) and a 2,4-D/α-ketoglutarate dioxygenase (*tfdA*). These gene clusters are typically missing *tfdD* and *tfdF* genes encoding chloromuconate cycloisomerases and maleylacetate reductases, respectively. However, in strains analyzed in this aspect, *tfdD* genes are located upstream of the *tfdR* regulator gene, whereas *tfdF* genes are located downstream of *tfdA* (Pieper, 2005).

Even though analysis of chlorocatechol gene operons in proteobacteria had concentrated on β- and γ-*Proteobacteria*, chlorocatechol genes have been also characterized also in members of the α-*Proteobacteria* (Müller et al., 2004). Both types of newly discovered chlorocatechol dioxygenases from *Sphingomonas* isolates represent deep-branching lines in the dendrogram and suggest a different reservoir and reduced transfer of the respective genes in α-*Proteobacteria* compared with the ones in β- and γ-*Proteobacteria*. Only poor information is available on chlorocatechol genes in *Actinobacteria*, and thus far, only two modules from *Rhodococcus opacus* 1CP have been analyzed in detail (Maltseva et al., 1994a; Moiseeva et al., 2002).

Usually, chlorocatechol 1,2-dioxygenases are characterized by their broad substrate specificity and can convert several higher chlorinated catechols. As an example, the chlorocatechol gene products of *P. knackmussii* B13 were capable of dealing with higher chlorinated catechols such as 3,5-dichloro- or 3,6-dichlorocatechol, whereas 3,4-dichlorocatechol was only a poor substrate (Dorn and Knackmuss, 1978; Oltmanns et al., 1988). In contrast, the enzyme of *Pseudomonas* sp. strain P51 was capable of transforming 3,4-dichlorocatechol (van der Meer et al., 1991). Recently, amino acids determining such differences in substrate specificity could be identified (Liu et al., 2005). In the case of chlorocatechol 1,2-dioxygenases from *Rhodococcus*, only one of the thus-far described enzymes exhibits broad substrate specificity (Moiseeva et al., 2002), whereas 4-chlorocatechol 1,2-dioxygenase prefers 4-chlorocatechol and has only negligible activity with 3-chlorocatechol (Maltseva et al., 1994a).

All proteobacterial CMCIs obviously share common features, which discriminate them from the *Rhodococcus* enzymes, but also from MCIs. In agreement with their special biochemical properties, a phylogenetic comparison depicted proteobacterial CMCIs to constitute a separate subfamily, whereas the rhodococcal CMCIs, like the respective chlorocatechol 1,2-dioxygenases, appear to represent isolated evolutionary lines (*Fig. 8*). As already described, proteobacterial CMCIs are specific dehalogenases, and capable of dehalogenating even higher substituted chloromuconates. In contrast, both CMCIs described from *R. opacus* 1CP exhibited extraordinary properties and catalyzed the formation of 5-chloromuconolactone from 2-chloromuconate but were not capable of dehalogenating (Moiseeva et al., 2002; Solyanikova et al., 1995) (*Fig. 7*).

Only some of the DLHs encoded in chlorocatechol gene clusters such as TfdEI from *C. necator* JMP134, and especially ClcD from *P. knackmussii* B13, have been described in more detail. These enzymes are active against both the *cis*- and *trans*-isomer, a feature that is indispensable for the mineralization of differently substituted chloroaromatics by proteobacteria as already described. Structure elucidation of the *P. knackmussii* B13 enzyme revealed that the protein belongs to the α/β hydrolase fold enzymes (Ollis and Nai, 1985). Analysis of

DLHs from various chloroaromatic degraders showed that even though some of these DLHs share only low sequence identity (down to 13%), they all belong to the same family with a conserved Cys-His-Asp triad and highly conserved residues flanking the triad; however they all differ from enol-lactone hydrolases (Schlömann, 1994). Unfortunately, only poor data are available on the substrate specificity of those DLHs; however, both DLHs from *Rhodococcus* are active only against the *cis*-isomer (Maltseva et al., 1994b; Moiseeva et al., 2002), contrasting DLHs from proteobacterial chlorobenzoate or chlorobenzene degraders.

2.2.3 The Alternative Pathway of 3-Chlorocatechol Degradation in *Rhodococcus opacus* 1CP

Among the Gram-positive organisms, chlorocatechol catabolism has mainly been investigated in *R. opacus* 1CP. 4-chloro- and 3,5-dichlorocatechol are transformed similar to the metabolism in proteobacteria, whereas 2-chloromuconate formed from 3-chlorocatechol is not dehalogenated during cycloisomerization. In contrast, dehalogenation is catalyzed by a muconolactone isomerase-related enzyme (Fig. 7). Despite its sequence similarity to muconolactone isomerase, the *Rhodococcus* enzyme does not show muconolactone-isomerizing activity and thus represents an enzyme dedicated to its new function as a 5-chloromuconolactone dehalogenase forming *cis*-dienelactone (Moiseeva et al., 2002). Thus, a major difference between chlorocatechol degradation in proteobacteria and in *Rhodococcus* lies in the fact that *cis*-dienelactone is the intermediate of both 4-chloro- and 3-chlorocatechol degradation in *Rhodococcus*, whereas *cis*- and *trans*-dienelactone, respectively, are formed by proteobacteria, therefore requiring a DLH with broad substrate specificity (Fig. 7).

2.2.4 The Alternative Pathway of 4-Halocatechol Degradation

Another variant of the chlorocatechol pathway has been described in *Pseudomonas reinekei* MT1 (Nikodem et al., 2003). In this strain 4-chlorocatechol formed from 4-chloro- and 5-chlorosalicylate is channeled into the 3-oxoadipate pathway by a catechol 1,2-dioxygenase and a MCI that showed unusual kinetic properties as being adapted for turnover of 4-chlorocatechol and 3-chloromuconate, respectively (Cámara et al., 2007). A *trans*-dienelactone hydrolase capable of transforming the *trans*- but not the *cis*-dienelactone isomer has been observed to be crucial for 4-chlorocatechol degradation by this strain. It was shown that the enzyme interacts with the MCI-catalyzed transformation of 3-chloromuconate and hydrolyzes the cycloisomerization intermediate 4-chloromuconolactone, thereby preventing the formation of protoanemonin in favor of maleylacetate (Nikodem et al., 2003) (Fig. 7). Maleylacetate is then transformed by maleylacetate reductase. DLHs active against *trans*-dienelactone only and not able to transform *cis*-dienelactone, the intermediate of 4-chlorocatechol degradation by enzymes of the chlorocatechol pathway, have initially been described to be involved in 4-fluorocatechol degradation by *C. necator* 335 and *C. necator* JMP222 and probably responsible for further degradation of intermediate 4-fluoromuconolactone (Schlömann et al., 1990).

2.2.5 Chlorocatechol Degradation via the *Meta*-Cleavage Pathway

Catechol *meta*-cleavage routes are widespread and usually involved in the degradation of methylsubstituted compounds such as toluene or methylphenols (See Chapter 4, Vol. 2, Part 2). For a long time the presence of such a *meta*-cleavage pathway was assumed to severely interfere with the degradation of chloroaromatics. One of the reasons of interference was assumed to be the formation of a reactive acyl chloride, e.g., from 3-chlorocatechol by the catechol 2,3-dioxygenase of *P. putida* mt-2 (XylE) (Bartels et al., 1984), resulting in irreversible inactivation of the ring cleavage enzyme (*Fig. 9*). In other cases, reversible inactivation was shown to be due to a rapid oxidation of the active site ferrous iron to its ferric form with concomitant loss of activity (Vaillancourt et al., 2002), and a general mechanism for the inactivation of extradiol dioxygenases during catalytic turnover involving the dissociation of superoxide from the enzyme-catechol-dioxygen ternary complex was suggested. In contrast to 3-chlorocatechol, 4-chlorocatechol is a moderate substrate for various catechol 2,3-dioxygenases (Bartels et al., 1984; Murray et al., 1972), among them catechol 2,3-dioxygenases of family I.2.A (Eltis and Bolin, 1996), which have been commonly observed to be involved in the degradation of methylaromatics. However, despite high sequence identity, members of this subfamily exhibit very different substrate specificities, and the capability to transform 4-chlorocatechol is not a general characteristic of catechol 2,3-dioxygenases of family I.2.A (Kitayama et al., 1996). Some publications postulate that compounds degraded via catechols chlorinated in the 4-position might be mineralized via a catechol degrading *meta*-cleavage pathway (Arensdorf and Focht, 1995) but information about the way in which the products are dechlorinated is missing.

In 1997, the first isolate, *P. putida* GJ31, capable of degrading a chloroaromatic compound (chlorobenzene) via 3-chlorocatechol using a *meta*-cleavage pathway was described (Mars et al., 1997). The CbzE chlorocatechol 2,3-dioxygenase of strain GJ31 productively converts 3-chlorocatechol, and stoichiometric displacement of chloride leads to the production of 2-hydroxymuconate (Kaschabek et al., 1998), which is further converted through the *meta*-cleavage pathway (*Fig. 9*). 3-Chlorocatechol was found to be the preferred substrate of CbzE. Additional pseudomonads using a *meta*-cleavage route for 3-chlorocatechol degradation were isolated in the meantime from various contaminated environments (Göbel et al., 2004), suggesting that productive *meta*-cleavage of 3-chlorocatechol is not atypical for chloroaromatic degradation. All the three isolates harbor chlorocatechol 2,3-dioxygenases, highly resistant to inactivation during 3-chlorocatechol turnover, sharing 97% amino acid sequence identity with the enzyme from strain GJ31, thus forming a distinct subfamily of catechol 2,3-dioxygenases in what was termed family I.2.C. Other extradiol dioxygenases of family 1.2.C do not share the capability of effectively transforming 3-chlorocatechol.

In all the chlorobenzene-degrading strains already mentioned, *cbzT* genes encoding ferredoxins are located upstream of *cbzE*. Similar ferredoxins have been observed to be encoded in various *meta*-cleavage pathways (Hugo et al., 2000). Catechol 2,3-dioxygenases are oxygen sensitive and unstable in vitro, particularly in the presence of substituted catechol substrates. This instability was shown to be due to the oxidation of active site Fe(II)–Fe(III) (Vaillancourt et al., 2002), and CbzT-like ferredoxins reactivate catechol 2,3-dioxygenases through the reduction of the iron atom in the active site of the enzyme (Hugo et al., 2000). It was thus proposed that the ability of strain GJ31 to metabolize both chlorobenzene and toluene might depend on the regeneration of the chlorocatechol dioxygenase activity mediated by CbzT (Tropel et al., 2002).

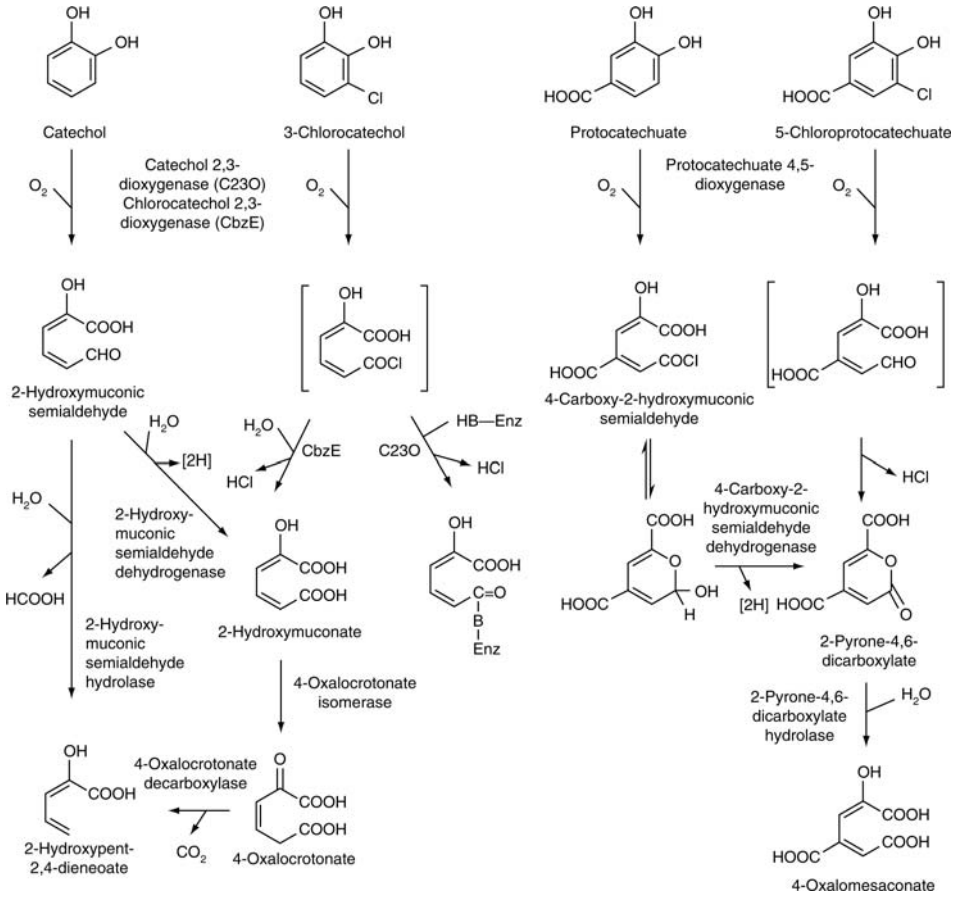

Figure 9

***Meta*-cleavage pathways for catechol, 3-chlorocatechol, protocatechuate and 5-chloroprotocatechuate. The acylchlorides are shown in brackets. The 3-chlorocatechol ring-cleavage product can react either with the ring cleavage enzyme resulting in suicide inactivation, or be rapidly hydrolyzed to give 2-hydroxymuconate. The 5-chloroprotocatechuate ring-cleavage product undergoes intramolecular rearrangement and dehalogenation.**

2.2.6 Degradation of 5-Chloroprotocatechuate via a *Meta*-Cleavage Pathway

A productive *meta*-cleavage without suicide effect has been known for many years. The extradiol cleavage of 5-chloroprotocatechuate by protocatechuate 4,5-dioxygenase (Kersten et al., 1985) results in the formation of 2-pyrone-4,6-dicarboxylate by nucleophilic displacement of chloride (*Fig. 9*). This indicates that cyclization entailing nucleophilic displacement of halogen provides an effective alternative to the enzyme suicide inactivation that occurs when a nucleophilic group of the extradiol dioxygenase undergoes acylation. An important aspect of this mechanism is that the ring fission product remains bound to the enzyme during the complete configuration change that precedes nucleophilic displacement.

2-Pyrone-4,6-dicarboxylate is a metabolite of the protocatechuate 4,5-dioxygenase pathway typically formed after the dehydrogenation of the ring-cleavage product (see *Fig. 9*), such that both protocatechuate and 5-chloroprotocatechuate degradation merge at this metabolic intermediate. So far, only the mineralization of 3-chlorobenzoate by *C. testosteroni* BR60 (Nakatsu et al., 1997) has been proposed to occur via such a route.

2.3 Ring Cleavage of (Chloro)Benzoquinols and (Chloro) Hydroxybenzoquinols

Ring cleavage dioxygenases involved in the turnover of (chloro) benzoquinols and (chloro) hydroxybenzoquinols has been identified from various microorganisms degrading γ-HCH, or chlorophenols (*Fig. 10*). Chlorobenzoquinol (but also benzoquinol) produced during the catabolism of γ-HCH in *S. japonicum* UT26 is subject to direct ring cleavage by a novel type of extradiol dioxygenase, designated chlorobenzoquinol/benzoquinol 1,2-dioxygenase (LinE), which preferentially cleaves aromatic rings with two hydroxyl groups at *para* positions (Miyauchi et al., 1999) and produces maleylacetate from either chlorobenzoquinol or benzoquinol (*Fig. 10*). Highly similar or identical dioxygenases (>99% sequence identity) have been identified in other γ-HCH-degrading organisms (Dogra et al., 2004). More distantly related extradiol dioxygenases (51–53% sequence identity) and designated 2,6-dichlorobenzoquinol 1,2-dioxygenases (PcpA) were observed in PCP degrading strains such as *S. chlorophenolicum* ATCC 39723 (Xu et al., 1999) and produce 2-chloromaleylacetate (2-CMA) from 2,6-dichlorobenzoquinol (*Fig. 10*). A protein highly similar to PcpA proteins and with activity with 2,6-dichlorobenzoquinol was also observed in *S. japonicum* UT26 (LinEb exhibiting 92% sequence identity to PcpA proteins (Endo et al., 2005)). However, its involvement in γ-HCH degradation responsible for cleavage of 2,5-dichlorobenzoquinol is unlikely, because such a cleavage would result in the formation of 3-chloromaleylacetate, which cannot be dehalogenated by maleylacetate reductase.

In contrast, members of the intradiol dioxygenase family are involved in the degradation of hydroxybenzoquinols formed from trichlorophenols, 2,4-dichloro- or 4-chlorophenol, and these (chloro)hydroxybenzoquinol dioxygenases form a defined phylogenetic group inside the intradiol dioxygenase family (See Chapter 39, Vol. 2, Part 6). Hydroxybenzoquinol is cleaved by TftH hydroxybenzoquinol 1,2-dioxygenase to yield maleylacetate during the mineralization of 2,4,5-T by *B. phenoliruptrix* AC1100 (Daubaras et al., 1996) (*Fig. 10*). In 2,4,6-TCP degradation by *C. necator* JMP134, TcpC 6-chlorohydroxyquinol 1,2-dioxygenase is responsible for direct cleavage of 6-chlorohydroxybenzoquinol producing 2-chloromaleylacetate (Louie et al., 2002) (*Fig. 10*). An important difference between the pathways of 2,4,5-TCP and 2,4,6-TCP degradation is the fact that 6-chlorohydroxybenzoquinol is directly cleaved instead of a preceding reductive dechlorination forming hydroxybenzoquinol. Interestingly, TftH hydroxybenzoquinol 1,2-dioxygenase from *B. phenoliruptrix* AC1100, which shares 53% amino acid sequence identity with TcpC, is unable to use 5-chloro- or 6-chlorohydroxybenzoquinol (Daubaras et al., 1996). The catabolism of (chloro)benzoquinols and (chloro)hydroxybenzoquinols is completed by the conversion of MA or 2-CMA to 3-oxoadipate (*Fig. 10*) by the action of maleylacetate reductases as already described (see Section 2.2.2).

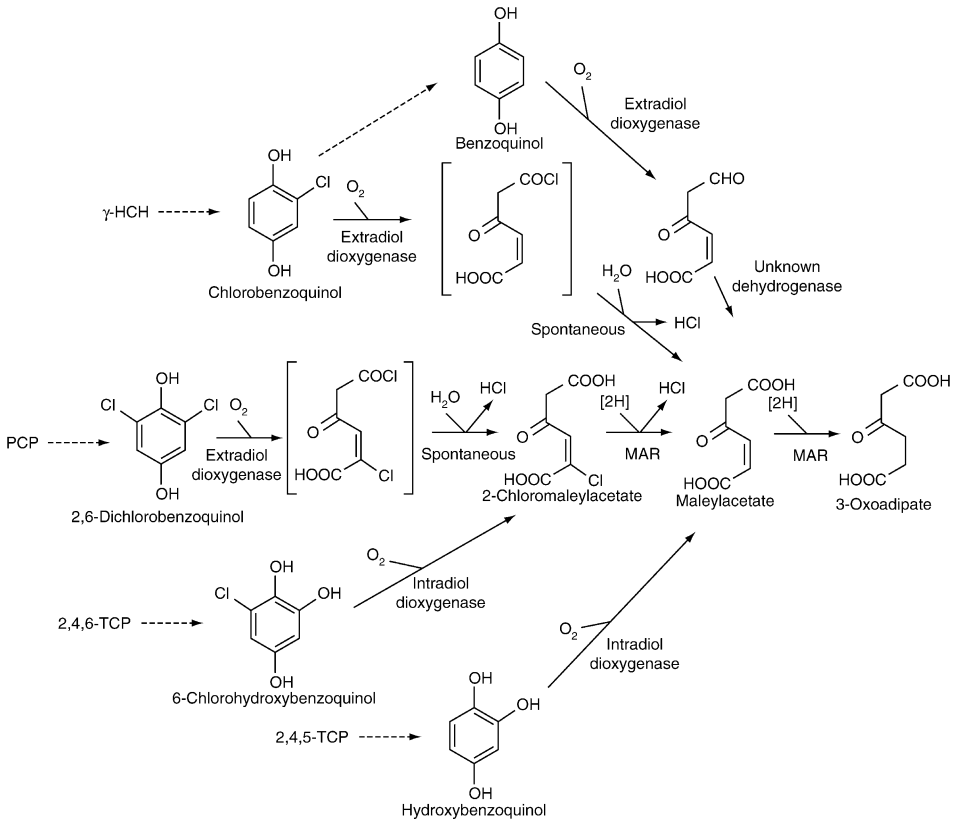

Figure 10

Catabolic pathways for (chloro)benzoquinols and (chloro)hydroxybenzoquinols. Unstable intermediates are enclosed in brackets. Broken arrows indicate reactions of the upper pathway detailed in *Fig. 6*.

3 Research Needs

Most of the current knowledge on biochemistry and genetics of chloroaromatic aerobic microbial metabolism has been obtained with bacterial strains isolated by traditional culture-dependent approaches. This is a severe drawback to understanding microbial degradation of chloroaromatics, as only a very minor proportion of microbes inhabiting the different ecosystems can be isolated by standard procedures and only a few reports are available on microbial associations (consortia) degrading this kind of compounds. Although it is possible that current knowledge covers a significant proportion of microbial strategies to degrade chloroaromatics, it would also be possible that new, unexpected ways to metabolize these compounds remain still unknown in the significant fraction of yet-uncultured microbes. Functional metagenomics approaches are required to address this point. Since natural haloaromatic formation is increasingly evident, studies including diverse ecosystems, not

necessarily exposed to chloroaromatic pollutants, will also provide a better idea on the diversity of metabolic pathways for aerobic degradation of chloroaromatics.

Several cases of adaptation or recruitment of pathways involved in the metabolism of natural aromatics for metabolism of the corresponding chloroaromatic counterparts are already reported. A further exploration of these possibilities would provide new insights into these phenomena. For instance, degradation of aromatic compounds through CoA derivatives seems to play a more significant role in aerobic degradation than initially expected (See ❯ Chapter 4, Vol. 2, Part 2). CoA derivatives have been reported as intermediates of 4-chlorobenzoate degradation, but additional routes are expected to be discovered.

Available are only a few studies of the degradation of mixed substituted chloroaromatic compounds, i.e., carrying additional amino/nitro/sulfonic substituents or heterocyclic aromatics, which without doubt, may give interesting new insights into aerobic microbial degradation of pollutants.

Even for the pathways already reported, there are several unclarified questions that deserve attention. For example, by which mechanism is the third or fourth chlorosubstituent eliminated during trichloro- or tetrachlorocatechol metabolism? What are the biochemical grounds for the degradation of 4-chlorocatechol via the *meta*-cleavage? By which mechanism is 3,6-dichlorogentisate mineralized in dicamba degrading microorganisms?

References

Arensdorf JJ, Focht DD (1995) A *meta* cleavage pathway for 4-chlorobenzoate, an intermediate in the metabolism of 4-chlorobiphenyl by *Pseudomonas cepacia* P166. Appl Environ Microbiol 61: 443–447.

Babbitt PC, Kenyon GL, Martin BM, Charest H, Sylvestre M, Scholten JD, Chang K-H, Liang P-H, Dunaway-Mariano D (1992) Ancestry of the 4-chlorobenzoate dehalogense: analysis of amino acid sequence identities among families of acyl: adenyl ligases, enoyl-CoA hydratases/isomerases, and acyl-CoA thiosterases. Biochemistry 31: 5594–5604.

Bartels I, Knackmuss H-J, Reineke W (1984) Suicide inactivation of catechol 2,3-dioxygenase from *Pseudomonas putida* mt-2 by 3-halocatechols. Appl Environ Microbiol 47: 500–505.

Beadle CA, Smith ARW (1982) The purification and properties of 2,4-dichlorophenol hydroxylase from a strain of *Acinetobacter* species. Eur J Biochem 123: 323–332.

Beil S, Mason JR, Timmis KN, Pieper DH (1998) Identification of chlorobenzene dioxygenase sequence elements involved in dechlorination of 1,2,4,5-tetrachlorobenzene. J Bacteriol 180: 5520–5528.

Belchik SM, Xun L (2008) Functions of flavin reductase and quinone reductase in 2,4,6-trichlorophenol degradation by *Cupriavidus necator* JMP134. J Bacteriol 190: 1615–1619.

Benning MM, Wesenberg G, Liu RQ, Taylor KL, Dunaway-Mariano D, Holden HM (1998) The three-dimensional structure of 4-hydroxybenzoyl-CoA thioesterase from *Pseudomonas* sp. strain CBS-3. J Biol Chem 273: 33572–33579.

Blasco R, Wittich R-M, Mallavarapu M, Timmis KN, Pieper DH (1995) From xenobiotic to antibiotic. Formation of protoanemonin from 4-chlorocatechol by enzymes of the 3-oxoadipate pathway. J Biol Chem 270: 29229–29235.

Bosch R, Moore ERB, GarciaValdes E, Pieper DH (1999) NahW, a novel, inducible salicylate hydroxylase involved in mineralization of naphthalene by *Pseudomonas stutzeri* AN10. J Bacteriol 181: 2315–2322.

Brinkmann U, Reineke W (1992) Degradation of chlorotoluenes by in vivo constructed hybrid strains: problems of enzyme specificity, induction and prevention of *meta*-pathway. FEMS Microbiol Lett 96: 81–88.

Cafaro V, Izzo V, Scognamiglio R, Notomista E, Capasso P, Casbarra A, Pucci P, Di Donato A (2004) Phenol hydroxylase and toluene/o-xylene monooxygenase from *Pseudomonas stutzeri* OX1: interplay between two enzymes. Appl Environ Microbiol 70: 2211–2219.

Crawford RL, Jung CM, Strap JL (2007) The recent evolution of pentachlorophenol (PCP)-4-monooxygenase (PcpB) and associated pathways for bacterial degradation of PCP. Biodegradation 18: 525–539.

Cámara B, Bielecki P, Kaminski F, dos Santos VM, Plumeier I, Nikodem P, Pieper DH (2007) A gene

cluster involved in degradation of substituted salicylates via *ortho* cleavage in *Pseudomonas* sp. strain MT1 encodes enzymes specifically adapted for transformation of 4-methylcatechol and 3-methylmuconate. J Bacteriol 189: 1664–1674.

Dai MH, Rogers JB, Warner JR, Copley SD (2003) A previously unrecognized step in pentachlorophenol degradation in *Sphingobium chlorophenolicum* is catalyzed by tetrachlorobenzoquinone reductase (PcpD). J Bacteriol 185: 302–310.

Danganan CE, Shankar S, Ye RW, Chakrabarty AM (1995) Substrate diversity and expression of the 2,4,5-trichlorophenoxyacetic acid oxygenase from *Burkholderia cepacia* AC1100. Appl Environ Microbiol 61: 4500–4504.

Daubaras DL, Saido K, Chakrabarty AM (1996) Purification of hydroxyquinol 1,2-dioxygenase and maleylacetate reductase: the lower pathway of 2,4,5-trichlorophenoxyacetic acid metabolism by *Burkholderia cepacia* AC1100. Appl Environ Microbiol 62: 4276–4279.

Dillon SC, Bateman A (2004) The Hotdog fold: wrapping up a superfamily of thioesterases and dehydratases. BMC Bioinformatics 12: 109.

Dogra C, Raina V, Pal R, Suar M, Lal S, Gartemann KH, Holliger C, van der Meer JR, Lal R (2004) Organization of *lin* genes and IS6100 among different strains of hexachlorocyclohexane-degrading *Sphingomonas paucimobilis*: evidence for horizontal gene transfer. J Bacteriol 186: 2225–2235.

Dong J, Carey PR, Wei YS, Luo LS, Lu XF, Liu RQ, Dunaway-Mariano D (2002) Raman evidence for Meisenheimer complex formation in the hydrolysis reactions of 4-fluorobenzoyl- and 4-nitrobenzoyl-coenzyme a catalyzed by 4-chlorobenzoyl-coenzyme A dehalogenase. Biochemistry 41: 7453–7463.

Dorn E, Knackmuss H-J (1978) Chemical structure and biodegradability of halogenated aromatic compounds. Substituent effects on 1,2-dioxygenation of catechol. Biochem J 174: 85–94.

Eltis LD, Bolin JT (1996) Evolutionary relationships among extradiol dioxygenases. J Bacteriol 178: 5930–5937.

Endo R, Kamakura M, Miyauchi K, Fukuda M, Ohtsubo Y, Tsuda M, Nagata Y (2005) Identification and characterization of genes involved in the downstream degradation pathway of gamma-hexachlorocyclohexane in *Sphingomonas paucimobilis* UT26. J Bacteriol 187: 847–853.

Ferraroni M, Seifert J, Travkin VM, Thiel M, Kaschabek S, Scozzafava A, Golovleva L, Schlömann M, Briganti F (2005) Crystal structure of the hydroxyquinol 1,2-dioxygenase from *Nocardioides simplex* 3E, a key enzyme involved in polychlorinated aromatics biodegradation. J Biol Chem 280: 21144–21154.

Fetzner S, Müller R, Lingens F (1992) Purification and some properties of 2-halobenzoate 1,2-dioxygenase, a two component enzyme system from *Pseudomonas cepacia* 2CBS. J Bacteriol 174: 279–290.

Fishman A, Tao Y, Rui L, Wood TK (2005) Controlling the regiospecific oxidation of aromatics via active site engineering of toluene para-monooxygenase of *Ralstonia pickettii* PKO1. J Biol Chem 280: 506–514.

Fukumori F, Hausinger RP (1993) *Alcaligenes eutrophus* JMP134 "2,4-dichlorophenoxyacetate monooxygenase" is an a-ketoglutarate-dependent dioxygenase. J Bacteriol 175: 2083–2086.

Furukawa K, Suenaga H, Goto M (2004) Biphenyl dioxygenases: functional versatilities and directed evolution. J Bacteriol 186: 5189–5196.

Gibson DT, Parales RE (2000) Aromatic hydrocarbon dioxygenases in environmental biotechnology. Curr Opin Biotechnol 11: 236–243.

Gisi MR, Xun L (2003) Characterization of chlorophenol 4-monooxygenase (TftD) and NADH:flavin adenine dinucleotide oxidoreductase (TftC) of *Burkholderia cepacia* AC1100. J Bacteriol 185: 2786–2792.

Greated A, Lambertsen L, Williams PA, Thomas CM (2002) Complete sequence of the IncP-9 TOL plasmid pWW0 from *Pseudomonas putida*. Environ Microbiol 4: 856–871.

Göbel M, Kranz OH, Kaschabek SR, Schmidt E, Pieper DH, Reineke W (2004) Microorganisms degrading chlorobenzene via a *meta*-cleavage pathway harbor highly similar chlorocatechol 2,3-dioxygenase-encoding gene clusters. Arch Microbiol 182: 147–156.

Hübner A, Danganan CE, Xun LY, Chakrabarty AM, Hendrickson W (1998) Genes for 2,4,5-trichlorophenoxyacetic acid metabolism in *Burkholderia cepacia* AC1100: Characterization of the *tftC* and *tftD* genes and locations of the *tft* operons on multiple replicons. Appl Environ Microbiol 64: 2086–2093.

Hugo N, Meyer C, Armengaud J, Gaillard J, Timmis KN, Jouanneau Y (2000) Characterization of three XylT-like [2Fe-2S] ferredoxins associated with catabolism of cresols or naphthalene: evidence for their involvement in catechol dioxygenase reactivation. J Bacteriol 182: 5580–5585.

Huong NL, Itoh K, Miyamoto M, Suyama K, Yamamoto H (2007) Chlorophenol hydroxylase activity encoded by TfdB from 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading *Bradyrhizobium* sp. strain RD5-C2. Biosci Biotechnol Biochem 71: 1691–1696.

Itoh K, Kanda R, Sumita Y, Kim H, Kamagata Y, Suyama K, Yamamoto H, Hausinger RP, Tiedje JM (2002) tfdA-like genes in 2,4-dichlorophenoxyacetic acid-degrading bacteria belonging to the *Bradyrhizobium-Agromonas-Nitrobacter-Afipia* cluster in

alpha-proteobacteria. Appl Environ Microbiol 68: 3449–3454.

Itoh K, Tashiro Y, Uobe K, Kamagata Y, Suyama K, Yamamoto H (2004) Root nodule *Bradyrhizobium* spp. harbor *tfdAalpha* and *cadA*, homologous with genes encoding 2,4-dichlorophenoxyacetic acid-degrading proteins. Appl Environ Microbiol 70: 2110–2118.

Kahng HY, Malinverni JC, Majko MM, Kukor JJ (2001) Genetic and functional analysis of the *tbc* operons for catabolism of alkyl- and chloroaromatic compounds in *Burkholderia* sp. strain JS150. Appl Environ Microbiol 67: 4805–4816.

Kaschabek SR, Kasberg T, Müller D, Mars AE, Janssen DB, Reineke W (1998) Degradation of chloroaromatics: purification and characterization of a novel type of chlorocatechol 2,3-dioxygenase of *Pseudomonas putida* GJ31. J Bacteriol 180: 296–302.

Kaschabek SR, Reineke W (1992) Maleylacetate reductase of *Pseudomonas* sp. strain B13: dechlorination of chloromaleylacetates, metabolites in the degradation of chloroaromatic compounds. Arch Microbiol 158: 412–417.

Kaulmann U, Kaschabek SR, Schlömann M (2001) Mechanism of chloride elimination from 3-chloro- and 2,4-dichloro-*cis,cis*-muconate: new insight obtained from analysis of muconate cycloisomerase variant CatB-K169A. J Bacteriol 183: 4551–4561.

Kersten P, Chapman PJ, Dagley S (1985) Enzymatic release of halogens or methanol from some substituted protocatechuic acids. J Bacteriol 162: 693–697.

Kitagawa W, Takami S, Miyauchi K, Masai E, Kamagata Y, Tiedje JM, Fukuda M (2002) Novel 2,4-dichlorophenoxyacetic acid degradation genes from oligotrophic *Bradyrhizobium* sp. strain HW13 isolated from a pristine environment. J Bacteriol 184: 509–518.

Kitayama A, Suzuki E, Kawakami Y, Nagamune T (1996) Gene organization and low regioselectivity in aromatic-ring hydroxylation of a benzene monooxygenase of *Pseudomonas aeruginosa* JI104. J Ferm Bioeng 82: 421–425.

Leahy JG, Batchelor PJ, Morcomb SM (2003) Evolution of the soluble diiron monooxygenases. FEMS Microbiol Rev 27: 449–479.

Ledger T, Pieper DH, Gonzalez B (2006) Chlorophenol hydroxylases encoded by plasmid pJP4 differentially contribute to chlorophenoxyacetic acid degradation. Appl Environ Microbiol 72: 2783–2792.

Lehrbach PR, Zeyer J, Reineke W, Knackmuss HJ, Timmis KN (1984) Enzyme recruitment in vitro: use of cloned genes to extend the range of haloaromatics degraded by *Pseudomonas* sp. strain B13. J Bacteriol 158: 1025–1032.

Liang P-H, Yang G, Dunaway-Mariano D (1993) Specificity of 4-chlorobenzoyl coenzyme A dehalogenase catalyzed dehalogenation of a halogenated aromatics. Biochemistry 32: 12245–12250.

Liu S, Ogawa N, Senda T, Hasebe A, Miyashita K (2005) Amino acids in positions 48, 52, and 73 differentiate the substrate specificities of the highly homologous chlorocatechol 1,2-dioxygenases CbnA and TcbC. J Bacteriol 187: 5427–5436.

Louie TM, Webster CM, Xun LY (2002) Genetic and biochemical characterization of a 2,4,6-trichlorophenol degradation pathway in *Ralstonia eutropha* JMP134. J Bacteriol 184: 3492–3500.

Makdessi K, Lechner U (1997) Purification and characterization of 2,4-dichlorophenol hydroxylase isolated from a bacterium of the alpha-2 subgroup of the Proteobacteria. FEMS Microbiol Lett 157: 95–101.

Maltseva OV, Solyanikova IP, Golovleva LA (1994a) Chlorocatechol 1,2-dioxygenase from *Rhodococcus erythropolis* 1CP. Kinetic and immunochemical comparison with analogous enzymes from Gram-negative strains. Eur J Biochem 226: 1053–1061.

Maltseva OV, Solyanikova IP, Golovleva LA, Schlömann M, Knackmuss H-J (1994b) Dienelactone hydrolase from *Rhodococcus erythropolis* 1CP: purification and properties. Arch Microbiol 162: 386–374.

Mars AE, Kasberg T, Kaschabek SR, van Agteren MH, Janssen DB, Reineke W (1997) Microbial degradation of chloroaromatics: use of the *meta*-cleavage pathway for mineralization of chlorobenzene. J Bacteriol 179: 4530–4537.

Matsumura E, Ooi S, Murakami S, Takenaka S, Aoki K (2004) Constitutive synthesis, purification, and characterization of catechol 1,2-dioxygenase from the aniline-assimilating bacterium *Rhodococcus* sp AN-22. J Biosci Bioeng 98: 71–76.

Matus V, Sánchez MA, Martínez M, González B (2003) Efficient degradation of 2,4,6-trichlorophenol requires a set of catabolic genes related to *tcp* genes from *Ralstonia eutropha* JMP134 (pJP4). Appl Environ Microbiol 69: 7108–7115.

McGowan C, Fulthorpe RR, Wright A, Tiedje JM (1998) Evidence for interspecies gene transfer in the evolution of 2,4-dichlorophenoxyacetic acid degraders. Appl Environ Microbiol 64: 4089–4092.

Mitchell KH, Studts JM, Fox BG (2002) Combined participation of hydroxylase active site residues and effector protein binding in a *para* to *ortho* modulation of toluene 4-monooxygenase regiospecificity. Biochemistry 41: 3176–3188.

Miyauchi K, Adachi Y, Nagata Y, Takagi M (1999) Cloning and sequencing of a novel *meta*-cleavage dioxygenase gene whose product is involved in degradation of gamma-hexachlorocyclohexane in

Sphingomonas paucimobilis. J Bacteriol 181: 6712–6719.

Miyauchi K, Suh SK, Nagata Y, Takagi M (1998) Cloning and sequencing of a 2,5-dichlorohydroquinone reductive dehalogenase gene whose product is involved in degradation of gamma-hexachlorocyclohexane by *Sphingomonas paucimobilis.* J Bacteriol 180: 1354–1359.

Moiseeva OV, Solyanikova IP, Kaschabek SR, Groning J, Thiel M, Golovleva LA, Schlömann M (2002) A new modified *ortho* cleavage pathway of 3-chlorocatechol degradation by *Rhodococcus opacus* 1CP: genetic and biochemical evidence. J Bacteriol 184: 5282–5292.

Müller TA, Byrde SM, Werlen C, van der Meer JR, Kohler HP (2004) Genetic analysis of phenoxyalkanoic acid degradation in *Sphingomonas herbicidovorans* MH. Appl Environ Microbiol 70: 6066–6075.

Müller TA, Fleischmann T, van der Meer JR, Kohler HP (2006) Purification and characterization of two enantioselective alpha-ketoglutarate-dependent dioxygenases, RdpA and SdpA, from *Sphingomonas herbicidovorans* MH. Appl Environ Microbiol 72: 4853–4861.

Murray K, Duggleby CJ, Sala-Trepat JM, Williams PA (1972) The metabolism of benzoate and methylbenzoates via the *meta*-cleavage by *Pseudomonas arvilla* mt-2. Eur J Biochem 28: 301–310.

Nagata Y, Endo R, Ito M, Ohtsubo Y, Tsuda M (2007) Aerobic degradation of lindane (gamma-hexachlorocyclohexane) in bacteria and its biochemical and molecular basis. Appl Microbiol Biotechnol 76: 741–752.

Nagata Y, Ohtomo R, Miyauchi K, Fukuda M, Yano K, Takagi M (1994) Cloning and sequencing of a 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase gene involved in the degradation of gamma-hexachlorocyclohexane on *Pseudomonas paucimobilis.* J Bacteriol 176: 3117–3125.

Nakatsu CH, Wyndham RC (1993) Cloning and expression of the transposable chlorobenzoate-3,4-dioxygenase genes of *Alcaligenes* sp. strain BR60. Appl Environ Microbiol 59: 3625–3633.

Nakatsu CH, Providenti M, Wyndham RC (1997) The *cis*-diol dehydrogenase *cbaC* gene of Tn5271 is required for growth on 3-chlorobenzoate but not 3,4-dichlorobenzoate. Gene 196: 209–218.

Nikodem P, Hecht V, Schlömann M, Pieper DH (2003) New bacterial pathway for 4- and 5-chlorosalicylate degradation via 4-chlorocatechol and maleylacetate in *Pseudomonas* sp. strain MT1. J Bacteriol 185: 6790–6800.

Nordin K, Unell M, Jansson JK (2005) Novel 4-chlorophenol degradation gene cluster and degradation route via hydroxyquinol in *Arthrobacter chlorophenolicus* A6. Appl Environ Microbiol 71: 6538–6544.

Nurk A, Kasak L, Kivisaar M (1991) Sequence of the gene (*pheA*) encoding phenol monooxygenae from *Pseudomonas* sp. EST1001: expression in *Escherichia coli* and *Pseudomonas putida.* Gene 102: 13–18.

Ollis DL, Nai KL (1985) Crystallization and preliminary x-ray crystallographic data of dienelactone hydrolase from *Pseudomonas* sp. B13. J Biol Chem 260: 9818–9819.

Oltmanns RH, Rast HG, Reineke W (1988) Degradation of 1,4-dichlorobenzene by enriched and constructed bacteria. Appl Microbiol Biotechnol 28: 609–616.

Pieper DH (2005) Aerobic degradation of polychlorinated biphenyls. Appl Microbiol Biotechnol 67: 170–191.

Pieper DH, Kuhm AE, Stadler-Fritzsche K, Fischer P, Knackmuss H-J (1991) Metabolization of 3,5-dichlorocatechol by *Alcaligenes eutrophus* JMP134. Arch Microbiol 156: 218–222.

Prucha M, Peterseim A, Timmis KN, Pieper DH (1996) Muconolactone isomerase of the 3-oxoadipate pathway catalyzes dechlorination of 5-chloro-substituted muconolactones. Eur J Biochem 237: 350–356.

Reineke W (2001) Aerobic and anaerobic biodegradation potentials of microorganisms. In The Handbook of Environmental Chemistry. O Hutzinger (ed.). Berlin: Springer, pp. 1–161.

Romanov V, Hausinger RP (1994) *Pseudomonas aeruginosa* 142 uses a three-component *ortho*-halobenzoate 1,2-dioxygenase for the metabolism of 2,4-dichloro- and 2-chlorobenzoate. J Bacteriol 176: 3368–3374.

Savard P, Charest H, Sylvestre M, Shareck F, Scholten JD, Dunaway Mariano D (1992) Expression of the 4-chlorobenzoate dehalogenase genes from *Pseudomonas* sp. CBS3 in *Escherichia coli* and identification of the gene translation products. Can J Microbiol 38: 1074–1083.

Schell U, Helin S, Kajander T, Schlömann M, Goldman A (1999) Structural basis for the activity of two muconate cycloisomerase variants toward substituted muconates. Prot Struct Funct Genet 34: 125–136.

Schlömann M (1994) Evolution of chlorocatechol catabolic pathways. Conclusions to be drawn from comparisons of lactone hydrolases. Biodegradation 5: 301–321.

Schlömann M, Schmidt E, Knackmuss H-J (1990) Different types of dienelactone hydrolase in 4-fluorobenzoate-utilizing bacteria. J Bacteriol 172: 5112–5118.

Schmidt E, Knackmuss H-J (1980) Chemical structure and biodegradability of halogenated aromatic compounds. Conversion of chlorinated muconic acids into maleoylacetic acid. Biochem J 192: 339–347.

Schmitz A, Gartemann K-H, Fiedler J, Grund E, Eichenlaub R (1992) Cloning and sequence analysis of genes for dehalogenation of 4-chlorobenzoate

from *Arthrobacter* sp. strain SU. Appl Environ Microbiol 58: 4068–4071.

Schofield CJ, Zhang Z (1999) Structural and mechanistic studies on 2-oxoglutarate-dependent oxygenases and related enzymes. Curr Opin Struct Biol 9: 722–731.

Seeger M, Timmis KN, Hofer B (1995) Degradation of chlorobiphenyls catalyzed by the bph-encoded biphenyl 2,3 dioxygenase and biphenyls 2,3-dihydrodiol 2,3-dehydrogenase of *Pseudomonas* sp. LB400. FEMS Microbiol Lett 133: 259–264.

Shingler V, Powlowski J, Marklund U (1992) Nucleotide sequence and functional analysis of the complete phenol/3,4-dimethylphenol catabolic pathway of *Pseudomonas* sp. strain CF600. J Bacteriol 174: 711–724.

Skiba A, Hecht V, Pieper DH (2002) Formation of protoanemonin from 2-chloro-*cis,cis*-muconate by the combined action of muconate cycloisomerase and muconolactone isomerase. J Bacteriol 184: 5402–5409.

Solyanikova IP, Malteva OV, Vollmer MD, Golovleva LA, Schlömann M (1995) Characterization of muconate and chloromuconate cycloisomerase from *Rhodococcus erythropolis* 1CP: indications for functionally convergent evolution among bacterial cycloisomerases. J Bacteriol 177: 2821–2826.

Teramoto M, Futamata H, Harayama S, Watanabe K (1999) Characterization of a high-affinity phenol hydroxylase from *Comamonas testosteroni* R5 by gene cloning, and expression in *Pseudomonas aeruginosa* PAO1c. Mol Gen Genet 262: 552–558.

Tropel D, Meyer C, Armengaud J, Jouanneau Y (2002) Ferredoxin-mediated reactivation of the chlorocatechol 2,3-dioxygenase from *Pseudomonas putida* GJ31. Arch Microbiol 177: 345–351.

Vaillancourt FH, Labbe G, Drouin NM, Fortin PD, Eltis LD (2002) The mechanism-based inactivation of 2,3-dihydroxybiphenyl 1,2-dioxygenase by catecholic substrates. J Biol Chem 277: 2019–2027.

Vallaeys T, Courde L, McGowan C, Wright AD, Fulthorpe RR (1999) Phylogenetic analyses indicate independent recruitment of diverse gene cassettes during assemblage of the 2,4-D catabolic pathway. FEMS Microbiol Ecol 28: 373–382.

van Berkel WJ, Kamerbeek NM, Fraaije MW (2006) Flavoprotein monooxygenases, a diverse class of oxidative biocatalysts. J Biotechnol 124: 670–689.

van der Meer JR, van Neerven ARW, de Vries EJ, de Vos WM, Zehnder AJB (1991) Cloning and characterization of plasmid-encoded genes for the degradation of 1,2-dichloro-, 1,4-dichloro-, and 1,2,4-trichlorobenzene of *Pseudomonas* sp. strain P51. J Bacteriol 173: 6–15.

Vollmer MD, Schlömann M (1995) Conversion of 2-chloro-*cis,cis*-muconate and its metabolites 2-chloro- and 5-chloromuconolactone by chloromuconate cycloisomerase of pJP4 and pAC27. J Bacteriol 177: 2938–2941.

Vollmer MK, Fischer P, Knackmuss H-J, Schlömann M (1994) Inability of muconate cycloisomerases to cause dehalogenation during conversion of 2-chloro-*cis,cis*-muconate. J Bacteriol 176: 4366–4375.

Westendorf A, Müller RH, Babel W (2003) Purification and characterisation of the enantiospecific dioxygenases from *Delftia acidovorans* MCI initiating the degradation of phenoxypropionate and phenoxyacetate herbicides. Acta Biotechnol 23: 3–17.

Worsey M, Williams PA (1975) Metabolism of toluene and xylenes by *Pseudomonas putida (arvilla)* mt-2: evidence for a new function of the TOL plasmid. J Bacteriol 124: 7–13.

Xu L, Resing K, Lawson SL, Babbitt PC, Copley SD (1999) Evidence that *pcpA* encodes 2,6-dichlorohydroquinone dioxygenase, the ring cleavage enzyme required for pentachlorophenol degradation in *Sphingomonas chlorophenolica* strain ATCC 39723. Biochemistry 38: 7659–7669.

Xun L, Topp E, Orser CS (1992a) Diverse substrate range of a *Flavobacterium* pentachlorophenol hydroxylase and reaction stoichiometries. J Bacteriol 174: 2898–2902.

Xun L, Topp E, Orser CS (1992b) Purification and characterization of a tetrachloro-p-hydroquinone reductive dehalogenase from a *Flavobacterium* sp. J Bacteriol 174: 8003–8007.

Xun L, Webster C (2004) A monooxygenase catalyzes sequential dechlorinations of 2,4,6-trichlorophenol by oxidative and hydrolytic reactions. J Biol Chem 279: 6696–6700.

Zhuang ZH, Song F, Zhang WH, Taylor K, Archambault A, Dunaway-Mariano D, Dong J, Carey PR (2002) Kinetic, Raman, NMR, and site-directed mutagenesis studies of the *Pseudomonas* sp. strain CBS3 4-hydroxybenzoyl-CoA thioesterase active site. Biochemistry 41: 11152–11160.