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Microbial Dehalogenation of Synthetic Organohalogen Compounds: Hydrolytic Dehalogenases

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Abstract. Hydrolytic removal of halogen substitutents is commonly the first step in the degradation of haloaliphatic compounds by aerobic bacteria, whereas initial dehalogenation of aryl halides is rare. Hydrolytic dehalogenations are catalyzed by specific dehalogenases, a group of enzymes which has been extensively studied in bacteria and which does not seem to occur in mammals. Questions pertaining to the origin and evolution of dehalogenases in soil bacteria have recently become tractable by the establishment of dehalogenase gene sequences. At the protein level, new dehalogenases are being discovered and known dehalogenases are being analyzed with respect to their mechanisms of catalysis. Finally, microbial dehalogenases, either as cells of dehalogenative bacteria or as enzyme preparations, have potential for applications in environmental biotechnology and biotransformation.

1. Introduction

Halogenated organic compounds are not only important industrial chemicals, but also significant environmental pollutants. They figure as the largest group of compounds on the U.S. Environmental Protection Agency's list of priority Pollutants [1]. Their wide distribution in the environment and their often toxic or carcinogenic potential have prompted extensive studies on their degradation by microorganisms, the principal agents for the ultimate breakdown of organic compounds in nature. Microbial organohalogen metabolism and its potential and limitations for applications in environmental biotechnology have been summarized and discussed in excellent reviews [2–7].

Microorganisms use three principal mechanisms for C–halogen bond breakage: oxygenation, reduction, and substitution. Oxygenative reactions figure most prominently in the degradation of haloaromatics. Many oxygenases non-specifically oxygenate haloaromatics and generate unstable intermediates from which halogen is spontaneously eliminated. Oxygenolytic dehalogenations are often fortuitous reactions of enzymes with broad substrate specificity. They may yield products which are not further metabolized by the dehalogenative microorganism. This metabolic pattern, in which the halogenated compounds are not utilized as carbon and energy sources, is termed cometabolism.

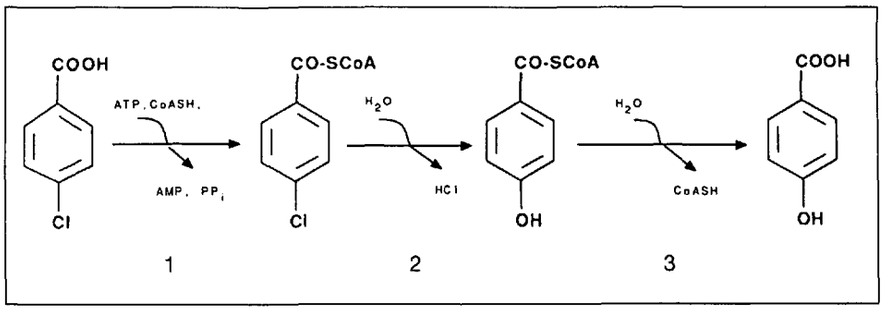
Reductive dehalogenation occurs primarily in anaerobic environments. For several highly chlorinated industrial compounds, including polychlorinated biphenyls, pentachlorophenol, and tetrachloroethene, it provides the only known biodegradation mechanism. Reductive dehalogenation results either from hydrogenolysis (replacement of a halogen substituent with an H-atom) or from dihaloelimination (removal of two halogen substituents from adjacent C-atoms with the formation of an additional bond between the C-atoms). Many bacterial pure cultures have been shown to catalyze the reductive dehalogenation of haloaliphatic compounds, but, so far, only one organism capable of anaerobic reductive dehalogenation of haloaromatics is available in pure culture [7]. Some reductive dehalogenations have been shown to be catalyzed by cobalt- and nickel-porphyrins [8] while others appear to require specific enzymes, and the biochemistry of the latter reactions remains to be explored.

Substitutive dehalogenation reactions are catalyzed by specific enzymes, the dehalogenases, which replace a halogen substituent with a OH group from H_2O. Hydrolytic dehalogenation is commonly observed as the first step in the aerobic degradation of halogenated aliphatic compounds. Initial removal of halogen substituents funnels haloaliphatics into central metabolism and enables a number of bacteria to utilize these halogenated substrates as carbon and energy sources.

2. Aromatic Dehalogenases

The hydrolytic removal of halogen substituents from halogenated aromatics would appear the most straightforward strategy for initiating the degradation of these compounds. Degradative pathways compatible with this mechanism, however, have rarely been described, and this is thought to reflect the resistance of haloaromatics to nucleophilic displacement reactions under physiological conditions [9]. An exception appears to be realized in a number of bacterial isolates representing the genera *Pseudomonas*, *Arthrobacter*, and *Corynebacterium* which were shown to initiate the degradation of 4-chlorobenzoate by displacement of Cl through OH. The fact that the enzymatic conversion of 4-chlorobenzoate to 4-hydroxybenzoate occurred in the absence of molecular O_2 [10–12], and incorporation experiments with $^{18}O_2$ and $H_2{}^{18}O$ [13][14] demonstrat-

Scheme 1. *Dechlorination of 4-Chlorobenzoate by* Pseudomonas *sp. CBS3* (from [17][18]). Reactions *1*, *2*, and *3* are catalyzed by 4-chlorobenzoate: CoA ligase, 4-chlorobenzoyl-CoA dehalogenase and 4-hydroxybenzoyl-CoA thioesterase, respectively. The system is currently registered as *EC 3.8.1.6.*



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ed that the OH group displacing Cl originated from H_2O and not from molecular O_2. The hydrolytic dehalogenation of 4-chlorobenzoate was studied in detail with *Pseudomonas* sp. *CBS3*. The 4-chlorobenzoate dehalogenase of this organism turned out to be a substrate-inducible multi-component system accepting 4-chloro-, 4-bromo-, and 4-iodobenzoate but not 4-fluorobenzoate as substrates. The genes encoding the dehalogenase proteins have been cloned and expressed in *E. coli* [15][16]. They encode a 57-, a 30- and a 16-kD polypeptide [16]. Purification of these proteins from recombinant *E. coli* led to the assignement of 4-chlorobenzoate CoA ligase, 4-chlorobenzoyl-CoA dehalogenase, and 4-hydroxybenzoyl-CoA thioesterase to one each of the three polypeptides and to the proposal of the 4-chlorobenzoate degradation pathway shown in *Scheme 1* [17]. The properties of the recently purified 4-chlorobenzoate CoA ligase from cell extract of *Pseudomonas* sp. *CBS3* [18] support this sequence of reactions.

Dehalogenation of 4-chlorobenzoate thus is based on an ATP-consuming reaction. Adenylylation of the carboxyl group of 4-chlorobenzoate, followed by displacement of the AMP with a thiol group from coenzyme A leads to the formation of a thioester. This activated intermediate facilitates nucleophilic attack by OH^- from H_2O at C(4) of the aromatic ring and leads to the displacement of the halogen substituent. Degradation of aromatic acids *via* the formation of coenzyme A esters is well known in anaerobic bacteria [19]. Its distribution among aerobic, 4-chlorobenzoate utilizing bacteria remains to be established. There is circumstantial evidence for its occurrence in at least two other bacteria [11][20]. On the other hand, labelling experiments with $H_2{}^{18}O$ suggest that hydrolytic dehalogenation of 4-chlorobenzoate in cell extracts of an *Arthrobacter* sp. does not proceed *via* a thioester [14].

3. Aliphatic Dehalogenases

The reactions catalyzed by aliphatic dehalogenases are shown in *Table 1*. Representatives of the enzymes listed have been purified to homogeneity and characterized to various extents. Gram-positive or Gram-negative soil bacteria capable to grow on a particular haloaliphatic compound served as enzyme sources. In some cases such organisms were readily isolated from ordinary soil, whereas in other cases isolates were obtained only from enrichment cultures inoculated with samples from polluted environments. One assumes that the ease with which organisms utilizing a haloaliphatic compound as a growth substrate are obtained, is correlated with the occurrence of this substrate in the environment, but there are no systematic studies on this aspect. In general, and where this has been examined, aliphatic dehalogenases react at increasing rates with the I-, Br-, and Cl-substituted analogs of a particular compound. Fluorinated compounds, however, are usually not attacked. These observations are in accordance with the stability of the C–halogen bond which increases in the order I < Br < Cl < F. The enzymology, biochemistry, and genetics of aliphatic dehalogenases have recently been reviewed by *Hardman* [5].

Table 1. *Aliphatic Dehalogenases*

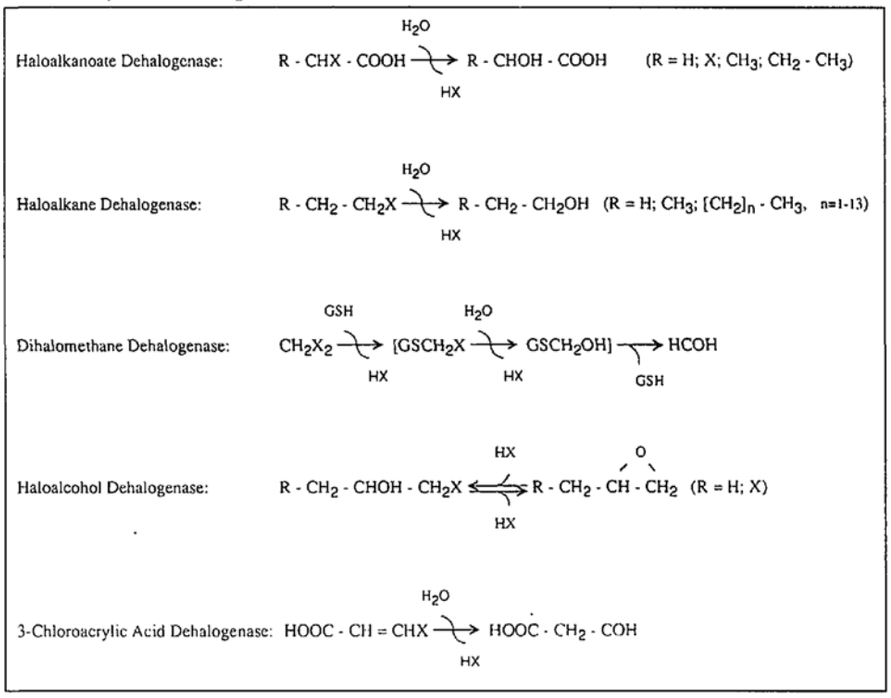


Table 2. *Haloalkanoic Acid Dehalogenases*

Enzyme	Substrates	Substrate/Product Configuration
Haloacetate Dehalogenase		
group *A1*	chloroacetate fluoroacetate	–
group *A2*	chloroacetate	–
2-Haloacid Dehalogenase		
group *B1*	L-2-haloacids	inversion
group *B2*	D- and L-2-haloacids	inversion
group *B3*	D- and L-2-haloacids	retention
group *B4*	D-2-haloacids	inversion

3.1. *Haloalkanoate Dehalogenases*

Haloalkanoate dehalogenases are the most widely studied hydrolytic dehalogenases. Their discovery dates back some 30 years [21]. There are two major groups of these enzymes: the haloacetate dehalogenases (*EC 3.8.1.3*) which are active on haloacetates only and the 2-haloacid dehalogenases (*EC 3.8.1.2.*) which act on haloacetates, 2-halopropionates and in some cases also on 2-haloalkanoic acids of higher C-chain length. As shown in *Table 2*, each of these groups is further divided into subgroups [5]. In the case of the haloacetate dehalogenases, the classification is based on the reactivity of the enzymes towards 2-fluoroacetate. Among the 2-haloacid dehalogenases, four subgroups can be recognized on the basis of their stereospecific action on D,L-2-monchloropropionate and the stereochemical configuration of the product [22]. As evident from *Table 3*, the subunit molecular mass of haloalkanoate dehalogenases ranges between 25 and 34 kD. Depending on the enzyme, monomeric, dimeric, and tetrameric tertiary structures have been reported.

The details of the dehalogenation reactions catalyzed by 2-haloacid dehalogenases have not been elucidated, but possi-

ble mechanisms have been proposed. Two different mechanisms are considered for enzymes that lead to inversion of configuration during the reaction (*Table 2*, groups *B1*, *B2* and *B4*). One model proposes that an enzyme active site with an electron-donating group leads to the activation of water which directly displaces the halogen in a nucleophilic substitution reaction. In the other model, a carboxylate group from an aspartate or a glutamate at the active site acts as the nucleophile. This would lead to the formation of an ester intermediate which is hydrolyzed by the attack of water on the carbonyl C-atom [5][22][23]. Enzymes whose activity results in retention of configuration (*Table 2*, group *B3*) are highly sensitive to sulfhydryl-blocking agents. This observation has led to a proposal involving a sulfhydryl group in the active site, displacement of halogen by formation of a thioether-enzyme intermediate and retention of configuration through a double inversion [22][24]. Comparison of the conserved regions in the haloalkanoate dehalogenase gene sequences available so far (*Table 3*) do not reveal the details of the dehalogenation mechanisms realized in the different systems. The gene sequences do, however, open possibilities for approaching this question by *in vitro* site-directed mutagenesis.

Bacteria utilizing haloalkanoic acids as growth substrates often contain two or even three [31] haloalkanoic acid dehalogenases, specific for either a single stereoisomer of the halogenated substrate or active against both isomers (*Table 3*). It has been suggested [5] that possession of more than one dehalogenase gene confers flexibility under fluctuating environmental conditions and thereby selective advantages on an organism. The significance of multiple dehalogenases is currently being explored by studying the expression of dehalogenase genes at the molecular level and by assessing their evolutionary relatedness. The majority of the haloalkanoate dehalogenases is substrate-inducible, but several cases of constitutive enzyme formation have also been reported. Little is known about the regulatory mechanisms involved in dehalogenase expression. In one instance, preliminary evidence suggests negative control by a repressor [26], in an other there are indications for positive control by an *rpoN*-dependent activator and for transcription from a -24/-12-type promoter [29]. The recent esthablishment of eight nucleotide sequences encoding haloalkanoate dehalogenases provides information on the evolutionary relatedness of these enzymes. *Table 4* shows the amino acid identities of eight haloalkanoic acid dehalogenases observed in pairwise alignments of the deduced amino-acid sequences. The enzymes fall clearly into three classes which exhibit low (12–21%) identity to each other and, thus, have probably evolved form different ancestral proteins. One class is represented by the group *A1* haloacetate dehalogenase of *Moraxella* sp., strain B. This enzyme shares two conserved regions in the N-terminal part with three hydrolases of *P. putida* and with the haloalkane dehalogenase of *Xanthobacter autotrophicus* [25]. The second class comprises six enzymes, namely the one group *A2* and the five group *B1* dehalogenases that have been sequenced so far. Percent identity within this class ranged from 33–67%, and the sequences shared amino acid identities at 46 positions. The group *B4* dehalogenase of *P. putida AJ1* represents the third distinct class of dehalogenases. None of the haloalkanoate dehalogenases in the second and the third class exhibited sequence homology with haloalkane or dichloromethane dehalogenase or with other proteins whose sequences are stored in databases [23][26–28][30]. It is interesting to note that in two of the three cases, where an organism contains two haloalkanoate dehalogenases, these enzymes belong to different evolutionary classes. Nevertheless the corresponding genes are arranged adjacently [27] or in close proximity [25] to each other.

3.2. *Haloalkane Dehalogenases*

Haloalkane dehalogenases (*EC 38.1.5*) form another important group of halidohydrolases that so far has exclusively been observed in bacteria. These enzymes hydrolytically convert halogenated aliphatic hydrocarbons and related compounds to the corresponding alcohols (*Table 1*). Organisms producing haloalkane dehalogenases have been obtained from soil by enrichment cultures using 1,2-dichloroethane, 1-chlorobutane, 1-chlorohexane, or 1,6-dichlorohexane as the sole source of carbon and energy. The isolation and the characterization of haloalkane dehalogenases have been reported from the Gram-negative *X. autotrophicus GJ10* [32] and from the gram-positive bacteria *Rhodococcus* sp. *HA1* [33] (reassigned from *Arthrobacter* after chemotaxonomy by DSM), *Corynebacterium* sp. strain *m15-3* [34], strain *GJ70* [35] and *Rhodococcus erythropolis Y2* [36]. Common properties of these enzymes include their monomeric structure and their relative molecular mass which ranges between 28 and 36 kD. With the exception of the *X. autotrophicus* enzyme, the haloalkane dehalogenases were found to be inducible. The preferred substrates of these enzymes as a group are mono- and biterminally halogenated al-

Table 3. *Haloalkanoic Acid Dehalogenases and Their Genes in Soil Bacteria*

Organism	Dehalogenase (group)[a]	Gene (designation)	Location of gene	Deduced protein molecular mass[b] (kD)	Reference
Moraxella sp. strain B	*A1*	*dehH1*	plasmid	33.3	25
	A2	*dehH2*	plasmid	26.0	25
Pseudomonas sp. CBS3	*B1*	*dehCI*	chromosome	25.4	26
	B1	*dehCII*	chromosome	25.6	26
Pseudomonas putida AJ1	*B1*	*hadL*	chromosome	25.7	27
	B4	*hadD*	chromosome	33.6	28
Pseudomonas putida PP3	*B3*	*dehI*	transposon	–	29
	B2	*dehII*	transposon	–	29
Pseudomonas cepacia MBA4	*B1*	*hdlIVa*	chromosome	25.9	30
Xanthobacter autotrophicus GJ10	*B1*	*dhlB*	chromosome	27.4	23

[a]) *cf. Table*; [b]) Molecular mass is given only for those enzymes whose structural genes have been sequenced.

Table 4. *Percent Identity*[a]) *between the Deduced Amino-Acid Sequences of Bacterial Haloalkanoate Dehalogenases*

Enzymes group[c])	Gene[b])	*hadD*	*dhlB*	*hdl*IVa	*hadL*	*deh*CII	*deh*CI	*dehH2*	*dehH1*
A1	*dehH1*	16	15	19	16	16	12	19	100
A2	*dehH2*	17	44	33	49	49	35	100	
B1	*deh*CI	19	42	67	38	36	100		
B1	*deh*CII	13	42	37	50	100			
B1	*hadL*	19	43	36	100				
B1	*hdl*IVa	21	40	100					
B1	*dhlB*	14	100						
B4	*hadD*	100							

[a]) Percent identity was determined by the program GAP (Genetics Computer Group, University of Wisconsin, Madison).
[b]) For references of the nucleotide sequences, see *Table 3*.
[c]) *Cf. Table 2*.

kanes of chain lengths up to 16 C-atoms. Unsaturated or branched haloalkanes are also accepted, as are secondary haloalkanes, haloalcohols, and the environmentally relevant compounds MeBr, 1,2-dibromoethane, 1,2-dichloroethane, and bis(2-chloroethyl) ether [5][35]. None of the enzymes dehalogenates alkanes with more than two C-atoms carrying vicinal Cl substituents, and none of them exhibits chiral specificity.

According to their substrate range the bacterial haloalkane dehalogenases characterized so far appear to fall in two classes. One class is formed by the *X. autotrophicus* enzyme which is active toward C_1–C_4 haloalkanes, particularly toward the important pollutant 1,2-dichloroethane. The other class encompasses the enzymes of the Gram-positive organisms which dehalogenate 1,2-dichloroethane at a negligible rate, but are active toward long chain haloalkanes. Similarity in the N-terminal amino acid sequences (at least 17 of the first 18 amino-acid residues are identical) has been observed between the enzymes from *Rhodococcus* sp. *HA1* and *R. erythropolis Y2* [33][36]. This may indicate evolutionary relatedness of these two enzymes and the possible distribution of a haloalkane dehalogenase structural gene among Gram-positive bacteria by horizontal transfer. The haloalkane dehalogenase from *X. autotrophicus* has been studied at the molecular level. Its structural gene has been sequenced [37], the protein has been crystallized, and its three-dimensional structure has been determined at 2.4-Å resolution [38]. The structural data suggest that none of the four cysteine residues in the polypeptide chain is involved in catalysis. Rather an aspartate at the putative active site seems to act as the nucleophilic residue essential for catalysis. This system, thus, has been developed to a point where further work should lead to a detailed understanding of the enzyme's reaction mechanism as well as to the engineering of enzyme variants with altered substrate specificity.

3.3. *Dichloromethane Dehalogenase*

Dichloromethane dehalogenase is a strongly inducible enzyme enabling facultatively methylotrophic bacteria to grow on CH_2Cl_2 as the sole carbon and energy source. It transforms CH_2Cl_2 to inorganic chloride and formaldehyde, a central metabolite of methylotrophic growth. In contrast to the haloalkane dehalogenases, nucleophilic displacement of Cl by dichloromethane dehalogenase is not based on the direct attack by OH^- or by a carboxylate group at the enzyme active site, but on the thiol group of the tripeptide glutathione (GSH) (*Table 1*). Dichloromethane dehalogenase was purified and characterized from five dichloromethane utilizing methylotrophs [39][40]. The dehalogenases were strictly GSH-dependent, hexameric enzymes with a subunit molecular mass of between 33 and 35 kD. They exhibited a narrow substrate range, reacting with dihalomethanes only, and their relatively low catalytic activity was compensated for by their high intracellular concentrations (up to 20% of total soluble protein).

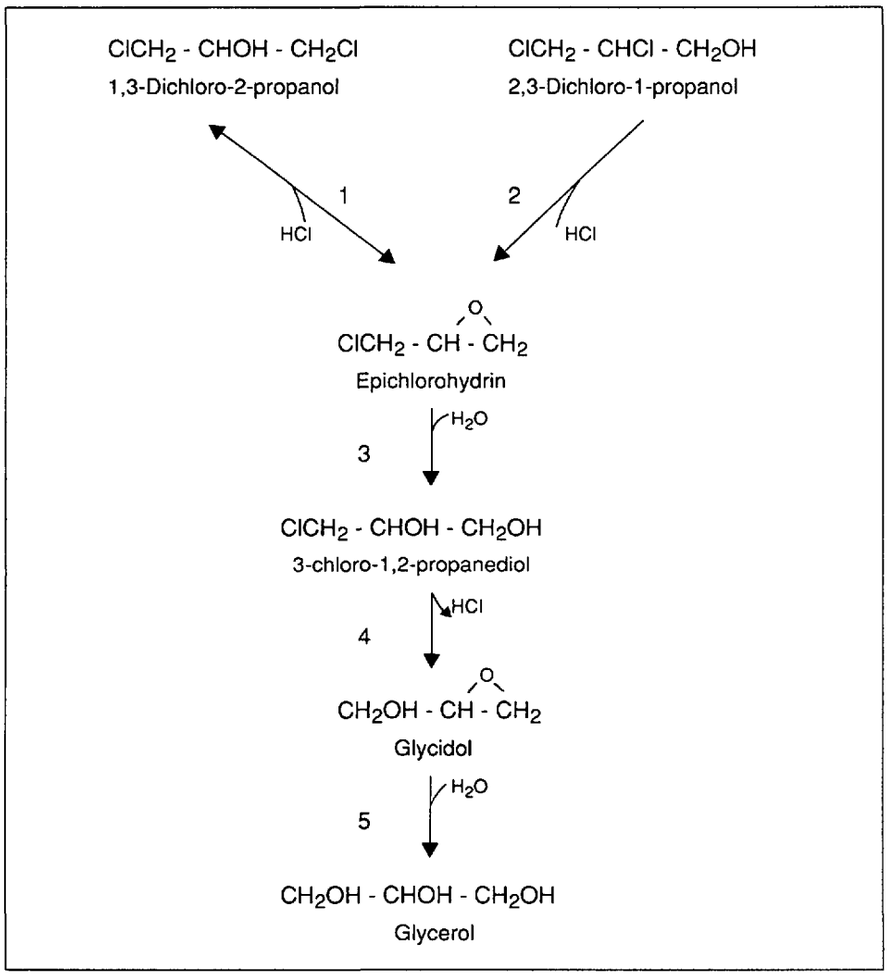
The genes for dichloromethane utilization were studied in *Methylobacterium* sp. *DM4*. They are encoded by a 2.8 kb chromosomal DNA fragment whose nucleotide sequence has been determined [41]. This DNA region contains *dcmA*, the structural gene of dichloromethane dehalogenase, and *dcmR*, the regulatory gene responsible for inducibility of the dehalogenase by dichloromethane. *dcmR* encodes a trans-acting factor which negatively controls dichloromethane dehalogenase formation at the transcriptional level [42]. Analysis of the amino acid sequence deduced from *dcmA* showed that dichloromethane dehalogenase belongs to the glutathione S-transferase (GST, *EC 2.5.1.18*) enzyme family [41].The same holds true for the dichloromethane dehalogenase of the restricted facultative methylotroph *Methylophilus* sp. DM11 whose structural gene we have recently sequenced (*R. Bader*, unpublished).

Soluble GSTs are a supergene family of proteins which catalyze the conjugation of GSH to a variety of electrophiles, and these enzymes have been extensively studied in eukaryotes [43]. Based on amino-acid sequence similarity, eukaryotic GSTs have been placed into four classes, namely Alpha, Mu, Pi, and Theta [44]. GSTs have also been purified and characterized from bacteria, but dichloromethane dehalogenase is the only prokaryotic GST for which an association with the GST family has been shown at the functional as well as at the structural level. Sequence comparisons indicate a particularly close relation of the two bacterial dichloromethane dehalogenases with the Theta class of eukaryotic GSTs. This observation has given rise to speculations [44] on the evolution of GSTs and on a possible role of bacterial dichloromethane dehalogenase as the archetypal enzyme of the GST enzyme family.

3.4. *Haloalcohol Dehalogenases*

Haloalcohol dehalogenases or halohydrin hydrogen-halide lyases have been purified and characterized from *Arthro-*

Scheme 2. *Bacterial Degradation of Halohydrins and Epichlorohydrin.* Steps *1* to *5* are catalyzed by the following enzymes or bacterial cultures: *1*) Haloalcohol dehalogenase from *Arthrobacter* sp. *AD2* [45], haloalcohol dehalogenase from *Corynebacterium* sp. *N-1074* [46][48]. *2*) Utilization of (*R*)-2,3-dichloropropan-1-ol by *Pseudomonas* sp. *DS-K-29* [15]. Utilization of (*S*)-2,3-dichloropropan-1-ol by *Alcaligenes* sp. *DS-K-S38* [52]. *3*) Epoxide hydrolase in crude extracts of *Corynebacterium* sp. *N-1074* [48] and *Pseudomonas* sp. *AD1* [53]. *4*) Haloalcohol dehalogenase from *Arthrobacter* sp. AD2 [45], utilization of (*R*)-3-chloropropane-1,2-diol by *Alcaligenes* sp. *DS-S-7G* [54], utilization of (*S*)-3-chloropropanedi-1,2-ol by *Pseudomons* sp. *DS-K-2DI* [55]. *5*) Epoxide hydrolase in crude extracts of *Pseudomonas* sp. *AD1* [53].



bacter sp. strain *AD* [45] and from *Corynebacterium* sp. *N-1074* (46). These inducible enzymes catalyze the dehalogenation of vicinal mono- and dichlorinated alcohols to their corresponding epoxides (*Table 1*). Substrates accepted by the enzymes included, in the order of decreasing activity, 1,3-dichloropropan-2-ol, 2-bromoethanol, 1-chloropropan-2-ol, 3-chloropropane-1,2-diol, and chloroacetone. No dehalogenase activity was detected with epichlorohydrin (3-chloro-1,2-epoxypropane) or 2,3-dichloropropan-1-ol. Both enzymes catalyzed the reverse reaction of dehalogenation, *i.e.* the halogenation of epoxides to the corresponding haloalcohols. The *Arthrobacter* and the *Corynebacterium* enzymes have a subunit molecular mass of 29 and 28 kD, respectively. The former is reported to be a dimer [45], while the latter appears to be a tetramer [46]. Studies with the pure enzyme from *Corynebacterium* have revealed a hitherto undetected catalytic reaction of this protein, namely its ability to transform, in the presence of cyanide, epoxides into β-hydroxynitriles [47]. Neither of these enzymes exhibited enantioselectivity. However, more recently a second haloalcohol dehalogenase has been purified from *Corynebacterium* sp. *N-1074*. This enzyme is a heterotetramer composed of 32- and 35-kD polypeptides. It showed some enantioselectivity and converted 1,3-dichloropropan-2-ol to (*R*)-epichlorohydrin of 75% optical purity [48]. Like many of the haloalkanoate degrading bacteria (*Table 3*) *Corynebacterium* sp., thus, also contains two dehalogenases catalyzing the same reaction but differing from each other with respect to enantioselectivity, molecular mass, substrate specificity and immunological properties [48].

3.5. *3-Chloroacrylic Acid Dehalogenases*

3-Chloroacrylic acid dehalogenases have been detected in two unidentified coryneform bacteria [49][50] and in a *Pseudomonas cepacia* strain [49]. These organisms were obtained by enrichment on media containing 3-chloroacrylic acid as the sole carbon source. The hydrolytic dehalogenases discussed so far do not accept substrates possessing halogen substituents on unsaturated C-atoms. Inducible enzymes exhibiting this type of activity, however, were detected in crude extracts of the 3-chloroacrylic acid utilizing bacteria. Each of the two coryneform bacteria produced two dehalogenases, one specific for *cis*- and the other for *trans*-3-chloroacrylic acid. The *P. cepacia* strain was capable of growth exclusively with the *cis*-isomer and accordingly contained a *cis*-specific enzyme only. The enzymes form malonate semialdehyde (3-oxopropionic acid) from their respective substrate (*Table 1*). The dehalogenation of 3-chloroacrylic acid is thought to proceed by hydration of the C=C bond, yielding the unstable intermediate 3-chloro-3-hydroxypropanoic acid from which HCl is eliminated to give malonate semialdehyde [49][50].

The enzymes of the coryneform bacterium *FG41* were purified and characterized. *cis*-3-Chloroacrylic acid dehalogenase was found to consist of two 16.2 kD polypeptide chains whereas *trans*-3-chloroacrylic acid dehalogenase was composed of 7.4 and 8.7 kD subunits whose arrangement in the native enzyme is not known. Each enzyme was completely specific for its respective isomer of 3-chloroacrylic acid, and no other substrates have been found so far [49].

4. Applications

The application of bacteria possessing hydrolytic dehalogenases comprises two major fields: Treatment processes for industrial waste streams and biotransformations for the production of chiral building blocks.

Biotechnological processes have been developed to remove dichloromethane from industrial effluents and waste gases. In aerobic fluidized bed reactors inoculated with dichloromethane utilizing methylotrophic bacteria and fed with model [56] or process wastewater [57], this compound was mineralized at rates of up to 1.6 $g/l/h^{-1}$. Waste gases containing CH_2Cl_2 have been treated successfully in aerobic trickling-bed reactors and biofilters [58][59]. 1,2-Dichloroethane is the other important environmental chemical whose

bacterial degradation in bioreactors has been studied [60][61]. As in the case of CH_2Cl_2, the results obtained in pilot studies were encouraging, and these systems show promise for large scale application in the cost-effective treatment of contaminated groundwater and process water.

Microbial dehalogenation reactions have also been used for the production of optically active compounds. For example, both enantiomers of lactic acid can be prepared separately from racemic 2-chloropropionic acid by successive treatment with L-2-haloacid dehalogenase and D,L-2-haloacid dehalogenase [62]. More recently the microbial production of chiral glycerol derivatives such as optically active epichlorohydrin, 3-chloro-1,2-propanediol and glycidol has been studied (compare *Scheme 2*). These compounds are important building blocks for the synthesis of chiral pharmaceuticals.

A first approach for the microbial preparation of chiral glycerol derivatives is based on the microbial resolution of racemic mixtures of 2,3-dichloropropan-1-ol or 3-chloropropanedi-1,2-ol by stereospecifically assimilating bacteria. It has the inherent disadvantage of yielding 50% or less of the desired enantiomer. Growth of an (*R*)-2,3-dichloropropan-1-ol assimilating *Pseudomonas* strain on the racemate yielded pure (100% enantiomeric excess) (*S*)-2,3-dichloropropan-1-ol from which, by treatment with aqueous NaOH, (*R*)-epichlorohydrin was prepared [51]. Using an (*S*)-2,3-dichloropropan-1-ol assimilating *Alcaligenes* strain, optically pure (*R*)-2,3dichloropropan-1-ol and (*S*)-epichlorohydrin were prepared in a similar manner [52]. Bacteria which sterospecifically attack either (*R*)- or (*S*)-3-chloropropane-1,2-diol have also been isolated and used to prepare optically active (*S*)- and (*R*)-3-chloropropane-1,2-diol as well as the chemically derived (*S*)-and (*R*)-glycidols [54][55].

A second approach for the preparation of optically active (*R*)-3-chloropropane-1,2-diol makes use of the prochiral symmetric compound 1,3-dichloropropan-2-ol as starting material (*Scheme 2*). *Corynebacterium* sp. *N-1074* contains an enantioselective haloalcohol dehalogenase which converts 1,3-dichloropropan-2-ol to *R*-rich epichlorohydrin, and the latter compound is hydrolyzed by a sterospecific epoxide hydrolase [48]. Systems based on these reactions may thus lead to processes for the direct enzymatic production of (*R*)-epichlorohydrin and (*R*)-3-chloropropane-1,2-diol from 1,2-dichloropropan-2-ol.

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