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Utilization of Trihalogenated Propanes by *Agrobacterium radiobacter* AD1 through Heterologous Expression of the Haloalkane Dehalogenase from *Rhodococcus* sp. Strain m15-3

TIJBBE BOSMA, EDWIN KRUIZINGA, ERIK J. DE BRUIN, GERRIT J. POELAREND, AND DICK B. JANSSEN*

Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9747 AG Groningen, The Netherlands

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Trihalogenated propanes are toxic and recalcitrant organic compounds. Attempts to obtain pure bacterial cultures able to use these compounds as sole carbon and energy sources were unsuccessful. Both the haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 (DhlA) and that from *Rhodococcus* sp. strain m15-3 (DhaA) were found to dehalogenate trihalopropanes to 2,3-dihalogenated propanols, but the kinetic properties of the latter enzyme are much better. Broad-host-range dehalogenase expression plasmids, based on RSF1010 derivatives, were constructed with the haloalkane dehalogenase from *Rhodococcus* sp. strain m15-3 under the control of the heterologous promoters $P_{lac}$, $P_{dhaA}$, and $P_{rec}$. The resulting plasmids yielded functional expression in several gram-negative bacteria. A catabolic pathway for trihalopropanes was designed by introducing these broad-host-range dehalogenase expression plasmids into *Agrobacterium radiobacter* AD1, which has the ability to utilize dihalogenated propanols for growth. The recombinant strain AD1(pTB3), expressing the haloalkane dehalogenase gene under the control of the *dhlA* promoter, was able to utilize both 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane as sole carbon sources. Moreover, increased expression of the haloalkane dehalogenase resulted in elevated resistance to trihalopropanes.

In the present study, we describe the steady-state kinetics of the conversion of trihalopropanes by the haloalkane dehalogenases from *Xanthobacter autotrophicus* GJ10 (DhlA) and *Rhodococcus* sp. strain m15-3 (DhaA). The gene encoding the latter dehalogenase was engineered to be under the control of different heterologous promoters and was functionally expressed in several gram-negative bacteria. High expression of the haloalkane dehalogenase was obtained in *A. radiobacter* AD1, allowing this strain to grow on 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane.

**Materials and Methods**

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this work are listed in Table 1. T. Omori kindly provided *Rhodococcus* sp. strain m15-3, formerly *Corynebacterium* sp. strain m15-3 (39). Based on 16S rRNA gene sequence analysis, strain m15-3 was identified as a *Rhodococcus* sp. (25a). *Escherichia coli* strains were grown in Luria-Bertani (LB) medium (30) at 30°C. The synthetic mineral (MMY) medium used in all growth experiments with *A. radiobacter* AD1, derivatives thereof, and *Pseudomonas* strains contained (per liter) 5.4 g of Na$_2$HPO$_4$ · 12H$_2$O, 1.4 g of KH$_2$PO$_4$, 0.5 g of (NH$_4$)$_2$SO$_4$, 0.2 g of MgSO$_4$ · 7H$_2$O, 5 ml of trace element metal solutions (15), and 5 mg of yeast extract. For the preparation of crude extracts for enzyme assays, *A. radiobacter*
TABLE 1. Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Relevant characteristics a</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>E. coli HB101</td>
<td>recA mutant, used for transformation of RSF1010 derivatives</td>
<td>6</td>
</tr>
<tr>
<td>A. radiobacter AD1</td>
<td>Utilizes dihalogenated propanols</td>
<td>34</td>
</tr>
<tr>
<td>Pseudomonas sp. strain GJ1</td>
<td>Utilizes 2-chloropropanol</td>
<td>14</td>
</tr>
<tr>
<td>P. putida US2</td>
<td>Utilizes 2-chloroethanol</td>
<td>28</td>
</tr>
<tr>
<td>P. putida GJ31</td>
<td>Utilizes long-chain alcohols</td>
<td>33</td>
</tr>
<tr>
<td>Rhodococcus sp. strain m15-3</td>
<td>DhaA-producing strain</td>
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<table>
<thead>
<tr>
<th>Plasmids</th>
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<tr>
<td>pKK233-2</td>
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<td>1</td>
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<tr>
<td>pGEl</td>
<td></td>
<td>28</td>
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<tr>
<td>pKKdhaA</td>
<td>dhaA gene cloned into the NcoI site of pKK233-2</td>
<td>This work</td>
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<tr>
<td>pDSK519</td>
<td>RSF1010 derivative, Km, P sec</td>
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<tr>
<td>pJR2D15</td>
<td>RSF1010 derivative, Km</td>
<td>9</td>
</tr>
<tr>
<td>pRK600</td>
<td>Cm, transfer functions for mobilization of RSF1010 derivatives</td>
<td>11</td>
</tr>
<tr>
<td>pTB1</td>
<td>dhaA inserted as a 0.9-kb BamHI fragment in pDSK519</td>
<td>This work</td>
</tr>
<tr>
<td>pTB3</td>
<td>1.5-kb HinII-BamHI fusion fragment of P vec-dhaA-rmb</td>
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</tr>
<tr>
<td></td>
<td>transcription terminator inserted in pJR2D15</td>
<td>This work</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics a</th>
<th>Reference</th>
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<td>Rhodococcus sp. strain m15-3</td>
<td>DhaA-producing strain</td>
<td>25</td>
</tr>
</tbody>
</table>

a Ap, ampicillin; Km, kanamycin; Cm, chloramphenicol.
on pH indicator plates with 1,2-dibromoethane as the substrate according to the procedure described by Schanstra et al. (31).

Protein concentrations of crude extracts were measured with Coomassie brilliant blue using bovine serum albumin as the standard. The concentration of the purified enzymes was measured spectrophotometrically at 280 nm. An absorbance of 1 corresponds to 0.54 and 0.72 mg of protein/ml for DhaA and DhlA, respectively, as calculated with the DNASTAR program (DNASTAR, Inc., Madison, Wis.).

Gas chromatography. Concentrations of halogenated compounds were determined quantitatively by gas chromatography. Samples (4.5 ml) were extracted with diethylether (1.5 ml) containing 0.05 mM 1-bromohexane as the internal standard. The ether layer was analyzed by split injection of 1-μl samples on a Chrompack 438S gas chromatograph equipped with a flame ionization detector and an HP-5 capillary column (length, 25 m; inner diameter, 0.2 mm; film thickness, 0.2 μm) (Hewlett Packard). The carrier gas was nitrogen (50 kPa), and the temperature program was 3-min isothermal at 45°C followed by an increase to 220°C at a rate of 10°C/min.

Gas chromatographic mass spectrometry was performed on an HP 5890 gas chromatograph with an HP5 capillary column (length, 25 m; inner diameter, 0.2 mm; film thickness, 0.2 μm) (Hewlett Packard) connected to a flame ionization detector and a type 5971 mass-selective detector. Helium was used as a carrier gas (0.9 mm/min), and the temperature program was the same as described above.

Chemicals. All organic compounds used were obtained from commercial suppliers (Acros, Merck, Aldrich, Jansen Chimica, and Lancaster). Restriction enzymes, T4 DNA ligase, Tag DNA polymerase, High Pure Plasmid Isolation kit, and the High Pure PCR Product Purification kit were all purchased from Boehringer.

RESULTS

Specificity of haloalkane dehalogenase for trihalogenated propanes. To evaluate the activity of the haloalkane dehalogenase from *X. autotrophicus* GJ10 (DhaA) and *Rhodococcus* sp. strain m15-3 (DhaA) on trihalogenated propanes, we used the recombinant enzymes expressed in *E. coli*. The quantity of both enzymes comprised up to 50% of the total soluble protein in *E. coli* BL21(DE3) when the expression was under the control of the T7 promoter using the expression vector pGEM*- (28). Recent work in our laboratory (25a) showed that the haloalkane dehalogenase gene from strain m15-3 is identical to that of the corresponding gene from *Rhodococcus rhodochrous* NCIMB 13064 (21). Based on sequence comparisons between DhIA and DhIA, these proteins are expected to have similar structures and catalytic mechanisms, but the substrate ranges are different (20, 39).

The steady-state kinetics of the conversion of trihalogenated propanes were studied using purified DhIA and DhIA. The halogenated products of these reactions were identified by gas chromatography and gas-chromatographic mass spectrometry analyses (Table 2). Both enzymes hydrolyzed trihalogenated propanes with a concomitant and stoichiometric accumulation of 2,3-dihalogenated propanol and halide. However, the kinetic properties of DhIA for trihalogenated propanes are much better (Table 2). For the trihalogenated propanes tested, DhIA had higher $k_{cat}$ and lower $K_m$ values than DhIA. The enzyme specificity ($k_{cat}/K_m$) of DhIA for these chemicals is at least ninefold higher. Therefore, this enzyme was selected for the construction of a catabolic pathway for trihalogenated propanes. The $k_{cat}$ and $K_m$ values of DhIA were comparable for 1,2,3-trihalomethanes and 1,2,3-dichloro-1-propanol and 2,3-dibromo-1-propanol. However, its affinity for 1,2-dibromo-3-chloropropane was somewhat lower. The chlorinated analog 1,2,3-trichloropropanone was a poor substrate for the dehalogenase, as the enzyme had a high $K_m$ and a low $k_{cat}$ for this compound.

The activity of both enzymes towards 1,2-dibromoethane was striking. This compound is the best known substrate for DhIA in terms of $k_{cat}$ and $K_m$. The $k_{cat}$ and $K_m$ values for DhIA were much higher than the values for DhIA.

**Utilization of halopropanols by *A. radiobacter* AD1.** In previous studies, it was shown that *A. radiobacter* AD1 exhibited good growth kinetics for the conversion of 1,3-dichloro-2-propanol. The organism had a high affinity for this compound, which was correlated with a low $K_m$ value of the haloalcohol dehalogenase, the first catabolic enzyme in the pathway, for 1,3-dichloro-2-propanol (34, 35). Therefore, we used *A. radiobacter* AD1 as a possible host organism for the construction of a degradation pathway for trihalopropanes. Utilization of 2,3-dichloro- and 2,3-dibromo-1-propanol by strain AD1 was tested, since these compounds were produced during hydrolytic conversion of trihalopropanes by haloalkane dehalogenase. The results showed that strain AD1 was able to grow on both 2,3-dichloro- and 2,3-dibromo-1-propanol with similar generation times (Table 3). The highest growth rate was obtained on 1,3-dichloro-2-propanol with a generation time of 4 h. Strain AD1 completely utilizes both 2,3-dichloro-1-propanol and 1,3-dichloro-2-propanol. In contrast, approximately 50% conversion was found for 2,3-dichloro-1-propanol. Chiral gas chromatography of the culture fluid with a Chiraldex B-TA capillary column (30 m) (Asteck) showed that only the (S)-enantiomer of 2,3-dichloro-1-propanol remained after growth had ceased, indicating that the conversion of 2,3-dichloro-1-propanol was stereospecific (32a), whereas both enantiomers of the brominated analog supported growth.

**Construction of a broad-host-range dehalogenase expression plasmid.** Expanding the substrate range of *A. radiobacter* AD1 by introducing the dhaA gene requires an efficient vector system and sufficient expression of the dehalogenase, since both the kinetic properties of the first catabolic enzyme and the dehalogenase content are important (35). We therefore constructed three different broad-host-range expression vectors.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Generation time (h)</th>
<th>Halide produced (mM)</th>
<th>Substrate remaining (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3-Dichloro-1-propanol</td>
<td>7</td>
<td>5.2</td>
<td>2.5</td>
</tr>
<tr>
<td>2,3-Dibromo-1-propanol</td>
<td>6</td>
<td>10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1,3-Dichloro-2-propanol</td>
<td>4</td>
<td>11</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
containing different promoters to direct expression of DhaA. The resulting expression vectors, pTB1, pTB3, and pTB5, are all based on the RSF1010 replicon, because the mobilization and replication of RSF1010 derivatives have been reported for Agrobacterium (3, 22). Figure 1 shows schematically the promoter dehalogenase regions of the constructed expression vectors.

In plasmid pTB1, the dhaA gene was fused to the 5′ part of the lacZa gene and placed under the control of the lac promoter. This resulted in the formation of a fusion protein composed of the first 18 amino acids of LacZa followed by DhaA.

Plasmid pTB3 contained the dhaA gene under the control of the dhlA promoter from X. autotrophicus GJ10, which was previously shown to operate efficiently in several gram-negative bacteria (16). The dhaA gene, including its own transcription terminator (21), was attached by PCR fusion to a fragment containing both promoter sequences of the dhlA gene (16). Overlap between primers p4 and p5 resulted in the precise attachment of the promoter to the dehalogenase gene. The dhlA promoter fragment provided a strong ribosome binding site four nucleotides upstream from the start codon (16).

The E. coli hybrid trp-lac promoter, P_{trc}, was used to direct the expression of DhaA in plasmid pTB5. The vector carries the dhaA gene translationally fused to the trc promoter, the lacZ ribosome binding site, and the rrnB transcription terminators.

Kanamycin-resistant transformants of E. coli HB101 containing the different broad-host-range expression vectors were identified by screening for dehalogenase activity.

PCR-amplified segments of all constructs were sequenced. Only in plasmid pTB3 was a base substitution (C→T) at position −13 found in the spacer region between the −35 and −10 promoter sequences of the first dhlA promoter.

**Expression of the dhaA gene in different gram-negative bacteria.** To evaluate the use of the constructs for the expression of the dehalogenase, the recombinant plasmids were introduced by triparental mating into different gram-negative bacteria. A. radiobacter AD1, Pseudomonas sp. strain GJ1, and P. putida US2 and GJ31 were used as recipients because these organisms could grow on various halogenated alcohols. Dehalogenase activities were measured in cell-free extracts with 1,2-dibromoethane as the assay substrate (Table 4). The broad-host-range plasmids are all based on the same replicon, RSF1010, so the efficiency of the different promoters can be compared without regard to copy number effects.

Low dehalogenase activities were obtained in strains containing plasmid pTB1, where the dhaA gene is under the control of the lac promoter. In all of these cases, the expression level of the dehalogenase was approximately 1% or less of the total soluble cellular protein. Higher specific activities were found in strains containing plasmid pTB3 or pTB5. The expression levels of the dehalogenase were comparable for these constructs, but levels varied with the host organism. Only small differences were observed between the efficiencies of the dhlA and trc promoters in various Pseudomonas species. P. putida US2 (pTB3) exhibited the highest dehalogenase activity. The enzyme amounted to almost 20% of the total soluble cellular protein in crude extracts. In A. radiobacter AD1, the dhlA and trc promoters generated similar dehalogenase levels. The haloalkane dehalogenase was present at approximately 7 to 8% of the total soluble cellular protein. The strength of the dhlA promoter is similar to that of the trc promoter, which is one of the strongest hybrid E. coli promoters (29). Introducing

**TABLE 4. Haloalkane dehalogenase activities towards 1,2-dibromoethane (10 mM) in crude extracts of various host plasmid systems**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Carbon source</th>
<th>Sp act (mU/mg of protein)*</th>
<th>pTB1</th>
<th>pTB3</th>
<th>pTB5</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli HB101</td>
<td>LB medium</td>
<td>150</td>
<td>1,100</td>
<td>1,800</td>
<td></td>
</tr>
<tr>
<td>A. radiobacter AD1</td>
<td>Citrate</td>
<td>320</td>
<td>1,900</td>
<td>2,000</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas sp. strain GJ1</td>
<td>Citrate</td>
<td>80</td>
<td>530</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>P. putida GJ31</td>
<td>Citrate</td>
<td>190</td>
<td>860</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>P. putida US2</td>
<td>Citrate</td>
<td>240</td>
<td>5,100</td>
<td>2,200</td>
<td></td>
</tr>
</tbody>
</table>

* The specific activities are the means of two experiments with differences of ≤15%.
a NdeI restriction site at the ATG translation initiation codon of \textit{dhaA} in pTB3 would facilitate the exchange of \textit{dhaA} with other dehalogenase genes. However, the expression level of the dehalogenase in \textit{E. coli} HB101 was almost fivefold lower (data not shown). Due to the introduction of this NdeI site, the triplet preceding the start codon has been changed from TCT to CAT. This may explain the decrease in dehalogenase activity, since it is known that this triplet can affect the efficiency of translation (13). The recombinant strain \textit{A. radiobacter} AD1(pTB3) was further characterized with respect to utilization of trihalogenated propanes.

**Utilization of trihalopropanes by \textit{A. radiobacter} AD1(pTB3).**

The introduction of halogen substituents into organic compounds may increase toxicity. Therefore we tested which concentrations of trihalogenated propanes were tolerated by strain AD1 and its derivatives (Fig. 2). The addition of increasing concentrations of trihalogenated propanes inhibited the growth of strains AD1, AD1(pTB1), and AD1(pTB3). Both 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane exhibited a high toxicity. Addition of these chemicals to a growing culture of strain AD1, AD1(pTB1), or AD1(pTB3) caused strong inhibition of growth and even cell death. No viable cells were obtained on nutrient broth plates from cultures that were previously incubated with the highest concentrations of 1,2,3-tribromopropane (1.5 mM) and 1,2-dibromo-3-chloropropane (1.6 mM). However, for the recombinant strain AD1(pTB3), the tolerance towards these compounds was increased due to a high expression level of DhaA. Of the trihalogenated propanes tested, 1,2,3-trichloropropane was least toxic. Moreover, the presence of DhaA did not increase the tolerance towards 1,2,3-trichloropropane, due to the low conversion rates of the dehalogenase for this compound.

Growth of the recombinant strain AD1(pTB3) with 1,2,3-tribromopropane, 1,2-dibromo-3-chloropropane, or 1,2,3-

![FIG. 2. Effect of increasing concentrations of trihalogenated propanes on growth of \textit{A. radiobacter} strains AD1 (○), AD1(pTB1) (▲), and AD1(pTB3) (■) growing on 5 mM 1,3-dichloro-2-propanol. The turbidity (OD$_{450}$) of the cultures was measured after 7 days of cultivation at 30°C. Initial OD$_{450}$ values ranged from 0.5 to 0.7. (A) 1,2,3-tribromopropane; (B) 1,2-dibromo-3-chloropropane; (C) 1,2,3-trichloropropane.](image)

![FIG. 3. Growth and degradation of trihalogenated propanes by \textit{A. radiobacter} AD1(pTB3). Cultures were grown aerobically at room temperature in MMY medium supplemented with 1 mM 1,2,3-tribromopropane (4 pulses), 1.2 mM 1,2-dibromo-3-chloropropane (2 pulses), or 1.6 mM 1,2,3-trichloropropane as carbon source. (A) 1,2,3-Tribromopropane (■), 2,3-dibromo-1-propanol (▲), OD$_{450}$ (○); (B) 1,2-dibromo-3-chloropropane (○), 2-bromo-3-chloro-1-propanol (■), OD$_{450}$ (▲); (C) 1,2,3-trichloropropane (■), 2,3-dichloro-1-propanol (▲), OD$_{450}$ (○); (D) (sterile controls) 1,2,3-tribromopropane (■), 1,2-dibromo-3-chloropropane (○), 1,2,3-trichloropropane (▲).](image)
Rhodococcus introduced the haloalkane dehalogenase from 1,2,3-trichloropropane, which is a poor substrate for DhaA, and 1,2-dibromo-3-chloropropane to the corresponding haloalcohols, which were subsequently utilized. The chlorinated analog 1,2-dibromo-3-chloropropane was able to rapidly dehalogenate 1,2,3-tribromopropane and 1,2-dichloro-1-propanol remained present in the culture. The results thus showed that the recombinant strain AD1(pTB3) was still able to convert about 46% of the 1,2,3-trichloropropane, whereas AD1 could use the environmental chemicals 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane as sole carbon and energy sources. The proposed degradation pathway of strain AD1(pTB3) is shown in Fig. 4. The complete dehalogenation of trihalogenated propanes involves the combined activities of haloalcohol dehalogenase, haloalcohol dehalogenase, and epoxide hydrolase, finally yielding glycerol, which is further metabolized by the organism (34). The conversion of 2,3-dihalogenated propanols by the host strain AD1 is similar to that of a Flavobacterium sp. utilizing 2,3-dibromo-1-propanol (7).

For growth on trihalogenated propanes, the kinetic properties ($K_m$ and $K_{cat}$) of DhaA for trihalopropanes are of major importance. The best substrate for DhaA, 1,2,3-tribromopropane, was rapidly degraded by strain AD1(pTB3), whereas degradation of 1,2-dibromo-3-chloropropane by AD1(pTB3), for which the dehalogenase has a lower affinity, was somewhat slower. This is probably due to a lower conversion rate of 1,2-dibromo-3-chloropropane by DhaA, since the concentration tolerated was about 0.7 mM (data not shown).

To obtain bacterial growth on trihalogenated propanes, we introduced the haloalkane dehalogenase from Rhodococcus sp. strain m15-3 (DhaA) into A. radiobacter AD1. The resulting strain could use the environmental chemicals 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane as sole carbon and energy sources. The proposed degradation pathway of strain AD1(pTB3) is shown in Fig. 4. The complete dehalogenation of trihalogenated propanes involves the combined activities of haloalcohol dehalogenase, haloalcohol dehalogenase, and epoxide hydrolase, finally yielding glycerol, which is further metabolized by the organism (34). The conversion of 2,3-dihalogenated propanols by the host strain AD1 is similar to that of a Flavobacterium sp. utilizing 2,3-dibromo-1-propanol (7).

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A high expression level of DhaA in strain AD1 increased the conversion rates of trihalogenated propanes. Due to the low expression level of DhaA in strain AD1(pTB1), growth was only observed on 1,2,3-tribromopropane, and the maximum concentration tolerated was about 0.7 mM (data not shown).

The conversion of 1,2,3-trichloropropane proceeded at a much lower rate. After 25 days of incubation, approximately 0.7 mM of 1,2,3-trichloropropane was converted, yielding a very small increase in OD. Transformation of the trihalogenated propanes was due to enzymatic activity, since its concentration decreased much more slowly in sterile controls (Fig. 3). During conversion of the trihalogenated propanes, the corresponding halopropanols accumulated. The brominated propanols were completely converted, while a low amount of 2,3-dichloro-1-propanol remained present in the culture. The results thus showed that the recombinant strain AD1(pTB3) was still able to convert about 46% of the 1,2,3-trichloropropane, whereas AD1 could use the environmental chemicals 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane as sole carbon and energy sources. The proposed degradation pathway of strain AD1(pTB3) is shown in Fig. 4. The complete dehalogenation of trihalogenated propanes involves the combined activities of haloalcohol dehalogenase, haloalcohol dehalogenase, and epoxide hydrolase, finally yielding glycerol, which is further metabolized by the organism (34). The conversion of 2,3-dihalogenated propanols by the host strain AD1 is similar to that of a Flavobacterium sp. utilizing 2,3-dibromo-1-propanol (7).

For growth on trihalogenated propanes, the kinetic properties ($K_m$ and $K_{cat}$) of DhaA for trihalopropanes are of major importance. The best substrate for DhaA, 1,2,3-tribromopropane, was rapidly degraded by strain AD1(pTB3), whereas degradation of 1,2-dibromo-3-chloropropane by AD1(pTB3), for which the dehalogenase has a lower affinity, was somewhat slower. This is probably due to a lower conversion rate of 1,2-dibromo-3-chloropropane by DhaA, since the concentration tolerated was about 0.7 mM (data not shown). Therefore, the dehalogenase produced by strain AD1 converted only the (R)-enantiomer of 2,3-dichloro-1-propanol, which also limited utilization of 1,2,3-trichloropropane. The stereospecific degradation of 2,3-dichloro-1-propanol by strain AD1 was similar to that of Pseudomonas sp. strain OS-K-29 (17).

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The fact that a catabolic route can be easily established indicates that the absence of organisms that utilize trihalogenated propane in the environment is due to the absence of dehalogenases for haloalcohols and for haloalkanes in a single organism. This could be due to the relatively recent entry of these compounds into the environment, giving microorganisms insufficient time to evolve appropriate pathways. The only example of an isolate which can produce both enzymes is a recently described isolate of Mycobacterium sp. strain GP1 which grows on 1,2-dibromoethane. This organism was obtained after prolonged adaptation and selection in batch culture (27).

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